



Please cite this report as: Anonymous 2015
CRI Group Annual Research Report for April 2014 to March 2015
Citrus Research International, Nelspruit

2014/15

TABLE OF CONTENTS

		Page
1	MARKET ACCESS TECHNICAL COORDINATION	1
	1.2 Japan	2
	1.3 USA	2
	1.4 China	3
	1.5 South Korea	3
	1.6 Vietnam	3
	1.7 Indonesia	3
	1.8 India	4
	1.9 Malaysia	4
	1.10 New Markets	4
	1.10.1 The Philippines	4
	1.10.2 Australia and Lebanon	4
	1.10.3 Imports	4
	1.10.3.1 Import conditions	4
2	BIOSECURITY AND REGULATIONS	4
3	PORTFOLIO: INTEGRATED PEST MANAGEMENT	5
	3.1 Portfolio summary	5
	3.2 Programme: False Codling Moth	8
	3.2.1 Programme summary	8
	3.2.2 Progress report: Development of mechanisms for the postharvest detection of cryptic pests in citrus fruit	12
	3.2.3 FINAL REPORT: Late season releases of <i>Trichogrammatoidea cryptophlebiae</i> for suppression of FCM	12
	3.2.4 PROGRESS REPORT: The use of entomopathogenic fungi to control the soil-dwelling life stages of false codling moth	27
	3.2.5 PROGRESS REPORT: Impact of abbreviated and complete cold-treatment on survival and fitness of FCM larvae	29
	3.2.6 PROGRESS REPORT: Large scale field trials with entomopathogenic nematodes for control of FCM, fruit fly and thrips	29
	3.2.7 FINAL REPORT: Gene expression analysis of <i>Thaumatotibia leucotreta</i> as result of different isolates of <i>Cryptophlebia leucotreta</i> granulosis virus	30
	3.2.8 FINAL REPORT: Behaviour, biology and survival of pupating false codling moth	34
	3.2.9 PROGRESS REPORT: Evaluation of 7-Vinyl-Decyl Acetate 1 for mating inhibition in FCM	38
	3.2.10 Final report: A survey of Lepidoptera infesting citrus fruit in the C. Cape Midlands	39
	3.2.11 FINAL REPORT: Determination of reapplication frequency of the <i>Cryptophlebia leucotreta</i> granulovirus to provide protection against FCM infestation of citrus	46
	3.2.12 FINAL REPORT: Using the larval parasitoid, <i>Agathis bishopi</i> , for detection of FCM infested fruit	48
	3.2.13 PROGRESS REPORT: A feasibility study on the use of sniffer dogs for detecting FCM infested fruit post-harvest	50
	3.2.14 PROGRESS REPORT: Classical biocontrol introduction of <i>Agathis bishopi</i> into the Western Cape	50
	3.2.15 PROGRESS REPORT: Laboratory handling and quality control for SIT: an experimental assessment of FCM chilling and flight performance with respect to the improvement of moth production parameters, particularly pertaining to improved cold-tolerance	51

TABLE OF CONTENTS

	Page	
3.2.16	PROGRESS REPORT: Novel approaches to mating disruption of FCM	51
3.2.17	PROGRESS REPORT: Movement of false codling moth (FCM) and fruit flies (FF) in multi-crop (citrus, stone fruit, grape, pomegranate) systems	52
3.2.18	PROGRESS REPORT: Improving the cold tolerance of false codling moth (<i>Thaumatotibia leucotreta</i>) for improved performance in a sterile insect release Programme	53
3.2.19	PROGRESS REPORT: Verification of proposed inspections standards within an FCM systems approach	54
3.2.20	FINAL REPORT: FCM infestation of packed lemons destined for export	55
3.2.21	PROGRESS REPORT: Identifying volatile emissions associated with false codling moth infestation of citrus fruit	62
3.2.22	FINAL REPORT: An audit of the efficacy of the sterile insect technique programme for false codling moth, <i>Thaumatotibia leucotreta</i> (Meyrick) (Lepidoptera: Tortricidae), in the Sundays River Valley	63
3.3	Programme: Fruit Fly	73
3.3.1	Programme summary	73
3.3.2	Progress report: Fruit fly rearing	75
3.3.3	PROGRESS REPORT: A new bait for more effective control of all <i>Ceratitidis</i> fruit flies	76
3.3.4	FINAL REPORT: Surveillance of <i>Bactrocera dorsalis</i> in commercial citrus orchards in South Africa	77
3.3.5	FINAL REPORT: Developmental threshold and critical thermal limits for two <i>Ceratitidis rosa</i> types in South Africa	90
3.3.6	FINAL REPORT: Develop a yeast autolysate attractant for fruit fly bait that is safe with copper and more palatable than hydrolysate	99
3.3.7	PROGRESS REPORT: Dispersal capacity of <i>Bactrocera dorsalis</i>	103
3.3.8	Progress report: Invasion and expansion of <i>Bactrocera dorsalis</i> in South Africa: a genetic analysis	104
3.3.9	PROGRESS REPORT: Utilisation of citrus and other fruit grown in South Africa by <i>Bactrocera dorsalis</i> previously recognized as <i>B. invadens</i>	104
3.3.10	PROGRESS REPORT: Detection methods for fruit flies of economic significance to fruit and vegetable production in Africa and Indian Ocean islands	105
3.3.11	PROGRESS REPORT: Evaluation of male annihilation treatments for control of <i>Bactrocera dorsalis</i>	106
3.4	Programme: Mealybug and other Market Access pests	106
3.4.1	Programme summary	106
3.4.2	FINAL REPORT: The morphology and ecology of the Carob moth in citrus orchards	107
3.4.3	PROGRESS REPORT: Evaluating GRAS post-harvest fumigants for phytosanitary pests	114
3.4.4	PROGRESS REPORT: The association of a lepidopteran borer complex between pecan nuts (<i>Carya illinoensis</i>) and citrus (<i>Citrus sinensis</i>) in the Vaalharts region	115
3.4.5	PROGRESS REPORT: Establishment of a monitoring system and control practices for carob moth on citrus	116
3.5	Programme: Non-Phytosanitary Key Pests	117
3.5.1	Programme summary	117
3.5.2	PROGRESS REPORT: Evaluation of entomopathogenic fungi against thrips and mealybug	117
3.5.3	PROGRESS REPORT: Short residual treatments for thrips, psylla, leafhoppers and woolly whitefly for late season usage	118
3.5.4	PROGRESS REPORT: Mating disruption for red scale control	119

TABLE OF CONTENTS

	Page	
3.6	Programme: Minor Pests and Mites	120
3.6.1	Programme summary	120
3.6.2	PROGRESS REPORT: Importing and releasing <i>Cales noacki</i> for the control of woolly whitefly	120
3.6.3	FINAL REPORT: Non-target effect updates	121
3.6.4	PROGRESS REPORT: Using banana odour as an attractant for monitoring fruit piercing moth in citrus orchards	130
4	PORTFOLIO: DISEASE MANAGEMENT	131
4.1	Portfolio summary	131
4.2	Programme: Graft Transmissible Diseases	133
4.2.1	Programme summary	133
4.2.2	PROGRESS REPORT: Cross-protection of Star Ruby using Beltsville sub-isolates of Nartia mild strain for the Orange River Valley	135
4.2.3	PROGRESS REPORT: The effect of different CTV sources in Valencias on different rootstock combinations for the Orange River Valley	136
4.2.4	PROGRESS REPORT: Cross-protection of Marsh and Star Ruby grapefruit by using the best field isolates collected in the different grapefruit production areas of southern Africa	136
4.2.5	PROGRESS REPORT: Identification of suitable <i>Citrus tristeza virus</i> sources for pre- immunising Turkey Valencia	137
4.2.6	PROGRESS REPORT: Searching for a <i>Citrus tristeza virus</i> source suitable for cross-protecting soft citrus	138
4.2.7	PROGRESS REPORT: Dynamics of <i>citrus tristeza virus</i> mild and severe strains in mild strain cross-protection strategies	138
4.2.8	FINAL REPORT: Differential cultivar selection or suppression of <i>Citrus tristeza virus</i> (CTV) genotypes	139
4.2.9	PROGRESS REPORT: Characterisation of <i>Citrus tristeza virus</i> variants and their influence on the symptom expression in the grapefruit host	160
4.2.10	PROGRESS REPORT: Evaluation of citrus material for greening resistance	161
4.2.11	PROGRESS REPORT: Further studies on alternative hosts of “ <i>Candidatus Liberibacter africanus</i> ” and related Liberibacters on tree members of indigenous Rutaceae	162
4.2.12	PROGRESS REPORT: Comparison of shoot tip grafted citrus with old clone Material	162
4.3	Programme: Fruit and Foliar Diseases	163
4.3.1	Programme summary	163
4.3.2	PROGRESS REPORT: Evaluation of new spray programmes for the control of <i>Alternaria</i> brown spot in the summer rainfall regions of South Africa	165
4.3.3	FINAL REPORT: Optimisation of fungicide spray applications in citrus orchards	165
4.3.4	FINAL REPORT: Control of <i>Botrytis cinerea</i> Pers. on lemons	202
4.3.5	PROGRESS REPORT: Development of a tree canopy characteristic calibration formula for reduced volume fungicide application in citrus orchards	218
4.3.6	PROGRESS REPORT: The use of adjuvants to improve fungicide foliar spray deposition and control of <i>Alternaria</i> brown spot on citrus	218
4.4	Programme: Soilborne Diseases	219
4.4.1	Programme summary	219
4.4.2	PROGRESS REPORT: The evaluation of different pre-plant products for the control of the citrus nematode, as part of an integrated nematode control approach in citrus replant situations	221

TABLE OF CONTENTS

	Page	
4.4.3	FINAL REPORT: Investigation into edaphic factors and their interactions on citrus tree decline	221
4.4.4	PROGRESS REPORT: Evaluation of alternative products for control of citrus nematode and <i>Phytophthora</i> spp. in citrus	235
4.4.5	PROGRESS REPORT: The status of <i>Armillaria</i> root rot and its management in South African citrus orchards	241
4.4.6	PROGRESS REPORT: Preventative and curative management of soilborne pathogens in citrus nurseries	242
4.5	Programme: Postharvest Pathology	242
4.5.1	Programme summary	242
4.5.2	FINAL REPORT: The JBT heated flooder as an alternative application method for fungicides in citrus packhouses	243
4.5.3	PROGRESS REPORT: Further optimisation of in-line aqueous fungicide application in citrus packhouses	255
4.5.4	PROGRESS REPORT: Optimisation of postharvest drench application of fungicides on citrus fruit	256
4.5.5	FINAL REPORT: Quantification of imazalil resistance in <i>Penicillium digitatum</i> populations in citrus packhouses	257
4.5.6	FINAL REPORT: Use of hot water, potassium silicate and biological control agents to reduce postharvest disease and chilling injury in citrus fruit	268
4.5.7	PROGRESS REPORT: Provision of an industry service whereby new packhouse treatments are comparatively evaluated, fungicide resistance is monitored and standardised recommendations are provided	269
4.5.8	FINAL REPORT: Identification and modelling of postharvest decay risk Indicators	289
4.6	Programme: Citrus Black Spot	297
4.6.1	Programme summary	297
4.6.2	FINAL REPORT: Monitoring ascospore releases in the Eastern Cape to determine the critical period for CBS infection	299
4.6.3	PROGRESS REPORT: Development of new spray programmes for the control of citrus black spot	313
4.6.4	FINAL REPORT: The global population structure and reproductive biology of the fungal pathogen, <i>Phyllosticta citricarpa</i> Kiely	317
4.6.5	FINAL REPORT: Improving the retention of suspension liquid phosphonate fungicides on citrus fruit and leaves	321
4.6.6	PROGRESS REPORT: Epidemiology and pest risk assessment of <i>Phyllosticta citricarpa</i>	327
4.6.7	FINAL REPORT: Identifying the fungi that cause CBS-like disease symptoms on citrus fruit	334
4.7	CRI Diagnostic Centre	339
5	PORTFOLIO: HORTICULTURE	340
5.1	Portfolio summary	340
5.2	Programme: Rind condition	340
5.2.1	Programme summary	340
5.2.2	PROGRESS REPORT: Studies on aspects concerning rind pitting/staining citrus fruit	341
5.2.3	PROGRESS REPORT: Effect of different chemical applications on development of Peteca spot in lemons	341
5.2.4	FINAL REPORT: The development of a rind disorder prediction model for citrus fruits based on climatic conditions	342

TABLE OF CONTENTS

	Page
5.2.5	PROGRESS REPORT: Investigating cold storage potential of new mandarin citrus selections/cultivars and the effect of ethylene degreening on rind disorders 345
5.3	Programme: Fruit Production and Quality 345
5.3.1	Programme summary 345
5.3.2	PROGRESS REPORT: A novel approach to water and nutrient management in citrus 346
5.3.3	FINAL REPORT: Study on the effect of humic and fulvic acids on fertiliser application in citrus 347
5.3.4	PROGRESS REPORT: Determining the time and duration of flower induction in early vs late mandarin cultivars and evaluating the effect of hand thinning, pruning and girdling on leaf and root carbohydrate levels, fruit size, vegetative regrowth and alternate bearing in Nadorcott mandarin 355
5.3.5	PROGRESS REPORT: Effect of shade net on fruit production of mandarin Citrus 356
5.3.6	PROGRESS REPORT: Effect of pruning on fruit production of Nadorcott mandarin 357
5.4	PROGRAMME: CULTIVAR EVALUATION 340
5.4.1	Programme summary 340
5.4.2	PROGRESS REPORT: Evaluation of Valencia selections in hot humid inland areas (Onderberg) 341
5.4.3	PROGRESS REPORT: Evaluation of Valencia selections in the hot dry inland areas (Letsitele & Hoedspruit) 345
5.4.4	PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the hot inland areas (Letsitele & Malelane) 354
5.4.5	PROGRESS REPORT: Evaluation of Valencia selections in the hot inland areas (Swaziland) 359
5.4.6	PROGRESS REPORT: Evaluation of Valencia selections in the intermediate production areas (Tom Burke) 362
5.4.7	PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the intermediate production areas (Marble Hall & Tom Burke) 363
5.4.8	PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the hot dry inland areas (Tshipise) 367
5.4.9	PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the cool inland areas (Burgersfort) 369
5.4.10	PROGRESS REPORT: Evaluation of Delta Valencia on new imported rootstocks in the Marble Hall area 372
5.4.11	PROGRESS REPORT: Evaluation of Valencias on new imported rootstocks in the Malelane area 378
5.4.12	PROGRESS REPORT: Evaluation of various Navel selections on different rootstocks in the Burgersfort and Marble Hall area 381
5.4.13	PROGRESS REPORT: Evaluation of various Valencia selections on different rootstocks in the Komatipoort area 384
5.4.14	PROGRESS REPORT: Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region (Western Cape) 390
5.4.15	PROGRESS REPORT: Cultivar characteristics and climatic suitability of Clementine mandarins in a cold production region (Western Cape) 393
5.4.16	PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (East Cape Midlands) 394
5.4.17	PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Sundays River Valley) 396
5.4.18	PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Gamtoos River Valley) 398
5.4.19	PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Western Cape) 403
5.4.20	PROGRESS REPORT: Cultivar characteristics and climatic suitability of navel oranges in a cold production region (Sundays River Valley) 407

TABLE OF CONTENTS

	Page
5.4.21 PROGRESS REPORT: Cultivar characteristics and climatic suitability of experimental navel oranges in a cold production region (Gamtoos River Valley)	409
5.4.22 PROGRESS REPORT: Evaluation of Valencia selections in a semi-desert production area (Kakamas)	411
5.4.23 PROGRESS REPORT: Cultivar characteristics and climatic suitability of experimental Navel oranges in a semi-desert region (Kakamas)	413
5.4.24 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a semi-desert production region (Kakamas)	414
5.4.25 PROGRESS REPORT: Cultivar characteristics and climatic suitability of lemons in a semi-desert production region (Kakamas)	416
5.4.26 FINAL REPORT: Establishment of a molecular citrus genotype reference database for citrus cultivar verification within the Citrus Improvement Scheme	418
5.5 Climatic Regions of Southern Africa and cultivars being evaluated	470
5.6 Approximate maturity periods	473
6 CITRUS IMPROVEMENT SCHEME (CIS)	481
6.1 Budwood	481
6.2 Seed	487
6.3 Production	488
6.4 Tree Certification	488
6.5 Nursery Certification	491
6.6 Statutory Improvement Scheme	493
6.7 Protective zone surrounding the Citrus Foundation Block	493
6.8 Shoot tip grafting (STG), pre-immunisation and nucleus block management	493
6.9 Diagnostic services for graft transmissible diseases	497
6.10 Citrus Biosecurity Activities	507
6.10.1 African Greening Citrus surveys	507
6.10.2 Asiatic Citrus Greening (HLB) and Asian Citrus Psyllid (ACP) surveys	507
6.10.3 Citrus Biosecurity in Africa	508
7 INTERNATIONAL VISITS	509
7.1 G.C. Schutte	509
7.2 A. Manrakhan	523
8 VOORLIGTING / EXTENSION	537
8.1 Transformation Managers' Annual Report	540
8.2 Research Priorities	543
8.3 Study Group Chairmen 2014-15	556
8.4 The relative funding support for research Portfolios and Programmes for 2014-15	557
8.5 Extension presentations by CRI researchers in 2014-15	559
8.6 Other means of Technology Transfer	563
8.6.1 S.A. Fruit Journal	563
8.6.2 CRI Website	563
8.6.3 CRInet	564
8.6.4 CRI Cutting Edge	564
9 PUBLICATIONS IN 2014-15	565
9.1 Refereed Publications (or ISI ranked journals)	565
9.2 Semi-scientific publications	566
10 PRESENTATIONS AT SOCIETAL AND INTERNATIONAL CONGRESSES	566

1 MARKET ACCESS TECHNICAL COORDINATION

By Vaughan Hattingh and Elma Carstens - CRI

1.1 EUROPE (EU)

The 2014 export season started with the threat that DG SANCO might strengthen the CBS import conditions applicable to the export of citrus fruit from South Africa to the EU for the 2014 citrus export season. The threat that citrus exports may be banned if the threshold of five interceptions for CBS is exceeded also remained. Additional measures were developed and on 27 May 2014 the EU Standing Committee on Plant Health voted in favour of introducing these additional measures. Technical inputs were made to avoid some of the more problematic aspects introduced into the various draft versions. The new set of measures was published in the EC's Official Journal on 03 July 2014 for application as of 24 July 2014 and the additional measures included compulsory pre-harvest ethephon testing of Valencia oranges from areas where CBS occurs, specified sampling and inspection intensities and specific additional declarations on the phytosanitary certificates. A series of grower meetings were held to inform affected growers and facilitate arrangements between growers and DAFF to ensure the industry's ability to comply with these measures. The CBS-RMS for the 2014 season was also amended to include the new set of additional measures.

South Africa received the first notification of a CBS interception for the 2014 season in July. The EU however informed South Africa that only CBS interceptions for citrus imported under the new conditions of Decision 2014/422 (effective from 24 July) will be taken into account for the 2014 export season (as far as the threshold of 5 interceptions is concerned). South Africa was however requested to provide an evaluation report on all the interceptions. Concerns were raised by the industry regarding the identification of CBS in the EU and the methodology/procedures used in the laboratories especially with regard to the viability of the pathogen and Dr Tian Schutte was sent to the EU to investigate. A report of the visit was provided to SA-DAFF and CGA for further engagement with the EU regulators.

In September when the fourth notification of a CBS interception was received the industry decided to suspend further exports of citrus fruit from areas not declared CBS-free (other than Mandarins) to the EU. As of 8 September only the following fruit was permitted for export to the EU – consignments that were inspected and certified before the date, Mandarins and fruit from CBS PFA areas officially recognised within South Africa by SA-DAFF. To further reduce the risks of CBS interceptions in the EU, an on-arrival phytosanitary inspection procedure was implemented in Netherlands ports for shipments that were already on the water when this decision was taken.

A total of 28 CBS interceptions in the EU were recorded for the 2014 export season. Concerns were again raised about the viability of the pathogen and technical arguments around the need to verify viability in CBS regulatory inspections were developed for use in engagements with EU regulators. These concerns bring into question the reliability of the 28 CBS interceptions reported by the EU. Inputs were made to all of the 28 CBS interception reports that were provided to EU by SA-DAFF.

In September 2014 a request was received from EFSA to enter into discussion with the International CBS Expert Panel on scientific issues pertaining to the EFSA CBS Scientific Opinion. Inputs on how to proceed with this request were obtained from the Regional Coordinators of the CBS Expert Panel and EFSA was advised that the view of the CBS Expert Panel is that although the Panel is not opposed to further dialogue, the timeframe and framework given by EFSA is problematic for entering into a meaningful dialogue. Furthermore EFSA's terms of reference for dialogue "to reduce uncertainty" but not to revisit the PRA precludes meaningful engagement, unless EFSA is prepared to revise the PRA. In January 2015 EFSA published a response to the International CBS Expert Panel's comments on the EFSA CBS PRA. EFSA continues to dismiss the comments made by the international expert panel.

In October 2014 industry meetings took place to prepare for the next export season. The CBS Risk Management System was amended to include additional measures pertaining to the inspections of orchards from which fruit will be degreened, the re-instatement of blacklisted PUCs and the special handling of the first crop of lemons in the Eastern Cape province. A spray program for the control of CBS in each of the citrus production areas per fruit type was developed and submitted to SA-DAFF in October 2014 as a tool to be used during the orchard verification process. In January further inputs were made to the CBS Risk Management System for the 2015 citrus export season and the final document was distributed by SA-DAFF on 23 January 2015 for implementation.

Steps were taken by the Secretariat of the IPPC to get the CBS dispute process between the EU and SA going by calling for experts to serve on the Expert CBS Committee. Three experts were nominated by SA and the relevant information was submitted via the SA official IPPC contact point. Progress with the IPPC

CBS dispute resolution process has been frustrated by objections that EU has lodged regarding the process and terms of reference. The IPPC secretariat proposed 3 names for the IPPC Expert Panel, but the EU objected. The IPPC launched another call for nominations of experts and the IPPC secretariat has facilitated bilateral discussions between SA and the EU to make progress, but little has been achieved to date. By the end of this reporting period no agreement could be reached by the two countries on the constitution of the Expert CBS committee. Meetings were held with members of the CBS Core Drafting Team to develop the documentation in anticipation of international CBS dispute settlement procedures.

A talk on "Citrus Black Spot – a global perspective" was presented by Dr Paul and his co-authors (included CBS specialists from South Africa, Australia, China, Brazil and USA) at the CLAM meeting in October 2014 in Madrid.

In December a FCM Disaster Management Committee meeting was held as a result of the 18 FCM interceptions that were reported in the EU in 2014. A document titled - A proposal for the regionalised implementation of plant health regulations for citrus fruit imported into the European Union from countries where specified citrus pests and diseases occur and that are of plant health concern to citrus producing Member States of the EU – was drafted after the FCM Disaster Management Committee held in December 2014. The implementation of such a system of regionalised implementation would provide a solution to the phytosanitary regulations that threaten continued access to the EU market, but there is resistance to the concept within the EU.

An EU-FVO delegation visited South Africa to audit the SA-CBS Risk management system from 23 February - 6 March 2015. Production units and packhouses in Limpopo, Mpumalanga and Eastern Cape were visited. A wrap-up meeting between the FVO delegation, CGA, CRI and SA-DAFF was held and further technical information was requested by the FVO on the Ethephon testing. By the end of this reporting period a final report from the EU-FVO team was still outstanding (subsequently the report was released and acknowledged the good system operated by South Africa).

1.2 JAPAN

Two issues remained pending for this market. The first outstanding issue was the broadening of access for soft citrus cultivars to include all other SA mandarins in addition to Clementines. In May 2014 information was submitted to SA-DAFF to address the unreasonable request by Japan-MAFF to submit Mediterranean fruit fly cold treatment efficacy data for each of the 32 mandarin cultivars. SA-DAFF submitted this information to Japan-MAFF in August 2014. The second outstanding issue was the adoption of an improved cold treatment condition for the export of all citrus types. The report on the experimental work requested by Japan (repeat of phase 2) to clarify aspects of the experimental results previously supplied by SA was provided to SA-DAFF in June 2014. This report was submitted to Japan-MAFF in September 2015. No feedback was received from Japan-MAFF on both issues by the end of this reporting period despite follow up requests made by SA-DAFF.

1.3 USA

During the previous reporting period one of the long standing issues, the reversion of the FCM cold treatment period from 24d to 22d, has been addressed. Citrus fruit was exported during the 2014 season within a pilot program which entailed the following: a 22-day cold treatment with the current biometric sampling and inspection procedures and the current FCM threshold of 7 larvae per consignment, but with an additional 200 asymptomatic fruit that needs to be cut per consignment to further determine the infestation level of FCM. Meetings were held between all role players to revise the workplan for the 2014 season to include the details of the pilot program and the revised document was provided by SA-DAFF to USDA-APHIS in May 2014. Information has also been provided to SA-DAFF in order to revise the USA list of actionable pests that are included in the workplan. A report on the pilot protocol revealed that the program was successfully completed and that no FCM was found during the cutting of the asymptomatic fruit. Trial consignments were also successfully sent through the Houston port.

The other three long outstanding issues namely (1) equivalence between USA domestic CBS regulations and USA import regulations (access to USA for all SA production areas), (2) expansion of CBS pest free areas to include the whole of the W Cape in the work plan and (3) adoption of CBS pest free places of production (N Limpopo region) remain pending. There was however progress on the request for equivalence between USA domestic CBS regulations and USA import regulations (access to USA for all SA production areas) - on 28 August USDA-APHIS published a proposed rule to allow fresh citrus fruit from areas in South Africa where CBS is present, under conditions equivalent to the USA domestic CBS regulations. This publication provided for submission (by 27 October) of public comments on the proposed rule. CRI

generated a science-based comment that was submitted by CGA. However by the end of this reporting period no decision had been taken by USDA-APHIS.

In July 2014 the USA intercepted live wood borers in pallet bases on citrus exports from SA. Meetings were held between WCCPF, CRI, CGA, SA-DAFF and USDA-APHIS to solve this problem. In order to off-load the fruit, USDA-APHIS requested that re-palletizing be done in the USA for two shipments. In South Africa it was decided to use plastic pallets for all further shipments from South Africa. A pallet task team was appointed to investigate the problem and a confidential report was compiled and submitted to SA-DAFF. SA-DAFF revealed that the problem was not due to re-infestation but to incomplete treatment of the pallets. All service providers (manufacturers and disinfection organisations) in the country were audited by SA-DAFF to check compliance with ISPM15 and some were deregistered. A meeting was held between CRI, WCCPF and SA-DAFF to consider proposals for an accredited wooden USA pallet of superior quality and phytosanitary status, to be utilized to safeguard this program in 2015 and onwards.

1.4 CHINA

The acceptance of non-containerised bulk shipping and a systems approach for FCM remains the outstanding issues for this market. In order to address this long outstanding issue pertaining to the systems approach for FCM, CRI engaged with the Chinese Academy of Agricultural Science to investigate the prospect of revitalising a dormant research cooperation agreement which was signed in 2005 between CRI-South Africa and China's Citrus Research Institute. In industry meetings held during this reporting period, it was agreed that further development and validation of the systems approach for FCM that was not accepted by the Chinese authorities in 2012 could be undertaken as a research collaboration research project between the two Institutes. A revised MOU between CRI and China-CRI was signed in February 2015 and CRI informed SA-DAFF about this signed contract and the proposed projects. The projects will focus on alternatives to the FCM cold treatment that is currently required for export of fresh citrus fruit to China which is too harsh to export lemons. The request for the acceptance of non-containerised bulk shipping is still pending and information was again provided to SA-DAFF. They indicated that more technical information is needed and by the end of this reporting period CRI was in the process of addressing these concerns.

1.5 SOUTH KOREA

Two issues remained problematic for this market - the clarification/interpretation of point 5.1 of the amended export protocol and the inclusion of mandarins in the range of citrus types that can be exported to this market. There were further discussions between industry, SA-DAFF and the South Korean authorities on the problematic clause 5.1 in the amended export citrus protocol. In August 2014 the South Korean authorities advised that they accept the SA proposals and that wrapping of the pallets in the packing house for transport to the port is no longer a requirement. Packhouses are now required to intensify trapping for fruit flies and FCM by installing insectors and more traps. SA's application for inclusion of mandarins in the S Korean export program remained pending by the end of this reporting period.

1.6 VIETNAM

In August 2014 the first Draft PRA Report on Importation of Fresh Sweet Orange Fruit *Citrus sinensis* from South Africa into Vietnam was received after the pest list was submitted in August 2013. The draft PRA lists 6 quarantine pests, 5 with high risk and 1 with medium risk rating potentials. However some of these pests are not pests of citrus fruit and therefore not associated with the pathway (fresh citrus fruit). Information was provided to SA-DAFF in September 2014 to get these pests removed from the Vietnamese quarantine list for fresh citrus fruit from South Africa. In December 2014 SA again received feedback from the Vietnam authorities declining South Africa's requests. CRI provided further information to SA-DAFF in December 2014 to support the removal of these pests from the quarantine list. This information was provided to the Vietnamese Authorities by SA-DAFF in February 2015. By the end of this reporting period no further feedback was received from Vietnam.

1.7 INDONESIA

South Africa exported fresh citrus fruit to this market on a permit system. In June 2014 an urgent request was received from SA-DAFF to provide technical details on integrated pest management systems pertaining to specific citrus pests in South Africa. In September 2014 the Indonesian Authorities requested more information about FCM especially with regard to the host status of lemons.

1.8 INDIA

SA-DAFF submitted a request to India in the previous reporting period to remove *Phyllosticta citricarpa* from their import conditions pertaining to fresh citrus fruit from South Africa as scientific articles indicated that this pest does occur in India. In May 2015 feedback was received from the Indian authorities indicated that they will not remove this pest from their import conditions as according to their records *Phyllosticta citricarpa* is not present in India.

1.9 MALAYSIA

Information was obtained that Malaysia has reviewed their import conditions and that as from the next export season a Phytosanitary Certificate and an Import Permit will be required. This information was provided to producers and exporters at various industry meetings.

1.10 NEW MARKETS

1.10.1 The Philippines

A Philippine delegation visited the South African Citrus Industry in August 2014. Orchards and packhouses were visited in the Limpopo, Mpumalanga and Western Cape Provinces. Outstanding issues pertaining to pests and pathogens were discussed as in June 2014 the Philippines indicated that they don't agree with the requests from SA to delete some of the pests and pathogens from the quarantine list pertaining to import of fresh citrus fruit from SA. It was made clear during the visit that South Africa does not currently have access to this market and that no consignments will be allowed to enter until the market is officially opened. In January 2015 the Philippine Authorities requested further information on pest management strategies. They specifically asked for information on fruit fly trapping and strategies pertaining to pests other than fruit flies and FCM. CRI provided the information to SA-DAFF in January and in February 2015 a revised draft PRA was received from the Philippines. CRI studied the document and identified that the draft PRA needs considerable revision. These concerns were highlighted in feedback provided to SA-DAFF in March 2015 with a recommendation that SA-DAFF indicate to the Philippine Authorities that phytosanitary requirements need to be scientifically justified.

1.10.2 Australia and Lebanon

No feedback was received from these countries during the last four years, despite several follow ups made by SA-DAFF. On the basis that none of these markets are identified by producers as new market opportunities and on confirmation by FPEF, these two markets are no longer discussion points at the Market Access Working Group for Fresh Fruit and Vegetables.

1.10.3 Imports

1.10.3.1 Import conditions

By the end of this reporting period revision of the import conditions for Citrus vegetative propagation material was still pending. A draft set of import conditions for seed was received for inputs in February 2015.

2 BIOSECURITY AND REGULATIONS

The exotic fruit fly *Bactrocera dorsalis* (previously known as *Bactrocera invadens*) is considered to be present in specified regions, actionable and under official control in South Africa. In February 2015 a report was sent to the IPPC on detection of incursions of *B. invadens* in South Africa in three municipal districts of the Northern Cape province. Quarantine and eradication measures were implemented in accordance with the relevant national action plan. No further detections of *B. dorsalis* were found for more than 12 weeks, or three life cycles, after the last fruit fly had been detected. The status in these districts is now Absent: Pest eradicated or no longer present.

In January 2015 a monitoring survey was conducted in some of the magisterial districts of the Western Cape province to maintain official recognition of the CBS pest free status of the region. At the end of the report period the laboratory report was still outstanding from SA-DAFF.

In June 2014 the following amendments to Regulation R110 in terms of the Agricultural Pests Act 1983 (Act No.36 of 1983) was published. The amendments included the compulsory notification of the occurrence of listed quarantine pests in pest free areas, the declaration of the whole Western Cape province as a CBS pest free area, the inclusion of the presence of *B. dorsalis* in the Vhembe district of the Limpopo province and the establishment of buffer zones to protect the Citrus Greening free status of the Eastern Cape

province. Amendments to Regulation R1013 were not published by the end of this reporting period. The new Plant Health (Phytosanitary) Bill, that is a revision of the Agricultural Pests Act 1983 (Act No.36 of 1983) was published in the Government Gazette in October 2014.

In a detection survey conducted in 2013 in some of the magisterial districts of the Western Cape to monitor the spread of African citrus greening disease within the province, positive trees were found in the Malmesbury district. These trees were removed and in 2014 a delimiting survey was conducted. By the end of this reporting period no report was available yet but some of the samples did test positive for African citrus greening disease. A delimiting survey was also conducted in the Eastern Cape magisterial districts of OR Tambo, Alfred Nzo to determine the spread of the pathogen in these areas and no positive trees were found. A project planning meeting was held to prepare a submission to SA-DAFF's Disaster Management division in order to get funding for early warning detection surveys in South Africa for Asian citrus greening disease. The plan was submitted in February 2015.

In 2012 South Africa was notified by the Chinese Authorities that *Xanthomonas axonopodis* (Citrus canker) had been detected in seed consignments imported from South Africa. The seed was returned to South Africa. Laboratory tests were conducted on the seeds and the nursery from which the seed originated was inspected, sampled and tested. The results confirmed the continued absence of this pathogen in SA. SA-DAFF provided the test report to the Chinese Authorities in June 2014.

3 PORTFOLIO: INTEGRATED PEST MANAGEMENT

3.1 PORTFOLIO SUMMARY

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

With each year that passes, the entomological challenges that the citrus industry faces seem to increase and expand. Granted, the current headline act in southern African citrus research is Citrus Black Spot (CBS). However, it would be naïve to simply focus on immediate hurdles and not be sufficiently foresighted to prepare for the impediments of tomorrow. This is the philosophy that the IPM Portfolio has strived to adopt over the last several years. For example, it is highly likely that more stringent export protocols pertaining to FCM will be the next challenge to follow CBS. Within the IPM research portfolio, CRI and its strategic partners (particularly Rhodes, Stellenbosch, Pretoria and Free State Universities) have dedicated a tremendous amount of effort, not only to increasing FCM research output but more importantly, strategically devising viable alternatives to current phytosanitary export protocols for FCM.

Another significant entomological challenge has been the arrival, regional establishment and continuing spread of the oriental fruit fly, *Bactrocera dorsalis*. The discovery that the fly is not *B. invadens*, as originally believed, does somewhat reduce its phytosanitary status, but certainly not its economic pest status and thus the need to monitor for and control it diligently.

A third challenge has been the erosion of IPM, inflicted by routine sprays for CBS. Not only does the most commonly used CBS fungicide, mancozeb, suppress predatory mite levels (the most important thrips natural enemy), but growers will often include a complement of insecticides with CBS sprays in order to save spraying costs, thus reducing commitment to monitoring based decision making, a cornerstone of IPM. This negative trend has been exacerbated by the arbitrary demand from some export market retailers for lower MRLs than the scientifically determined ones regulated by their governments and for fewer residues on fruit. This not only undermines IPM, but also responsible pesticide resistance management.

CRI is also preparing for the imminent threat of the Asian citrus psyllid, *Diaphorina citri*, which transmits Asian citrus greening, which is far more virulent than African citrus greening, transmitted by the African trioza, *Trioza erytraea*. This is being conducted through the appointment of a Biosecurity Manager and by scheduling collaborative research in countries where Asian greening and its vector occur.

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

As has been the case for the last few years, the FCM programme has expectedly attracted more funding than the others. During the past year, 21 projects were conducted within the programme. Seven of these very importantly focussed on post-harvest research. Of these, six were focussed on post-harvest detection and only one focussed on post-harvest control. When the results of this last project, which focussed on partial cold-treatments, were combined with an inspection standards (pre- and post-harvest) project, a systems approach was developed, which offered a higher level of risk mitigation than required by a post-harvest disinfestation treatment, such as cold-sterilisation.

The 14 pre-harvest focussed research projects within the FCM programme covered four major areas: biological control (including microbial control), sterile insect technique (SIT), mating disruption (or inhibition) and ecology of the pest. Although many of these projects were exciting and promise meaningful contribution to FCM control in the future, possibly the shining light was the project that investigated the use of entomopathogenic fungi for control of soil-dwelling life stages of FCM, revealing consistently significant efficacy.

The focus of the fruit fly programme in the year 2014-2015 was on optimising the pre-harvest control measures for the different fruit fly pests and gathering data on the biology and ecology of these pests. The projects within the programme were well balanced between the Oriental fruit fly and the *Ceratitis* fruit flies, based on their current importance and gaps in our knowledge, particularly pertaining to monitoring and control. A few of the important findings within the programme were that all available male annihilation (MAT) products available for Oriental fruit fly control were of similar efficacy; that GF-120 was inclined to cause burn on Nadorcott Mandarins when the fruit were green; and that distinct warm and cold type Natal fly groups existed, possibly being different species.

Within the mealybug and other phytosanitary pests programme, most projects focussed on carob moth. Although carob moth is not a phytosanitary pest for most markets, this is not the case for China. This focus on carob moth is an indication of the recent realisation that a) levels of this pest are sometimes higher than expected and occasionally even higher than FCM, and b) differentiation between carob moth and FCM larvae is not always easy. However, the project within this programme that produced the most exciting findings during the last year is one that examined GRAS compounds for post-harvest fumigation of a range of phytosanitary pests on fruit, with particularly promising results against FCM and grain chinch bug.

The project on non-phytosanitary key pests examined the use of entomopathogenic fungi against thrips and mealybug, mating disruption against red scale and the use of short residual chemicals against woolly whitefly. The latter is to be expanded against thrips, psylla and leafhoppers, with the objective of identifying IPM compatible options that can be used late in the season.

The final programme within the IPM portfolio is that dedicated to minor pests and mites. In this programme a parasitoid was imported and released for classical biological control of the woolly whitefly. Positively, some recovery of the parasitoid from the field was made. Non-target bioassays with some relatively new insecticides were conducted, revealing some rather detrimental effects with Pynex and Delegate. Finally, work continued on the development of a monitoring system for fruit piercing and sucking moths.

During the research year in question CRI entomologists and many of the other entomologists working within this programme, participated actively in scientific meetings both locally (including the Citrus Symposium) and internationally, emphasising to the international scientific community the quality and relevance of research coming out of this team. Additionally, a number of papers were published in top international scientific peer-reviewed journals and in our local fruit journal. CRI entomologists also participated actively in carrying the important messages emanating from their research over to the grower community – this particularly through study group meetings and Cutting Edge publications.

PORTEFEULJEOPSOMMING

Met elke jaar wat verbygaan, vermeerder die entomologiese uitdagings vir die sitrusbedryf. Dit is seker 'n feit dat die huidige hoofrol in suidelike Afrika se sitrus navorsing tans deur Sitruswartvelk geneem word. Dit sal egter naïef wees om eenvoudig op onmiddellike struikelblokke te fokus en nie genoegsaam voorsigtig te wees om vir die hindernisse van more voor te berei. Hierdie is die filosofie wat die IPM Portefeulje gepoog het om te omhels oor die laaste paar jare. Byvoorbeeld, dit is hoogs waarskynlik dat strenger uitvoer protokolle wat VKM betref, die volgende uitdaging na Sitruswartvlek sal wees. Binne die IPM navorsings portefeulje het CRI en sy strategiese vennote (veral Rhodes, Pretoria, Stellenbosch en Vrystaat Universiteite) 'n geweldige poging ingesit om nie net VKM navorsings uitsette te vermeerder nie, maar mees belangrik, om strategiese billike alternatiewe vir huidige fitosanitêre uitvoer protokolle vir VKM te ontwikkel.

Nog 'n beduidende entomologiese uitdaging is die aankoms, streeksverbonde vestiging en aanhoudende verspreiding van die Oosterse vrugtevlieg, *Bactrocera dorsalis*. Die ontdekking dat die vlieg nie *B. invadens* is nie, soos oorspronklik geglo, verminder tot 'n mate sy fitosanitêre status, maar beslis nie sy ekonomiese plaagstatus nie en daarom bestaan daar nogsteeds 'n sterk behoefte om daarvoor te montior en die vlieg te beheer.

’n Derde uitdaging is die erosie van IPM wat deur die roetiene bespuitings vir Sitruswartvlek veroorsaak word. Die mees algemene swamdoder wat teen swartvlek gebruik word, mankozeb, is baie nadelig vir roofmyte (die belangrikste natuurlike vyand van sitrusblaaspootjie). Nog ’n probleem is dat produsente soms ’n verskeidenheid insekdoders saam met swartvlek bespuitings insluit om bespuitings kostes te bespaar. Hierdie verminder die behoefte vir monitering-gebaseerde besluitneming, ’n hoeksten van IPM. Hierdie negatiewe tendens word verder vererg deur die arbitrêre vereistes van sekere uitvoer mark handelaars vir laer MRLe (maksimum residu beperkings) as die wetenskaplik bepaalde standaard wat deur hulle regerings gereguleer is, en ook ’n vermindering in die hoeveelheid residue op vrugte. Hierdie ondermyn nie net IPM nie maar ook verandwoordelike weerstandbiedendheids bestuur.

CRI berei ook voor vir die nakende dreiging van die Asiatiese sitrubladvloeï, *Diaphorina citri*, wat Asiatiese vergroenings siekte oordra. Dit is dramaties meer virulent as Afrika sitrus vergroenings siekte wat deur die Afrika bladvloeï, *Trioza erytraea*, oorgedra word. Hierdie word aangepak deur die aanstelling van ’n Biosekuriteits Bestuurder en deur beplanning van navorsings samewerking in lande waar Asiatiese vergroening en sy vektor voorkom.

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

Soos wat die geval oor die laaste paar jaar was het die VKM program, soos verwag, meer bevondsing as die ander programme gelok. Gedurende die laaste jaar is 21 projekte in die program uitgevoer. Sewe van hierdie het baie belangrik op na-oes navorsing gefokus. Van hierdie is ses gefokus op na-oes opsporing en net een het op behandeling gefokus. Wanneer die resultate van hierdie laasgenoemde projek, wat op onvoldoende koue behandelinge gefokus het, met ’n inspeksie standaard (voor- en na-oes) projek gekombineer is, is ’n stelsels benadering ontwikkel, wat ’n hoër vlak van risiko vermindering aangebied het as wat van ’n na-oes ontsmettings behandeling, soos koue-sterilisasie, vereis word.

Die 14 voor-oes gefokusde navorsings proewe binne die VKM program het vier hoof areas gedek: biologiese beheer (insluitend mikrobiële beheer), steriele insek tegniek (SIT), paringsontwrigting (of inhibisie) en ekologie van die plaag. Alhoewel baie van hierdie projekte opwindend is en belowe beduidende bydraes tot VKM beheer in die toekoms, waarskynlik tans die helderste lig is die projek wat die gebruik van entomopatogeniese swamme vir beheer van ondergrondse lewensstadiums van VKM ondersoek het, wat konstant goeie werking gewys het.

Die fokus van die vrugtevlëë program in die 2014-15 jaar was die optimalisering van die voor-oes beheer maatreëls vir die verskillende vrugtevlëë plae en versameling van data op die biologie en ekologie van hierdie plae. Die projekte binne die program is goed gebalanseer tussen Oosterse vrugtevlëë en die *Ceratitidis* vrugtevlëë, gebasseer op huidige belangrikheid en tekortkominge in ons kennis, veral wat monitering en beheer betref. ’n Paar van die belangrike bevindinge binne die program is byvoorbeeld: alle beskikbare mannetjie uitwissings tegniek produkte vir Oosterse vrugtevlëë het soortgelyke doeltreffendheid gewys; GF-120 is geneig om brand op Nadorcott Mandaryne te veroorsaak wanneer vrugte nog groen was; en duidelike warm en koue tipe Natalse vrugtevlëë groepe bestaan, heel moontlik verskillende spesies.

Binne die witluis en ander fitosanitêre plae program, het meeste projekte op karobmot gefokus. Alhoewel karobmot nie ’n fitosanitêre plaag vir meeste markte is nie, is hierdie nie vir China die geval nie. Die fokus vir karobmot is ’n aanduiding van die onlangse waarneming dat a) vlakke van hierdie plaag is soms hoër as wat verwag word en soms selfs hoër as VKM, en b) onderskeiding tussen karobmot en VKM larwes is nie altyd maklik nie. Die projek in hierdie program wat in die laaste jaar egter die mees opwindende bevindinge tot vore gebring het is een wat GRAS samestellings vir na-oes berokking van ’n reeks fitosanitêre plae op vrugte ondersoek het, met veral belowende resultate teen VKM en graanstinkbesie.

Die projek op nie-fitosanitêre sleutel plae het die gebruik van entomopatogeniese swamme teen blaaspootjie en witluis ondersoek, ook paringsontwrigting teen rooidopluis en die gebruik van kort residuele chemikalië teen wollerige witluis. Laasgenoemde projek gaan uitgebrei word om ook blaaspootjie, bladvloeï en bladspringsers te dek, met die doel om IPM verenigbare opsies te identifiseer wat laat in die seisoen gebruik kan word.

Die laaste program in die IPM portefeulje is dié wat aan minder belangrike plae en myte toegewy word. In hierdie program is ’n parasiet ingevoer en losgelaat vir klassieke biologiese beheer van wollerige witvlëë. Die goeie nuus is dat van die parasiete wel al van die veld herwin is. Nie-teiken biotoetse met sekere relatief nuwe insekdoders is uitgevoer, en dié het ’n paar redelike nadelige effekte met Pynex and Delegate gewys. Ten slotte, is werk voortgesit met die ontwikkeling van ’n moniterings stelsel vir vrugte-steek en vrugte-suig motte.

Gedurende die laaste navorsingsjaar het CRI entomoloë en verskeie ander entomoloë wat binne die program werk, aktief deelgeneem aan plaaslike (insluitend die Sitrusposium) en internasionale wetenskaplike kongresse. Dit het die gehalte en relevansie van die navorsing wat uit dié navorsingsspan gekom het vir die internasionale wetenskaplike gemeenskap beklemtoon. Verder is 'n hele paar artikels in top internasionale wetenskaplike eweknie-resenseerde joernale asook in ons plaaslike vrugtejoernaal gepubliseer. CRI entomoloë het ook aktief deelgeneem in die oordra van belangrike informasie wat uit hulle navorsing gekom het aan die produsente gemeenskap. Hierdie is veral deur produsentestudiegroepe en Snykant publikasies gedoen.

3.2 PROGRAMME: FALSE CODLING MOTH

Programme coordinator: Sean D Moore (CRI)

3.2.1 Programme summary

FCM research remained the number one entomological research priority within the IPM Portfolio during the 2014/15 research cycle. With the completion of the European and Mediterranean Plant Protection Organisation (EPPO) Pest Risk Assessment (PRA) on FCM just over a year ago and the findings that South African citrus exports could pose a pathway risk for FCM for the EPPO region, expectations of more stringent regulatory export protocols in the near future have been elevated. Not wanting to be saddled with similar difficulties as are currently being experienced with Citrus Blackspot (CBS) and the European Union (EU), the FCM research programme has sought to develop potential solutions to pre- and post-harvest management of the pest as rapidly as possible, with a strong focus on options that can be presented to export markets as viable alternatives to conventional cold sterilisation.

Here we report on 21 different projects within the FCM programme, two of which were not funded by CRI, but were nevertheless conducted by students forming part of the greater citrus IPM research team. Seven of these projects entirely or partly investigate post-harvest solutions to FCM management. The remainder cover pre-harvest management research.

Of the seven post-harvest focussed projects, six examined detection or mechanisms for detection of FCM infested fruit. The first proposed to explore a number of possible technologies for detection of cryptically infested fruit, but focussed very strongly on X-ray detection (3.2.2). Refinement of micro-focus X-ray tomography succeeded in reducing scanning time per fruit to 34 seconds. The exact efficacy of the scan is still being evaluated. Another technology that was investigated was the use of the larval parasitoid, *Agathis bishopi*, to behaviourally indicate FCM infested fruit (3.2.12). In Y-tube olfactometer trials, female parasitoids responded positively to both infested fruit and certain associated volatiles, particularly when they already had oviposition experience. A similar concept was explored with the use of sniffer dogs for detecting infested fruit (3.2.13). A 3-year old German Shepherd demonstrated the ability to detect a single recently FCM-infested Navel orange in a carton of healthy oranges, when presented with several cartons of oranges. This was following an intensive imprint training period of a few months. A previous study, which identified several elevated volatiles associated with FCM-infested fruit, was reignited (3.2.21). The objective of the study is to refine the technology, currently based on GCMS. However, the study is still in a very early phase.

The remaining two post-harvest detection projects, were not technology related but pertained rather to the accuracy of inspection standards within a proposed systems approach for FCM (3.2.19) and the host status of lemons for FCM (3.2.20). The systems approach was developed as an alternative to cold sterilisation. A clear relationship between non-compliance of orchards with infestation standards pre- and post-harvest was evident, as was a progressively increasing level of discrimination at successive inspection steps. Appropriate sensitivity of the grading and inspection thresholds for application to successive steps in the systems approach were thus verified. In the lemon host status project, 30 346 fruit, packed for export, were sampled and dissected without detection of a single infested fruit. This demonstrated 99.99% non-host status of Eureka lemons at the 95.19% confidence level and provided strong justification for the exclusion of lemons from any cold-sterilisation protocols for FCM.

The final post-harvest project mainly examined partial cold-treatment protocols, as a step in a systems approach (3.2.5). A temperature of 2°C for 18 days caused 99.61% mortality of 4th and 5th instar larvae and reduced viability (ability to produce a subsequent generation) by 99.98%. Additionally, cold sterilisation trials were conducted, achieving probit 9 mortality of 5th instar larvae at -0.4°C for 18 days.

Of the pre-harvest trials, six were dedicated to investigating different aspects of biological control of FCM. Of these, four focussed on microbial control of FCM. The first, which was initiated about six years ago, investigated the use of locally isolated entomopathogenic fungi for control of FCM, by targeting the soil-

dwelling life stages (3.2.4). Field trials confirmed the potential observed during the previous season, indicating that a single application in spring or correctively late in the season, could induce significant control. *Beauveria bassiana* appeared more effective under microjet irrigation, whereas *Metarhizium anisopliae* appeared more effective under drip irrigation. The second microbial control project also targeted the soil-dwelling life stages of FCM, but with entomopathogenic nematodes (3.2.6). A late season corrective treatment showed that application of 20 IJs/cm² reduced both moth counts and fruit infestation relative to an untreated control. Two other projects focussed on different aspects of the *Cryptophlebia leucotreta* granulovirus. The aim of the first was to investigate gene expression of *T. leucotreta* in response to virus infection. The results showed that there is a dynamic gene expression response in FCM due to granulovirus infection under different conditions. These findings provide insight into better understanding and potentially improving the control of FCM. The second granulovirus project (3.2.11) investigated the rate of UV breakdown of the virus and sought to determine the necessary reapplication frequency. Activity of the virus was reduced more rapidly on the northern than southern sides of trees, indicating almost total breakdown after four weeks, when exposed directly to sunlight.

The final two biocontrol projects pertained to egg (3.2.3) and larval (3.2.14) parasitoids of FCM. The former, investigated the efficacy of late season (from January to harvest) augmentative releases of *Trichogrammatoidea cryptophlebiae* and concluded that such releases were minimally effective. The current recommendation of releasing this parasitoid as early as possible in the season therefore stands. The second project proposed to classically release *Agathis bishopi* in the Western Cape, where it (and all larval parasitoids) has been found to be absent. To date, insufficient laboratory reared parasitoids for release have been available.

Three of the pre-harvest FCM management trials looked at key aspects of the sterile insect technique (SIT) programme. One, a non-CRI funded project, sought to independently audit the efficacy of the SIT programme in the Sundays River Valley of the Eastern Cape (3.2.22) and concluded that wild male trap catches were lower in the SIT areas than the non-SIT areas for all the comparisons and hence, the results support the effectiveness of the SIT programme in the SRV. The study also concluded the urgency to prioritise improvement of cold tolerance of sterile moths, an issue being addressed by the other two SIT studies. One of them aimed to improve cold tolerance of laboratory reared FCM through improvement or manipulation of production parameters (3.2.15). For the lower levels of activity, or critical thermal minima (CT_{min}), there were significant effects of rearing and treatment temperature and the interaction thereof, with the intermediate acclimation temperature resulting in the lowest CT_{min}. Flight tests with these moths must still be conducted. The second cold tolerance project augmented larval diets with cryoprotectants (3.2.18) and found that the addition of trehalose enabled 40% flight at 15°C compared to 0% flight without trehalose. This was supported by a field trial in which the ratio of moth recaptures were 4.89:1 for trehalose moths to standard sterile moths.

The last two projects pertaining directly to control measures for FCM investigated mating disruption (MD). The one looked at novel approaches to conventional mating disruption, such as aerial applications and MD “overkill” (3.2.16). It was concluded that aerial application of Checkmate was significantly effective and that Isomate also significantly enhanced the efficacy of an SIT programme. The second MD project was referred to as mating inhibition rather than MD and investigated the efficacy of a novel compound, 7-vinyl decyl acetate-1 (7-VDA) (3.2.9). In a laboratory trial to examine the effect on mating, measured by reduction in oviposition, a combination treatment of 7-VDA and Isomate (FCM pheromone) reduced egg laying by 76%, compared to 42% with Isomate alone. Field cage studies have not yet been successful.

The final three projects within the FCM programme studied ecological aspects of the pest. Although not direct control measures, this improved understanding of the ecology of FCM in the citrus orchard environment is critical to achieving improved control of the pest. The first ecological project studied the pupation behaviour and biology of FCM (3.2.8) and found that very few abiotic factors had a clear influence on FCM pupation. Larval wandering time and distance were short and pupation depth was shallow. FCM eclosion success was highest in sandy soil. Air temperature limits of 15°C and 32°C had a strongly negative impact on eclosion success. When provided with a choice, FCM larvae showed a strong preference for pupating in areas with soil. These results may have implications for EPF and EPN application, survival and persistence in the soil for improved control of FCM. Another ecological study is examining the movement of FCM and fruit flies in multi-crop systems in the Western Cape (3.2.17). Peaks in FCM activity were not related to ripening of fruit type, unlike fruit fly, where this was clearly the case. In the case of both pests, highest trap catches were recorded in nectarines. The final ecological study conducted a survey of lepidopteran pests in general in the Eastern Cape Midlands (3.2.10) and compiled a genetic database to reliably identify a range of Lepidoptera that may be found infesting or otherwise associated with citrus.

Programopsomming

VKM navorsing was nogsteeds die nommer een entomologiese navorsings prioriteit binne die IPM Portefeulje gedurende die 2014/15 navorsings siklus. Met die voltooiing van die Europese en Mediterseanse Plant Beskermings Organisasie (EPPO) Plaag Risiko Analise (PRA) op VKM net oor 'n jaar gelede en die bevinding dat Suid-Afrikaanse sitrus uitvoere 'n pad kon skep vir VKM tot die EPPO streek, het verwagtinge van strenger uitvoer protokol regulasies binne die nabye toekoms verhoog. Omdat ons nie weer met dieselfde tipe probleme wil sit as wat tans met Sitruswartvlek en die Europese Unie ervaar word, is die doel van die VKM navorsings program om voor- en na-oes bestuurs oplossings so gou as moontlik te kan ontwikkel, met sterk fokus op opsies wat aan uitvoer markte voorgestel kan word as billike alternatiewe vir konvensionele koue-sterilisasië.

Hier berig ons oor 21 verskillende projekte binne die VKM program, waarvan twee nie deur CRI bevonds is nie, maar nietemin uitgevoer deur studente wat deel vorm van die groter sitrus IPM navorsings span. Sewe van hierdie projekte ondersoek gedeeltelik of uitsluitlik na-oes oplossings vir VKM bestuur. Die ander dek voor-oes bestuurs navorsing.

Van die sewe na-oes gefokusde projekte, het ses opsporing of megansimes vir opsporing van VKM-besmette vrugte ondersoek. Die eerste se voorstel was om 'n verskeidenheid moontlike tegnologieë vir opsporing van kripties besmette vrugte te ondersoek maar het sterk op X-straal tegnologie gefokus (3.2.2). Verfyning van mikro-fokus X-straal tomografie het skandering tydsduur tot net 34 sekondes verminder. Die presiese doeltreffendheid van die skandering word nog geëvalueer. Nog 'n tegnologie wat ondersoek is, is die gebruik van die larwe parasiet, *Agathis bishopi*, om deur sy gedrag VKM-besmette vrugte aan te dui (3.2.12). In Y-buisie olfaktometer proewe het wyfie parasiete 'n positiewe respons teen albei besmette vrugte en sekere vlugtige stowwe getoon, veral waar die wyfie vorige eierleggings ondervinding gehad het. 'n Soortgelyke konsep is met die gebruik van snuffelhonde vir opsporing van besmette vrugte ondersoek (3.2.13). 'n 3-jaar oue Duitse Skaaphond het die vermoë gewys om 'n enkele pas VKM-besmette Nawellemoen in 'n karton vol gesonde lemoene op te spoor, wanneer dit met 'n klomp kartonne vol vrugte voorsien is. Dit is gevolg deur 'n intensiewe opleidings tydperk van 'n paar maande. 'n Vorige studie wat verskeie verhoogde vlugtige stowwe geïdentifiseer het wat met besmette vrugte geassosieer is, is weer begin (3.2.21). Die doel van die studie is om die tegnologie, wat op GCMS gebaseer is, te verfyn. Die studie is egter nog in die begin fase.

Die oorblywende twee na-oes opsporings projekte is nie tegnologieë gekoppel nie maar het eerder te doen gehad met die akuraatheid van inspeksie standaarde binne 'n voorgestelde stelselsbenadering (3.2.19) en die gasheer status van suurlimoene vir VKM (3.2.20). Die stelselsbenadering is as 'n alternatief vir koue sterilisasië ontwikkel. 'n Duidelike verhouding tussen nie-nakoming van boorde met besmettings standaarde voor- en na-oes was duidelik, so ook 'n progressiewe ontwikkeling van onderskeiding met agtereenvolgende inspeksie stappe. Geskikte sensitiwiteit van die gradering en inspeksie drempelwaardes vir toepassing vir agtereenvolgende stappe in die stelsels benadering is dus geverifieer. In die suurlimone gasheer status studie is 30 346 vrugte, gepak vir uitvoer, gesny en ondersoek, en is heeltemaal vry van VKM besmetting gevind. Hierdie het 'n 99.99% nie-gasheer status van Eureka suurlimoene teen 'n 95.19% vlak van vertrouwe gewys en het sterk regverdiging voorsien vir uitsluiting van suurlimoene van enige koue-sterilisasië protokol vir VKM.

Die finale na-oes projek het hoofsaaklik onvolledige koue behandeling protokolle ondersoek, as 'n stap in 'n stelsels beandering (3.2.5). 'n Temperatuur van 2°C vir 18 dae het 99.61% mortaliteit van 4de en 5de instar larwes veroorsaak en het lewensvatbaarheid (vermoë om 'n volgende generasie te produseer) met 99.98% verminder. Boonop is koue-sterilisasië proewe uitgevoer en probit 9 mortaliteit van 5de instar larwes is na 18 dae teen -0.4°C gekry.

Van die voor-oes proewe het ses op verskillende aspekte van biologiese beheer van VKM gefokus. Uit hierdie het vier op mikrobiëse beheer van VKM gefokus. Die eerste, wat omtrent 6 jaar gelede begin is, het die gebruik van plaaslik geïsoleerde entomopatogeniese swamme vir beheer van VKM ondersoek, deur om die ondergrondse lewensstadiums te teiken (3.2.4). Veldproewe het die vermoë wat verlede seisoen opgelet is bevestig, wat aangedui het dat 'n enkele behandeling, toegedien in die lente of korrektyf laat in die seisoen, beduidende beheer kon gee. *Beauveria bassiana* het meer doeltreffend onder drupbesproeiing geblyk, waar *Metarhizium anisopliae* meer doeltreffend onder drupbesproeiing voorgekom het. Die tweede mikrobiëse beheer projek het ook gefokus op die ondergrondse lewensstadiums van VKM, maar met entomopatogeniese nematodes (3.2.6). 'n Laat-seisoen korrektywe behandeling het gewys dat toediening van 20 IJs/cm² albei mot getalle en vrugbesmetting verminder het teenoor die onbehandelde kontrole. Twee projekte het gefokus op verskillende aspekte van die *Cryptophlebia leucotreta* granulovirus. Die doel van die eerste een was om die gene uitdrukking van *T. leucotreta* in reaksie op virus besmetting te ondersoek. Die

resultate het 'n dinamiese gene uitdrukking respons in VKM getoon teen granulovirus besmetting onder verskillende omstandighede. Hierdie bevindinge skep insig tot 'n verbeterde begrip en potensiële verbetering in die beheer van VKM. Die tweede granulovirus projek (3.2.11) het die UV-afbraak koers van die virus ondersoek en het gepoog om die nodige hertoedienings intervalle te bepaal. Virus aktiwiteit het vinnger afgeneem op die noordelike as die suidelike aspekte van bome, en waar direk aan sonlig blootgestel was daar naby aan totale afbraak binne vier weke.

Die laaste twee biologiese beheer projekte het met eier (3.2.3) en larwe (3.2.14) parasiete van VKM gewerk. Die eerste projek het die doeltreffendheid van laat-seisoen (van Januarie tot oestyd) byvoegende loslatings van *Trichogrammatoidea cryptophlebiae* ondersoek en het die gevolgtrekking gemaak dat sulke loslating minimaal doeltreffend was. Die huidige aanbeveling om hierdie parasiet so vroeg as moontlik in die seisoen los te laat bly dus staan. Die doel van die tweede projek was om klassieke biologiese beheer loslatings van *Agathis bishopi* in die Wes-Kaap te doen. Dit is gevind dat dit (en alle larwe parasiete) nie in die Wes-Kaap teenwoordig is nie. Tot op datum was daar nog nie genoeg laboratorium geteelde parasiete om los te laat nie.

Drie van die voor-oes VKM bestuur proewe het gekyk na sleutel aspekte van die steriele insek tegniek (SIT) program. Een van hulle, wat nie deur CRI bevonds was nie, het 'n onafhanklike audit van die werking van die SIT program in die Sondagsrivier Vallei van die Oos-Kaap gedoen (3.2.22) en het die gevolgtrekking gemaak dat wilde mannetjie lokvalvangstes laer was in SIT areas as nie-SIT areas vir alle vergelykings en dus ondersteun die resultate die doeltreffendheid van die program in die SRV. Die studie het ook die belangrikheid en dringendheid van navorsing om koue toleransie van steriele motte te verbeter beklemtoon, iets wat deur die twee ander SIT projekte aangepak word. Een van hulle het gepoog om koue toleransie van laboratorium geteelde VKM te verbeter deur verbetering of manipulasie van produksie parameters (3.2.15). Vir die laer aktiwiteitsvlakke, of kritiese termiese minima (CT_{min}), was daar beduidende effekte van teling en behandelingstemperature en die interaksie tussen die twee aspekte, met die intermediêre akklimatisasiestemperature wat die laagste CT_{min} gehad het. Vlugtoetse met hierdie motte moet nog uitgevoer word. Die tweede koue toleransie projek het larwe diete met kouebeskerms bygevoeg (3.2.18) en het gevind dat die byvoeging van trehalose tot 40% vlug teen 15°C gelei het in vergelyking met 0% vlug sonder trehalose. Hierdie is deur 'n boordproef ondersteun waar die verhouding van mot hervangs 4.89:1 was vir trehalose motte tot standaard steriele motte.

Die laaste twee projekte wat direk te doen gehad het met beheermaatreels vir VKM, het paringsontwrigting (PO) aangeraak. Die een het gekyk na nuwe benaderings tot konvensionele PO, soos lugtoediening en PO oorvoeding (3.2.16). Dit is opgelet dat lugtoedienings van Checkmate beduidend doeltreffend was en dat Isomate VKM beheer binne 'n SIT program ook beduidend verbeter het. Die tweede PO projek was eintlik paringsverhoeding eerder as PO en het die effektiwiteit van 'n nuwe samestelling, 7-viniel desiel asetaat-1 (7-VDA) ondersoek (3.2.9). In 'n laboratorium proef om die effek op paring te ondersoek, gemeet deur eierlegging, het 'n kombinasie behandeling van 7-VDA en Isomate (VKM feromoon) eierlegging met 76% verminder, in vergelyking met 42% met Isomate alleen. Veld-hok proewe is nog nie suksesvol uitgevoer nie.

Die finale drie projekte in die VKM program het ekologiese aspekte van die plaag ondersoek. Alhoewel nie direkte beheermaatreels nie, het hierdie die begrip van die ekologie van VKM in die sitrusboord omgewing verbeter, wat krities is vir verbeterde beheer van die plaag. Die eerste ekologiese projek het die papievormings gedrag en biologie van VKM bestudeer (3.2.8) en het gevind dat daar baie min abiotiese faktore was wat 'n duidelike invloed op VKM papievorming gehad het. Tydsduur en afstand wat larwes gedwaal het was kort en papies het baie oppervlakkig gevorm. Ontpoppings sukses (van die mot uit die papie uit) was hoogste in sanderige grond. Lug temperatuur perke van 15°C en 32°C het 'n sterk negatiewe impak op ontpoppings sukses gehad. Waar hulle 'n keuse gehad het, het VKM larwes 'n sterk voorkeur gewys vir papievorming waar daar grond beskikbaar was. Hierdie resultate kan dalk implikasies inhou vir EPF en EPN toediening, oorlewing en nawerking in die grond vir verbeterde beheer van VKM. Nog 'n ekologiese studie ondersoek die beweging van VKM en vrugtevlieë in veelvuldige gewas sisteme in die Wes-Kaap (3.2.17). Pieke in VKM aktiwiteit het geen verhouding gewys met rypwording van vrugteplae nie, in teenoorstelling met vrugtevlieë waar hierdie duidelik die geval was. In die geval van albei plae is die hoogste lokvalvangstes in nektariene gekry. Die finale ekologiese studie het 'n opname van Lepidoptera in die algemeen op plase in die Oos-Kaapse middellande gedoen (3.2.10) en het 'n genetiese databasis opgestel om met volle betroubaarheid 'n reeks Lepidoptera te identifiseer wat sitrus kon besmet of enige verhouding met sitrus kon hê.

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

Project 976 (April 2010 – March 2015) by Wayne Kirkman, Sean Moore (CRI), Frikkie de Beer, Kobus Hoffmann, Lunga Bam and Robert Nshimirimana (NECSA)

Summary

The objective of this study is to investigate post-harvest techniques to detect cryptic pests in citrus fruit. In previous studies using the Nuclear Energy Corporation of South Africa's (NECSA) Microfocus X-ray unit, the quality of the images was optimised. Subsequent trials have shown that abbreviated cold treatment kills most of the smaller larvae, so it might not be necessary to detect the very small areas of damage caused by these larvae. Emphasis was placed on speed of the scans. Last year, the time per scan was reduced from 35 minutes to 1 minute and 26 seconds, further refining of the technique resulted in a scan lasting only 34 seconds. The efficacy of this scan will be evaluated. Imaging algorithms are being developed in collaboration with NECSA, Maf-Roda and Greefa. Trials were conducted to evaluate the ability of the Greefa IPix presorting unit to detect FCM. Results were promising, especially on well-developed infestations using image saturation. One more visit is planned to NECSA to complete the X-ray study, and the collaboration with Greefa, Maf-Roda and Compac will continue under project 1120.

Opsomming

Die doel van die studie is om tegnieke te ondersoek wat insekplae binne sitrusvrugte na-oes kan opspoor. In vorige proewe met die Mikrofokus X-straleenheid van die Nuclear Energy Corporation of South Africa (NECSA) is beeldkwaliteit geoptimaliseer. Onlangse proewe het getoon dat verkorte kouebehandeling meeste van die kleiner larwes doodmaak. Dit is dus moontlik nie nodig om die klein penetrasies wat deur hulle veroorsaak word, op te spoor nie. Daar is op die spoed van skanderings gefokus. Verlede seisoen is die tyd per skandering verkort van 35 minute tot 1 minuut 26 sekondes. Die tegniek is verder verfyn, wat skanderingstyd tot 34 sekondes verminder het. Die effektiwiteit van dié skandering sal geëvalueer word. Beeld algoritmes word in samewerking met NECSA, Maf-Roda en Greefa ontwikkel. Toetse is uitgevoer om die vermoë van Greefa se IPIX eenheid te bepaal. Resultate lyk belowend met beeld-saturasie, veral waar besmetting goed ontwikkel is. Nog een besoek aan NECSA word beplan om die X-straal werk klaar te maak, en samewerking met Greefa, Maf-Roda en Compac sal onder projek 1120 voortgaan.

3.2.3 **FINAL REPORT: Late season releases of *Trichogrammatoidea cryptophlebiae* for suppression of FCM**

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

Summary

Studies on the effectiveness of the FCM egg parasitoid, *Trichogrammatoidea cryptophlebiae*, were conducted a number of years ago, showing that FCM infestation could be reduced by up to 61%. However, this was a result of early season parasitoid releases. Parasitism by naturally occurring *T. cryptophlebiae* generally builds up from December and reaches a peak in January or February. Any releases at and shortly before this time gave negligible benefit over and above that of the naturally occurring parasitoids. Currently such early releases are nearly impossible in the industry, due to the application of a series of pesticides for control of thrips and other pests and diseases during the first half of the season. *Trichogrammatoidea cryptophlebiae* is very sensitive to many of these pesticides. Where a chemical orientated pest control programme is followed, an FCM parasitoid vacuum is often created, exacerbating this increase in FCM infestation shortly before harvest. This study aimed to determine whether mid to late season releases would still be of benefit in reducing FCM infestation, even if the effect was only seen shortly before harvest. During the 2012/13 season parasitoids were released at four sites – all Navel oranges – at 100 000 parasitoids per hectare, as monthly releases of 25 000 parasitoids from January to April. Thereafter, FCM presence (moths, eggs and larval infestation of fruit) and egg parasitism were evaluated weekly in release and comparable control blocks. At three out of the four trial sites, FCM levels were too low to obtain meaningful results. At the fourth site, FCM levels were very high. However, so too was natural parasitism, thus obscuring any impact which the released parasitoids might have had. The study was repeated during the 2013/14 season at four different sites in the same region. FCM egg parasitism and fruit infestation were evaluated weekly from 7 February until 19 June in release and control blocks at all sites. At two of the sites parasitism was significantly higher in the release block. However, this did not lead to any detectable reduction in FCM infestation. This project has been terminated.

Opsomming

Studies oor die doeltreffendheid van die VKM eier parasiet, *Trichogrammatoidea cryptophlebiae*, is 'n paar jaar gelede uitgevoer en het gewys dat VKM besmetting met tot 61% verminder kon word. Hierdie is egter as gevolg van vroeë-seisoen loslatings. Parasitisme deur *T. cryptophlebiae* wat natuurlik voorkom, bou gewoonlik van omtrent Desember op en bereik 'n piek in getalle in Januarie of Februarie. Enige loslating op hierdie stadium en kort voor hierdie stadium het baie min voordeel gegee oor dié van die parasiete wat natuurlik voorgekom het. Tans is sulke vroeë loslatings amper onmoontlik in die bedryf as gevolg van toediening van 'n reeks plaagdoders vir beheer van blaaspootjie en ander plae en siektes gedurende die eerste helfte van die seisoen. *Trichogrammatoidea cryptophlebiae* is baie sensitief vir baie van hierdie plaagdoders. Waar 'n chemies-geïntegreerde plaagbestrydingsprogram uitgevoer word, veroorsaak dit gereeld 'n VKM-parasiet-vakuum, wat 'n styging in VKM besmetting kort voor oes kan veroorsaak. Die doel van hierdie studie was om te bepaal of middel-tot-laag-seisoen loslatings nogsteeds VKM besmetting kon verminder, selfs as die effek eers kort voor oes gesien word. Gedurende die 2012/13 seisoen is parasiete by vier persele losgelaat – alles Nawellemoene – teen 100 000 parasiete per hektaar. Maandeliks van Januarie tot April is 25 000 parasiete per hektaar losgelaat. VKM teenwoordigheid (motte, eiers en larwe besmetting van vrugte) en eier parasitisme is daarna weekliks in loslatings blokke en vergelykbare onbehandelde kontrole blokke geëvalueer. By drie uit die vier proefpersele is VKM vlakke te laag om waardevolle resultate te kry. By die vierde perseel is VKM vlakke baie hoog. Natuurlike parasitisme was egter ook hoog, en het dus enige impak wat die losgelate parasiete kon gehad het verduister. Die studie is gedurende die 2013/14 seisoen by vier verskillende persele in dieselfde streek herhaal. VKM eierparasitisme en vrugbesmetting is van 7 Februarie tot 19 Junie ontleed in albei loslatings en kontrole blokke by alle persele. By twee van die persele is parasitisme beduidend hoër in die loslatingsblok. Hierdie het egter nie tot laer vlakke van VKM besmetting van vrugte gelei nie. Hierdie projek is beëindig.

Introduction

Studies on the effectiveness of the FCM egg parasitoid, *Trichogrammatoidea cryptophlebiae*, were conducted during the 1970s and 1980s (Newton, 1988, Newton & Odendaal, 1990, Schwartz, 1980). Parasitoids totalling 1.5 - 3.8 million were released per hectare on a weekly basis. Results were variable, but larval populations were reduced by up to 70%. Such high density and frequent releases are impractical and expensive if conducted on a commercial scale.

Consequently, from 1998 to 2002 a study was conducted to test inoculation with more realistic numbers (Moore & Fourie, 1999; Moore & Richards, 2000, 2001 & 2002). Releases of as few as 100 000 parasitoids per hectare per season proved successful, with parasitism being increased by up to 42% and FCM infestation reduced by up to 61%. Another important finding was the relationship between time and efficacy of releases. Releases initiated in October were more effective than those initiated in November. Releases initiated only in December were the least effective. Moore & Richards (2000 & 2002) showed that parasitism by naturally occurring *T. cryptophlebiae* generally builds up from December and reaches a peak in January or February. Any releases at this time – and even during the few weeks leading up to this time – gave negligible benefit over and above that of the naturally occurring parasitoids. The corollary of this is that if natural parasitism has not built up by this time then releases would still be superfluous, as they would then be released into what is most likely an unfavourable environment.

One potential problem with these early releases is the application of a series of pesticides for control of thrips, mealybug, citrus blackspot and other pests and diseases during the first half of the season (i.e. from spring to mid-summer). *Trichogrammatoidea cryptophlebiae* is a small parasitoid and has thus proved very sensitive to many of these pesticides (Grout et al., 2002). Initiation of releases during the time that these pesticides are being applied and while their residues are still on the trees, would be ineffective.

This raises the question of whether releases after this time would still be of benefit in reducing FCM infestation, even if the effect is seen only much later in the season. It is recognised that even when FCM is under good control, infestation of fruit tends to increase shortly before harvest, as fruit ripens and becomes more susceptible to infestation. Where a chemical orientated pest control programme is followed, an FCM parasitoid vacuum could be created, exacerbating this increase in FCM infestation shortly before harvest. Due to the decreasing phytosanitary tolerance for FCM by almost all of South Africa's export markets, this is an untenable situation.

The possibility of such a situation is no longer speculative. In a study conducted from 1999 to 2002, very high levels of naturally occurring parasitism of FCM eggs in citrus orchards were recorded, often well in excess of 80% (Moore & Fourie, 1999; Moore & Richards, 2000, 2001 & 2002). Additionally, there was a strongly significant positive correlation between an increase in parasitism and a decline in FCM infestation of

fruit. In recent seasons, albeit without formal surveys, very little parasitism has been observed – none at all in most orchards in which FCM research trials have been conducted. This is attributed to the change in spray programme adopted in recent years, whereby thripicides are sprayed more frequently, including the use of IPM-incompatible thripicides in mid-summer, black spot sprays are applied regularly and pyrethroids are used for FCM control during the last few months before harvest. There is therefore no respite for the pesticide sensitive egg parasitoids. Consequently, FCM levels at harvest are now sometimes higher than they were years ago before the current comprehensive control programmes were initiated.

Something urgently needs to be done to curtail this devastating trend. Consequently, CRI is embarking on a concerted drive to change this approach. It is accepted that the use of hard thripicides may be necessary early in the season. However, effective control of thrips pre-blossom and during the first month after petal fall, should allow for the use of softer more IPM-compatible thripicides for the remainder of the season. The use of pyrethroids against FCM, other than during the last five weeks before harvest, must be avoided. By so doing, an environment for survival of egg parasitoids can be created. However, due to the use of disruptive pesticides earlier in the season, augmentation of *T. cryptophlebiae* will be necessary between January and harvest. The intention is that this will serve to suppress FCM levels at harvest.

Objectives

1. To test whether late season (from January onwards) releases of *T. cryptophlebiae* could be of benefit, after a chemically orientated control programme, in reducing FCM pressure shortly before harvest. This could potentially reduce phytosanitary risk.
2. To compare levels of natural parasitism and the success of parasitoid augmentation in trial sites which were used almost 10 years ago.

Materials and methods

Trial sites

During 2012/13, four farms were selected on which to conduct trials. Two of the farms had been used approximately 10 years previously for *T. cryptophlebiae* augmentation trials and thus comparisons could be made. Both of these farms, Sun Orange and Lupus Den, were considered relatively IPM-adoptive. The other two farms, Falcon Ridge and Kleinplaas, were considered to adopt more conventional spray programmes and were selected due to their recent history of FCM problems. Details for trial sites are provided in Table 3.2.3.1. All orchards used were Navel oranges. A section of not more than 1 ha was used for release and another hectare as the untreated control (either in the same or a nearby orchard of the same cultivar). The control block was not adjacent to the release block i.e. separated by at least a short distance.

Table 3.2.3.1. Details of sites used for *T. cryptophlebiae* release trials.

Farm	Coordinates	Orchard no.	Navel variety	Year planted	Spacing (rows x trees)
Sun Orange	33°28'17"S 25°39'03"E	73 & 74	Fukumoto	2003	5.75 x 2
Lupus Den	33°27'00"S 25°34'23"E	2	Newhall	1998	5.7 x 2
Falcon Ridge	33°25'22"S 25°30'06"E	16	Cambria	2006	5.5 x 3
Kleinplaas	33°29'40"S 25°41'39"E	5	Newhall	2005	5.2 x 3

During 2013/14, four farms were again selected on which to conduct trials (Table 3.2.3.2). All orchards used were again Navel oranges with a recent history of conspicuous FCM infestation of fruit. The trials were laid out as during the previous season.

Table 3.2.3.2. Details of sites used for *T. cryptophlebiae* release trials.

Farm	Coordinates	Orchard no.	Navel cultivar	Year planted	Spacing (rows x trees)
Halaron	33°28'17"S 25°39'03"E	51	Cara cara	2000	5.5 x 3.0
Eluhlaza	33°28'57"S 25°39'16"E	42	Palmer	2006	5.5 x 2.5
Addo Research Station	33°34'12"S 25°41'30"E	7	Cara cara	1998	6 x 2

Pezula	33°27'33"S 25°30'19"E	2	Palmer	2008	6 x 3
--------	--------------------------	---	--------	------	-------

Source and quality control of parasitoids

Parasitoids were obtained from Du Roi IPM, who reared and supplied them as parasitized FCM eggs on either wax paper sheets or smooth cardboard squares (cards). The cards contained an estimated 1000 parasitised eggs each and were crafted with a hook to hang over a branch. Wax sheets were cut into pieces each containing at least 1000 eggs. On each occasion that eggs were received for release, a sample of a few sheets and/or cards were placed separately into sealed glass jars. After parasitoids had eclosed and died, numbers of parasitized eggs and numbers of eclosed parasitoids were counted. This provided an indication of actual numbers released and percentage eclosion.

Parasitoid releases

As quality control on actual numbers was only conducted post-release, these numbers differed slightly in some cases, as during 2012/13 (Table 3.2.3.3) and substantially in other cases, as during 2013/14 (Table 3.2.3.4). The objective was to release an estimated total of at least 100 000 parasitoids per hectare at each site. This was to be divided into monthly releases of 25 000 parasitoids per hectare per month. Twenty five release points were marked at regular intervals throughout each of the 1 ha release blocks. An estimated 1000 parasitised eggs were placed at each release point on each occasion.

Table 3.2.3.3. Estimated actual numbers of parasitoids released per hectare during 2012/13.

Date	Parasitoids released per ha (extrapolated from QC data)
25 Jan 2013	26052
22 Feb 2013	33169
22 Mar 2013	26309
12 Apr 2012	21575
Total	107105

Table 3.2.3.4. Estimated actual numbers of parasitoids released per hectare during 2013/14.

Date	Parasitoids released per ha (extrapolated from QC data)
29 Jan 2014	20325
27 Feb 2014	25350
26 Mar 2014	9875
24 Apr 2014	10025
Total	65575

Trial evaluation

Weekly, from 25 January in 2013 and 7 February in 2014, assessments of FCM egg presence on fruit, parasitism of eggs, and FCM infestation of fruit were conducted at each trial site, in both the release block and the adjacent or nearby control block (also 1 ha in size). This was done by marking 10 data trees diagonally through each release and control block and inspecting 10 randomly selected fruit on each tree on each occasion for FCM presence, recording whether eggs were parasitized or not. All hatched eggs and parasitized eggs from which the parasitoid had already eclosed were ignored. Simultaneously, all fruit which had dropped (during the preceding week) under each data tree were collected and inspected (by cutting the fruit) for FCM infestation. Fruit were recorded as clean, presently infested (with an FCM larva) or historically infested (larva had already exited fruit, but frass and burrow remained).

Results and discussion

2012-13

Quality control of parasitoids

Eclosion of parasitoids from FCM eggs ranged from 37% to 83% and averaged 54% on cards and 63% on wax sheets (Table 3.2.3.5). This was not very good and did not compare favourably with eclosion rates recorded in similar trials several years previously, where eclosion averaged 76.5% and 91.0% in two consecutive seasons (Moore & Richards, 2001 & 2002). This low rate of eclosion may have to do with transportation, refrigeration (storage) or clumping of eggs. However, the parasitoid numbers produced per

card or sheet were far better (Table 3.2.3.5), the objective being 1000 parasitoids. This obviously means that cards and sheets had well over 1000 parasitised eggs each.

Table 3.2.3.5. Quality control data for samples from each released batch of parasitoids: eclosion rate and numbers of parasitoids per card or sheet (1000 being the standard).

Date	Card or sheet	Number	Average eclosion (%) ¹	Average parasitoids/card
25 Jan	Card	2	37.13	348.50
	Sheet	9	82.92	1504.44
22 Feb	Card	4	69.77	964.00
	Sheet	4	61.99	1689.50
22 Mar	Card	4	62.43	920.00
	Sheet	4	65.88	1184.75
12 Apr	Card	4	46.37	594.00
	Sheet	4	40.93	1132.00
Overall	Card	14	53.92	706.62
	Sheet	21	62.93	1377.67

¹Ratio of eclosed parasitoids to parasitized eggs. It is possible that an egg could be parasitized by more than one parasitoid.

Field trials

At three of the four sites (Falcon Ridge, Lupus Den and Sun Orange), FCM levels were extraordinarily low (Tables 3.2.3.6, 3.2.3.7 & 3.2.3.8) – far lower than any recent previous season. This unfortunately, prevented us from being able to determine whether the releases could be effective or not. FCM levels at the fourth site – Kleinplaas – were extremely high (Table 3.2.3.9). However, so too was natural parasitism (in both the control and release blocks), obscuring any possible impact that the released parasitoids might have had.

Table 3.2.3.6. Fruit infestation, egg presence and parasitism of eggs in release and non-release blocks at Falcon Ridge, Sundays River Valley 2013.

Date	Release block				Control block			
	Fruit with eggs (%)	Eggs parasitized (%)	Effective parasitism (%)	FCM fruit drop/tree/week	Fruit with eggs (%)	Eggs parasitized (%)	Effective parasitism (%)	FCM fruit drop/tree/week
25/01	3	0.0	0.0	-	3	0.0	0.0	-
01/02	2	0.0	0.0	-	1	0.0	0.0	-
08/02	0	0.0	0.0	0	1	0.0	0.0	0
15/02	5	66.7	60.0	0	1	0.0	0.0	0.1
22/02	5	57.1	40.0	0.1	1	0.0	0.0	0
01/03	1	100.0	100.0	0.1	1	0.0	0.0	0
08/03	1	100.0	100.0	0	1	0.0	0.0	0
15/03	1	100.0	100.0	0.1	0	0.0	0.0	0.1
21/03	1	0.0	0.0	0	0	0.0	0.0	0
28/03	3	66.7	66.7	0	1	0.0	0.0	0.1
03/04	3	0.0	0.0	0.2	0	0.0	0.0	0
12/04	0	0.0	0.0	0	1	0.0	0.0	0
20/04	1	100.0	100.0	0	0	0.0	0.0	0
26/04	2	100.0	100.0	0	0	0.0	0.0	0.2
02/05	2	100.0	100.0	0	0	0.0	0.0	0.1
10/05	1	0.0	0.0	0.4	0	0.0	0.0	0.3
17/05	1	0.0	0.0	1.0	3	0.0	0.0	1.0
24/05	0	0.0	0.0	0.5	1	0.0	0.0	0.4
30/05	2	100.0	100.0	0.2	2	0.0	0.0	0.3
05/06	1	0.0	0.0	0.3	1	100.0	100.0	0.9
13/06	1	100.0	100.0	0.4	1	0.0	0.0	0.7
20/06	0	0.0	0.0	1.1	0	0.0	0.0	0.3
26/06	0	0.0	0.0	0.5	1	0.0	0.0	0.2
Mean	1.57±0.30	43.07±9.89	42.03±9.84	0.23±0.07	0.87±0.18	4.35±4.35	4.35±4.35	0.22±0.07

Table 3.2.3.7. Fruit infestation, egg presence and parasitism of eggs in release and non-release blocks at Lupus Den, Sundays River Valley 2013.

Date	Release block				Control block			
	Fruit with eggs (%)	Eggs parasitized (%)	Effective parasitism (%)	FCM fruit drop/tree/week	Fruit with eggs (%)	Eggs parasitized (%)	Effective parasitism (%)	FCM fruit drop/tree/week
25/01	0	0.0	0.0	-	0	0.0	0.0	-
01/02	0	0.0	0.0	-	0	0.0	0.0	-
08/02	0	0.0	0.0	0	0	0.0	0.0	0
15/02	0	0.0	0.0	0	0	0.0	0.0	0
22/02	1	100.0	100.0	0	0	0.0	0.0	0

01/03	0	0.0	0.0	0	0	0.0	0.0	0
08/03	1	100.0	100.0	0	0	0.0	0.0	0
15/03	1	100.0	100.0	0	0	0.0	0.0	0
21/03	1	100.0	100.0	0	0	0.0	0.0	0
28/03	2	0.0	0.0	0	0	0.0	0.0	0.1
03/04	1	0.0	0.0	0	0	0.0	0.0	0
12/04	0	0.0	0.0	0	1	0.0	0.0	0
20/04	0	0.0	0.0	0	0	0.0	0.0	0
26/04	0	0.0	0.0	0	0	0.0	0.0	0
02/05	0	0.0	0.0	0	0	0.0	0.0	0.2
10/05	0	0.0	0.0	0	1	0.0	0.0	0
Mean	0.44±0.16	25.00±11.18	25.00±11.18	0.00±0.00	0.13±0.09	0.00±0.00	0.00±0.00	0.02±0.02

Table 3.2.3.8. Fruit infestation, egg presence and parasitism of eggs in release and non-release blocks at Sun Orange, Sundays River Valley 2013.

Date	Release block				Control block			
	Fruit with eggs (%)	Eggs parasitized (%)	Effective parasitism (%)	FCM fruit drop/tree/week	Fruit with eggs (%)	Eggs parasitized (%)	Effective parasitism (%)	FCM fruit drop/tree/week
25/01	0	0.0	0.0	-	0	0.0	0.0	-
01/02	0	0.0	0.0	-	0	0.0	0.0	-
08/02	1	0.0	0.0	0	0	0.0	0.0	0
15/02	1	0.0	0.0	0.1	0	0.0	0.0	0.2
22/02	1	100.0	100.0	0.1	0	0.0	0.0	0
01/03	1	0.0	0.0	0	0	0.0	0.0	0.3
08/03	0	0.0	0.0	0	0	0.0	0.0	0.3
15/03	1	0.0	0.0	0.2	0	0.0	0.0	0
21/03	1	75.0	50.0	0.1	0	0.0	0.0	0
28/03	1	100.0	100.0	0	0	0.0	0.0	0
03/04	1	0.0	0.0	0.1	1	0.0	0.0	0.3
12/04	0	0.0	0.0	0.1	0	0.0	0.0	0
20/04	0	0.0	0.0	0.4	0	0.0	0.0	0.2
26/04	1	0.0	0.0	0	1	0.0	0.0	0
02/05	0	0.0	0.0	0	0	0.0	0.0	0
10/05	0	0.0	0.0	0.3	0	0.0	0.0	0
Mean	0.56±0.13	17.19±9.33	15.63±8.80	0.10±0.09	0.13±0.09	0.00±0.00	0.00±0.00	0.09±0.04

Table 3.2.3.9. Fruit infestation, egg presence and parasitism of eggs in release and non-release blocks at Kleinplaas, Sundays River Valley 2013.

Date	Release block				Control block			
	Fruit with eggs (%)	Eggs parasitized (%)	Effective parasitism (%)	FCM fruit drop/tree/week	Fruit with eggs (%)	Eggs parasitized (%)	Effective parasitism (%)	FCM fruit drop/tree/week
25/01	46	0.0	0.0	-	39	0.0	0.0	-
01/02	27	8.7	3.7	-	25	13.9	8.0	-
08/02	33	16.7	9.1	3.6	20	15.4	5.0	2.0
15/02	31	21.3	9.7	2.7	22	31.6	9.1	1.7
22/02	29	48.0	31.0	1.3	26	70.0	46.2	0.2
01/03	29	52.5	41.4	1.5	33	78.3	63.6	0.7
08/03	18	80.3	72.2	1.0	20	85.7	80.0	0.5
15/03	13	94.4	92.3	0.6	12	80.0	75.0	0.9
21/03	14	85.7	85.7	0.9	14	53.3	50.0	0.7
28/03	7	58.3	42.9	0.6	8	90.0	87.5	0.6
03/04	6	100.0	100.0	2.2	3	100.0	100.0	2.2
12/04	4	75.0	75.0	0.2	9	81.8	55.6	0.2
20/04	6	16.7	16.7	0.6	5	10.0	0.0	1.4
Mean	20.23±3.65	50.58±9.68	44.59±10.08	1.38±0.32	18.15±3.03	54.62±9.86	44.62±10.05	1.01±0.21

Results from the four 2012/13 trial sites are summarised in Table 3.2.3.10.

Table 3.2.3.10. Summary of *T. cryptophlebiae* parasitism of FCM eggs in release and control blocks for the period 21 January to 26 June and FCM infestation of fruit for the period 8 February to 26 June 2013.

Farm	Orchard	Total			Mean % eggs parasitized	Mean infested fruit per tree per week
		Fruit with eggs	Eggs	Parasitised		
Falcon Ridge	Release	36	71	21	43.07±9.89	0.23±0.07
	Control	24	24	1	4.35±4.35	0.22±0.07
Lupus Den	Release	9	17	11	25.00±11.18	0.00±0.00
	Control	2	2	0	0.00±0.00	0.02±0.02
Sun Orange	Release	8	11	5	17.19±9.33	0.10±0.09
	Control	2	3	0	0.00±0.00	0.09±0.04
Kleinplaas	Release	266	439	145	50.58±9.68	1.38±0.32
	Control	241	387	174	54.62±9.86	1.01±0.21

If one looks at a graphic display of parasitism data at Kleinplaas (Fig. 3.2.3.1), it is clear that there was a lot of natural parasitism, which began to appear within one week of beginning trial evaluation. This is similar to the situation which was observed in *T. cryptophlebiae* augmentation trials conducted over 10 years ago (Moore & Fourie, 1999; Moore & Richards, 2000, 2001 & 2002). It was concluded that where an orchard environment was conducive to biological control and where natural parasitism was at a high level (usually peaking in February), augmentation after February almost became superfluous.

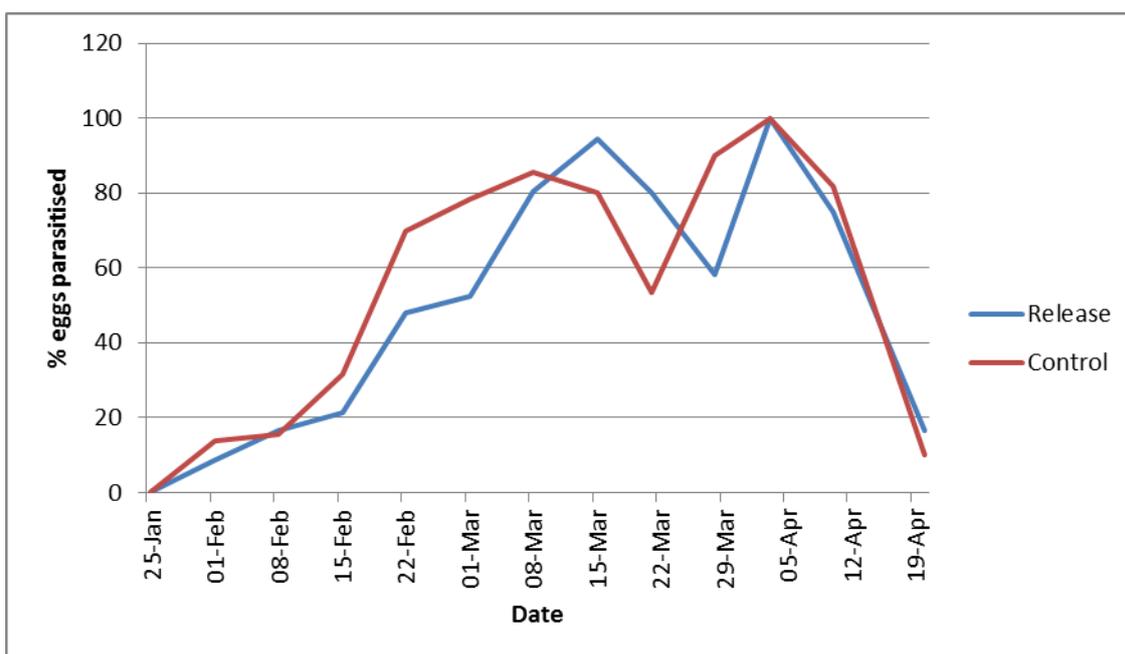


Fig. 3.2.3.1. Weekly FCM egg parasitism in release and control blocks at Kleinplaas from 26 January to 20 April 2013.

At Kleinplaas, initially FCM infestation of fruit in the release block was higher than in the control block (Fig. 3.2.3.2). This difference disappeared after a few weeks. However, there is insufficient evidence to link this to improvement in the level of parasitism in the release block.

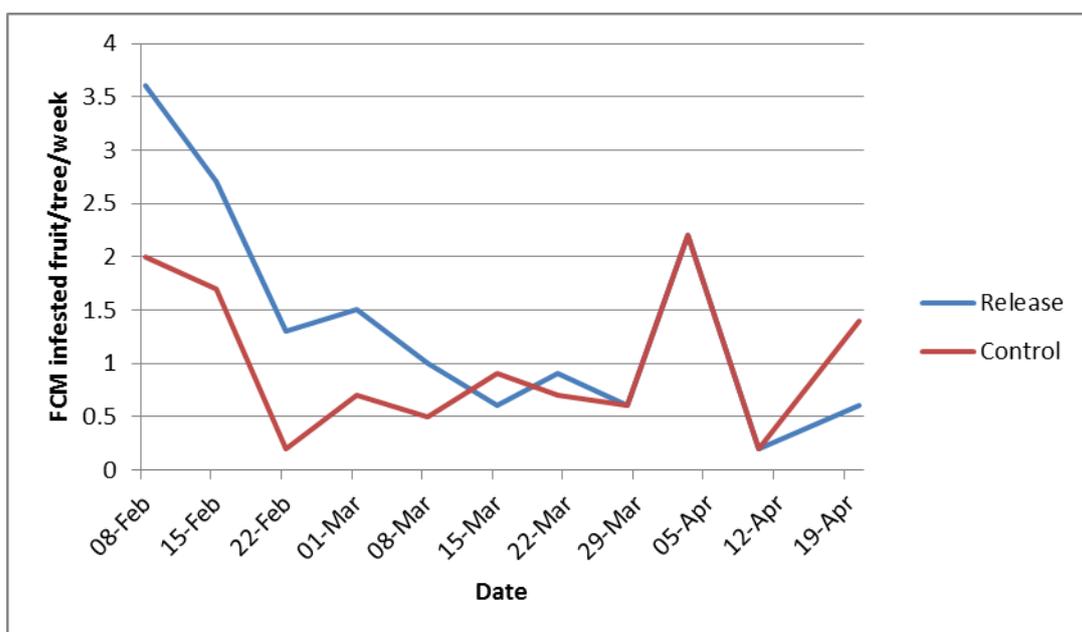


Fig. 3.2.3.2. Weekly FCM fruit infestation in release and control blocks at Kleinplaas, evaluated from 8 February to 20 April 2013.

2013-14

Quality control of parasitoids

Eclosion of parasitoids from FCM eggs ranged from 75% to 30% and averaged 52% (Table 3.2.3.10). Although parasitoid eclosion from the first two batches was acceptably high (67% and 75%), eclosion from the final two batches was unacceptably poor (36% and 30%). Hence, eclosion was generally poorer than recorded in similar trials during the previous year (average between 54% and 63%) and even more so than the good rates recorded in trials several years previously, where eclosion averaged 76.5% and 91.0% in two consecutive seasons (Moore & Richards, 2001 & 2002). It is not clear why eclosion rates were so poor for the final two consignments, as there were no apparent differences in transportation or refrigeration of eggs. Similar observations of parasitoids obtained from the same source were made during non-target effect tests (Tim Grout, personal communication). Possibly this was indicative of genetic bottle-necking and the need for introduction of new field collected parasitoid material. Mean parasitoid numbers produced per card for all four releases was 656, somewhat lower than the projected 1000 (Table 3.2.3.11).

Table 3.2.3.11. Quality control data for samples from each released batch of parasitoids: eclosion rate and numbers of parasitoids per card or sheet (1000 being the standard).

Date	Number of cards	Average eclosion (%) ¹	Average parasitoids/ card
29 January 2014	3	66.7	813
25 February 2014	4	75.28	1014
25 March 2014	4	35.57	395
25 April 2014	4	30.49	401
Overall		52.01	656

¹Ratio of eclosed parasitoids to parasitized eggs. It is possible that an egg could be parasitized by more than one parasitoid.

Field trials

At both Pezula (Table 3.2.3.12) and the Addo Research Station (Table 3.2.3.15), parasitism of FCM eggs was significantly higher in release blocks than control blocks. However for the other two sites there was little difference in levels of parasitism between release and control blocks. Despite the significantly higher parasitism levels in release blocks at Pezula (Fig 3.2.3.3), and the Addo Research Station (Fig 3.2.3.4), there was no reduction in FCM infestation.

Table 3.2.3.12. Fruit infestation, egg presence and parasitism of eggs in release and non-release blocks at Pezula, Sundays River Valley 2014.

Date	Release block				Control block			
	Fruit with eggs (%)	Eggs parasitized (%)	Effective parasitism (%)	FCM fruit drop/tree/week	Fruit with eggs (%)	Eggs parasitized (%)	Effective parasitism (%)	FCM fruit drop/tree/week
07/02	2	50	50.0	-	1	0	0	-
13/02	3	0	0.0	-	4	0	0	-
20/02	2	50	50.0	-	2	0	0	-
26/02	4	16.7	25.0	0	6	28.6	16.7	0
06/03	9	0	0.0	0.5	4	0	0.0	0.7
13/03	4	0	0.0	0.9	6	0	0.0	1.1
21/03	5	11.1	20.0	0.9	6	11.1	16.7	0.6
28/03	5	0	0.0	0.9	2	33.3	50.0	1.0
03/04	4	25.0	25.0	0.4	7	14.3	14.3	0.1
11/04	3	40.0	33.3	0.2	6	14.3	16.7	0.1
18/04	1	100.0	100.0	0	3	20.0	33.3	0
26/04	5	85.7	80.0	1.1	5	0	0.0	0.3
01/05	1	100.0	100.0	1.2	1	0	0.0	1.3
09/05	5	92.3	80.0	2.1	2	100.0	100.0	1.6
15/05	3	100	100.0	1.6	4	28.6	0.0	0.9
23/05	1	100.0	100.0	4.3	1	100.0	100.0	0.6
29/05	2	66.7	50.0	0.6	0	0.0	0.0	3.8
04/06	2	0	0.0	1.5	4	16.7	25.0	1.4
13/06	1	100.0	100.0	0.8	2	0.0	0.0	0.2
19/06	3	71.4	33.3	0.5	1	100.0	100.0	0.6
Mean	3.25±0.44	50.45±9.20	47.33±8.79	1.03±0.25	3.35±0.48	23.35±7.79	23.64±7.96	0.84±0.22

Table 3.2.3.13. Fruit infestation, egg presence and parasitism of eggs in release and non-release blocks at Eluhlaza, Sundays River Valley 2014.

Date	Release block				Control block			
	Fruit with eggs (%)	Eggs parasitized (%)	Effective parasitism (%)	FCM fruit drop/tree/week	Fruit with eggs (%)	Eggs parasitized (%)	Effective parasitism (%)	FCM fruit drop/tree/week
07/02	2	33.3	50.0	-	0	0.0	0.0	-
13/02	3	0.0	0.0	-	1	0.0	0.0	-
20/02	3	33.3	33.3	-	5	60.0	60.0	-
26/02	4	0.0	0.0	0.4	3	0.0	0.0	0.1
06/03	5	86.7	60.0	0.6	3	66.7	66.7	0.8
13/03	5	66.7	60.0	1.5	2	50.0	50.0	1.8
21/03	7	30.0	14.29	0.5	4	83.3	75.0	0.8

28/03	9	57.1	22.2	1.0	2	33.3	0.0	1.1
03/04	0	0.0	0.0	0.4	0	0.0	0.0	0.5
11/04	3	60.0	33.3	0.3	5	85.7	80.0	0.5
18/04	4	85.7	75.0	0.0	3	25.0	33.3	0.1
26/04	2	100.0	100.0	0.4	3	100.0	100.0	0.8
01/05	6	87.5	66.7	0.3	2	66.7	50.0	0.3
09/05	1	100.0	100.0	0.9	2	100.0	100.0	0.8
15/05	0	0.0	0.0	0.2	3	66.7	66.7	0.3
23/05	5	100.0	100.0	0.0	3	100.0	100.0	0.3
29/05	2	75.0	50.0	0.2	1	100.0	100.0	0.1
04/06	2	0.0	0.0	0.1	0	0.0	0.0	0.1
13/06	2	50.0	50.0	0.1	1	50.0	0.0	0.2
19/06	2	100.0	100.0	0.2	3	100.0	100.0	0.3
Mean	3.35±0.51	53.27±8.63	45.74±8.25	0.42±0.10	2.30±0.33	54.37±8.71	49.09±9.24	0.52±0.11

Table 3.2.3.14. Fruit infestation, egg presence and parasitism of eggs in release and non-release blocks at Halaron, Sundays River Valley 2014.

Date	Release block				Control block			
	Fruit with eggs (%)	Eggs parasitized (%)	Effective parasitism (%)	FCM fruit drop/tree/week	Fruit with eggs (%)	Eggs parasitized (%)	Effective parasitism (%)	FCM fruit drop/tree/week
07/02	1	100.0	100.0	-	2	0.0	0.0	-
13/02	3	0.0	0.0	-	3	33.3	33.3	-
20/02	4	50.0	50.0	-	3	66.7	66.7	-
26/02	2	0.0	0.0	0.0	3	0.0	0.0	0.0
06/03	3	0.0	0.0	1.0	5	16.7	20.0	0.9
13/03	5	33.3	40.0	0.5	6	37.5	16.7	0.2
21/03	4	0.0	0.0	0.4	5	55.6	20.0	1.5
28/03	6	33.3	33.3	1.3	8	41.7	25.0	0.2
03/04	0	0.0	0.0	1.6	0	0.0	0.0	1.2
11/04	9	50.0	55.6	0.1	6	75.0	83.3	0.3
18/04	4	100.0	50.0	0.8	2	100.0	100.0	1.5
26/04	5	80.0	80.0	0.1	8	70.0	62.5	0.4
01/05	2	50.0	50.0	0.3	1	100.0	100.0	0.4
09/05	7	77.8.	57.1	0.2	6	100.0	100.0	1.1
15/05	0	0.0	0.0	0.0	1	100.0	100.0	0.0
23/05	4	100.0	125.0	0.1	1	100.0	100.0	0.1
Mean	3.69±0.61	39.77±10.33	40.06±9.77	0.49±0.15	3.75±0.64	56.03±9.65	51.72±10.27	0.60±0.16

Table 3.2.3.15. Fruit infestation, egg presence and parasitism of eggs in release and non-release blocks at Addo Research Station, Sundays River Valley 2014.

Date	Release block				Control block			
	Fruit with eggs (%)	Eggs parasitized (%)	Effective parasitism (%)	FCM fruit drop/tree/week	Fruit with eggs (%)	Eggs parasitized (%)	Effective parasitism (%)	FCM fruit drop/tree/week
07/02	2	0.0	0.0	-	3	33.3	33.3	-
13/02	0	0.0	0.0	-	1	0.0	0.0	-
20/02	3	33.3	33.3	0.2	3	0.0	0.0	0.2
26/02	5	40.0	40.0	0.1	6	0.0	0.0	0.0
06/03	2	50.0	0.0	0.3	3	33.3	33.3	0.2
13/03	2	0.0	0.0	0.8	3	0.0	0.0	0.6
21/03	3	0.0	0.0	0.5	7	12.5	14.29	1.1
28/03	2	50.0	50.0	0.1	1	0.0	0.0	0.7
03/04	0	0.0	0.0	0.2	0	0.0	0.0	0.3
11/04	0	0.0	0.0	0.0	2	0.0	0.0	0.3
18/04	0	0.0	0.0	0.3	0	0.0	0.0	0.1
26/04	2	100.0	100.0	2.3	1	0.0	0.0	2.1
01/05	1	100.0	100.0	0.1	1	100.0	100.0	0.0
09/05	2	100.0	0.0	0.2	1	0.0	0.0	0.3
15/05	1	100.0	0.0	0.0	0	0.0	0.0	0.0
23/05	2	100.0	0.0	0.6	1	100.0	0.0	0.1
29/05	0	0.0	0.0	0.1	1	0.0	0.0	0.1
04/06	1	100.0	0.0	0.0	1	0.0	0.0	0.0
13/06	0	0.0	0.0	0.0	0	0.0	0.0	0.1
19/06	0	0.0	0.0	0.2	1	0.0	0.0	0.3
Mean	1.40±0.30	38.67±9.97	16.17±7.25	0.33±0.13	1.80±0.43	13.96±6.97	9.04±5.33	0.36±0.12

Results from the four 2012/13 trial sites are summarised in Table 3.2.3.16.

Table 3.2.3.16. *Trichogrammatoidea cryptophlebiae* parasitism of FCM eggs in release and control blocks for the period 7 February to 19 June and FCM infestation of fruit for the period 20 February to 19 June 2014.

Farm	Orchard	Total			Mean % eggs parasitized	Mean infested fruit per tree per week
		Fruit with eggs	Eggs	Parasitised		
Pezula	Release	65	92	41	50.45±9.20	1.03±0.25
	Control	67	87	14	23.35±7.79	0.84±0.22
Eluhlaza	Release	67	101	63	53.27±8.63	0.42±0.10
	Control	46	58	40	54.37±8.71	0.52±0.11
Halarton	Release	59	74	37	39.77±10.33	0.49±0.15
	Control	60	81	46	56.03±9.65	0.60±0.16
Addo Research Station	Release	28	30	15	38.67±9.97	0.33±0.13
	Control	36	43	5	13.96±6.97	0.36±0.12

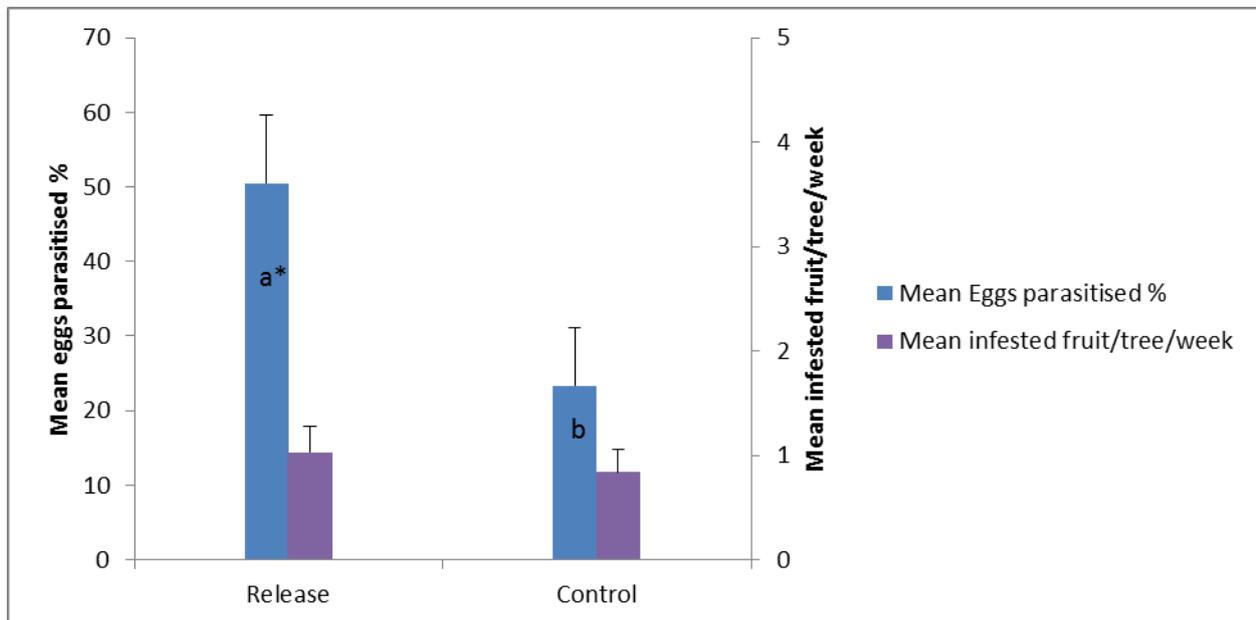


Fig 3.2.3.3. *Trichogrammatoidea cryptophlebiae* parasitism of FCM eggs in release and control blocks for the period 07 February to 19 June and FCM infestation of fruit for the period 26 February to 19 June 2014 on Pezula Farm in the Sundays River Valley. (*Different letters in the same series denote significant differences between values (P=0.0001, Wilks multivariate test of significance (Statistica 2015)).

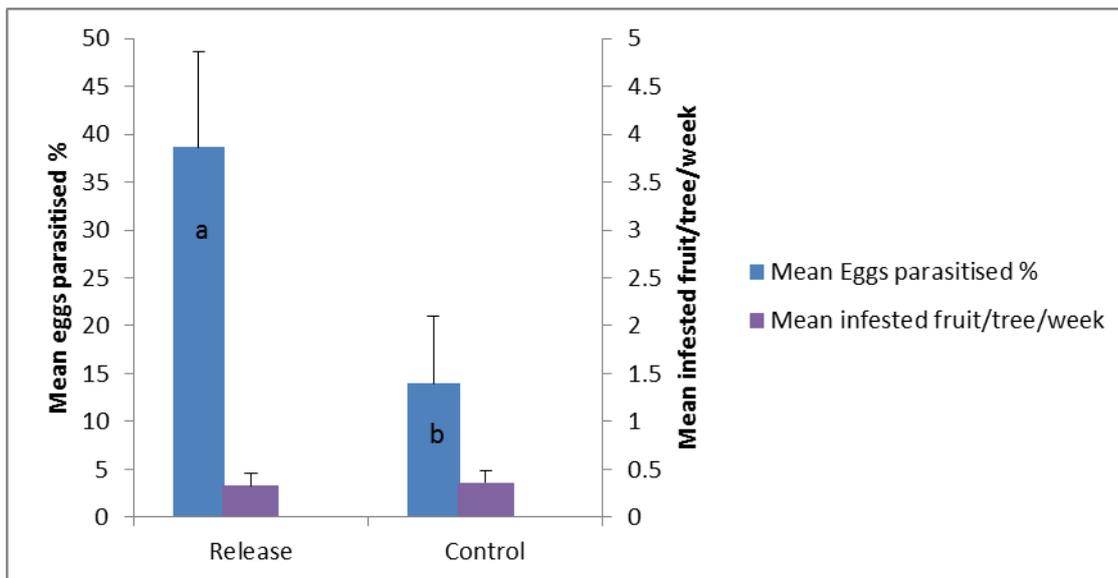


Fig 3.2.3.4. *Trichogrammatoidea cryptophlebiae* parasitism of FCM eggs in release and control blocks for the period 07 February to 19 June and FCM infestation of fruit for the period 26 February to 19 June 2014 at the Addo Research Station in the Sundays River Valley. (*Different letters in the same series denote significant differences between values (P=0.005, Wilks multivariate test of significance (Statistica 2015)).

Conclusions

Augmentation of *T. cryptophlebiae* did not result in lower FCM infestation levels in any of the eight trials conducted over two years. In the first year, FCM levels were very low in three sites. In the fourth, it was concluded that where an orchard environment was conducive to biological control and natural parasitism was at a high level (usually peaking in February), augmentation after February almost became superfluous. In the second year, parasitism was significantly higher in release blocks, but this did not lead to lower FCM infestation levels. Poor parasitoid eclosion rates and thus lower numbers of parasitoids released may have reduced the chances of success, but based on the trial results, late season releases of *T. cryptophlebiae* for the control of FCM should not be recommended.

Technology Transfer

No technology transfer has been conducted within this project, as results have not been sufficiently compelling.

References cited

- Grout, T.G., Stoltz, K.C. and Tate, B.A. 2002. *Database of non-target impact ratings (similar to percentage mortality but taking persistence into account) against five key natural enemies in citrus*. Citrus Research International, Nelspruit.
- Moore, S.D. and Fourie, J.G. 1999. Assessment and development of the augmentation technique for FCM control with the parasitoid *Trichogrammatoidea cryptophlebiae*. *Outspan Citrus Centre Annual Research Report 1999*, pp. 51-58.
- Moore, S.D., Kirkman, W., Mommsen, W. & Beetge, L. 2013. Late season releases of *Trichogrammatoidea cryptophlebiae* for suppression of FCM. *Citrus Research International Annual Research Report 2013*, p
- Moore, S.D. & Richards, G.I. 2000. Assessment and development of the augmentation technique for FCM control with the parasitoid *Trichogrammatoidea cryptophlebiae*. In: *Citrus Research International Annual Research Report*, pp. 23-29.
- Moore, S.D. & Richards, G.I. 2001. Assessment and development of the augmentation technique for FCM control with the parasitoid *Trichogrammatoidea cryptophlebiae*. In: *Citrus Research International Annual Research Report*, pp. 68-74.
- Moore, S.D. & Richards, G.I. 2002. Assessment and development of the augmentation technique for FCM control with the parasitoid *Trichogrammatoidea cryptophlebiae*. In: *Citrus Research International Annual Research Report*, pp.
- Newton, P.J. 1988. Movement and impact of *Trichogrammatoidea cryptophlebiae* Nagaraja (Hymenoptera: Trichogrammatidae) in citrus orchards after inundative releases against the false codling moth,

Cryptophlebia leucotreta (Meyrick) (Lepidoptera: Tortricidae). *Bulletin of Entomological Research* 78: 85-99.

Newton, P.J. & Odendaal, W.J. 1990. Commercial inundative releases of *Trichogrammatoidea cryptophlebiae* (Hym: Trichogrammatidae) against *Cryptophlebia leucotreta* (Lepidoptera: Tortricidae) in Citrus. *Entomophaga* 35(4): 545-556.

Schwartz, A. 1980. Eier-parasiet van valskodlingmot: evaluasie van 'n teel- en vrylaatprogram. *The Citrus and Subtropical Fruit Journal*, January 1980: 6-8.

Statistica. 2015. Statsoft, version 12.

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

Project 1024 (Jan 2011 – Dec 2015) by Candice Coombes, Martin Hill, Jo Dames (RU) and Sean Moore (CRI)

Summary

Over the 2014/2015 financial year, two field trials were completed with a further three initiated. Results of completed field trials have been reported in previous annual reports. Major findings indicated that *B. bassiana* isolate Bb1 (G Ar 17 B3) was more effective in reducing FCM infestation in citrus orchards employing micro-jet irrigation than *M. anisopliae* isolates Ma1 (G 11 3 L6) and Ma2 (FCM Ar 23 B3) (field trial 1). Further analysis of Bb1 (field trial 2) indicated that as a late application spray, a reduction in FCM infestation of 50% could be achieved. Of the three field trials, two were initiated in October 2014 (field trial 3 and 4), the other in March 2015 (field trial 5). Based on a completed cage trial, the results of which are reported in previous annual reports, the poorer performance of Ma1 in reducing FCM eclosion of artificially introduced FCM fifth instars after fungal application, resulted in its exclusion in any further field analysis. In field trial 3, application of Ma2 and Bb1 occurred as in field trial 1, but to treatment blocks under drip irrigation. Preliminary analysis of fruit drop results suggests that under drip irrigation, Ma2 is more effective in reducing FCM infestation than Bb1 in comparison to the control; 57% versus 39% respectively. Field trial 4 was initiated to determine whether application of Ma2 and Bb1 could be applied at lower rates (1×10^{12} and 1×10^{13} as opposed to the current field rate of 1×10^{14} spores/ha). Low fruit drop coupled with even lower FCM infestation rates in both treatment and control blocks, has however prohibited any conclusions from being drawn. The highest number of infected fruit has however been obtained from blocks to which Ma2 was applied. Field trial 5 was initiated as a late application spray. Only isolate Bb1 was applied to the treatment area. Application occurred via the irrigation system (micro-jet sprinklers). No results are yet available, but the presence of Bb1 in the soil was confirmed by CFU analysis. All field trials are still in progress and will be terminated upon harvest.

Previous trials had indicated the persistence of Ma1, Ma2 and Bb1 in a citrus orchard to be good; all isolates persisted in sterile soil for six months. Given the methodology used, the true persistence of these isolates was unknown and has since been confirmed. Under micro-jet irrigation, CFU analysis of soil samples collected from within each treatment block showed that all three isolates persisted for six months. In addition, following an initial decline, approximately three months post-application fungal titre was found to increase. This is thought to be related to the infection and mycosis of FCM within the soil. These results are reported in previous annual reports. The persistence of isolates Ma2 and Bb1 under drip irrigation is now being monitored over the 2014/2015 citrus growing season.

During field trials, dried spores of the fungal isolates were suspended in water supplemented with the surfactant Breakthru[®] S240. It is known that surfactants can impact the physical (e.g. dispersion and retention of spores within the medium) and biological (=viability) properties of fungi differently. To determine if the use of an alternate surfactant could be used to enhance field application by improving the emulsification characteristics (e.g. retention of spores in suspension) and viability of Bb1 and Ma2, five different surfactants (Breakthru[®] S240, Breakthru[®] S233, Breakthru[®] OE 446, Breakthru[®] Advance and BP Medium Oil) were evaluated. Emulsification characteristics were analysed according to the protocol outlined for chemical pesticide formulation testing in the CIPAC Handbook Volume F (Dobrat & Martjin 1995). Viability was measured according to Inglis *et al.* (2012). Major findings highlighted that BP medium oil is not suitable as a wetting agent unless immediate application occurs due to the clumping of spores upon re-emulsification. Oils are typically used in foliar application to prevent desiccation of the spores and provide protection from UV radiation. Of the other four Breakthru[®] products investigated, no major differences were apparent with regard to both their physical characteristics as wetting agents and their toxicity towards spores of Bb1 and Ma2. Breakthru[®] S240 was however slightly toxic to the spores of Ma2 compared to the other wetting agents after 24 hours exposure. However, the concentration of wetting agents used in these

experiments, was higher than that used in the field. Viability was always above 70%. More research is however necessary to determine the use of these wetting agents in medium and long term storage as well as their potential benefits in protecting spores from environmental conditions in the field. In addition, as surfactants may alone be toxic to the target insects, bioassays examining this may be warranted.

Opsomming

Oor die 2014/2015 finansiële jaar is twee veldproewe voltooi, en 'n verdere drie geïnisieer. Resultate van voltooide veldproewe is in vorige verslae berig. Belangrike bevindinge dui daarop dat die *B. bassiana* isolaat Bb1 (G Ar 17 B3) meer effektief was in die vermindering van VKM as die *M. anisopliae* isolate Ma1 (G 22 3 L6) en Ma2 (FCM Ar 23 B3) in situsboorde waar mikro-besproeiing gebruik is (veldproef 1). Verdere analise van Bb1 (veldproef 2) dui daarop dat met 'n laat aanwend, 'n reduksie in VKM infestasië van 50% bereik kan word. Van die drie veldproewe, is twee geïnisieer in Oktober 2014 (veldproef 3 en 4) en die ander een in Maart 2015 (veldproef 5). Gebaseer op 'n voltooide hokproef, waarvan die resultate in vorige jaarlikse verslae berig is, het die swakker prestasie van Ma1 in die vermindering van VKM ontpopping van kunsmatig aangewende VKM vyfde instar larwes na swam aanwending, gelei tot die uitsluiting van enige verdere veldproewe. In veldproef 3, was die toediening van Ma2 en Bb1 dieselfde as in veldproef 1, maar in blokke onder drup-besproeiing. Voorlopige analises van vrugval resultate dui daarop dat onder drup-besproeiing, Ma2 meer effektief is in die vermindering van VKM besmettings as Bb1 in vergelyking met die kontrole: 57% teenoor 39% onderskeidelik. Veldproef 4 is geïnisieer om vas te stel of Ma2 en Bb1 teen 'n laer konsentrasie toegedien kan word (1×10^{12} en 1×10^{13} teenoor die huidige konsentrasie van 1×10^{14} spore/ha). Lae vrugval tesame met selfs laer VKM besmetting in beide die behandelde en kontrole blokke, het egter enige gevolgtrekkings verhoed. Die hoogste aantal besmette vrugte is egter verkry in 'n blok waar Ma2 aangewend is. Veldproef 5 is geïnisieer as 'n laat toediening bespuiting. Slegs Bb1 is toegedien in die behandelings area. Toediening is deur middel van die besproeiings sisteem (mikro-besproeiing) toegedien. Geen resultate is tot dusver beskikbaar nie, maar die teenwoordigheid van Bb1 in die grond is bevestig deur CFU analises. Alle veldproewe is nog aan die gang, en sal teen oestyd beëindig word.

Vorige proewe het getoon dat die nawerking van Ma1, Ma2 en Bb1 in 'n sitrusboord goed is; alle isolate is volhou in steriele grond vir ses maande. Gegewe die metodiek wat gebruik is, was die ware nawerking van die isolate onbekend, en is sedertdien bevestig. Onder mikro-besproeiing wys CFU analises van grondmonsters, geneem binne elke behandelings blok, dat alle isolate vir ses maande voortgeduur het. Daarbenewens, ná 'n aanvanklike afname, is gevind dat swam titer ongeveer drie maande na toediening verhoog het. Dit hou vermoedelik verband met die infeksie en mikose van VKM in die grond. Die resultate is berig in vorige jaarlikse verslae. Die nawerking van die isolate Ma2 en Bb1 onder drupbesproeiing word in die 2014/2015 groei seisoen gemonitor.

Gedurende veldproewe, is gedroogte spore van die swam isolate in water opgelos met die benatter Breakthru[®] S240. Dit is bekend dat benatters verskillende impakte kan hê op die fisiese (dws dispersie en retensie van spore in die medium) en biologiese eienskappe (=lewensvatbaarheid) van swamme. Om te bepaal of 'n alternatiewe benatter toediening in veldproewe kon verbeter, deur die verbetering van die emulsifisering eienskappe (dws retensie van spore in suspensie) en die lewensvatbaarheid van Bb1 en Ma2, is vyf verskillende benatters (Breakthru[®] S240, Breakthru[®] S233, Breakthru[®] OE 466, Breakthru[®] Advance en BP Medium Olie) geëvalueer. Emulsifiserings eienskappe is geanaliseer volgens die protokol vir chemiese plaagdoder formulasie toetse in die CIPAC Handbook Volume F (Dobrat & Martjin 1995). Lewensvatbaarheid is gemeet volgens Inglis *et al.* (2012). Belangrike bevindinge beklemtoon dat BP Medium Olie nie geskik is as 'n benattingsmiddel nie tensy toediening dadelik plaasvind weens die verklompings van spore by her-emulsifisering. Olies word tipies gebruik vir blaas toediening om uitdroging van die spore te voorkom en om beskerming te bied teen UV-bestraling. Van al vier die ander Breakthru[®] produkte wat ondersoek is, is geen belangrike verskille duidelik met betrekking tot beide hul fisiese eienskappe as benattingsmiddel en hul toksisiteit teenoor die Bb1 en Ma2 spore nie. Breakthru[®] S240 was wel effens giftig vir die spore van Ma2 vergeleke met die ander benattingsmiddels na 24 uur blootstelling, alhoewel die konsentrasie benattingsmiddel gebruik in die eksperimente hoër was as die gebruik in die veld. Lewensvatbaarheid was altyd bo 70%. Meer navorsing is nodig om vas te stel of hierdie benattingsmiddels gebruik kan word vir medium- en langtermyn berging, sowel as die potensieële voordele vir die beskerming van spore van omgewings toestande in die veld. Omdat benatters alleen giftig kan wees vir die teiken insekte, word biotoetse regverdig.

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

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

Summary

Due to a Pest Risk Assessment (PRA) having been recently conducted by the European Plant Protection Organisation (EPPO) on FCM and the impending debilitating outcome of this PRA for export of southern African citrus to Europe, a study on the effect of abbreviated cold treatments on FCM survival was initiated in 2012. This study was continued in 2013 and 2014. In order to support the validity of the findings, a number of supporting trials were conducted. It was concluded that cold-susceptibility of laboratory reared larvae in artificial diet was comparable to that of wild larvae in fruit and therefore larvae in diet could be used for all further trials. It was also concluded that cold susceptibility of larvae in different citrus cultivars was comparable and that larvae from the Eastern Cape could be used in trials, as they were found to be the most cold tolerant amongst all regions, although usually not significantly so. A temperature of 2°C for 18 days caused 99.61% mortality of 4th and 5th instar larvae and reduced viability (ability to produce a subsequent generation) by 99.98%. Additionally, cold sterilisation trials were conducted, achieving probit 9 mortality of 5th instar larvae at -0.4°C for 18 days. From field collected infested fruit from different regions, up to 67% of larvae in one of the samples were carob moth. Cold treatment trials showed carob moth larvae to be significantly more cold-susceptible than FCM larvae.

Opsomming

As gevolg van 'n Plaag Risiko Analise (PRA) wat pas deur die Europese Plantbeskermings Organisasie (EPPO) op VKM uitgevoer is en die dreigende uitkoms van die PRA vir die uitvoer van sitrus van suidelike Afrika Europa toe, is 'n studie op die effek van verkorte koue behandelings op VKM oorlewing in 2012 geïnisieer. Hierdie studie is in 2013 en 2014 voortgesit. Om die waarde van die bevindinge te ondersteun is verskeie ondersteunende proewe uitgevoer. Dit is gevind dat koue gevoeligheid van laboratorium geteelde larwes in kunsmatige dieet vergelykbaar was met wilde larwes in vrugte en daarom kon larwes in dieet vir alle verdere proewe gebruik word. Dit is ook gevind dat koue gevoeligheid van larwes in verskillende sitrus kultivars vergelykbaar was en dat larwes van die Oos-Kaap in proewe gebruik kon word, omrede hulle die meeste koue bestand was van alle streke, al gewoonlik nie betekenisvol so nie. 'n Temperatuur van 2°C vir 18 dae het 99.61% mortaliteit van 4de en 5de instar larwes veroorsaak en het lewensvatbaarheid (vermoë om 'n volgende generasie te produseer) met 99.98% verminder. Daarbenewens is koue-sterilisatie proewe uitgevoer en probit 9 mortaliteit is met 5de instar larwes bereik met -0.4°C vir 18 dae. Van veld versamelde besmette vrugte van verskillende streke is tot 67% van die larwes in een monster karobmot. Koue behandelings proewe het gewys dat karobmot larwes beduidend meer koue-gevoelig as VKM larwes was.

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

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

Summary

One late season corrective trial was conducted with *Heterorhabditis bacteriophora* against FCM in citrus orchards in the Eastern Cape during each of the 2013/14 and 2014/15 seasons. The first trial was initiated on 6 March in a Newhall Navel orchard in Sundays River Valley which was heavily attacked by FCM. The EPNs were applied at 10 and 20 IJs/cm². FCM caught in traps and fruit infestation were monitored for eight weeks until 15 May. Fewest moths were caught in the 20 IJs/cm² orchard and most moths were caught in the untreated control. However, the differences were not great. Fruit infestation was also lowest for the 20 IJs/cm² treatment, but there was no difference between the lower treatment and the untreated control. FCM infestation was surprisingly and disappointingly low. A high-pressure corrective trial was also planned for Mpumalanga, but FCM levels never picked up, so the trial was cancelled. The trial was repeated in the Eastern Cape during the 2014/15 season; results will be reported during the next cycle.

Opsomming

Een laat-seisoen korrektiewe proef is met *Heterorhabditis bacteriophora* teen VKM in sitrusboorde in die Oos-Kaap gedurende elk van die 2013/14 en 2014/15 seisoene uitgevoer. Die eerste proef is op 6 Maart in

'n Newhall Nawel boord in die Sondagsrivier Vallei uitgevoer waar 'n hoë teenwoordigheid van VKM vermoed is. Die EPNs is teen 10 en 20 IJs/cm² toegedien. VKM lokval vangstes en vrugbesmetting is vir agt weke tot 15 Mei geëvalueer. Die minste motte is in die 20 IJs/cm² behandeling gevang en die meeste motte is in die onbehandelde boorde gevang, alhoewel die verskille nie groot was nie. Vrugbesmetting is ook die laagste vir die 20 IJs/cm² behandeling maar daar is geen verskil tussen die laagste behandeling en die onbehandelde kontrole nie. VKM besmetting is verbasend en teleurstellend laag. 'n Hoë druk korrektiewe proef is ook vir Mpumalanga beplan maar VKM vlakke het nooit toegeneem nie en dus is die proef gekanselleer. Die proef is gedurende die 2015/15 seisoen in die Oos-Kaap herhaal; resultate sal gedurende die volgende siklus berig word.

3.2.7 FINAL REPORT: Gene expression analysis of *Thaumatotibia leucotreta* as result of different isolates of *Cryptophlebia leucotreta* granulosis virus Project 1049 (2012/01 – 2014/12) by A.E. Timm & J. Ridgeway (CRI)

Summary

Gene expression studies provide baseline information on the interactions of insects with their environment. Despite the importance of this information, limited gene expression data are available for most insect pests, including the family Tortricidae (Lepidoptera), which includes *Thaumatotibia leucotreta* (Meyr), an important agricultural pest in Africa. Because *T. leucotreta* can be controlled successfully by a granulovirus, this system is a good model for exploring insect-virus susceptibility. The main aim of this study was to investigate gene expression of *T. leucotreta* in response to virus infection. However, before pursuing this aim, two objectives required completion. First, the most suitable RNA extraction method for insects needed to be determined and second, the most suitable reference genes for qPCR for Tortricidae pests needed to be identified. Once these objectives were accomplished, the response of *T. leucotreta* to its granulovirus was evaluated at different temperatures and points after infection. Four RNA extraction methods, the RNeasy® Mini Kit, SV Total RNA isolation system, TRIzol® reagent, and a CTAB-based method, were compared using two beetle and two moth species, including *T. leucotreta*. The quality of extracted RNA was similar for all four species for all extraction methods. Based on several criteria, the best RNA extraction method was the SV Total RNA isolation system. Six candidate reference genes were evaluated for qPCR using different tissue types of *T. leucotreta* and two other Tortricidae pests. Additionally, reference genes were evaluated for *T. leucotreta* with and without its granulovirus at different temperatures. Reference gene stability was found to be dependent on species and tissue type. Overall the most suitable combination of reference genes for *T. leucotreta* were α -actin, arginine kinase and elongation factor 1- α . Gene expression of *T. leucotreta* in response to granulovirus infection at different temperatures and intervals after infection was evaluated by qPCR using 13 target genes associated with the infection process. Most genes were down-regulated after 24 and 48 h.p.i. However, after 72 h.p.i most genes were up-regulated. The same trend was observed at different temperatures, where most genes were down-regulated at 15°C and 25°C but up-regulated at 35°C. These results show that there is a dynamic gene expression response in *T. leucotreta* due to granulovirus infection under different conditions. Not only do these findings provide insight into the control of this tortricid pest, they also contribute further to our knowledge of insect-virus interactions.

This full report is available in the form of an MSc thesis by Jarryd Ridgeway entitled "Gene expression analysis of *Thaumatotibia leucotreta* in response to the *Cryptophlebia leucotreta* granulosis virus", available through Rhodes University.

Opsomming

Gene uitdrukings studies voorsien basislyn inligting oor die interaksie van insekte met hulle omgewing. Ondanks die belangrikheid van hierdie inligting, is beskikbare gene uitdrukings inligting beperk vir meeste insek spesies, insluitend die Tortricidae (Lepidoptera) familie, wat *Thaumatotibia leucotreta* (Meyr) insluit, 'n belangrike landbou plaag in Afrika. Omdat *T. leucotreta* deur 'n granulovirus suksesvol beheer kan word, is hierdie stelsel 'n goeie model vir 'n ondersoek aan insek-virus vatbaarheid. Die hoofdoel van hierdie studie was om gene uitdrukking van *T. leucotreta* in reaksie teenoor virus besmetting te bestudeer. Voor hierdie doel egter aangepak kon word, moes twee ander mikpunte eers voltooi word. Eerstens moes die mees geskikte RNA ekstraksie metode vir insekte bepaal word en tweedens moes die mees geskikte verwysings genes vir qPCR vir Tortricidae plaeg geïdentifiseer word. Sodra hierdie mikpunte voltooi was, is die reaksie van *T. leucotreta* teenoor sy granulovirus geëvalueer teen verskillende temperature en tye na infeksie. Vier RNA ekstraksie metodes is vergelyk met die gebruik van twee kewer en twee mot spesies, insluitend *T. leucotreta*: die RNeasy® Mini Kit, SV Total RNA isolasie stelsel, TRIzol® reagent, en 'n CTAB-gebaseerde metode. Die gehalte van geëkstrakteerde RNA was vir al vier spesies en alle metodes vergelykbaar.

Gebaseer op verskeie kriteria, was die beste RNA ekstraksie metode die SV Total RNA isolasie stelsel. Ses kandidaat verwysings genes is vir qPCR geëvalueer met die gebruik van verskillende weefsel tipes van *T. leucotreta* en nog twee ander Tortricidae plae. Bonop is verwysings genes vir *T. leucotreta* geëvalueer met en sonder sy granulovirus teen verskillende temperature. Dit is gevind dat verwysings gene stabiliteit afhangend is van spesie en weefsel tipe. Oor die algemeen is die mees geskikte kombinasie van verwysings genes vir *T. leucotreta* α -actin, arginine kinase en verlengings faktor 1- α . Gene uitdrukking van *T. leucotreta* in respons teenoor granulovirus besmetting teen verskillende temperature en intervale na besmetting is deur qPCR geëvalueer met die gebruik van 13 teiken genes wat met die besmettings proses geassosieer is. Meeste genes is na 24 en 48 ure na besmetting afgereguleer, maar na 72 ure na besmetting is meeste genes opgereguleer. Dieselfde tendens is teen verskillende temperature opgelet, waar meeste genes teen 15°C en 25°C afgereguleer is maar teen 35°C opgereguleer is. Hierdie resultate wys dat daar 'n dinamiese gene uitdrukings respons in *T. leucotreta* is as gevolg van granulovirus besmetting onder verskillende omstandighede. Hierdie bevindings voorsien nie net insig vir die beheer van hierdie Tortricidae plaag nie maar dra ook by tot verdere kennis van insek-virus interaksies.

Hierdie volle verslag is beskikbaar as 'n MSc tesis deur Jarryd Ridgeway onder die titel "Gene expression analysis of *Thaumatotibia leucotreta* in response to the *Cryptophlebia leucotreta* granulosus virus", en beskikbaar deur Rhodes Universiteit.

Results and discussion

Table 3.2.7.1. Fold change difference between control and CrleGV infected *T. leucotreta* at different time points post infection, with values shown for infected larvae. Bold values identify significant fold changes above the 2-fold threshold. P-values represent the likelihood of the value occurring at random.

Gene name	Gene function	24hpi		48hpi		72hpi	
		change	p-value	change	p-value	change	p-value
Cell Death	Defence	1.14	0.814	-1.26	0.126	3.22	0.000
Gelsolin	Cytoskeletal maintenance	-2.21	0.416	-2.72	0.000	1.12	0.755
Cytochrome oxidase 1	Metabolism	1.38	0.019	-2.03	0.002	1.28	0.065
Dicer	Defence	1.86	0.036	2.10	0.000	2.84	0.000
Enolase	Metabolism	-1.92	0.048	-2.31	0.013	-1.1	0.466
Juvenile hormone epoxide hydrolase (a)	Juvenility	-2.14	0.001	-3.36	0.000	2.66	0.003
Juvenile hormone epoxide hydrolase (b)	Juvenility	-2.20	0.000	-4.20	0.000	2.75	0.010
Glutathione peroxidase	Metabolism	-1.58	0.028	-3.58	0.000	1.25	0.004
Heat Shock Protein 70	Cell stabilization	-1.21	0.461	-1.53	0.015	2.05	0.001
Heat Shock Protein 90	Cell stabilization	-1.06	0.949	-1.59	0.000	1.17	0.450
Initiation factor 5a ref AF109731	Cell maintenance	1	0.942	-1.7	0.000	1.2	0.137
Mitochondrial aldehyde dehydrogenase	Metabolism	-1.04	0.976	-2.48	0.000	1.23	0.163
α-Tubulin	Cytoskeletal maintenance	1.36	0.246	1.18	0.199	1.1	0.795

Table 3.2.7.2. Fold change difference between control and CrleGV infected *T. leucotreta* at different temperatures, with values shown for infected larvae. Bold values identify fold changes above the 2-fold threshold. P-value represents the likelihood of the value occurring at random.

Gene name	Gene function	15°C		25°C		35°C	
		change	p-value	change	p-value	change	p-value
Cell Death	Defence	-1.59	0.074	-1.26	0.126	-1.19	0.000
Gelsolin	Cytoskeletal maintenance	1.52	0.248	-2.72	0.000	4.69	0.000
Cytochrome oxidase 1	Metabolism	1.09	0.452	-2.03	0.002	2.28	0.000
Dicer	Defence	-2.04	0.003	2.1	0.000	1.59	0.000
Enolase	Metabolism	-1.01	0.641	-2.31	0.013	5.32	0.003
Juvenile hormone epoxide hydrolase(a)	Juvenility	-1.85	0.247	-3.36	0.000	2.62	0.000
Juvenile hormone epoxide hydrolase (b)	Juvenility	-1	0.924	-4.2	0.000	2.3	0.000
Glutathione peroxidase	Metabolism	-1.41	0.002	-3.58	0.000	1.49	0.000
Heat Shock Protein 70	Cell stabilization	-1.16	0.294	-1.53	0.015	1.47	0.000
Heat Shock Protein 90	Cell stabilization	1.02	0.874	-1.59	0.000	-1.04	0.000
Initiation factor 5a	Cell maintenance	1.34	0.037	-1.7	0.000	1.38	0.000
Mitochondrial aldehyde dehydrogenase	Metabolism	-1.02	0.865	-2.48	0.000	1	0.000
α- Tubulin	Cytoskeletal maintenance	2.21	0.000	1.18	0.199	2.11	0.000

Technology Transfer

The findings of the study were presented as posters at the 2012 and 2014 Citrus Research Symposiums.

The study has also been published in two peer-review scientific journals as:

Ridgeway JA, Timm AE (2015) Reference Gene Selection for Quantitative Real-Time PCR Normalization in Larvae of Three Species of Grapholitini (Lepidoptera: Tortricidae). PLoS ONE 10(6): e0129026. doi:10.1371/journal.pone.0129026

Brits D, Ridgeway JA, Timm AE (2015) Laboratory evaluation of temperature effects on the efficacy of *Cryptophlebia leucotreta* granulovirus (CrLeGV-SA) on fourth-instar false codling moth larvae. African Entomol 23. doi: 10.4001/003.023.0106

3.2.8 FINAL REPORT: Behaviour, biology and survival of pupating false codling moth

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

Summary

Promising developments in the field of microbial control of false codling moth (FCM) through the use of entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPNs) have highlighted the need for research regarding pupation biology, behaviour and survival of FCM, as a good understanding of biology of the target organism is an important component of any biological control programme. The aim of this study was to improve the current understanding of FCM pupation habits through the manipulation of soil texture class, groundcover, shading, soil compaction, air temperature, and soil moisture in the laboratory. These findings would then be used to aid the biological control programmes using EPF and EPNs against FCM in the soil. Three soil texture classes (sandy loam, silt loam and silty clay loam) were obtained from orchards for use in the study. FCM larvae were allowed to drop into the soil of their own accord and the pupation behaviour that followed was then captured on film with pupae formed in the soil being kept in order to measure adult eclosion.

In general, very few abiotic factors had a clear influence on FCM pupation. Larval wandering time and distance was short, but also variable between individuals. Distance did increase when soils were moist. Pupation depth was shallow, with pupal cocoons generally being formed on the soil surface. Depth of pupation was less than one centimetre for all abiotic conditions, with little burrowing into soil. When comparing just the three different soil texture classes, FCM eclosion success was highest in sandy soil and this further increased when the sandy loam soil moisture content was maintained at field capacity. FCM was sensitive to desiccation when the soils were dry. Air temperature limits of 15°C and 32°C had a strongly negative impact on eclosion success. Preferences for particular abiotic conditions were limited to only certain soil texture classes when comparing dry soils and soils at field capacity moisture levels. When provided with a choice of areas with or without soil, FCM larvae showed a strong preference for pupating in areas with soil. No preference was found for pupation in sandy loam soil over the other soil texture classes when the soils were dry, despite eclosion success being highest for this soil texture class under these conditions. It was noted that when using this experimental design, individuals pupated in close proximity to one another. Viable direct habitat manipulation for FCM control could not be identified. These results and all of the abiotic variables measured have important implications for EPF and EPN application, survival and persistence in the soil in order to improve the ability of these biological control agents to control FCM.

The full report of this study can be found in the MSc thesis, "The biology, behaviour and survival of pupating false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), a citrus pest in South Africa", by Claire Love, available through Rhodes University library.

Opsomming

Belowende ontwikkelinge in die veld van mikrobiese beheer van valskodlingmot (VKM) deur die gebruik van entomopatogeniese swamme (EPF) en entomopatogeniese nematodes (EPNs) het die noodsaaklikheid van navorsing oor die biologie, gedrag en oorlewing van die VKM papie stadium beklemtoon, omrede 'n goeie begrip van die biologie van die teiken organisme 'n belangrike komponent van enige biologiese beheer program is. Die doel van hierdie studie was om die huidige begrip van die VKM papie stadium gewoontes deur die manipulasie van grondtekstuur klas, grondbedekking, skadering, grondverdigting, lugtemperatuur, en grondvog in die laboratorium te verbeter. Hierdie bevindinge sal dan gebruik word om biologiese beheer programme deur die gebruik van EPFs en EPNs teen VKM in die grond te verbeter. Drie grondtekstuurklasse (sandleem, sliikleem en slikkleileem) was uit die boorde versamel vir gebruik in die studie. VKM larwes is toegelaat om uit hulle eie op die grond af te sak en die gedrag van die papie stadium is daarna verfilm. Papias wat in die grond gevorm het is behou om ontpopping van volwassenes te meet.

Oor die algemeen het baie min abiotiese faktore 'n duidelike invloed op die VKM papie stadium gehad. Tydsduur en afstand wat larwes gedwaal het was kort, maar daar was ook baie variasie tussen individue. Afstand het vermeerder wanneer die grond klam was. Diepte waar papievorming plaasgevind het was vlak, met meeste papie kokonne op die grondoppervlak. Vir alle abiotiese toestande is papievormings diepte minder as een millimeter, met min penetrasie tot in die grond. Ontpoppings sukses was hoër vir sanderiger gronde waar hulle droog en nie gekompakteer was nie, maar die byvoeging van beide vog en grondverdigting het VKM ontpoppings sukses verhoog. VKM was geneig om uit te droog wanneer die grond droog was. Temperatuur perke van 15°C en 32°C het 'n sterk negatiewe impak op ontpoppings sukses gehad. Voorkeure vir spesifieke abiotiese toestande is beperk tot net sekere vogtoestande wanneer droë gronde en gronde teen veld-kapasiteit vog vlakke vergelyk is. Daar was geen duidelike voorkeur vir spesifieke grondteksture nie, ten spyte van die sterk invloed op ontpoppings sukses. Dit het geblyk dat papies nie naby aan mekaar gevorm het nie. Geen praktiese geleentheid vir direkte habitat manipulasie vir verbeterde beheer van VKM kon geïdentifiseer word nie. Hierdie resultate en al die abiotiese faktore wat gemeet is het belangrike implikasies vir EPF en EPN toediening, oorlewing en nawerking in die grond om die vermoë van hierdie biologiese beheer agente vir VKM beheer te verbeter.

Die volle verslag van hierdie studie is beskikbaar in die MSc tesis "The biology, behaviour and survival of pupating false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), a citrus pest in South Africa", deur Claire Love, deur Rhodes Universiteit biblioteek.

Results and discussion

A significant relationship was found between soil texture class and eclosion success of FCM adults, with sandy loam having a significantly higher number of successfully eclosed adults than silt loam, but not silty clay loam (Chi-square = 10.058, df = 2, p = 0.006). Silt loam had the lowest eclosion success with only 57% of the pupae produced in this soil giving rise to viable adult moths, while sandy loam soils had the highest amount of successful eclosion with 90% and silty clay loam with 75% (Fig. 3.2.8.1). Increased FCM control measures may be required in drier, high sand content soils.

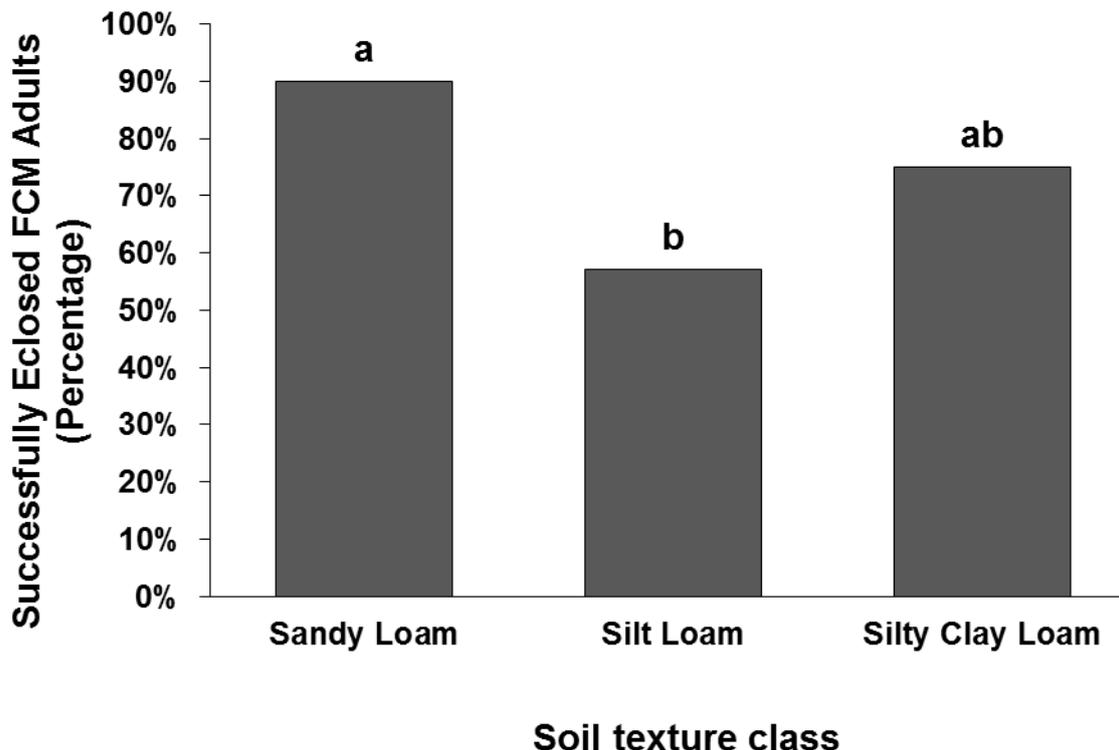


Figure 3.2.8.1. The percentage of successfully eclosed FCM adults from pupae formed in the three different soil texture classes (n = 63). Different letters denote significant differences (Chi-square test, p < 0.05).

Pupation depth was significantly shallower in uncompacted silt loam soil and silty clay loam soil when compared to the sandy loam compacted soil treatment and all three compacted soil treatments ($H_{(5,180)} = 29.54$, p < 0.0001) (Fig. 3.2.8.2). Since the sandy loam soil does not compact well to begin with, no difference was found between the compacted and uncompacted sandy loam soils. FCM larvae made little effort to physically burrow into the soil, with the increase in pupation depth being largely due to changes in

the physical structure of the soil once compacted. This shallow pupation depth could potentially increase the likelihood of contact between FCM and EPFs or EPNs being applied to the soil.

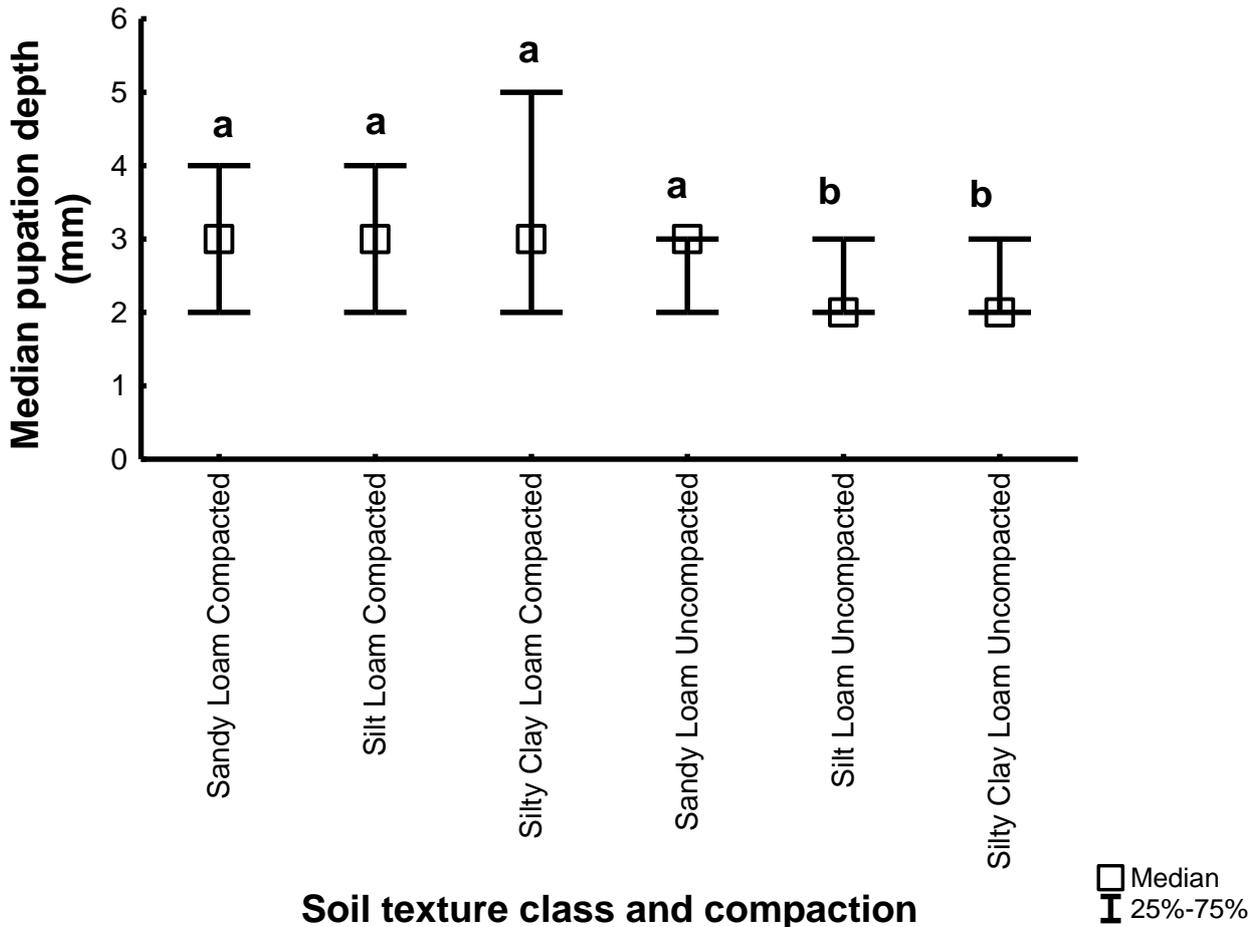


Figure 3.2.8.2. Median depth of FCM pupation in three different soil texture classes with compacted and uncompacted soil (n = 180). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The effect of air temperature on the eclosion success of FCM was clearly demonstrated with a significant association being found between air temperature and FCM eclosion. The 25°C air temperature had a significantly higher eclosion success rate (73.3%) than either the 15°C (26.7%) or 32°C (25.6%) experiments (Chi-square = 55, df = 2, $p < 0.000$). Both high and low temperatures had a negative impact on FCM eclosion success (Fig. 3.2.8.3).

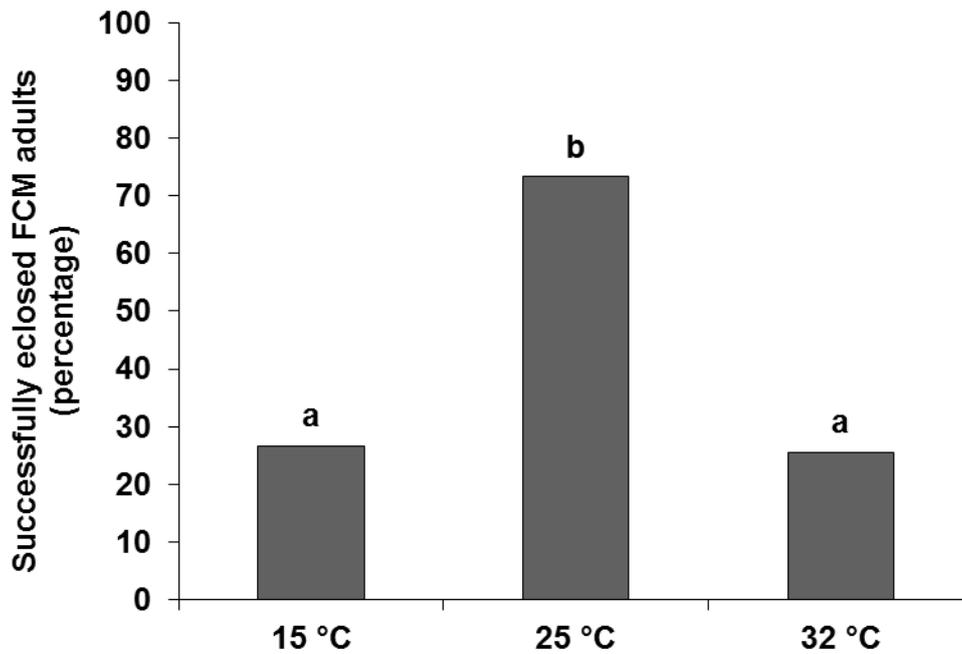


Figure 3.2.8.3 The percentage of successfully eclosed FCM adults from pupae formed in soil of the three different soil texture classes combined at temperatures of 15 °C (low temperature), 25 °C or 32 °C (high temperature) (n = 113). Different letters denote significant differences (Chi-square test, $p < 0.05$).

For a comparison of the overall effect of soil moisture, irrespective of soil texture, all three textures were combined. The amount of time spent by FCM larvae wandering on the soil surface was significantly higher for soils when at field capacity (FC) moisture than at the refill point (RP) or when dry ($H_{(2,270)} = 17.849$, $p = 0.0001$) (Fig. 3.2.8.4). This increase in wandering time will increase exposure to EPFs and EPNs. Furthermore, moisture is an essential component for both biological control agents and is expected to assist in EPF and EPN survival.

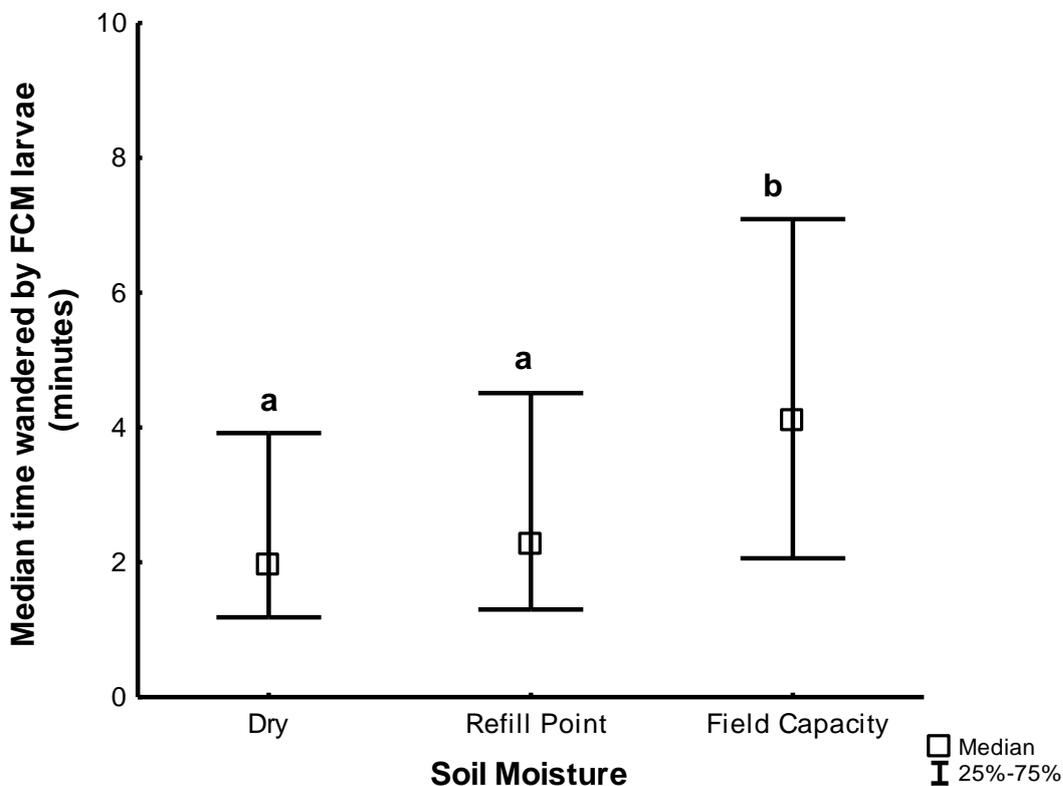


Figure 3.2.8.4. The median amount of time wandered by FCM larvae on the soil surface of the three different soil texture classes prior to pupation site selection with soils of varying moisture content (dry, refill

point or field capacity) (n = 270). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

When comparing all three texture classes amongst all three soil moistures the eclosion success of the silt loam soil was consistently significantly lower than that of all the sandy loam treatments, regardless of soil moisture content (Chi-square = 34.224; df = 8; $p < 0.0001$) (Fig. 3.2.8.5). Increased soil moisture does result in higher eclosion success rates of FCM adults, but is also expected to provide more optimal conditions for EPFs and EPNs, thus also potentially providing improved control.

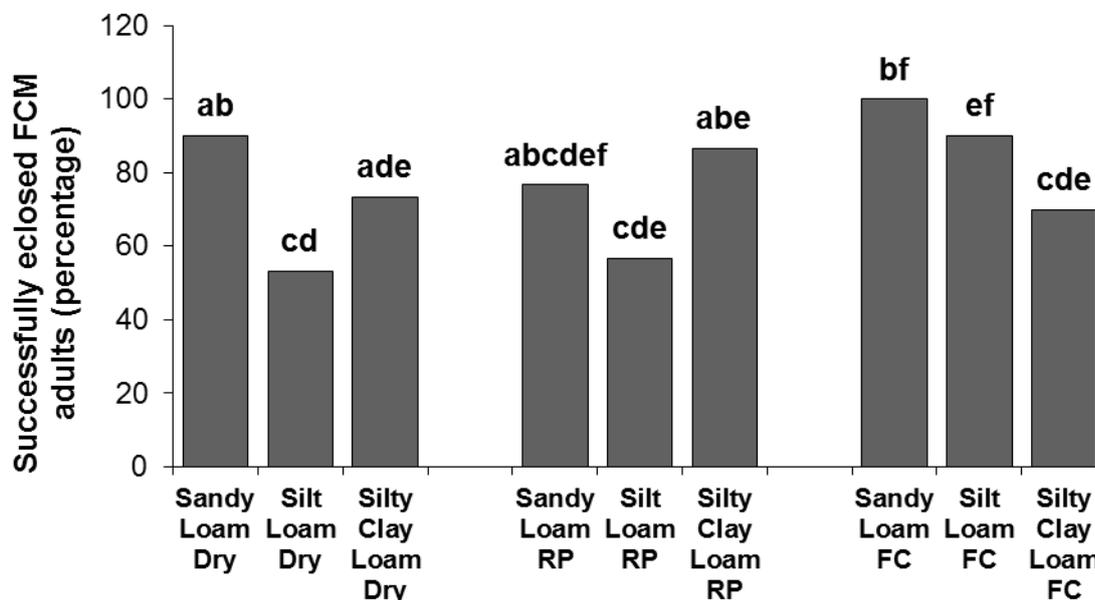


Figure 3.2.8.5. The percentage of successfully eclosed FCM adults from pupae formed in each of the soil texture classes at each soil moisture content (n = 207). Different letters denote significant differences (Chi-square test, $p < 0.05$).

Technology Transfer

Talks or presentations:

Love, C.N., Hill, M.P. and Moore, S.D. (2014, August). *The biology, behaviour and survival of pupating false codling moth*. Presented at the 8th Citrus Research Symposium, Drakensberg, South Africa.

Love, C.N., Hill, M.P. and Moore, S.D. (2014, October). *The biology, behaviour and survival of pupating false codling moth under varying soil environments*. Presented at the Postgraduate Symposium, Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa.

To be attended: Entomological Society of SA Congress 2015. *Know your enemy: investigating the pupation of false codling moth*.

Prospective publications:

Refereed paper: Effects of citrus orchard soil characteristics on pupation behaviour and biology of *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae)

SAFJ article: FCM pupation behaviour and biology

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

Project 1063 (Sep 2011 – March 2015) BY Sean Moore, Wayne Kirkman (CRI) and Ben Burger (SU)

Summary

Several years ago it was discovered, almost accidentally, that 7-vinyldecyl acetate 1 (7-VDA) was capable of preventing adult false codling moth (FCM) males from locating virgin females. Consequently, we decided to examine this further with a view to developing a novel mating disruption, or rather a mating inhibition, technology. A novel polyethylene dispenser was developed, which allowed a consistent release rate of 7-VDA, comparable or better than that of FCM pheromone from an Isomate dispenser. Laboratory mating inhibition trials indicated superior efficacy with FCM pheromone to 7-VDA in reducing numbers of eggs laid. However, where a combination treatment of 7-VDA and Isomate (FCM pheromone) were used, egg laying

was reduced by 76%, compared to 42% with Isomate alone. Contrary to previous results, 7-VDA on its own was now also more effective than Isomate in this trial, reducing egg laying by 68%. A field cage trial was conducted late during the 2013/14 season. Five treatments were used (two cages per treatment): untreated control, Isomate (two dispensers per cage), and 7-VDA at 2, 4 and 8 dispensers per cage and 10 virgin pairs of moths were released into each cage. Loss of Isomate per dispenser was on average 3.2 times the loss of 7-VDA per dispenser (old type dispenser). Therefore, the fairest comparison of 7-VDA with the efficacy of Isomate was that of 8 dispensers per cage. Over a 4 week period, 6 infested fruit were recorded in the control cages, zero in the Isomate cages and between 1 and 2 in the 7-VDA cages. Another field cage trial was initiated in December 2014. Three cages were allocated to each of 4 treatments: untreated control, Isomate, 7-VDA x 1 and 7-VDA x2. No meaningful results in egg laying or fruit infestation were obtained. The field trial protocol will thus have to be revised.

Opsomming

Jare gelede is dit ontdek, amper toevalig, dat 7-vinieldesielasetaat 1 (7-VDA) die vermoë het om volwasse valskodlingmot (VKM) mannetjies te verhoed om ongepaarde wyfie motte te vind. Daarom het ons besluit om hierdie verder te ondersoek met die moontlikheid van 'n oorspronklike paringsontwrigting – of liever paringsverhoeding – tegnologie te ontwikkel. 'n Oorspronklike poliëtileen vrysteller is ontwikkel, wat 'n konstante vrystellings tempo van 7-VDA toegelaat het, vergelykbaar of beter as dié van VKM feromoon van 'n Isomate vrysteller. Laboratorium paringsontwrigtings proewe het aangedui dat VKM feromoon meer doeltreffend as 7-VDA was, gemeet deur vermindering in eierlegging. Waar 'n kombinasie van 7-VDA en Isomate (VKM feromoon) gebruik is, is eierlegging egter met 76% verminder in vergelyking met 42% met Isomate alleen. Teengesteld met vorige resultate, is 7-VDA op sy eie meer doeltreffend as Isomate in hierdie proef, met 'n eierleggings vermindering van 68%. 'n Veld-hok proef is laat gedurende die 2013/14 siesoen uitgevoer. Vyf behandelings is gebruik (twee hokke per behandeling): onbehandelde kontrol, Isomate (2 vrystellers per hok), en 7-VDA teen 2, 4 en 8 vrystellers per hok, en 10 ongepaarde mot pare is in elke hok losgelaat. Isomate vrystelling was gemiddeld 3.2 maal hoër as 7-VDA (ou tipe vrysteller). Daarom is die mees regverdig vergelyking van 7-VDA met die werking van Isomate, die 8 vrystellers per hok. Oor 'n 4 weke tydperk is 6 besmette vrugte in die kontrole hokke waargeneem, nil in die Isomate hokke, en tussen 1 en 2 in die 7-VDA hokke. Nog 'n veld-hok proef is in Desember 2014 geïnisieer. Drie hokke is gebruik vir elk van 4 behandelings: onbehandelde kontrol, Isomate, 7-VDA x1 and 7-VDA x2. Geen betekenisvolle resultate is verkry, albei wat eierlegging en vrugbesmetting betref. Daarom moet die veldproef protokol aangepas word.

3.2.10 FINAL REPORT: A survey of Lepidoptera infesting citrus fruit in the Eastern Cape Midlands

Project RU1064 (Mar 2013-Apr 2014) by Tammy Marsberg, Alicia Timm, Martin Hill (RU) and Sean Moore (CRI)

Summary

A number of insects, primarily Lepidoptera, cause damage to citrus in South Africa. A major limitation to the management and control of these pests is their correct identification. The aim of this study was to develop a database of gene sequences to aid in the identification of these Lepidoptera. Multiple specimens of 12 species were sequenced for the ~650 bp of the cytochrome oxidase I gene. These sequence data were supplemented and validated using sequences available in public databases. Results showed that each species could be unambiguously identified, and neighbour-joining analysis based on K2P distances formed highly supported, distinct clusters for each species, *i.e.* the maximum intraspecific genetic distance was less than that of the minimum interspecific genetic distances. Thus, this data set provides a molecular means to successfully identify the most important Lepidoptera associated with citrus in South Africa.

This studied has been published in a peer reviewed scientific journal under the following reference: Marsberg, T., Hill, M.P., Moore, S.D. & Timm, A.E. 2015. DNA-based identification of Lepidoptera associated with citrus in South Africa. *African Entomology* 23(1): 165-171.

Opsomming

Verskeie insekte, veral Lepidoptera, is verandwoordelik vir skade op sitrus in Suid-Afrika. 'n Belangrike beperking tot die bestuur en beheer van hierdie plaë is hulle korrekte identifikasie. Die doel van hierdie studie was om 'n databasis van gene reekse te ontwikkel om met die identifikasie van hierdie Lepidoptera te help. Verskeie monsters van 12 spesies is gesekwenseer vir die ~650 bp van die sitokroom oksidase 1 gene. Hierdie sekwensie data is gesupplementeer en gevalideer met gebruik van reekse wat in publieke databasise beskikbaar is. Resultate het gewys dat elke spesie sonder enige twyfel geïdentifiseer kon word,

en buurman-koppel analise gebaseer op K2P afstande het duidelike groepe vir elke spesie gevorm dws die maksimum binne-spesie genetiese afstand was minder as dié van die minimum interspesie genetiese afstande. Daarom voorsien die datastel 'n molekulêre metode om die belangrikste Lepidoptera wat met sitrus in Suid-Afrika geassosieer is te identifiseer.

Hierdie studie is in 'n wetenskaplike joernaal gepubliseer met die verwysing: Marsberg, T., Hill, M.P., Moore, S.D. & Timm, A.E. 2015. DNA-based identification of Lepidoptera associated with citrus in South Africa. *African Entomology* 23(1): 165-171.

Introduction

Citrus fruit is a major source of revenue for South Africa and in 2012 the country ranked second in the exportation of fresh citrus worldwide, generating a gross value of R4 billion (CGA 2013). However, this value would be greater were it not for the wide range of pests that attack citrus, which can reduce yields if not controlled properly, and some of which may even pose phytosanitary risk. Globally, citrus has been documented as a host for approximately 875 insects and mites (Smith & Peña 2002). Of these, approximately 100 are found in South Africa, including scale insects, mealybugs, aphids, whiteflies, leafhoppers, mites, beetles, thrips, flies and Lepidoptera (Bedford *et al.* 1998; Smith & Peña 2002). The high number of pests reflects the fact that South Africa has a favourable climate, including tropical and sub-tropical regions (Urquhart 1999).

A number of lepidopteran species are associated with citrus in South Africa. Of these, only one is considered to be a major pest - *Thaumatotibia leucotreta* (Meyrick 1913) (Tortricidae, false codling moth). Minor or sporadic pests of citrus include *Papilio demodocus* (Esper 1798) (Papilionidae, citrus swallowtail), *Pa. nireus lyaeus* (Linnaeus 1758) (Papilionidae), *Pa. dardanus* (Brown 1776) (Papilionidae) *Lozotaenia capensana* (Walker 1863) (Tortricidae, apple leaf roller), *Ectomyelois ceratoniae* (Zeller 1839) (Pyralidae, carob moth), *Phyllocnistis citrella* (Stainton 1856) (Gracillariidae, citrus leaf miner), *Prays citri* (Millière 1873) (Yponomeutidae, citrus flower moth), *Choristoneura occidentalis* (Walsingham 1891) (Tortricidae, citrus leaf roller), *Helicoverpa armigera* (Hübner 1809) (Noctuidae, cotton bollworm), *Ascotis selenaria reciprocaria* (Denis & Schiffermüller, 1775) (Geometridae, citrus looper), *Serrodus partita* (Fabricius 1775) (Noctuidae), *Sphingomorpha chlorea* (Cramer 1777) (Noctuidae), *Hypanua xyliana* (Distant 1898) (Noctuidae), *Ophiusa tirhaca* (Cramer 1773) (Noctuidae), *Eudocima divitosa* (Walker 1869) (Noctuidae), *Eudocima materna* (Linnaeus 1767) (Noctuidae), *Oraesia emarginata* (Fabricius 1794) (Noctuidae), *Pericyma atrifusa* (Hampson 1902) (Noctuidae) and *Pe. mendax* (Walker 1858) (Noctuidae). Secondary citrus pests include *Achaea echo* (Walker 1858) (Noctuidae), *A. finita* (Guenée 1852) (Noctuidae), *A. infinita* (Guenée 1852) (Noctuidae), and *A. lienardi* (Boisduval 1833) (Noctuidae), fruit sucking moths (Bedford *et al.* 1998; Smith & Peña 2002; Timm 2008; Rentel 2013). In addition, a number of insects have been historically associated with citrus, although they no longer have pest status. Such insects include *T. batrachopa* (Meyrick 1908) (Tortricidae, macadamia nut borer), which has only been recorded once on citrus (Bedford *et al.* 1998), *Egybolis vaillantina* (Stoll 1790) (Noctuidae), *Odites sucinea* (Meyrick 1915) (Oecophoridae) and *Salagena* species (Walker 1865) (Cossidae). Identification of these lepidopteran pests is critical for effective control because the identity of the species determines the control method, and associated biosecurity measures (Armstrong & Ball 2005, Jinbo *et al.* 2011).

Morphological keys or identification guides exist for only some of the Lepidoptera infesting citrus in South Africa (Timm 2007, Moore & Manrakhan 2012, Rentel 2013). For most of these, identification is based primarily on adult morphology, which limits identification since larvae or eggs are often collected in the field and must be reared to adults to be identified. This practice is time-consuming and often unsuccessful. In addition, expert knowledge is often needed to interpret many of the identification keys, which is problematic because the number of moth taxonomists in South Africa is limited. DNA identification offers a useful alternative to morphological identification, and provides advantages such as the ability to identify any life stage, rapidly and accurately (Hebert *et al.* 2005; Briski *et al.* 2011; Von Cräutlein *et al.* 2011; Jinbo *et al.* 2011). For DNA identification to be successful, a database of DNA sequences for each species is essential. Although the recent application of DNA barcoding has led to a rapid increase in DNA sequence data, limited DNA sequences are available for species of African origin. The aim of this study was to compile DNA sequences of Lepidoptera infesting citrus in South Africa to aid their identification.

Materials and methods

Insect material

Insect material was obtained by field collection or donation. Specimens of 12 species were sequenced for this study and are shown in Table 3.2.10.1. Where fresh specimens could not be obtained for DNA analysis, data were supplemented with sequences obtained from the BOLD or Genbank databases. With both collected and supplemented specimens, all current major and minor citrus lepidopteran sequences were included in the analyses, with the exception of *Pa. dardanus*, *Or. provocans*, *Or. triobliqua*, *Eu. fullonia* and *Pe. scandulata*. Lepidoptera historically associated with citrus were not included in the analyses, with the exception of *T. batrachopa* because fresh specimens were available for this species.

Table 3.2.10.1. Collection details of specimens sequenced to establish a COI database for Lepidoptera associated with citrus.

Family	Species	No. of specimens	Lab number	Collection locality	Geographic coordinates	GenBank accession number
Noctuidae	<i>A. lienardi</i>	3	L1–L3	Fort Beaufort, South Africa	32°45'44"E, 26°36'50"S	KP083413 KP083414 KP083415
	<i>A. echo</i>	3	L4–L6	Fort Beaufort, South Africa	32°45'44"E, 26°36'50"S	KP083416 KP083417 KP083418
	<i>A. infinita</i>	3	L7–L9	Fort Beaufort, South Africa	32°45'44"E, 26°36'50"S	KP083419 KP083420 KP083421
	<i>S. partita</i>	2	L10–L11	Fort Beaufort, South Africa	32°45'44"E, 26°36'50"S	KP083422 KP083423
	<i>S. chlorea</i>	3	L12–L14	Fort Beaufort, South Africa	32°45'44"E, 26°36'50"S	KP083424 KP083425 KP083426
	<i>H. xyliana</i>	3	L15–L17	Fort Beaufort, South Africa	32°45'44"E, 26°36'50"S	KP083427 KP083428 KP083429
	<i>O. tirhaca</i>	3	L18–L20	Fort Beaufort, South Africa	32°45'44"E, 26°36'50"S	KP083430 KP083431 KP083432
Tortricidae	<i>L. capensana</i>	3	L21–L23	Stellenbosch, South Africa	18°51'52"E, 33°55'48"S	KP083433 KP083434 KP083435
	<i>T. batrachopa</i>	2	L24–L25	Bathurst, South Africa	33°30'24"E, 26°50'40"S	KP083436 KP083437
	<i>T. leucotreta</i>	1	L26	Fort Beaufort, South Africa	32°41'32"E, 26°36'50"S	KP083438
	<i>T. leucotreta</i>	1	L27	Kenya		KP083439
Pyrilidae	<i>E. ceratoniae</i>	2	L28–L29	Stellenbosch, South Africa	18°51'52"E, 33°55'48"S	KP083440 KP083441
	<i>E. ceratoniae</i>	1	L30	Adelaide, South Africa	32°47'39"E, 26°17'39" S	KP083444
	<i>E. ceratoniae</i>	2	L31–L32	Stellenbosch, South Africa	18°51'52"E, 33°55'48"S	KP083442 KP083443
Papilionidae	<i>P. demodocus</i>	3	L33–L35	Grahamstown, South Africa	26°31'10"E, 33°18'49"S	KP083445 KP083446 KP083447

DNA extraction

DNA was extracted from the whole body of a larva or single leg of adults using the salting-out protocol described in Sunnucks & Hales (1996). DNA quality and quantity was analysed using a Nanodrop 2000 spectrophotometer (Thermo Scientific®).

Polymerase chain reaction

The polymerase chain reaction (PCR) was performed using standard protocols, with the primers LCO and HCO to amplify ~650 bp of the cytochrome oxidase I (COI) gene (Folmer 1994). Reactions were performed in a total volume of 50 µl, using 2µl DNA (50 ng), 25 µl *Taq* Master Mix (Promega), 40 pmol of each primer and 4 µl magnesium chloride. A BioRad® T100 thermocycler was used for amplification, using the following cycling programme: 95°C for 5 minutes, 35 cycles of 95°C for 45 seconds, 45°C for 45 seconds, 72°C for 1 minute and a final extension of 72°C for 5 minutes. The PCR products were analysed on a 1% agarose gel, using a 100 bp DNA Ladder (Promega®) to estimate fragment lengths. PCR products were sequenced by Macrogen using the same primers used for PCR.

Sequence analysis

Sequences were manually edited and assembled using BioEdit® sequencing alignment editor (Hall 1999). To supplement and verify generated sequences, COI sequences were obtained from GenBank and BOLD databases for relevant species and included in analyses. All sequences were aligned using BioEdit®. MEGA® version 5 (Tamura et al. 2011) was used to translate nucleotide sequences to check alignments, and to determine base composition. This software was also used to calculate genetic distances based on the Kimura 2-parameter model with uniform rates and to construct neighbour-joining trees using these genetic distances, with 1000 bootstrap replications. *Papilio demodocus* sequences were used to root the tree. Haplotypes, interspecific diversity and intraspecific diversity were identified using DnaSP version 5.1 (Librado & Rozas 2009).

Results and discussion

A fragment of ~650 bp at the 5' end of the COI gene was generated for 35 individuals, comprising 12 Lepidoptera species associated with citrus in South Africa. The sequences were aligned unambiguously, because they contained no insertions or deletions. All sequences were translated into amino acids and it was confirmed that there were no sequencing errors and no pseudogenes were present. For the sequences generated, the average base composition was 39.9% thymine (T), 15.1% cytosine (C), 30.5% adenine and 14.4% guanine (G) (GC content 29.5%).

To supplement the sequence database and to validate results obtained, sequences of relevant species obtained from the public databases Genbank and BOLD were included in analysis. The addition of these sequences allowed the analysis of a total of 26 insect species associated with citrus in South Africa. Sequences were also used to validate results, by comparing sequences generated in this study with sequences available in public databases. In the neighbour-joining tree (Fig. 3.2.10.1), sequences were most similar to those from the same species, with high bootstrap support, indicating the reliability of the dataset.

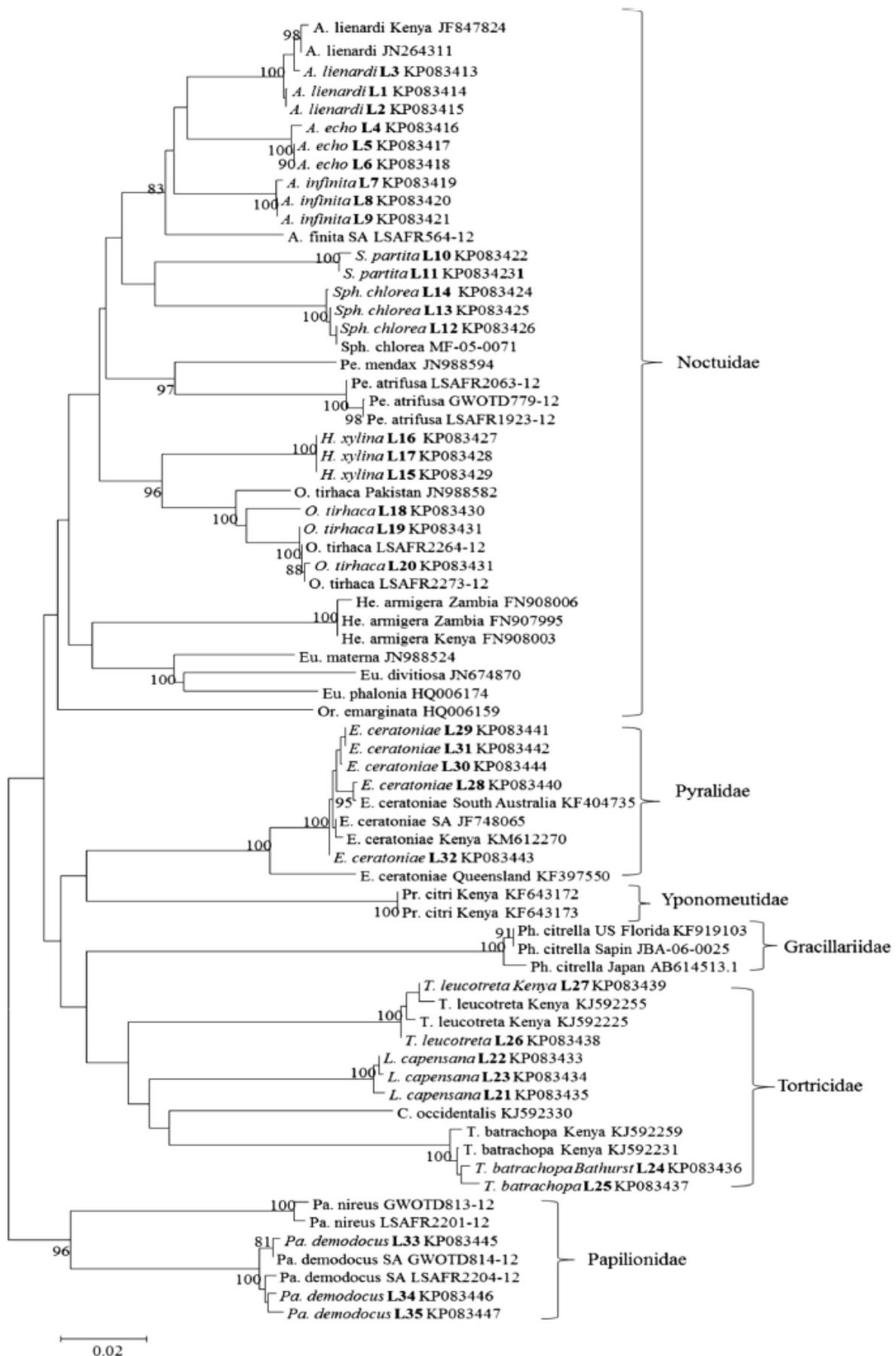


Figure 3.2.10.1. Neighbour-joining (K2P) phylogenetic tree representing COI barcoding sequences of Lepidoptera that are associated with citrus. Sequences generated for this study are numbered L1-35 and italicized, and are indicated in bold.

As further confirmation of species identification, nucleotide variation and divergence was calculated. Sequence analysis showed that a high interspecific nucleotide variation, which is essential for unambiguous species identification, was present. There was a clear gap between the greatest intraspecies (0.026±0.013, *A. infinita*) and minimum interspecies nucleotide diversity (0.049± 0.0005, *A. echo* and *A. lienardi*). Interspecific K2P divergence ranged from 2.5 % to 11.6 %, with an average of 7.24 %. The intraspecific K2P divergence ranged from 0 to 1.6 %, with an average of 0.44 %. Thus, COI sequences included in analysis are sufficient to distinguish unambiguously among Lepidoptera infesting citrus in South Africa.

Neighbour-joining analysis (Fig. 3.2.10.1) showed that species generally clustered according to the six families to which they belong. Phylogenetic relationships among species and families were not recovered. However, phylogenetic determination is required for accurate species identification and will not be considered in this study.

Fifteen Noctuidae species were included in the analyses. These were mainly fruit-sucking and fruit-piercing moths. Species within each genus could be clearly distinguished, with high bootstrap support. Four species of *Achaea* were analysed – *A. lienardi*, *A. echo*, *A. finita* and *A. infinita*. Three haplotypes were present in six sequences of *A. lienardi*, whereas two haplotypes were found for both *A. infinita* and *A. echo*, where only three sequences of each were included. This pattern of high haplotype diversity was reflected in the remaining Noctuidae species. Relatively high numbers of haplotypes were found for *Sph. chlorea* (3 haplotypes), *S. partita* (2 haplotypes), *Pe. atrifusa* (2 haplotypes), *O. tirhaca* (3 haplotypes), and *He. armigera* (2 haplotypes) even though specimen numbers were low (4, 2, 2, 6 and 3 respectively). In contrast, a single haplotype was observed in *H. xyliana*, where three sequences were included in analysis. Species within the genus *Eudocima* could not be clearly separated. However, only single sequences of each of the three species were included. To clarify results, it would be necessary to increase the number of sequences for this genus, but obtaining specimens of these are difficult because they are not common in South African orchards.

Nine sequences of *E. ceratoniae* were analysed, yielding seven haplotypes. A single *E. ceratoniae* from Queensland was distantly related to remaining sequences. The specimen may be misidentified or represent a cryptic species, which may be a result of host plant use (Mozaffarian et al. 2007). Carob moth is an important pest of pomegranates, dates, walnuts and pecan nuts and can attack a number of other fruit types and nuts in many regions throughout the world (Cox 1976; Al-Izzi et al. 1985; Alrubeai 1987). However, it is only a minor or even secondary pest on citrus (Honiball & Catling, 1998).

Four species of Tortricidae, *T. leucotreta*, *To. capensana*, *C. occidentalis* and *T. batrachopa* were used in the analysis, all of which could be clearly distinguished. The major citrus pest, *T. leucotreta*, was found to have three haplotypes from four specimen sequences. Three sequences of *To. capensana* were found to consist of two haplotypes and four sequences of *T. batrachopa* were found to consist of four haplotypes. *Thaumatotibia batrachopa* was included in the analyses, because its host range and distribution overlap those of *T. leucotreta* in South Africa. However, it has been recorded only once in a citrus packhouse (Bedford et al. 1998). It has been suggested that *T. batrachopa* may be mistaken for *T. leucotreta* (Bedford et al. 1998) and thus having sequences of both will aid their identification or differentiation.

Three species belonging to the family Papilionidae are associated with citrus in South Africa – *Pa. nireus lyaeus*, *Pa. demodocus* and *Pa. dardanus*. Specimens of *Pa. dardanus* could not be obtained, as the insect is seldom observed in orchards. However, sequences of *Pa. demodocus* and *Pa. nireus lyaeus* were included, and the two could be clearly distinguished. High haplotype diversity was seen in both species: two sequences of *Pa. nireus lyaeus* yielded two haplotypes and five sequences of *Pa. demodocus* yielded four haplotypes.

This study produced partial COI sequences for 12 lepidopteran pests that can infest citrus in South Africa and were placed in a public database. Previously, sequences from the 5' end of the COI gene from South African specimens were available for only one of these species (*E. ceratoniae*). Analyses included sequences of relevant species obtained from public databases, resulting in identification of 26 lepidopteran species. Thus, a large representative database of citrus Lepidoptera pests is available for further identification purposes, with only five minor pests missing.

Acknowledgements

We would like to thank Rhodes University and Citrus Research International (CRI) for funding the study. Candice Coombes, Mathew Goddard and Tanya Fullard provided advice and support during this project. We

would also like to thank all the citrus farmers (Shaun Brown, Rudi van der Meulen, Llewelyn Roberts, Isabel Sparks, Johnny du Preez, Robert Moss and Dave Murray) for allowing us to use their orchards and to Mathew Goddard, Pia Anderson, Gail Morland and Tertia Grové for supplying specimens to sequence. Hermann Staude identified specimens of *Achaea* and Henk Geertsema identified specimens of *E. ceratoniae*.

References cited

- AL-IZZI, M.A.J., AL-MALIKY, S.K., YOUNIS, M.A. & JABBO, N.F. 1985. Bionomics of *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) on pomegranates in Iraq. *Environmental Entomology* **14**: 149–153.
- ALRUBEAI, H.F. 1987. Growth and development of *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) on pomegranates in Iraq. *Environmental Entomology* **14**: 149–153.
- ARMSTRONG, K.F. AND BALL, S.L. 2005. DNA barcodes for biosecurity: invasive species identification. *Philosophical Transactions of the Royal Society* **360**: 1813-1823.
- BEDFORD, E.C.G., VAN DEN BERG, M.A. AND DE VILLIERS, E.A. 1998. *Citrus pests in the Republic of South Africa*. 2nd edition. Agricultural research council. Republic of South Africa 92 – 232 pp.
- BRISKI, E., CRISTESCU, M.E., BAILEY, S.A., AND MACISAAC, H.J. 2011. Use of DNA barcoding to detect invertebrate invasive species from diapausing eggs. *Biological Invasions* **13**: 1325-1340.
- CITRUS GROWERS ASSOCIATION (CGA). 2013. Key Industry Statistics for Citrus Growers 48 pp.
- COX, P.D. 1976. The influence of temperature and humidity on the life-cycle of *Ectomyelois*. *Environmental Entomology* **14**: 149-153.
- FOLMER, O., BLACK, M., HOEH, W., LUTZ, R., VRIJENHOEK, R. 1994 DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**: 294–299.
- GOBLE, T.A., DAMES, J.F., HILL, M.P., AND MOORE, S.D. 2011. Investigation of native isolates of entomopathogenic fungi for the biological control of three citrus pests. *Biocontrol Science and Technology* **21**: 1193-1211.
- HALL, TOM A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95-98.
- HEBERT, P.D.N. AND GREGORY, T.R. 2005. The promise of DNA barcoding for taxonomy. *Systematic Biology* **54**: 852-859.
- HONIBALL, F. AND CATLING, H.D. 1998. Carob moth, *Ectomyelois ceratoniae* (Zeller) (= *Spectrobates ceratoniae* (Zeller)), pp. 210-211. In E.C.G. Bedford, M.A. Van den Berg & E.A. De Villiers (eds), *Citrus Pests in the Republic of South Africa*, Second edition (revised). Dynamic Ad, South Africa.
- JINBO, U., KATO, T. AND ITO, M. 2011. Current progress in DNA barcoding and future implications for entomology. *Entomological Science* **14**: 107-124.
- LIBRADO, P., AND ROZAS, J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**: 1451-1452.
- MOORE, S.D. AND MANRAKHAN, A. 2012. Identification of insect larvae infesting citrus fruit. CRI Cutting Edge: Research news from citrus research international. *Citrus Research International* **139**: 1-4.
- MOZAFFARIAN, F., SARAFRAZI, A., & GANBALANI, G. N. (2007). Host plant-associated population variation in the carob moth *Ectomyelois ceratoniae* in Iran: A geometric morphometric analysis suggests a nutritional basis. *Journal of Insect Science*, **7**: 1-11.
- RENTEL, M. 2013. Morphology and taxonomy of tortricid moth pests attacking fruit crops in South Africa. MSc Thesis, Stellenbosch University, 113 pp.
- SMITH, D. AND PEÑA, J.A. 2002. Tropical citrus pests. *Tropical Fruit Pests And Pollinators. Biology, Economic Importance, Natural Enemies and Control*. CABI Publishing. New York: 57-82 pp.
- SUNNUCKS, P. AND HALES, D.F. 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the Genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology of Evolution* **13**: 510-524.
- TAMURA, K., PETERSON, D., PETERSON, N., STECHER, G., NEI, M., & KUMAR, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**: 2731-2739.
- TIMM, A. E., WARNICH, L., & GEERTSEMA, H. (2007). Morphological and molecular identification of economically important Tortricidae (Lepidoptera) on tropical and subtropical fruit in South Africa. *African Entomology*, **15**., 269-286.
- URQUHART, P. 1999. IPM and the citrus industry in South Africa. *International Institute for Environment and Development* **86**: 1-23.
- VON CRÄUTLEIN, M., KORPELAINEN, H., PIETILÄINEN, M. AND RIKKEN, J. 2011. DNA barcoding: a total for improved taxon identification and detection of species diversity. *Biodiversity Conservation* **20**: 373 – 389.

Technology transfer

This study has been published in a peer-reviewed scientific journal:

Marsberg, T., Hill, M.P., Moore, S.D. & Timm, A.E. 2015. DNA-based identification of Lepidoptera associated with citrus in South Africa. *African Entomology* 23(1): 165-171.

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

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

Summary

Cryptophlebia leucotreta granulovirus (CrleGV-SA) is a baculovirus specifically pathogenic to the citrus pest false codling moth, *Thaumatotibia leucotreta*. CrleGV-SA is formulated as a commercial biopesticide, Cryptogran[®] (River Bioscience, South Africa). The virus has a stable, proteinaceous crystalline occlusion body (OB) that protects the nucleocapsid. The major limitation to the use of baculoviruses is their susceptibility to the ultraviolet (UV) component of sunlight, which rapidly and greatly reduces their efficacy as biopesticides. The UVA and UVB components are the most destructive to biological organisms. To date no publication has reported the effect of UV on the structure and virulence of CrleGV, or the effectiveness of the OB as a UV protectant. In this study the effect of UV irradiation on the structure and infectivity of pure CrleGV-SA and Cryptogran[®] was investigated using scanning electron microscopy (SEM), Raman spectroscopy, qPCR, and bioassays. The project included laboratory and field studies. In the laboratory, CrleGV-SA and Cryptogran[®] were exposed to either UVA or UVB for periods of 24 hours to 7 days before analysis. In the field, Cryptogran[®] was applied to trees in a citrus orchard with young fruit. The fruit were collected from 24 hours to 28 days after application and bioassays conducted to assess the effect of sunlight over time on virus structure and efficacy when applied to the northern or southern sides of the trees. No surface morphological changes to the virus were detected using SEM. However, small compositional changes were detected by Raman spectroscopy. qPCR and bioassays demonstrated that UV irradiation damaged the viral DNA, greatly reducing the infectivity of pure CrleGV-SA and Cryptogran[®]. Exposure to UVB reduced the virulence of the virus more than UVA. The field studies revealed that the activity of CrleGV-SA decreased more on the northern side of the trees than on the southern side.

The full report of this study appears in the MSc thesis by Patrick Mwanza, entitled "Determination of the effects of sunlight and UV irradiation on the structure, viability and reapplication frequency of the biopesticide *Cryptophlebia leucotreta* granulovirus in the protection against false codling moth infestation of citrus crops" and available through Nelson Mandela Metropolitan University Library.

Opsomming

Cryptophlebia leucotreta granulovirus (CrleGV-SA) is 'n bakulovirus wat spesifiek patogenies is vir die sitrusplaag valskodlingmot, *Thaumatotibia leucotreta*. CrleGV-SA is as 'n kommersiële biologiese plaagdoder geformuleer, naamliks Cryptogran[®] (River Bioscience, Suid-Afrika). Die virus het 'n stabiele proteïen-kristal okklusie partikel wat die nukleokapsied beskerm. Die hoof beperking met die gebruik van bakuloviruse is hulle vatbaarheid vir die ultraviolet (UV) komponent van sonlig, wat vinnig en dramaties hulle werking as plaagdoders verminder. Die UVA en UVB komponente is die mees skadelik vir biologiese organismes. Tot op datum het geen publikasie die effek van UV op die struktuur en virulensie van CrleGV gerapporteer of die doeltreffendheid van die okklusie partikel as 'n UV beskermer. In hierdie studie is die effek van UV bestraling op die struktuur en infektiwiteit van suiwer CrleGV-SA en van Cryptogran[®] ondersoek met die gebruik van skandeer elektron mikroskopie (SEM), Raman spektroskopie, qPCR, en biotoetse. Die projek het laboratorium en veldstudies ingesluit. In die laboratorium is CrleGV-SA en Cryptogran[®] aan of UVA of UVB blootgestel vir tydperke van 24 ure tot 7 dae voor analise. In die veld is Cryptogran[®] op bome met jong vrugte in 'n sitrusboord toegedien. Die vrugte is na 24 uur tot 28 dae na toediening versamel en biotoetse is uitgevoer om die effek van sonlig oor tyd op virus struktuur en effektiwiteit op die noordelike en suidelike kante van bome te bepaal. Geen oppervlakkige morfologiese veranderings aan die virus is met SEM opgelet nie. Klein komposisionele veranderings is deur Raman spektroskopie egter opgelet. qPCR en biotoetse het gewys dat UV bestraling die DNA beskadig het, wat dramaties die infektiwiteit van suiwer CrleGV-SA en Cryptogran[®] verminder het. Blootstelling aan UVB het die virulensie van die virus meer beïnvloed as UVA. Veld studies het gewys dat aktiwiteit van CrleGV-SA meer op die noordelike kant van bome as die suidelike kant verminder het.

Die volle verslag van hierdie studie verkyn in die MSc tesis deur Patrick Mwanza, getiteld "Determination of the effects of sunlight and UV irradiation on the structure, viability and reapplication frequency of the

biopesticide *Cryptophlebia leucotreta* granulovirus in the protection against false codling moth infestation of citrus crops” en is beskikbaar deur Nelson Mandela Metropolitaanse Universiteit Biblioteek.

Results and discussion

Table 3.2.11.1 Estimated LD₅₀ and LD₉₀ values of CrleGV-SA and Cryptogran from laboratory experiments after UV irradiation

Treatment	24 hours		72 hours		Day 7	
	*LD ₅₀	LD ₉₀	LD ₅₀	LD ₉₀	LD ₅₀	LD ₉₀
Control	0.02	31.74	0.02	66.76	0.02	59.03
CrleGV+UVA	0.28	162.58	0.88	3403.03	8.41	5316.41
CrleGV+UVB	0.06	190.42	3.30	6335.01	55.67	11346.10
Cryptogran+UVA	0.08	214.92	0.69	2478.88	1.28	7641.27
Cryptogran+UVB	0.09	505.73	4.26	11134.53	26.03	17567.35

* All LD units are OBs/larva

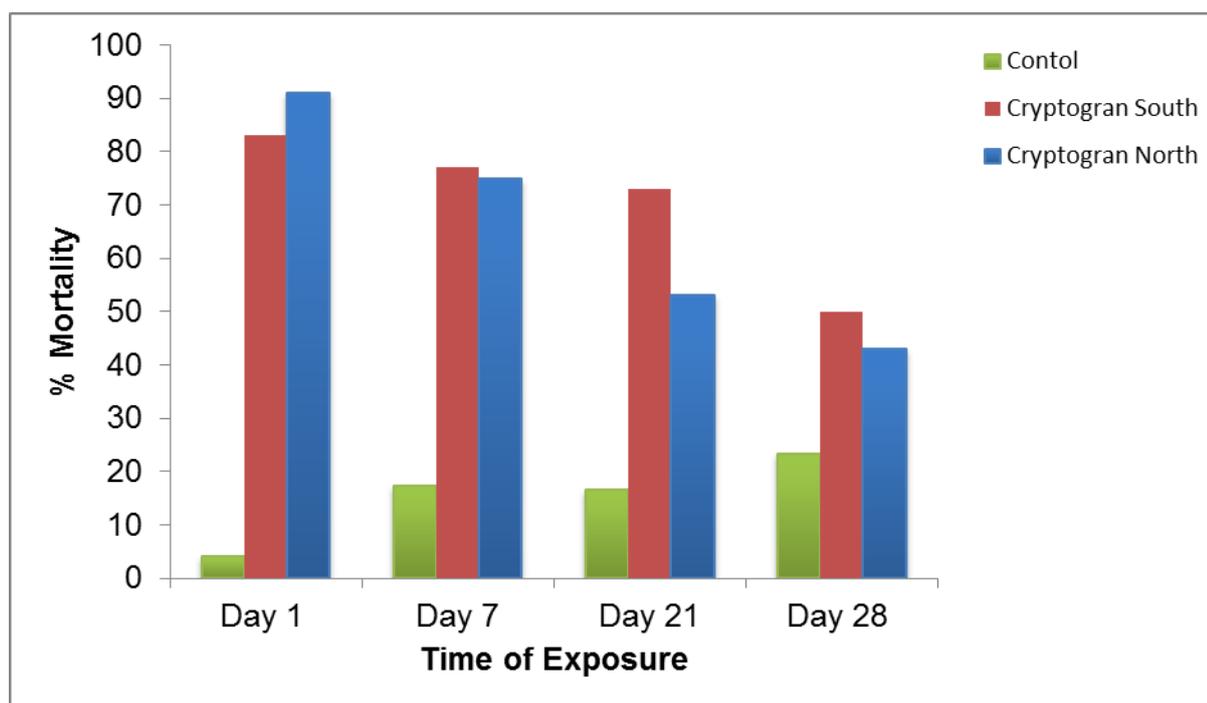


Figure 3.2.11.1 Response of FCM larvae to Cryptogran sprayed on fruit obtained from the northern and southern aspects of trees in the orchard. The green bars represent the control unsprayed fruit, red represents mortality of larvae on fruit from the south facing side, while blue represents mortality of larvae on fruit from the north facing side of the trees.

Table 3.2.11.2 Estimated LD₅₀ and LD₉₀ of Cryptogran from field experiments

Treatment	Cryptogran (North)		Cryptogran (South)	
	*LD ₅₀	LD ₉₀	LD ₅₀	LD ₉₀
Day 1	0.0614	730	0.0914	949
Day 7	2.15	4420	0.270	448
Day 14	19.8	33500	2.63	7800
Day 21	180	123000	11.6	26400
Day 28	ND	ND	180	123000

* All LD units are OBs/larva; ND-Not determined

Technology Transfer

Findings of the study were presented at the 2014 Citrus Research Symposium, the IOBC WPRS Working Group for Microbial and Nematode Control in Latvia in June 2015 and will be presented at the Entomological Society of southern Africa Congress in July 2015.

3.2.12 FINAL REPORT: Using the larval parasitoid, *Agathis bishopi*, for detection of FCM infested fruit

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

Summary

Thaumatotibia leucotreta (Meyrick) (Lepidoptera: Tortricidae) is one of the major citrus pests of economic importance for South Africa's citrus industry. It is endemic to Africa, and therefore a phytosanitary pest for some export markets. The cryptic nature of *T. leucotreta* makes visual inspection a inefficient method for detecting neonate larvae in fruit in the packhouse. Therefore, a more accurate method for sorting infested fruit at the packhouse, particularly for newly infested fruit, could ensure market access. A recent study showed that fruit infested by *T. leucotreta* emit a chemical profile different from that of healthy fruit. Several studies provide evidence that parasitoids locate their hosts feeding on fruit by exploiting the novel chemical profiles produced due to host herbivory. The aim of this study was to evaluate the potential for using the naturally occurring behaviour of a larval parasitoid, *Agathis bishopi* (Nixon) (Hymenoptera: Braconidae), for detection of *T. leucotreta* infested fruit, by determining which compound in infested fruit is attractive to parasitoids. Y-tube olfactometer and flight-tunnel bioassays with healthy and *T. leucotreta* infested fruit showed a significantly stronger response of *A. bishopi* female parasitoids to infested fruit. Among the volatile compounds associated with *T. leucotreta* infested fruit, D-limonene elicited the strongest attraction to *A. bishopi* female parasitoids. Attraction of mated *A. bishopi* female parasitoids to *T. leucotreta* infested fruit and D-limonene significantly increased after oviposition experience. Behavioural responses of *A. bishopi* female parasitoids that were associated with *T. leucotreta* infested fruit were investigated to determine which behaviours are distinct and interpretable. Probing and oviposition behaviours were the most noticeable and were only elicited on infested fruit when parasitoids contacted *T. leucotreta* frass, indicating that chemical compounds in frass are short-range cues used for final host location. Since production of D-limonene by fruit is elevated due to herbivory by different pests and by mechanical injury on fruit, response of *A. bishopi* female parasitoids to compounds in frass offers a more specific and potentially useful mechanism for development of a detection system for *T. leucotreta* infested fruit. Chemical analysis of *T. leucotreta* frass and conditioning of *A. bishopi* parasitoids to respond behaviourally to compounds in frass is proposed.

This project has been completed and is comprehensively reported in the MSc thesis by Kennedy Zimba, entitled "Using the larval parasitoid, *Agathis bishopi*, for detection of False Codling Moth, *Thaumatotibia leucotreta* infested orange fruit" and available through Rhodes University library.

Opsomming

Thaumatotibia leucotreta (Meyrick) (Lepidoptera: Tortricidae) is een van die hoof sitrusplae van ekonomiese belang vir Suid-Afrika se sitrusbedryf. Dit is endemies tot Afrika, en dus 'n fitosanitêre plaag vir sekere uitvoer markte. Die kriptiese geaardheid van *T. leucotreta* maak visuele inspeksie 'n ondoeltreffende metode vir die opsporing van pasuitgebroeide larwes in vrugte in die pakhuis. Daarom sal 'n meer akkurate metode vir die sortering van besmette vrugte in die pakhuis voordelig wees, veral vir wat nuut besmette vrugte betref, en kan marktoegang verseker. 'n Onlangse studie het getoon dat *T. leucotreta* besmette vrugte 'n ander chemiese profiel afgee as die van gesonde vrugte. Verskeie studies bied bewyse dat parasitoïdes hul gasheer op vrugte opspoor deur die merkbare chemiese profiele veroorsaak deur gasheervoeding. Die doel van dié studie was om die potensiaal vir die gebruik van die natuurlike gedrag van 'n larvale parasitoïde, *Agathis bishopi* (Nixon) (Hymenoptera: Braconidae), vir die opsporing van *T. leucotreta* besmette vrugte, deur om vas te stel watter verbinding in die besmette vrugte aantreklik vir die parasitoïde is. Y-buis olfaktometer en vlug-tonnel biotoetse met gesonde en *T. leucotreta* besmette vrugte het 'n betekenisvolle sterker reaksie van *A. bishopi* wyfie parasitoïdes tot besmette vrugte getoon. Onder die vlugtige verbindings geassosieer met *T. leucotreta* besmette vrugte, het D-limonien die sterkste aantrekking van *A. bishopi* wyfie parasitoïdes ontlok. Aantrekking van gepaarde *A. bishopi* wyfie parasitoïdes tot *T. leucotreta* besmette vrugte en D-limonien het aansienlik toegeneem na ondervinding met eierlegging. Gedrags respons van *A. bishopi* wyfie parasitoïdes wat geassosieer is met *T. leucotreta* besmette vrugte is ondersoek om vas te stel watter gedrag duidelik en verklaarbaar is. Eierlegging en gepaardgaande gedrag was mees opmerklik en slegs ontlok deur die besmette vrugte wanneer parasitoïdes kontak met *T. leucotreta* uitwerpsels gemaak het, wat aandui dat chemiese verbindings in uitwerpsels die kort afstand gedragslyn is vir die finale gasheer opsporing. Aangesien produksie van D-limonien deur vrugte verhoog deur voeding van verskeie plae, asook meganiese skade aan vrugte, bied die respons van *A. bishopi* wyfie parasitoïdes tot verbindings in uitwerpsels 'n meer spesifieke en potensieel gebruikbare meganisme vir die ontwikkeling van 'n opsporing sisteem vir *T. leucotreta* besmette vrugte. Chemiese analise van *T. leucotreta* uitwerpsels en kondisionering van *A. bishopi* parasitoïdes vir gedrags response teenoor verbindings in die uitwerpsels word voorgestel.

Hierdie projek is voltooi en word ten volle berig in die MSc tesis deur Kennedy Zimba, genaamd "Using the larval parasitoid, *Agathis bishopi*, for detection of False Codling Moth, *Thaumatotibia leucotreta* infested orange fruit" en is beskikbaar deur Rhodes Universiteit biblioteek.

Results and discussion

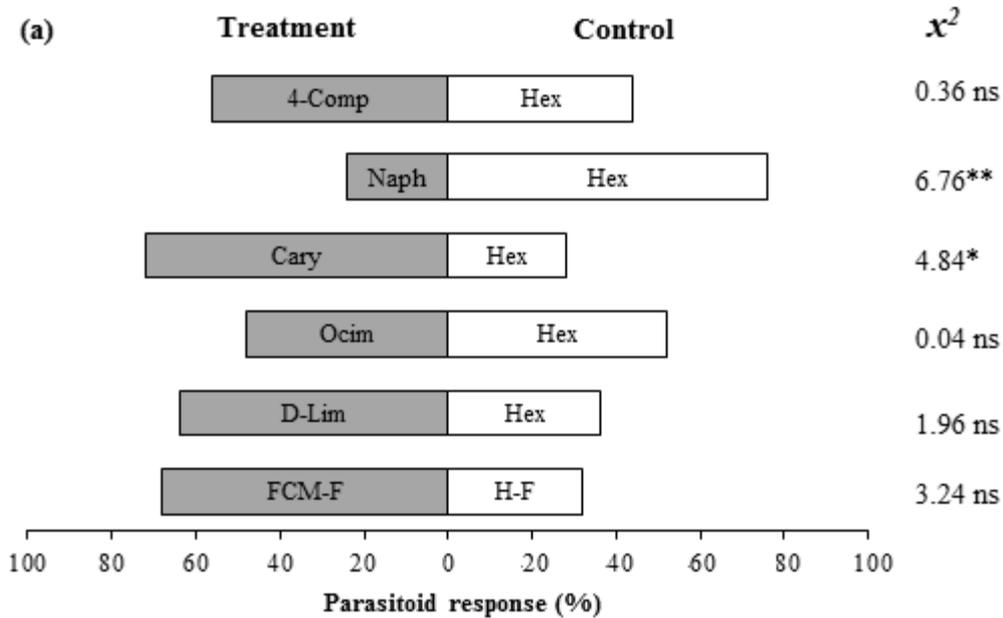


Figure 3.2.12.1. Attraction of mated (a) naïve and (b) experienced female *Agathis bishopi* to odour sources versus a control in a Y-tube olfactometer during a 10 min bioassay. Differences in the parasitoids' preferences for the options offered were analysed using a χ^2 statistical test. (* denotes $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and n.s non-significant $P > 0.05$); FCM-F = fruit infested by *Thaumatotibia leucotreta*; H-F = healthy fruit; Hex = Hexane; D-lim = d-limonene; Ocim = ocimene; Cary = caryophyllene; Naph = naphthalene; 4-Comp = 4 compound blend.

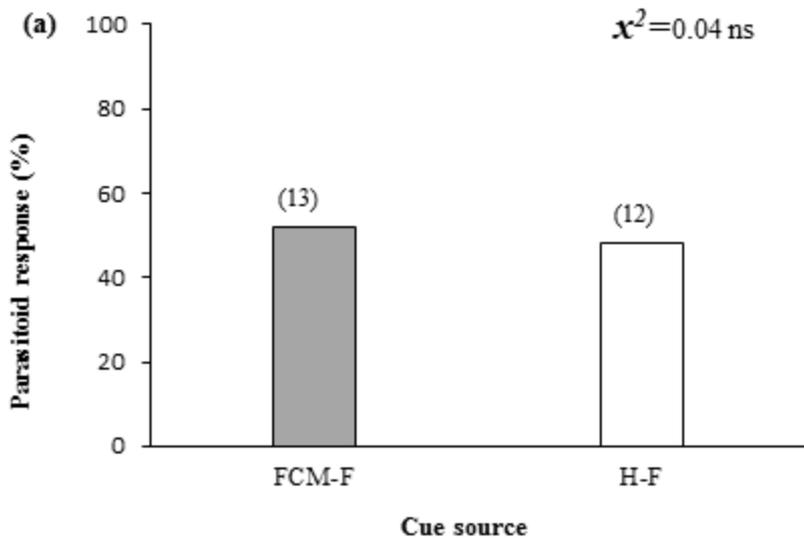


Figure 3.2.12.2. Preference of mated (a) naïve and (b) experienced female *Agathis bishopi* to *Thaumatotibia leucotreta* infested fruit versus healthy fruit in a flight tunnel. Percentage response of parasitoids to the treatment (FCM-F) and control (H-F) was recorded over a 5 min bioassay. Differences in the parasitoids' preferences for the options offered were analysed using χ^2 statistical test. (* denotes $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and n.s non-significant $P > 0.05$); FCM-F = fruit infested by *Thaumatotibia leucotreta*; H-F = Healthy fruit.

Technology transfer

Results of the study were presented as a poster at the 2013 Congress of the Entomological Society of southern Africa and at the 2014 Citrus Research Symposium.

3.2.13 **PROGRESS REPORT: A feasibility study on the use of sniffer dogs for detecting FCM infested fruit post-harvest**

Project 1071 (April 2013 – Mar 2014) by Sean Moore, Tim Grout, Wayne Kirkman (CRI), Stan Gillham (The Gillham Trust) and Pierre Olivier (SAPS)

Summary

This project proposes the use of dogs for detecting FCM-infested fruit post packhouse processing. The Gillham Trust is cooperating with us in conducting imprinting exercises with a dog on FCM infested fruit. Freshly harvested fruit were regularly infested with neonate larvae in the laboratory and sent to the dog handler, along with healthy fruit, for the imprinting exercises. Rapid progress was made in imprinting a 3-year old German Shepherd, Max. Max demonstrated the ability to detect a single recently FCM-infested Navel orange in a carton of healthy oranges, when presented with several cartons of oranges. However, it was clear that regular practice was necessary to keep Max reliable and that the dog handler had to be acutely aware of Max's body language to ensure that he was still adequately focussed. Max's performance was also better, when held on a leash by his handler, rather than moving freely. This trial will culminate in a large scale replicated trial at the end of the 2015 packing season, which can connect a degree of statistical confidence to the dog's ability to detect FCM-infested fruit in packed cartons.

Opsomming

Hierdie projek stel die gebruik van honde voor om VKM besmette vrugte na die pakhuis verwerkingsproses op te spoor. The Gillham Trust werk saam met ons op vroeë stadium inprent oefeninge met 'n hond en VKM besmette vrugte. Vars geplukte vrugte is gereeld besmet met pasuitgebroeide larwes in die laboratorium en na die honde hanteerder gestuur, saam met vars vrugte, vir inprentings oefeninge. Vinnige vordering is gemaak met die inprent van a 3-jaar oue Duitse Skaaphond, Max. Max het die vermoë gewys om 'n enkel pas VKM-besmette Nawellemoen in 'n karton van gesonde lemoene op te spoor, wanneer met verskeie kartonne lemoene voorgesien. Dit was egter duidelik dat gereelde oefening sessies nodig was om Max betroubaar te hou en dat die honde hanteerder baie bewus moes wees van Max se liggaamstaal om seker te maak dat hy nogsteeds genoegsaam gefokus was. Max se vertoning was ook beter wanneer hy op 'n leiband deur sy hanteerder gehou is, eerder as om vry te loop. Dit laaste proef in hierdie projek sal 'n grootskaalse herhaalde proef naby aan die einde van die 2015 pakseisoen wees, wat 'n mate van statistiese vertroue sal heg aan die hond se vermoë om VKM besmette vrugte in gepakte kartonne op te spoor.

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

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

Summary

Larval parasitoids of false codling moth have never been found in the Western Cape in previous studies. In 2013 sampling of sanitation fruit was begun to see whether previous observations regarding absence of larval parasitism were still valid. If absent, then *Agathis bishopi*, from a culture at Rhodes University, would be released in the Citrusdal area in an attempt to aid biological control of FCM. In 2013/14 no sign of *Agathis bishopi* was found in the Citrusdal area. In 2014/15 sanitation fruit was again sampled on a weekly basis, from two farms that are considered hotspots for FCM, within the same production area. Again, the presence of *A. bishopi* was not detected in any samples. The *A. bishopi* culture at Rhodes University experienced prolonged fungal contamination during 2014/15 and insects were not available for release in the Western Cape. New field-collected parasitized material has recently been incorporated into the culture. Hopefully, wasps will be available for release in the near future. If wasps cannot be released before winter then it is planned to carry out a release in Spring 2015.

Opsomming

Parasitoeïdes van die larwa van valskodlingmot (VKM) is nie vantevore in die Wes Kaap gevind nie. In 2013 is daar begin om monsters te neem van sanitasievrugte om te sien of vorige waarnemings (ten opsigte van hierdie afwesigheid van sulke parasitoeïdes) nog geldig is. Indien dit nog steeds afwesig is, sal *Agathis*

bishopi van Rhodes Universiteit in die Citrusdal area vrygelaat word in 'n poging om die biologiese beheer van VKM te verbeter. In 2013/14 is geen teken van *Agathis bishopi* in die Citrusdal area gevind nie. In 2014/15 is die ondersoek voortgesit op twee plase wat bekend is vir die hoë voorkoms van VKM. Daar is egter steeds geen *A.bishopi* in enige van die monsters gevind nie. Die teling van *A.bishopi* by Rhodes Universiteit gedurende 2014/15 het baie probleme met swam kontaminasie ondervind wat die gevolg gehad het dat insekte vir loslating in die Wes Kaap nie beskikbaar was nie. Nuwe gearasiteerde materiaal van die veld is onlangs in die laboratorium kultuur ingesluit. Hopelik sal wespers vir loslating in die nabye toekoms beskikbaar wees.

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

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

Summary

Sterile false codling moths (FCM) are known to be less active at low temperatures compared to their wild counterparts. This has implications for the efficacy of Sterile Insect Release (SIT) for the control of FCM particularly during the cooler months when harvest of citrus approaches. The project aim is to expose sterile FCM to different thermal conditions and to assess the effect of these on flight and mating ability. Changes could then be incorporated into the rearing process in order to improve the fitness of released sterile moths. Effects of FCM prior thermal history (thermal manipulation) on flight and mating ability were concluded. Treatment temperature, but not acclimation, had an effect on cumulative egg production with the intermediate test temperature producing the most eggs (15°C>20°C>10°C). There were also significant interactions between acclimation and treatment temperature. For CTmin, there were significant effects of rearing and treatment temperature and the interaction thereof, with the intermediate acclimation temperature resulting in the lowest CTmin (5.7°C) (15°C>25°C>20°C). Field tests of flight ability (trade-offs with longer flight producing less successful mating or fecundity decrease) were assessed. The results will be finalised in the coming weeks.

Opsomming

Dit is bekend dat steriele valskodlingmotte (VKM) minder aktief is by laer temperature as wat wilde motte is. Dit hou implikasies in vir die effektiwiteit van SIT in die beheer van VKM gedurende die koeler maande voor die plukseisoen van sitrus begin. Die projek het ten doel om steriele VKM aan verskillende temperatuur kondisies bloot te stel en om die effek van hierdie vlieg- en paringsvermoë te assesseer. Toetse om die effek wat vorige temperatuur geskiedenis op die vlieg- en paringsvermoë te bepaal, is afgehandel. Dit is bevind dat behandelingstemperatuur, maar nie akklimatisasie nie, 'n effek op kumulatiewe eierproduksie het. Die intermediaire toets temperatuur (15°C>20°C>10°C) het die meeste eiers geproduseer. Daar was ook beduidende interaksie tussen akklimatisasie behandelingstemperatuur. Vir CTmin was daar beduidende effekte van teling en behandelingstemperatuur en die interaksie tussen die twee aspekte. Intermediaire akklimatisasietemperatuur het die laagste CTmin (5.7°C) (15°C>25°C>20°C) tot gevolg gehad. Veldtoetse op vliegvermoë is geassesseer en dit is bevind dat waar motte verder vlieg, paring minder suksesvol is en vrugbaarheid afneem. Die resultate sal in die komende weke gefinaliseer word.

3.2.16 **PROGRESS REPORT: Novel approaches to mating disruption of FCM**

Project 1080 by Martin Gilbert (CRI)

Summary

The aim of this project is to investigate FCM control by the increased use of mating disruption products beyond levels that are presently registered. Another aspect of this is the use of mating disruption products in combination with Sterile Insect Release (SIT). Both of these aspects, if proved to have merit, would make the possibility of local FCM pheromone synthesis more viable by increasing the potential demand for the pheromone. During 2014/15 two farms, in the Citrusdal area, that had been identified by Xsit as hotspots for FCM were chosen as experimental sites for the application of Isomate (at Bo-Bergvlei) and aerial Checkmate (at Vrede). Problematic (hotspot) farms were chosen bearing in mind the lack of results obtained on other farms in the previous season due to low FCM pressure. At both farms aerial releases of sterile FCM were carried out as usual by Xsit as part of their commercial programme. At Bo-Bergvlei one half of a block of Washington Navels also received two applications of Isomate as per registration. At Vrede, multiple applications of Checkmate were applied by means of an Xsit autogyro at 132 ml per ha at 4-weekly intervals,

weather permitting. FCM yellow delta traps were placed out in the treated and untreated (SIT only) blocks and monitored weekly. In addition, 10 trees per treatment (and controls) were marked and all fallen fruit were collected weekly and examined for the presence of FCM. At Bo-Bergvlei from early January onwards, no wild FCM were caught in traps where Isomate + SIT were applied. In the control block, sporadic catches of wild FCM still occurred after early January although at low levels. As regards fruit infestation, 12 extra fruit per tree were lost due to FCM in the control block as compared to the SIT + Isomate block. At Vrede, during January and February fruit loss due to FCM was lower than at Bo-Bergvlei. Nevertheless, the Checkmate block lost a total of 3.5 fewer fruit per tree to FCM during these two months. During April 2015, at both farms, fruit loss due to FCM increased considerably. By the middle of April, fruit loss during the previous 3-month period in control blocks (SIT only) at Bo-Bergvlei had accumulated to nearly 18 fruit per tree and, at Vrede, was 11 fruit per tree, in comparison to the "mating disruption-treated plus SIT" blocks. Mating disruption products can therefore clearly enhance the results of SIT and improve the overall control of FCM particularly in orchards / on farms with high moth pressure. Experiments will also be done in the future on farms that are not included in the Xsit programme. As regards the local synthesis of FCM pheromone, initial research was not encouraging due to too small a volume being estimated as being required by the industry. However, contact has been made with an experienced organic chemist in the Eastern Cape who is busy researching suitable chemical pathways by way of which the pheromone might be synthesised. The economics will then be further investigated. Any trial results which indicate merit in increased use of FCM pheromone for mating disruption will help to make the synthesis project more viable.

Opsomming

Die doel van hierdie projek is om VKM beheer te ondersoek deur die verhoogde gebruik (meer as wat huidig geregistreer is) van paringsontwrigting produkte. Nog 'n aspek is die gebruik van paringsontwrigting produkte in kombinasie met die Steriele Insek Tegniek. Albei hierdie aspekte, (indien van waarde) kan die moontlikheid van die plaaslike vervaardiging van VKM feromoon verhoog deur die aanvraag van die feromoon te verhoog. In 2014 is twee plase in die Citrusdal area geïdentifiseer wat bekend is as "hotspots" vir VKM aktiwiteit, en gekies vir die toediening van Isomate (by Bo-Bergvlei plaas) en lugtoedienings van Checkmate (by Vrede plaas). Op albei plase het die normale loslaatings van steriele VKM voortgegaan as deel van Xsit se kommersiële program. By Bo-Bergvlei het een helfte van 'n Washington nawel blok ook twee toedienings van Isomate (soos geregistreer) ontvang. Op Vrede is meervoudige toedienings van Checkmate toegedien op 'n maandelikse basis deur Xsit se autogiros teen 132 ml per ha. Twee geel VKM delta lokvalle is gebruik om motte weekliks te monitor in behandelde en onbehandelde blokke. Al die sanitasie vrugte is op 'n weeklikse basis onder 10 bome opgetel in behandelde en kontrole blokke. Die vrugte is ondersoek vir die teenwoordigheid van VKM. By Bo-Bergvlei is daar van Januarie af geen wilde VKM gevang waar Isomate + SIT toegedien is nie. In die kontrole blok (SIT alleen) het sporadiese vangstes van wilde VKM nogsteeds voorgekom na Januarie, maar op 'n lae vlak. Wat sanitasie betref, is 12 ekstra vrugte per boom verloor as gevolg van VKM in die kontrole blok (SIT alleen) in vergelyking met die SIT + Isomate blok vanaf Januarie tot Maart. By Vrede plaas is vrugverlies laer as by Bo-Bergvlei en die Checkmate + SIT blok het 3.5 minder VKM vrugte per boom gehad. Gedurende April 2015 het vrugverlies as gevolg van VKM geweldig toegeneem op albei plase. By Bo-Bergvlei is die verskil tussen die behandelde blok (Isomate + SIT) en die kontrole blok (SIT alleen) 18 vrugte per boom vanaf Januarie tot April. By Vrede was die verskil 11 vrugte per boom. Paringsontwrigtingprodukte kan dus duidelik die beheer van VKM verbeter veral in boorde / op plase waar VKM voorkoms hoog is. In die toekoms sal proewe gedoen word op plase buite die Xsit toedienings area. Wat die plaaslike vervaardiging van VKM feromoon betref, was die aanvanklike ondersoek teleurstellend as gevolg van die feit dat daar te klein volumes benodig sal word in die industrie. Kontak is egter wel gemaak met 'n ervare organiese chemikus in die Oos Kaap wat geskikte prosesse vir die vervaardiging van VKM feromoon ondersoek. Enige proefresultate wat daarop dui dat verhoogde gebruik van VKM feromoon wel meriete het, sal help om die projek meer lewensvatbaar te maak.

3.2.17 **PROGRESS REPORT: Movement of false codling moth (FCM) and fruit flies (FF) in multi-crop (citrus, stone fruit, grape, pomegranate) systems** Project 1081 (2013/14 – 2015/6) by Martin Gilbert (CRI)

Summary

Fruit-fly and false codling moth are polyphagous pests infesting many different cultivated fruit types as well as wild hosts. The aim of this project was to investigate these pests on farms where differing fruit types were grown close to each other. The 13 blocks from which results were obtained in 2013/14 continued to be monitored on a weekly basis in 2014/15. In addition, a farm where citrus and pomegranates are grown close together was included in the project for the purposes of monitoring FCM. There were marked differences in the patterns of activity between fruit fly and FCM. Regarding fruit fly, peaks in trap counts were closely

related to the ripening of each successive fruit type. Regarding FCM, the peaks in male flight activity were not related to the ripening of fruit type. The results obtained from Riebeek Kasteel were particularly clear in that peaks of moth activity from October 2014 to April 2015 were very similar in their timing irrespective of which orchard type the traps were placed in. This would seem to indicate that FCM is far less restricted to a particular crop than fruit-fly and is able to disperse throughout a farm at any particular time. The nocturnal nature of FCM is obviously a factor in governing the pattern of its flight activity. These results indicate that other factors are governing flight activity rather than just host (fruit) maturity. This may have important consequences with regard to future pest management. After 7 months of trapping, the cumulative totals of FCM trapped in citrus, plum and peach orchards at Riebeek Kasteel were very similar, ranging from 57 – 60 moths per trap. Nectarines showed the maximum with 66 moths per trap. Numbers in grapes were significantly lower at only 10.5 FCM per trap (cumulative total). This contrasts with fruit-fly, where variation was far greater, with cumulative totals of Medfly on citrus, plum and peach of 43.3, 30.8 and 51.0 respectively. Nectarines showed the maximum number of Medfly at 122.5 per trap and grapes were at a cumulative total of 41.8 per trap.

Opsomming

Vrugtevlieg en valskodlingmot (VKM) is plaes wat verskillende aangeplante vrugtesoorte sowel as wilde gasheerplante aanval. Die doel van hierdie projek is om hierdie plaes te ondersoek op plase waar verskillende vrugtesoorte naby aan mekaar voorkom. Die 13 blokke waarvan resultate in 2013/14 verkry is, word steeds op 'n weeklikse basis in 2014/15 gemonitor. 'n Plaas waar sitrus en granate naby aan mekaar verbou word, is ook in die projek ingesluit ten einde VKM te monitor. 'n Duidelike verskil is waargeneem tussen die aktiwiteitspatrone van vrugtevlieg en VKM. Daar is bevind dat daar 'n nou verband is tussen die pieke in vrugtevliegtellings (in valletjies) en die rypwordings-stadium van die verskillende vrugtesoorte. Wat VKM betref, is dit gevind dat die vlugaktiwiteit van mannetjie motte nie verband hou met die rypwordings-stadium van vrugte nie. Die resultate wat van Riebeek Kasteel area verkry is, wys duidelik dat pieke in motaktiwiteit van Oktober 2014 tot April 2015, soortgelyk was wat tyd aanbetref, ongeag die boord en vrugtesoorte waar valletjies gemonitor is. Dit wil dus voorkom of hierdie statistieke daarop dui dat VKM baie minder beperk is tot 'n spesifieke vrugtesoort (soos in die geval van vrugtevlieg) en die vermoë het om op enige stadium oor 'n plaas te versprei. Omdat VKM gedurende die nag aktief is, beïnvloed dit hulle vlugaktiwiteitspatrone. Die resultate van die ondersoek dui dus daarop dat ander faktore, behalwe net gasheerryphheid, hulle vlugaktiwiteit beïnvloed en beheer, en dit mag belangrike implikasies hê vir die beheer van plaes. Na 7 maande, die kumulatiewe totale van VKM wat in sitrus, pruime en perske boorde by Riebeek Kasteel gevang is, is baie soortgelyk en strek van 57 – 60 motte per val. Hoogste getalle is in nektarien boorde gevang met 66 motte per val. Getalle in wingerde was betekenisvol minder met 'n kumulatiewe totaal van net 10.5 VKM per val. Met vrugtevlieg, was getalle meer visselvallig, met kumulatiewe totale van Medvlieg op sitrus, pruim en perske van 43.3, 30.8 en 51.0. Hoogste getalle is in nektarien boorde gevang met 122.5 per val, en druie die laagste met 41.8 per val.

3.2.18 PROGRESS REPORT: Improving the cold tolerance of false codling moth (*Thaumatotibia leucotreta*) for improved performance in a sterile insect release programme
Project 1083 (Apr 2014 – Dec 2015) by Claire Daniel, Martin Hill (RU) and Sean Moore (CRI)
(This is not a CRI funded project)

Summary

An SIT programme for the control and/or suppression of false codling moth (FCM), *Thaumatotibia leucotreta*, in citrus was implemented in South Africa in 2007. The programme is generally successful, but there is a defect in the SIT programme, where wild moths can theoretically be active down to a temperature of 12°C and laboratory reared sterilized moths, appear to be inactive below 20°C. This means that the sterile males are out-competed by wild males during the cooler months of the year. Therefore, it is vital to increase the cold tolerance of the sterile males to maximize the effect of the SIT programme, even in the cooler months. Studies have shown that adding cryoprotectants to the basic laboratory diets increases the cold tolerance of certain insects. Improving the cold tolerance of FCM could be done in a similar way, via augmenting the basic diet by adding a cryoprotectant. The aim of this study is to determine if and what cryoprotectants will increase the cold tolerance of FCM. Results thus far have shown that by adding trehalose to the normal laboratory diets, allows the moths to fly at lower temperatures, with an average of 40% flight at 15°C compared with 0% flight for the control. Results have also shown that various additives result in a faster developmental rate. This was particularly evident when adding cholesterol to the normal laboratory diets whereby the time taken for moths reared on cholesterol augmented diets to develop from egg to adult, was an average of 16.3 days when compared with the control which took an average of 26 days. A field trial was conducted and results support the laboratory results whereby the ratio of moth recaptures were 4.89:1 for

trehalose moths when compared with the control. Field trials will be conducted again in winter and summer months.

Opsomming

(Hierdie is nie 'n CRI-bevondse projek nie)

'n SIT program vir die beheer en/of onderdrukking van valskodlingmot (VKM), *Thaumatotibia leucotreta*, in sitrus is in 2007 geïmplementeer in Suid-Afrika. Die program is grotendeels suksesvol, maar daar is 'n defek met die SIT program, waar wilde motte teoreties aktief kan wees tot 12°C, en laboratorium geteelde steriele motte onaktief is onder 20°C. Dit beteken dat steriele mannetjies uitmeegeding word deur wilde mannetjies gedurende die koue maande van die jaar. Dus is dit noodsaaklik om die koue toleransie van die steriele mannetjies te verhoog, en so die effek van die SIT program te maksimeer, selfs gedurende koeler maande. Studies het getoon dat deur die byvoeging van kouebeskermers tot die basiese laboratorium diëte, die koue toleransie van sekere insekte verhoog kan word. Verbetering van die koue toleransie van VKM kan in 'n soortgelyke manier verkry word, deur die toevoeging van 'n kouebeskermer tot die basiese diëet. Die doel van hierdie studie is om vas te stel of, en watter kouebeskermers die koue toleransie van VKM kan verhoog. Resultate toon dusver het gewys dat die byvoeging van trehalose tot die normale laboratorium diëte die motte toelaat om by laer temperature te vlieg, met 'n gemiddelde 40% vlug by 15°C teenoor 0% vlug vir die kontrole. Resultate het ook gewys dat verskeie bymiddels tot 'n vinniger ontwikkelings tempo lei. Dit was veral duidelik wanneer cholesterol tot die normale laboratorium diëet bygevoeg word waar die tyd wat dit vat vir die motte geteel op die cholesterol diëet om te ontwikkel vanaf eier tot mot ongeveer 16.3 dae geneem het, vergeleke met die kontrole wat ongeveer 26 dae geneem het. 'n Veldproef is uitgevoer en die resultate het die laboratorium resultate ondersteun, waar die verhouding van mot hervangste 4.89:1 was vir trehalose in vergelyking met die kontrole. Veldproewe sal tydens die winter en somer maande weer uitgevoer word.

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

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

Summary

The European Plant Protection Organisation (EPPO) has completed a Pest Risk Assessment (PRA) of FCM for Europe. This may lead to more stringent regulations on importation of southern African citrus (and other fresh produce from Africa that can host FCM). The worst case scenario is that cold sterilisation (shipping at -0.6°C for 22 days) will become mandatory for all citrus exports from South Africa to Europe. This will effectively make Europe an unviable destination, with devastating effects for the industry as a whole. Consequently, CRI has been proactive in drafting a systems approach for management of FCM as an effective alternative to cold sterilisation. The systems approach includes a series of mandatory management steps from pre-season to post-packing, including weekly inspections at orchard level up to the time of harvest, pre-packing and post-packing inspections. Infestation thresholds have been suggested which determine whether the fruit qualifies to move onto the next step in the system or if it should be rejected on the grounds of unacceptable risk. This study aims to scientifically validate these standards and to alter them if necessary in order to make the systems approach as accurately predictive and reliable as possible. Larval infestation of fruit was monitored weekly in fruit from 33 orchards over two seasons, until the time of harvest, post-picking and post-packing into export cartons. A significant correlation was evident between infestation of fruit on delivery to packhouse and infestation in the packed carton, thus emphasising the importance of thorough pre-packing inspections. Although there were no significant correlations between pre-harvest and post-harvest levels of infestation, there was a clear relationship between non-compliance of orchards with infestation standards pre- and post-harvest. A progressively increasing level of discrimination at successive inspection steps was evident. From pre-harvest to post-packing, 15 of the 33 orchards remained compliant with the systems approach; 13 were disqualified in the orchard; three were disqualified on delivery at the packhouse; and only two were disqualified after packing. These results verified the appropriate sensitivity of the grading and inspection thresholds for application to successive steps in the systems approach. The post-harvest non-destructive inspections detected 73.1% of infested fruit, whereas the pre-packing visual detectability of infested fruit was 77.8%. The packhouse grading process was shown to reduce the fruit infestation proportion by 21.1%.

Opsomming

Die Europese Plantbeskermingsorganisasie (EPPO) het 'n Plaag Risiko Analise (PRA) op VKM vir Europa voltooi. Dit kan tot strengere regulasies op die invoer van suidelike Afrikaanse sitrus (en ander vars produkte van Afrika wat gashere vir VKM kan wees) lei. Die ergste moontlike scenario is dat kouesterilisasie

(verskeping teen -0.6°C vir 22 dae) verpligtend vir alle sitrusuitvoere van Suid-Afrika Europa toe sal word. Die uiteinde is dat Europa 'n onwingsgewende bestemming sal word, met 'n teisterende effek op die bedryf as geheel. Daarom het CRI proaktief opgetree en 'n stelselsbenadering vir die bestuur van VKM as 'n doeltreffende alternatief vir koue sterilisasie opgestel. Die stelselsbenadering sluit in 'n reeks verpligtende bestuursstappe van voorseisoen tot na verpakking. Dit sluit in weeklikse boord inspeksies tot oestyd, en voor- en na-verpakking inspeksies. Besmettings drempelwaardes is voorgestel wat sal bepaal of die vrugte kwalifiseer om aan te skyf na die volgende stap toe of as hulle afgekeur moet word as gevolg van onaanvaarbare risiko. Die doel van hierdie studie is om hierdie standaard wetenskaplik te verifieer om die stelselsbenadering so akuraat en betroubaar as moontlik te maak. Larwe besmetting van vrugte van 33 boorde is weekliks gemonitor oor twee seisoene tot oestyd, na oes, en na verpakking in uitvoer kartonne. 'n Beduidende korrelasie is gekry tussen besmetting van vrugte op aflewering by die pakhuis en besmetting in die gepakte karton, wat die belangrikheid van deeglike voor-verpakkings inspeksies beklemtoon het. Alhoewel daar geen beduidende korrelasies tussen voor-oes en na-oes besmettings vlakke was nie, is daar 'n duidelike verhouding tussen nie-nakoming van boorde met besmettings standaard voor- en na-oes. 'n Progressief vermeerderende vlak van diskriminasie by agtereenvolgende inspeksie stappe was duidelik. Van voor-oes tot na verpakking het 15 van die 33 boorde nakomend met die stelsels benadering gebly; 13 is in die boord gediskwalifiseer; drie is op aflewering by die pakhuis gediskwalifiseer; en net twee is na verpakking gediskwalifiseer. Hierdie resultate het die gepaste sensitiwiteit van die gradering en inspeksie drempelwaardes gewys vir toepassing van die agtereenvolgende stappe in die stelselsbenadering. Die na-oes nie-destruktiewe inspeksies het 73.1% van besmette vrugte opgespoor, en die voor-verpakkings visuele opspoorbaarheid van besmette vrugte was 77.8%. Die pakhuis graderings proses het die proporsie van besmette vrugte met 21.1% verminder.

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

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

Summary

Entry of lemons into markets which require cold-sterilisation (-0.6°C for 22 days) of fruit cannot be achieved through current protocols. Lemons cannot be cold sterilised in transit due to their susceptibility to chilling damage and it is unlikely that many lemon orchards could be declared free of FCM on the basis of pheromone baited trap catches. Consequently, a two-year trial was conducted to determine whether any lemons packed for export were infested with FCM. A total of 30 346 fruit were sampled and dissected without detection of a single infested fruit. All but one of the 27 FCM traps hung in proximal orchards caught FCM, sometimes in high numbers, thus demonstrating the presence and activity of FCM in the region of the lemon orchards in question. This demonstrated 99.99% non-host status of Eureka lemons at the 95.19% confidence level. Hence, it is concluded that Eureka lemon fruit is a conditional non-host for FCM and at a state of maturity that is suitable for harvesting for commercial export. This provides technical justification for the exclusion of Eureka lemons from mandatory cold treatment as a FCM phytosanitary risk mitigation measure in citrus fruit export protocols. This project is now terminated and the outcomes will be published in a peer-reviewed scientific journal in September 2015.

Opsomming

Toegang van suurlemoene tot markte wat kouesterilisasie (-0.6°C vir 22 dae) vereis kan nie deur gebruik van huidige protokolle bereik word nie. Suurlemoene kan nie gedurende verskeping aan koue sterilisasie blootgestel word nie omrede hulle vatbaarheid vir koue skade. Dit is ook onwaarskynlik dat veel suurlemoenboorde as VKM vry verklaar sal kan word op die basis van feromoon-lokvalvangstes. Daarom is a twee-jaar proef uitgevoer om te bepaal of enige suurlemoene wat vir uitvoer gepak is, met VKM besmet is. 'n Totaal van 30 346 vrugte is ondersoek en gesny sonder enige teken van selfs 'n enkele besmette vrug. Elke lokval, behalwe een, van die 27 VKM lokvalle wat in nabygeleë boorde gehang is het VKM gevang, soms in hoë getalle, wat daarom die teenwoordigheid en aktiwiteit van VKM in die relevante suurlemoen boorde gewys het. Hierdie het 99.99% nie-gasheer status van Eureka suurlemoene gewys teen 'n vlak van vertroue van 95.19%. Daarom kan die gevolgtrekking gemaak word dat Eureka suurlemoene 'n voorwaardelike nie-gasheer vir VKM is teen rypheds status wat geskik is vir oes vir kommersieel uitvoer. Hierdie voorsien tegnieke regverdiging for uitsluiting van Eureka suurlemoene van verpligtende koue behandeling maatreels vir VKM fitosanitêre risiko vermindering in sitrusvrug uitvoer protokolle. Hierdie projek is nou voltooi en die bevindinge gaan in September 2015 in 'n wetenskaplike jernaal gepubliseer word.

Introduction

The South African citrus industry is dependent on export of fresh fruit to many markets around the world, with approximately 70% of South Africa's citrus crop being exported (CGA, 2013). The false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is recorded as a pest of citrus fruit in southern Africa (Newton, 1998; Grout and Moore, 2015). As a result of its endemism to sub-Saharan Africa (Moore, 2002), certain export markets of importance for the South African citrus industry, such as Peoples Republic of China, USA and South Korea, regulate it as a quarantine pest.

Control of the pest in the field can be highly effective, using a suite of integrated control options, applied with diligent management (Moore & Hattingh, 2012). These can succeed in reducing *T. leucotreta* infestation by 97% or more (Moore *et al.*, 2015). Such an integrated system, using the sterile insect technique as the mainstay of the programme, has succeeded in reducing moth catches by 99%, fruit infestation by 96% and export rejections by 89% in the Western Cape Province of South Africa, since the inception of the programme in 2007 (Barnes *et al.*, 2015).

Despite the potential efficacy of such control strategies, some export markets still require mandatory post-harvest cold treatment of citrus fruit as a phytosanitary risk mitigation measure for *T. leucotreta* (SA-DAFF, 2014). The cold treatment developed for *T. leucotreta* by Myburgh (1965) entails maintenance of the fruit at temperatures below 0°C for 22 days. Such treatment is not only costly to apply, but there is a high risk of the fruit developing chilling injury (Lafuente *et al.*, 2003; Cronje, 2007). Some citrus fruit types are able to tolerate such cold treatment, if carefully managed (Lafuente and Zacarias, 2006). However, some other citrus types are highly sensitive to cold damage and this cold treatment is not a feasible risk mitigation measure for commercial export of these types (Lafuente and Zacarias, 2006). Lemons are highly sensitive to cold damage and cannot withstand exposure to temperatures below 0°C for 22 days (Underhill *et al.*, 1999; Lafuente and Zacarias, 2006).

Although standalone postharvest quarantine treatments, such as cold treatment, are still the most commonly used phytosanitary risk mitigation measure (Paull and Armstrong 1994), a range of alternative options, including non-host status, are increasingly being adopted (Liquidó *et al.* 1995; Aluja *et al.* 2004; Follett and Neven 2006; Follett and Hennessey, 2007; Pringle *et al.*, 2015). Pringle *et al.* (2015) indicated that there are no definitions of host status of fruit specifically for tortricid pests, but referred to the following definitions that Aluja and Mangan (2008) applied to host status for Tephritidae: A natural host is one that is infested under totally natural field conditions. A non-natural host (or conditional host) is not known to be infested under natural field conditions, but there is experimental evidence that it can be infested and produce reproductive adults under laboratory (artificial) conditions. A non-host is one in which development cannot be completed. Pringle *et al.* (2015) also applied the following definitions provided by NAPPO (2008): A conditional host may be a host or non-host depending on suitability of conditions (e.g. stage of maturity, other physiological conditions or physical conditions), whereas a natural non-host does not become infested in nature. Most importantly, Pringle *et al.* (2015) followed NAPPO (2008) in its guidelines on determination of host status from natural infestation by evaluating infestation during the export harvest season, as a mandatory requirement in determining host status for phytosanitary purposes, regardless of data from field cage, glasshouse or laboratory trials.

This approach to establishing host status was adopted by Armstrong (1991), who declared Sharwil avocados non-hosts (when attached to the tree) for oriental fruit fly, *Bactrocera dorsalis* (Hendel), and Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), after inspection of 114 000 fruit during two seasons with no observed infestation. Mangosteen fruit was determined to be a non-host for oriental fruit fly from laboratory inspection of 40 000 fruit collected from orchards throughout Thailand during two years (Burikam *et al.*, 1992). Non-host status of Tahiti limes for Caribbean fruit fly, *Anastrepha suspensa* (Loew), was shown after inspection of 102 384 unsorted, ungraded packhouse fruit from 184 different orchards on 60 harvest dates and finding no infested fruit (Hennessey *et al.*, 1992). Pringle *et al.* (2015) inspected 17 799 apples for *T. leucotreta* shortly before harvest over an eight year period and thereby demonstrated that apple is a non-host for *T. leucotreta*.

Different citrus types are known to have different susceptibility to *T. leucotreta* attack and infestation (Love *et al.*, 2014). For example, certain cultivars of navel oranges, *Citrus sinensis* Osbeck, have been reported to be highly susceptible to *T. leucotreta* infestation (Newton, 1990; Love *et al.*, 2014). Several studies and reviews on the host plants of *T. leucotreta* have been conducted. Schwartz (1981) used 10 references, dating from 1901 to 1976 in compiling a list of 35 host plants, of which 21 were cultivated. On this list were orange (*C. sinensis*), grapefruit (*C. paradisi* (Macf.)) and mandarin (*C. reticulata* Blanco), with no inclusion of lemon (*C. limon* (L.) Burm.). Venette *et al.* (2003) used 15 references dating from 1972 to 2003 in compiling

their list of 70 host plants of *T. leucotreta*. Included on the list was *C. sinensis* and *Citrus* spp. Closer scrutiny of all 15 listed studies revealed no specific reference to lemons as a host.

It appears that the first reference to lemon being a host for *T. leucotreta* was USDA-APHIS-PPQ (1983), perpetuated in USDA-APHIS-PPQ (2010). However, no specific reference to lemons being a host for *T. leucotreta* appear in either of these reports. More recently, EPPO (2013) used some of the same and several other references, dating from 1958 to 2010 in compiling a list of 107 previously listed hosts. However, they questioned the validity of several of these and even outright refuted 36 of the listed host species. This was based on a thorough investigation of the literature and the conclusion that reporting was often ambiguous or there was no original source of substantiating data. Included in the list of refuted host plants previously listed were lemon and lime (*C. aurantiifolia* (Christm.)). This is in agreement with Economides (1979) and Newton (1998), who both stated that in lemons and limes larval development is rarely, if ever completed. Perhaps this is because of their greater acidity and excessive juice (Economides, 1979). Thus there is no reliable record in the literature of lemons being a host for *T. leucotreta*.

However, this absence of any reliable host record was not taken into consideration in the specification of mandatory *T. leucotreta* disinfestation treatments (for all citrus types) imposed by some countries importing citrus fruit from South Africa. As a consequence, lemons have been precluded from the export programmes to such markets and this seemingly imposes technically unjustified and unnecessary barriers to trade. This study was therefore undertaken to generate data of specific relevance to the phytosanitary host status of lemons for *T. leucotreta* with the prospect of potentially supporting the removal of unnecessarily trade restrictive disinfestation requirements for lemon exports.

Materials and methods

A trial was conducted to determine the host status of lemons (cultivar Eureka) for *T. leucotreta*, when the fruit is packed for export according to standard commercial packhouse inspection and grading procedures. Eureka was selected as the cultivar of choice, as the overwhelming majority of lemon trees planted in South Africa (around 70%) belong to this cultivar (CGA, 2013). In total, 30 346 Eureka lemons that had been packed for export were collected from four large communal packhouses in the Sundays River Valley (Eastern Cape Province, South Africa) between 5 September 2013 and 22 July 2014 and destructively inspected for infestation with *T. leucotreta* larvae. This was done by carefully peeling and dissecting every fruit while observing it through a head-loop or magnifier lamp. The ripeness colour standard (Anonymous, 1995) was also recorded for each inspected fruit.

Details were obtained for all of the orchards and farms from which the inspected lemons were obtained (Table 3.2.20.1). Pheromone traps for monitoring *T. leucotreta* male moths (Grout and Moore, 2015) were hung in proximal orchards of susceptible citrus types (Navel oranges, Valencia oranges, Mandarins or grapefruit) for all 27 lemon orchards. All but two of the traps were in the range of 22 to 391 m from the lemon orchards. Traps on Outback South and Outback West were 675 and 509 m respectively from the lemon orchards. These pheromone traps are known to be attractive over extensive distances, such that they are recommended to be placed at a maximum density of one trap per four hectares (Moore, 2012) and were therefore taken as indicative of FCM activity in and around the lemon orchards.

The traps were monitored for the entire growing seasons (2012-13 and 2013-14) leading up to harvest in 2013 and 2014.

Table 3.2.20.1. *Thaumatotibia leucotreta* moths caught per trap per week in pheromone monitoring traps hung in close proximity to the lemon orchards used for post-harvest sampling for *T. leucotreta* larval infestation in 2013 and 2014. Trap catches for the last 10 weeks preceding and including harvest are shown.

Farm name	Coordinates	Week harvested and inspected	Moths caught/trap/week for 10 weeks preceding and including harvest									
			10	9	8	7	6	5	4	3	2	1
Maureen	33°32'12"S 25°41'33"E	2 Sep 2013	7	1	3	3	0	1	2	1	0	1
Weltevreden	33°31'07"S 25°41'22"E	2 Sep 2013	0	0	0	0	0	2	1	1	1	0
Glegg	33°25'00"S 25°23'06"E	2 Sep 2013	1	1	0	0	2	0	0	1	2	1
Falcon Ridge	33°24'35"S 25°29'45"E	9 Sep 2013	3	1	12	8	11	8	6	8	13	7
Olifantsklip	33°23'47"S 25°24'22"E	16 Sep 2013	1	0	0	0	3	1	3	1	0	0
Halaron	33°29'44"S 25°40'58"E	23 Sep 2013	1	1	0	2	1	2	1	2	0	2
Nuutbegin	33°23'48"S 25°25'04"E	30 Sep 2013	2	0	0	0	0	*	-	-	-	-
Glentana	33°23'50"S 25°27'31"E	7 Apr 2014	0	1	2	1	1	0	0	0	1	0
Groenus	33°23'56"S 25°22'02"E	7 Apr 2014	0	0	4	0	0	0	0	0	0	0
Outback South	33°37'29"S 25°42'29"E	14 Apr 2014	0	0	2	0	1	0	1	0	0	0
Blaartjebrug	33°37'45"S 25°42'34"E	14 Apr 2014	0	5	0	5	0	0	1	0	0	0
Outback West	33°37'27"S 25°42'16"E	21 Apr 2014	0	2	0	1	0	1	0	0	0	0
Sackville	33°32'01"S 25°39'01"E	21 Apr 2014	0	0	0	0	0	0	0	0	0	0
Gholfland	33°23'59"S 25°27'28"E	21 Apr 2014	5	3	3	4	1	6	3	1	1	4
Penhill	33°34'52"S 25°42'06"E	21 Apr 2014	3	5	4	0	2	2	3	0	1	0
Lapland	33°23'31"S 25°21'07"E	12 May 2014	0	1	0	0	0	0	0	0	0	0
Ekhaya	33°23'50"S 25°27'31"E	12 May 2014	7	4	9	9	12	6	6	5	5	11
Pennyview	33°28'37"S 25°40'19"E	12 May 2014	0	0	0	1	3	7	9	8	4	5
Sun Orange	33°28'09"S 25°39'37"E	2 Jun 2014	0	1	0	0	0	0	0	0	0	0
Eluhlaza	33°28'46"S 25°39'21"E	9 Jun 2014	6	1	13	0	6	0	0	0	4	11
Warlen Court	33°29'45"S 25°35'49"E	7 Jul 2014	4	3	1	2	1	0	0	0	1	2
Tortello	33°26'13"S 25°34'57"E	7 Jul 2014	0	2	0	0	0	2	6	2	0	0
Willow Tree	33°32'02"S 25°39'31"E	14 Jul 2014	3	5	1	3	0	2	1	1	1	1
Mimosa	33°26'24"S 25°30'22"E	14 Jul 2014	4	1	0	0	1	0	1	1	1	0
Voetpadsклоof	33°25'38"S 25°20'54"E	14 Jul 2014	2	2	3	1	2	3	1	0	1	3
Arundel	33°30'52"S 25°39'30"E	14 Jul 2014	0	1	0	2	2	0	1	1	0	0
Boerboon	33°28'03"S 25°34'16"E	21 Jul 2014	0	1	0	0	0	0	0	1	8	0

*Trap was removed.

Results and discussion

Only data from the final 10 weeks before and including the harvest from which the inspected fruit were taken are shown (Table 3.2.20.1), as this was the critical period for risk of post-harvest infestation with *T. leucotreta* larvae, if fruit were susceptible. Moths were caught in 26 out of the 27 traps, and in some cases in relatively high numbers (Moore *et al.*, 2008), thus not only confirming *T. leucotreta* presence and activity in the vicinity of these lemon orchards but also in the broader Sundays River Valley citrus growing region. This was not surprising, as *T. leucotreta* infestation pressure has been known to be higher in the Eastern Cape Province than many other regions in South Africa (Grout and Moore, 2015), due to the preponderance of Navel oranges grown in this region (CGA, 2013) and *T. leucotreta*'s preference for Navel oranges (Love *et al.*, 2014). Despite this, no control measures were applied for *T. leucotreta* in any of the lemon orchards, as lemons are not considered to be a host.

The 30 346 lemon fruit inspected were graded according to industry export colour standards (Fig. 3.2.20.1). Not one of the lemons inspected was infested with any *T. leucotreta* larvae. Neither were there any signs of attempted penetration or historical infestation (e.g. tunnelling and frass). Likewise, none of the lemons were infested with fruit fly larvae (*C. capitata* or *C. rosa* Karsch (Diptera: Tephritidae)), the only other insect pests known to be able to internally infest citrus fruit in South Africa (Grout and Moore, 2015).

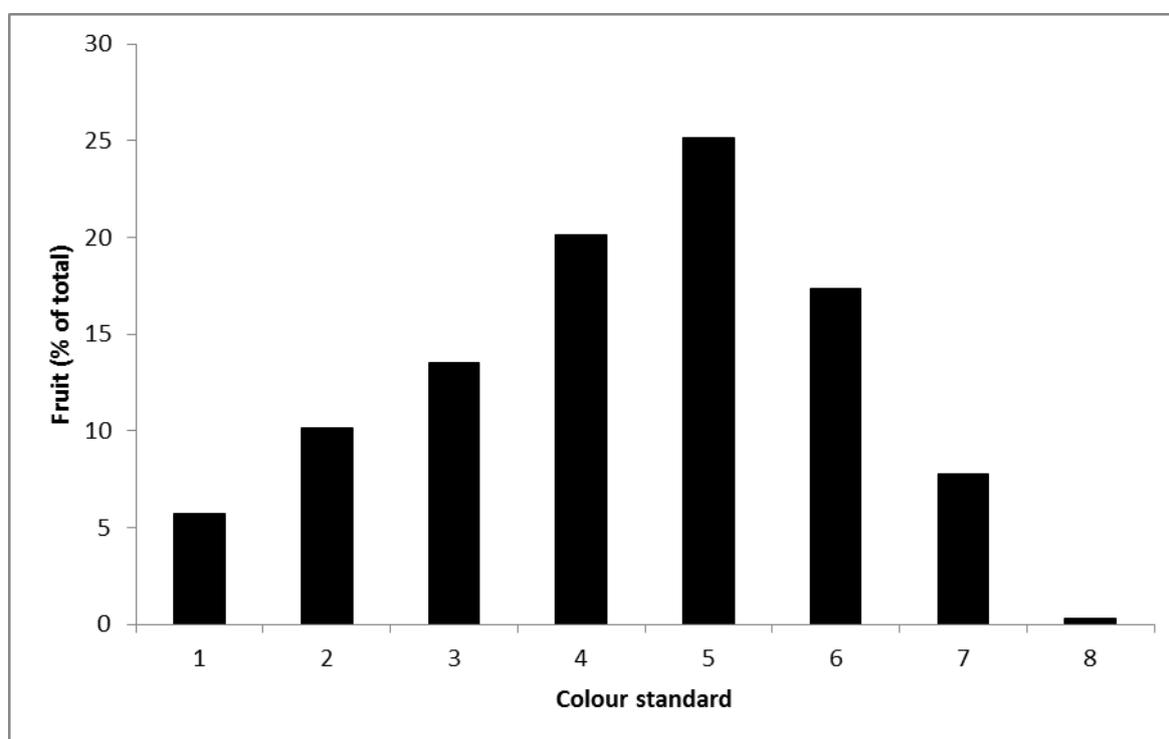


Figure 3.2.20.1. Frequency distribution of lemon fruit inspected for *T. leucotreta*, using colour standards 1 to 8.

For some countries, commodities and pests, probit 9 treatments have traditionally been required to provide an acceptable level of quarantine security (Liquido *et al.* 1995; Follett and Neven 2006). A probit 9 level of efficacy means zero survivors in a sample of at least 93 616 individuals (Couey and Chew, 1986; Schortmeyer *et al.*, 2011), which is a 99.9968% control at the 95% confidence level (Follett and Hennessey, 2007). However, Landolt *et al.* (1994) pointed out that the probit 9 standard may be too stringent for commodities that are rarely infested or are poor hosts, and hence a less severe postharvest treatment might still provide quarantine security. In this case, fewer insects may be needed during research to develop quarantine treatments (Follett and McQuate, 2001).

For example, for some countries, a 99.99% treatment efficacy at the 95% confidence level is sufficient to prove the safety of a consignment of a commodity for the phytosanitary organism in question. For instance, Japan, Australia, and New Zealand accept quarantine treatment efficacy at this level, which is obtained by applying the quarantine treatment to 29 956 insects with no survivors (Couey and Chew 1986). More specifically, Japan requires a total of 30 000 individuals in three to four trials (Sproul 1976), New Zealand

requires three replicates of 10 000 test insects each, and Australia accepts a cumulative total of 30 000 treated insects with no survivors after the quarantine treatment (Heather and Corcoran, 1992).

Follett & Neven (2006) equated non-host status to a quarantine measure for agricultural commodities. A commodity may be a non-host for only part of its life cycle (Greany, 1994; Armstrong, 1994) and most importantly, for confirmation of host status for phytosanitary purposes, data on natural infestation during the export harvest period is mandatory, regardless of data from other trials, such as field cage, glasshouse or laboratory trials (NAPPO, 2008). The absence of any infestation in 30 000 harvested fruit that were exposed to the pest in the field, establishes 99.99% non-host status of the fruit with a 95% confidence level (Follett & Hennessey, 2007; Pringle *et al.*, 2015).

Similarly in this study, we determined by thorough destructive sampling that there were no *T. leucotreta* infested fruit in 30 346 Eureka lemon fruit which had been exposed to the pest during growing under natural conditions and had been commercially harvested and packed for export. This demonstrated 99.99% non-host status of such fruit at a 95.19% confidence level. This result is supported by data from routine sampling and inspection of lemon fruit conducted by some citrus packhouses in the Sundays River Valley, as supplied by the independent evaluator of these samples, Fruit Quality Control Services (Gerber, pers. comm.). Samples were drawn from fruit packed for export and retained at 25°C for 14 days, such that any cryptic infestation with *T. leucotreta* or fruit fly larvae would become visually obvious, before inspection and dissection of all fruit showing any signs of decay or infestation. Over the period 2007 to 2014, 7 000 660 individual lemons were inspected and not a single lemon fruit was observed to be infested with *T. leucotreta* or fruit fly larvae. These findings support what is already considered common knowledge in the citrus industry (Newton, 1998; Grout and Moore, 2015) and provide technical justification for the exclusion of Eureka lemons from mandatory cold treatment as a *T. leucotreta* phytosanitary risk mitigation measure in citrus fruit export protocols.

Acknowledgements

Sundays River Citrus Company, Unifrutti and Sun Citrus are thanked for their generous donation of export lemons. Ian Share and Eugene Nepgen of Xsit and MJ van der Mescht of Sun Citrus are thanked for assisting with supplying of trapping data. Akona Zondi, Sinxolo Phindani, Siphesihle Gogodla, Siphosethu Ntisa, Zanele Mntambo, Nombulelo Mxubane, Aviwe Sontshi and Nelisa Mali are thanked for assisting with fruit inspections. Craig Chambers and Sean Thackeray are thanked for assistance with preparation of high resolution figures.

References cited

- ALUJA, M., & R. L. MANGAN. 2008. Fruit fly (Diptera: Tephritidae) host status determination: critical conceptual, methodological, and regulatory considerations. *Annual Review of Entomology* **53**: 473-502.
- ALUA, M., DÍAZ-FLEISCHER, F. & ARRENDONDO, J. 2004. Nonhost status of commercial *Persea americana* 'Hass' to *Anastrepha ludens*, *Anastrepha obliqua*, *Anastrepha serpentina*, and *Anastrepha striata* (Diptera: Tephritidae) in Mexico. *Journal of Economic Entomology* **97**: 293-309.
- ANONYMOUS, 1995. *Colour prints for blemish standards*. Outspan International, Centurion, South Africa.
- ARMSTRONG, J. W. 1991. 'Sharwil' avocado: quarantine security against fruit fly (Diptera: Tephritidae) infestation in Hawaii. *Journal of Economic Entomology* **84**: 1308-1315.
- ARMSTRONG, J. W. 1994. Commodity resistance to infestation by quarantine pests. In: Sharp, J.L. & Hallman, G.J. (Eds.) *Quarantine treatments for pests of food plants*. 199-211. Westview Press, Boulder, USA.
- BARNES, B.N., HOFMEYR, J.H., GROENEWALD, S., CONLONG, D.E. & WOHLFARTER, M. 2015. The sterile insect technique in agricultural crops in South Africa: a metamorphosis but will it fly? *African Entomology* **23**(1): 1-18.
- BURIKAM, I., SARNTHOY, O., CHARENSOM, K., KANNO, T. & HOMMA, H. 1992. Cold temperature treatment for mangosteens infested with the oriental fruit fly (Diptera: Tephritidae). *Journal of Economic Entomology* **85**: 2298-2301.
- CITRUS GROWERS' ASSOCIATION (CGA). 2013. *Key Industry Statistics*. Citrus Growers Association, South Africa.
- COUEY, H. M., & V. CHEW. 1986. Confidence limits and sample size in quarantine research. *Journal of Economic Entomology* **79**: 887-890.
- CRONJE P.J.R, 2007. *Postharvest Rind Disorders of Citrus Fruit*. Citrus Research International, Nelspruit, South Africa.

- ECONOMIDES, V.C. 1979. False codling moth, a serious pest of citrus in Zambia. *Farming in Zambia* May 1979: 4.
- EPPO. 2013. *Pest risk analysis for Thaumatotibia leucotreta*. EPPO, Paris. Available at http://www.eppo.int/QUARANTINE/Pest_Risk_Analysis/PRA_intro.htm
- FOLLETT, P. A. & McQUATE, G.T. 2001. Accelerated development of quarantine treatments for insect on poor hosts. *Journal of Economic Entomology* **94**: 1005-1011.
- FOLLETT, P. A. & NEVEN, L.G. 2006. Current trends in quarantine entomology. *Annual Review of Entomology* **51**: 359-385.
- FOLLETT, P.A. & HENNESSEY, M.K. 2007. Confidence limits and sample size for determining nonhost status of fruits and vegetables to tephritid fruit flies as a quarantine measure. *Journal of Economic Entomology* **100**: 251-57.
- GREANY, P. D. 1994. Plant host status and natural resistance. In: Armstrong, J.W. & Paull, R.E. (Eds.) *Insect pests of fresh horticultural products: treatments and responses*. 37-46. CAB International, Wallingford, United Kingdom.
- GROUT, T.G. & MOORE, S.D. 2015. Citrus. In: Prinsloo, G.L. & Uys, G.M. (Eds). *Insects of cultivated plants and natural pastures in Southern Africa*. 447-501. Entomological Society of Southern Africa, Pretoria, South Africa.
- HEATHER N. W. & CORCORAN, R.J. 1992. Effects of ionizing energy on fruit flies and seed weevil in Australian mangoes, In: *Panel Proceedings of the Final Research Coordination Meeting on Use of Irradiation as a Quarantine Treatment of Food and Agricultural Commodities, Kuala Lumpur, Malaysia*. 43-52. International Atomic Energy Agency, Vienna, Austria.
- HENNESSEY, M. K., BARANOWSKI, R. M. & SHARP, J. L. 1992. Absence of natural infestation of Caribbean fruit fly (Diptera: Tephritidae) from commercial Florida 'Tahiti' lime fruits. *Journal of Economic Entomology* **85**: 1843-1845.
- LAFUENTE, M.T. & ZACARIAS, L. 2006. Postharvest physiological disorders in citrus fruit. *Stewart Postharvest Review* **1**(2): 1-9.
- LAFUENTE, M.T., ZACARIAS, L., MARTÍNEZ-TÉLLEZ, M.A., SANCHEZ-BALLESTA, M.T. & GRANELL, A. 2003. Phenylalanine ammonia-lyase and ethylene in relation to chilling injury as affected by fruit age in citrus. *Postharvest Biology and Technology* **29**: 309-318.
- LANDOLT, P.J., CHAMBERS, D.L. & CHEW, V. 1994. Alternative to the use of probit 9 mortality as a criterion for quarantine treatments of fruit fly (Diptera: Tephritidae) infested fruit. *Journal of Economic Entomology* **77**: 285-287.
- LIQUIDO N. J., GRIFFIN, R.L. & VICK, K.W. 1995. *Quarantine security for commodities: current approaches and potential strategies*. U.S. Dep. Agric. Publ. Ser. 1996-04.
- LOVE, C.N., HILL, M.P. & MOORE, S.D. 2014. *Thaumatotibia leucotreta* and the Navel orange: ovipositional preferences and host susceptibility. *Journal of Applied Entomology*, **138**: 600-611.
- MOORE, S.D. 2002. The development and evaluation of *Cryptophlebia leucotreta* granulovirus (CrLeGV) as a biological control agent for the management of false codling moth, *Cryptophlebia leucotreta*, on citrus. PhD Thesis, Rhodes University, Grahamstown, South Africa.
- MOORE, S.D. 2012. Moths and butterflies: false codling moth. In: Grout, T.G. (Ed.) *Citrus Research International IPM Production Guidelines, Volume 3, Part 9.4*. Nelspruit, South Africa.
- MOORE, S.D., GROUT, T.G., HATTINGH, V. & HOFMEYR, J.H. 2008. Thresholds and guidelines for intervention against citrus pests. *South African Fruit Journal* **7**(4): 77-81.
- MOORE, S.D. & HATTINGH, V. 2012. A review of current Pre-harvest Control Options for False Codling Moth in Citrus in Southern Africa. *South African Fruit Journal* **11**(4): 82-85.
- MOORE, S.D., KIRKMAN, W., RICHARDS, G.I. & STEPHEN, P. 2015. The *Cryptophlebia leucotreta* granulovirus – 10 years of commercial field use. *Viruses* 2015, **7**: 1284-1312; doi:10.3390/v7031284.
- MYBURGH, A.C. 1965. Low temperature sterilisation of false codling moth, *Argyroploce leucotreta* Meyr., in export citrus. *Journal of the Entomological Society of southern Africa* **28**(2): 277 - 285.
- NAPPO. 2008. Guidelines for the determination and designation of host status of a fruit or vegetable for fruit flies (Diptera: Tephritidae). NAPPO Regional Standards for Phytosanitary Measures (RSPM). RSPM No. 30, Ottawa, Canada.
- NEWTON, P.J. 1990. Ovipositional preferences amongst navel sweet orange types by the false codling moth, *Cryptophlebia leucotreta*. *Annals of Applied Biology* **116**: 143-150.
- NEWTON, P.J. 1998. False codling moth *Cryptophlebia leucotreta* (Meyrick). In: Bedford, E.C.G., van den Berg, M.A. & de Villiers, E.A. (Eds.) *Citrus pests in the Republic of South Africa*. 194-200. Dynamic Ad, Nelspruit, South Africa, pp.
- PAULL R.E. & ARMSTRONG, J.W. 1994. *Insect Pests and Fresh Horticultural Products: Treatments and Responses*. CAB International, Wallingford, UK.

- PRINGLE, K.L., HEUNIS, J.M. & DE VILLIERS, M. 2015. Phytosanitary host status of apples as a host for false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae). *African Entomology* **23**(1): 234-238.
- SCHORTEMAYER, M., THOMAS, K., HAACK, R.A., UZUNOVIC, A., HOOVER, K., SIMPSON, J.A. & GRGURINOVIC, C.A. 2011. Appropriateness of Probit-9 in the Development of Quarantine Treatments for Timber and Timber Commodities. *Journal of Economic Entomology* **104**(3): 717-731.
- SCHWARTZ, A. 1981. 'n Bydrae tot die biologie en beheer van die valskodlingmot *Cryptophlebia leucotreta* (Meyr.) (Lepidoptera: Eucosmidae) op nawels. PhD thesis, University of Stellenbosch.
- SOUTH AFRICAN DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES (DAFF). 2014. Export protocols/programmes/directive. Online at: <http://www.daff.gov.za/daffweb3/Services/Exports>
- SPROUL, A.N. 1976. Disinfestation of Western Australian Granny Smith apples by cold treatment against the egg and larval stages of the Mediterranean fruit fly (*Ceratitidis capitata* (Wied.)) *Australian Journal of Experimental and Agricultural Animal Husbandry* **16**: 280-285.
- U.S. DEPARTMENT OF AGRICULTURE, ANIMAL, PLANT HEALTH INSPECTION SERVICE, PLANT PROTECTION AND QUARANTINE. 1983. Action Plan, False Codling Moth, *Cryptophlebia leucotreta* (Meyrick).
- U.S. DEPARTMENT OF AGRICULTURE, ANIMAL, PLANT HEALTH INSPECTION SERVICE, PLANT PROTECTION AND QUARANTINE, EMERGENCY AND DOMESTIC PROGRAMS. 2010. New Pest Response Guidelines: False Codling Moth *Thaumatotibia leucotreta*. Riverdale, Maryland [http://www.aphis.usda.gov/import_export/plants/manuals/online_manuals.shtml]
- UNDERHILL, S.J., DAHLER, J.M., McLAUCHLAN, R.L. & BARKER, L.R. 1999. Susceptibility of Lisbon and Eureka lemons to chilling injury. *Australian Journal of Experimental Agriculture* **39**: 757-60.
- VENETTE, R.C., DAVIS, E.E., DA COSTA, M., HEISLER, H. & LARSON, M. 2003. Mini Risk Assessment: false codling moth, *Thaumatotibia* (= *Cryptophlebia*) *leucotreta* (Meyrick) [Lepidoptera: Tortricidae]. University of Minnesota, Department of Entomology, CAPS PRA. 1-30. http://www.aphis.usda.gov/ppq/ep/pest_detection/practica/tleucotretapra.pdf. Accessed: 02/03/2012.

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

Project 1090 (April 2014 – March 2017) by Wayne Kirkman and Sean Moore (CRI)

Summary

A Solid Phase Microextraction (SPME) probe has been shown to effectively trap as well as concentrate headspace volatile compounds surrounding intact fruit. Volatile compound detection is achieved by inserting this probe into a Gas Chromatography-Mass Spectrometry (GCMS) system. A previous study showed that SPME detection of volatiles emitted by fruit and differences in emission profiles between healthy and infested fruit has great potential as a post-harvest screening option. Five major volatile compounds of interest were released by the infested oranges. These major volatile compounds are D-limonene, 3,7-dimethyl-1,3,6-octatriene, (E)-4,8-dimethyl-1,3,7-nonatriene, caryophyllene and naphthalene. Limonene was one of the most abundant volatile compounds released by the infested citrus fruit. Naphthalene, which is possibly produced due to larval feeding and development within the fruit, maintained higher concentrations than controls throughout the infestation within the fruit. Naphthalene would be a good indicator of FCM infestation, however, not primarily for early infestation detection. In this earlier study, a significantly higher concentration of D-limonene, 3,7-dimethyl-1,3,6-octatriene, (E)-4,8-dimethyl-1,3,7-nonatriene and naphthalene was detected using the SEP over the SPME technique. These results need to be verified, and processes refined, to avoid unwanted variables, and to build on the previous study. Wayne Kirkman registered for a PhD at Rhodes University. Trials on Satsuma mandarins were initiated. Preparations were made for a visit to the University of Leeds.

Opsomming

Soliede Fase Mikro-ekstraksie (SPME) het hoofruim vlugtigestowwe om vrugte effektief opgevang en gekonsentreer. Gaskromatografie-Massaspektrometrie (GCMS) analiese is gedoen om die vlugtigestowwe van besmette en gesonde vrugte te meet. 'n Vorige studie het gewys dat SPME opsporing van vlugtigestowwe goeie potesiaal het vir na-oes bepaling van vlugtigestowwe wat afgeskei word. Vyf hoof vlugtigestowwe van belang is deur besmette vrugte afgeskei, naamlik D-limonien, 3,7-dimethyl-1,3,6-oktatrien, (E)-4,8-dimethyl-1,3,7-nonatrien, kariofileen en naftaleen. Limonien is een van die mees menigvuldige vlugtigestowwe wat deur sitrusvrugte vrygestel word. Naftaleen, wat waarskynlik as gevolg van larwe voeding en ontwikkeling in die vrug geproduseer word, het aanhoudend hoër konsentrasies as gesonde vrugte gehandhaaf. Die vroeë studie het gewys dat naftaleen 'n goeie aanwyser van valskodlingmot

besmetting kan wees, maar egter nie om vroeë besmetting aan te wys nie. 'n Beduindende hoër konsentrasie van D-limonien, 3,7-dimetiel-1,3,6-oktatrieen, (E)-4,8-dimetiel-1,3,7-nonatrieen, kariofileen en naftaleen is met die SEP tegniek opgespoor in vergelyking met SPME. Die resultate moet geverifieer word, en tegnieke moet verfyn word, om ongewenste variasie te vermy, sodat daar op die vorige studie gebou kan word. Wayne Kirkman het by Rhodes Universiteit vir 'n PhD geregistreer. Proewe is op Satsuma mandaryne begin. Voorbereiding is vir 'n besoek aan die Universiteit van Leeds gedoen.

3.2.22 **FINAL REPORT: An audit of the efficacy of the sterile insect technique programme for false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), in the Sundays River Valley**

By Francois Joubert, Martin Hill (RU) and Sean Moore (CRI)

(This project was not funded by CRI)

Summary

The SIT programme in the Sundays River Valley (SRV) was implemented during 2011. Xsit Pty (Ltd) conduct all their own monitoring of FCM population numbers in the SRV. The aim of this study was to conduct an independent comparison to quantify the effects of SIT. The two study areas selected for comparison were Penhill and Dunbrody. For the Dunbrody comparison, trap catch data for the 2011/2012 and 2012/2013 season from the SIT and non-SIT areas were compared. This comparison was supplemented with fruit infestation data for the same orchards for the 2012/2013 season. For the Penhill comparison, trap catch data for the 2012/2013 season from the SIT and non-SIT areas were compared. The wild male trap catches were lower in the SIT areas than the non-SIT areas for all the comparisons. These results support the effectiveness of the SIT programme in the SRV. An increase in the trap catches during the winter months, the harvesting period of most citrus cultivars, raises concern. This increase in the wild FCM population was linked to colder temperatures during the winter months which decrease the mobility of sterile male moths reducing the efficacy of the SIT programme. The main recommendation from this study was that monitoring in non-SIT areas should be intensified (to facilitate more comparisons between SIT and non-SIT areas) and research that may lead to an increase in the cold tolerance of facility reared FCM should be prioritized.

Opsomming

Die SIT program in die Sondagsrivier Vallei (SRV) is gedurende 2011 geïmplementeer. Xsit Pty (Ltd) doen hulle eie monitering van VKM populasie getalle in die SRV. Die doel van hierdie studie was om 'n onafhanklike vergelyking te doen om te effektiwiteit van SIT te kwantifiseer. Die twee studie areas wat vir die vergelyking gekies was is Penhill en Dunbrody. Vir die Dunbrody vergelyking, lokval data vir die 2011/2012 en 2012/2013 seisoen van SIT en nie-SIT areas is vergelyk. Die vergelyking is met vrugbesmettings data vir dieselfde boorde vir die 2012/2013 seisoen gesupplementeer. Vir die Penhill vergelyking is lokval data vir die 2012/2013 seisoen vir SIT en nie-SIT areas vergelyk. Die wilde mannetjie lokval vangstes is laer in die SIT areas as die nie-SIT areas vir al die vergelykings. Hierdie resultate ondersteun die doeltreffendheid van die SIT program in die SRV. 'n Verhoging in die lokval vangstes gedurende die winter maande, oestyd vir meeste kultivars, is van kammernis. Hierdie verhoging in die wilde VKM populasie is gekoppel met kouer temperature gedurende die winter maande wat die vliegvermoë van steriele mannetjie motte verminder, wat die doeltreffendheid van die SIT program verminder. Die hoof voorstel van hierdie studie is dat monitering in nie-SIT areas geïntensifiseer moet word (om meer vergelykings tussen SIT en nie-SIT areas te fasiliteer) en navorsing wat dalk kan lei tot 'n verbetering in die koue toleransie van geteelde VKM moet geprioritiseer word.

Introduction

Numerous methods are used to control FCM in South Africa and they include inspection and monitoring (Moore *et al.* 2008), attract & kill (Nepgen 2014), biological control (*Cryptophlebia leucotreta* granulovirus) (Moore *et al.* 2011), cultural control (orchard sanitation) (Kirkman & Moore 2009), mating disruption (Nepgen 2014; Kirkman 2007), chemical control (Moore *et al.* 2011; Moore *et al.* 2004) and the sterile insect technique, which is a form of biological control. Biological methods have a lot of potential due to the development of resistance against chemical insecticides in FCM and the growing trend to decrease the residue of chemicals on citrus fruit (Moore *et al.* 2011; Moore *et al.* 2004).

The sterile insect technique (SIT) dates back to early 1930 (Klassen & Curtis 2005). The basic concept of SIT is to release sterile pest species of an insect into a wild population to induce sterility or dilute the number of fertile wild males in the population to ultimately control the numbers of the target pest species (Klassen &

Curtis 2005). Three researchers independently developed SIT in three very different environments, including A.S. Serebrovskii (Moscow State University), F.L. Vanderplank (tsetse field research station in what is now Tanzania) and E.F. Knipping (United States Department of Agriculture) (Klassen & Curtis 2005). The early years of SIT up to very recently were met with considerable controversy and speculation (Krafsur 1998). SIT has expanded in recent years and programmes to deal with pest species is becoming more widespread (Klassen & Curtis 2005). One of the well known SIT success stories is the eradication of the New World screwworm fly *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) from large portions of North America (Vargas-Teran *et al.* 2005).

Xsit (Pty) Ltd commercialized the use of SIT for FCM in SA during 2007. The method they employ relies on sterilized males to compete for mates with wild fertile males. Large numbers of sterilized males are released into an area on a regular basis frequented by a wild population. The ratio they aim on reaching is 10 sterile to 1 wild fertile male FCM. This is thus a method of diluting the population with sterile males. These sterile moths are still competitive and thus reduce the number of copulations that lead to a next generation being produced. Considerable success has been had controlling FCM population numbers in the Citrusdal area close to the Xsit sterilization facility. The method was introduced to the Sundays River Valley during 2011. The main aim of this study is 1) to conduct an independent post-release evaluation of the SIT programme in SRV and 2) ultimately contribute to the control of FCM.

Materials and methods

This study utilized data sourced from Xsit (Pty) Ltd, which was collected over a period of 3 years (2011-2013), sufficient to make conclusions on the programmes efficacy. The data included weekly trap catches of both wild and sterile male FCM and fruit infestation data from areas where SIT is implemented and areas where SIT is not implemented. The trap catches were carried out by placing one Delta trap in the target orchard baited with a pheromone lure which attracts male FCM. At the bottom of the trap is a sheet of sticky paper which the moths instantly stick to when they land in the trap. The number of trapped sterile FCM males can be determined by squashing the bodies of the moths, as the sterile moths have a colorant added to their diet during their rearing which gives their gut a distinct purple colour. For the fruit infestation, data was collected by examining fallen fruit from 10 trees from the selected target orchard. The fallen fruit were collected each Monday on a weekly basis. The fruit were dissected for any signs of larval infestation. The percentage of the fallen fruit infested by larvae give an indication of the FCM population numbers. Xsit analyze their own data to confirm that the ratio between wild and sterile male moths is sufficient and to determine if the programme is effective in reducing fruit infestation and wild moth population numbers. The analysis conducted by Xsit draws conclusions from large sets of data that includes multiple farms and cultivars.

The focus for this study was to make multiple small scale comparisons to decrease the amount of variables that may influence the results. Two farming areas in the SRV were selected from the Xsit data set to make comparisons between SIT and Non-SIT areas. These two areas, Dunbrody and Penhill, are both situated in the heart of the SRV citrus producing area. The farms selected for the comparisons were selected based on the control regimes in place for FCM. The goal was to select farms with very similar control regimes in place. All the farms (SIT and Non-SIT) use virus applications throughout the year (Cryptex or Cryptogran). SIT farms generally apply 3 virus treatments for 1st year of SIT programme, 2 virus treatments for 2nd year of SIT programme and 1 virus treatment for 3rd year of SIT treatment. Non-SIT farms utilize 1-3 virus treatments per year and generally 1 broad spectrum insecticide treatment before harvest (e.g. Delegate).

For the Dunbrody comparisons, the same areas were compared from the 2011 / 2012 season up to the 2012 / 2013 season. The comparisons were based on male FCM (wild and sterile) trap catches in the SIT and Non-SIT areas of Dunbrody and limited to Palmer navel orchards. The Penhill comparison could only be made for the 2012 / 2013 season. The comparison was also based on male FCM (wild and sterile) trap catches in the SIT and Non-SIT areas of Penhill. The comparison could not be limited to a single navel cultivar due to a lack of available replicates available for the Non-SIT farms.

Results

It is clear that the SIT programme in the SRV has been very effective in reducing the numbers of wild male FCM (Figure 3.2.22.1, 3.2.22.2 & 3.2.22.3). When comparing the SIT programme to more conventional approaches it is clear that the SIT programme has been very effective in reducing the numbers of wild male FCM.

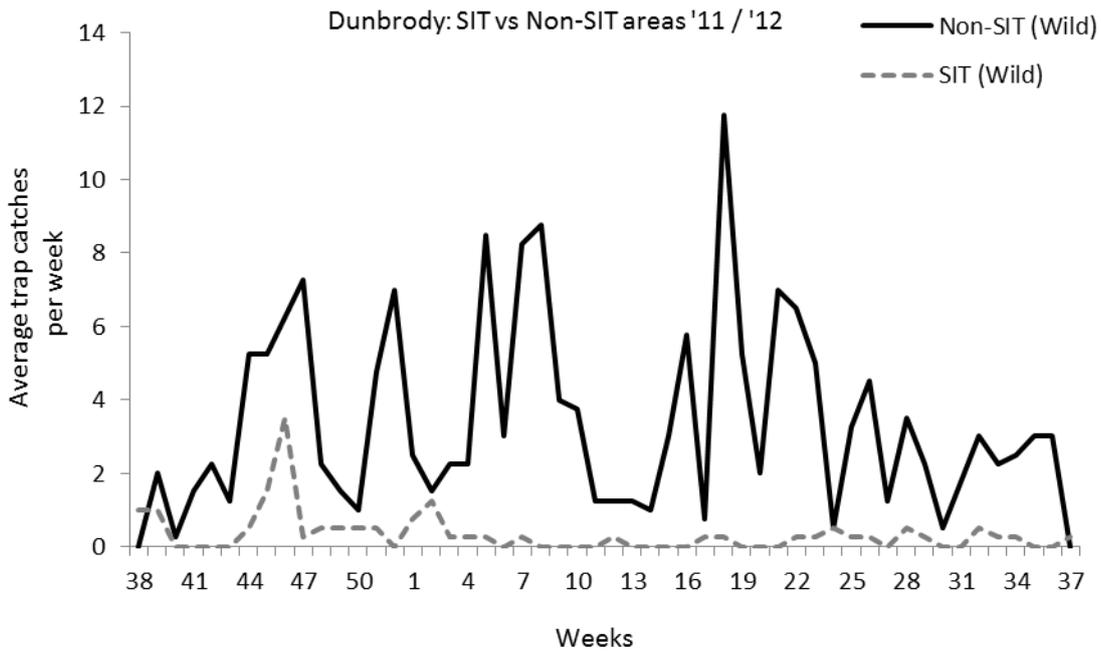


Figure 3.2.22.1 Average trap catches per week of wild male false codling moth, comparing the SIT and Non-SIT areas of Dunbrody for the 2011 / 2012 season.

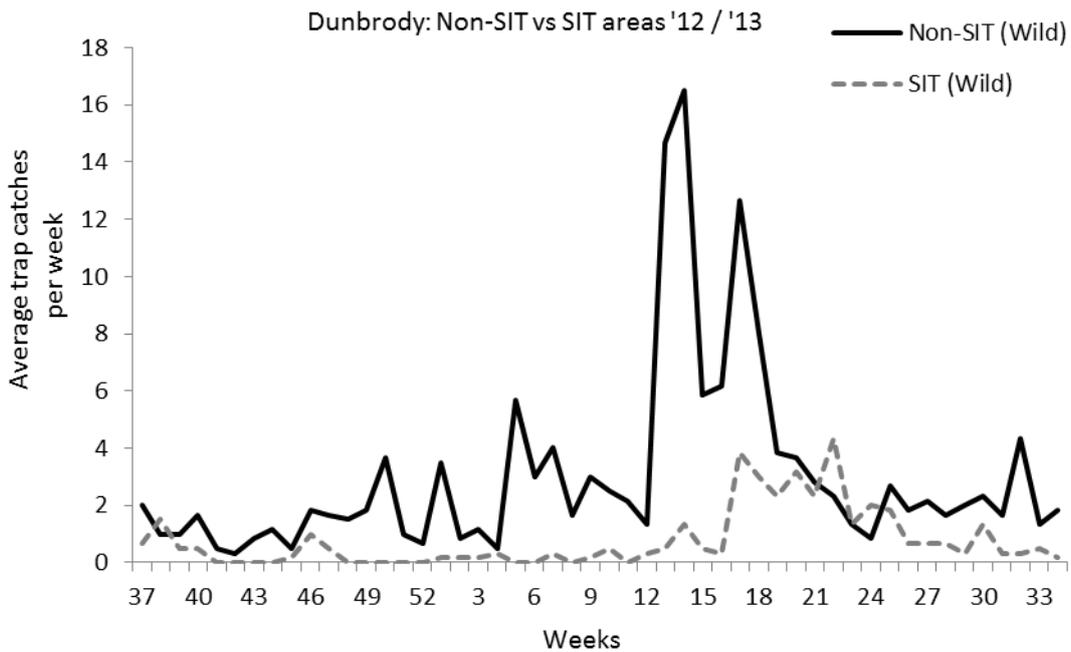


Figure 3.2.22.2 Average trap catches per week of wild male false codling moth, comparing the SIT and Non-SIT areas of Dunbrody for the 2012 / 2013 season.

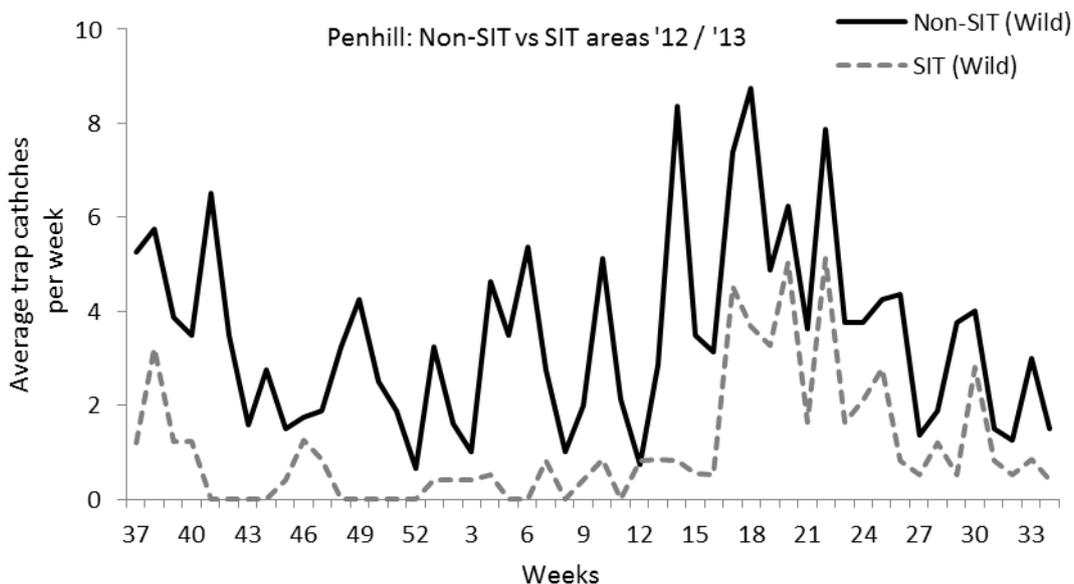


Figure 3.2.22.3 Average trap catches per week of wild male false codling moth, comparing the sterile insect technique and Non-SIT areas of Penhill for the 2012 / 2013 season.

Figure 3.2.22.4 represents a comparison of a SIT farm with a Non-SIT farm both situated in the Dunbrody area. The orchards selected from both farms consisted of Palmer navel orchards. The graph represents average weekly trap catches of sterile male FCM. The weeks throughout the year are arranged from the start to the end of the general navel citrus season in the SRV. This season starts around August / September (2011) when the trees start to bloom, around March (2012) the young immature fruit are visible and around the month of June (2012) the fruit are ready for harvest. There was minimal spill-over of sterile moths into the Non-SIT area. The trap catches of sterile male FCM in the SIT area was the highest during the summer months and very low during the winter months. The same areas were compared for Figure 3.2.22.1, however this graph represents weekly trap catches of wild male FCM. The graph represents the average weekly trap catches of wild FCM. During this first year of implementation the SIT programme was very affective in the Dunbrody area (Figure 3.2.22.1). Control of FCM on the SIT farm was sufficient throughout the season with a minor increase in trap catches from week 44 – 47 of 2011.

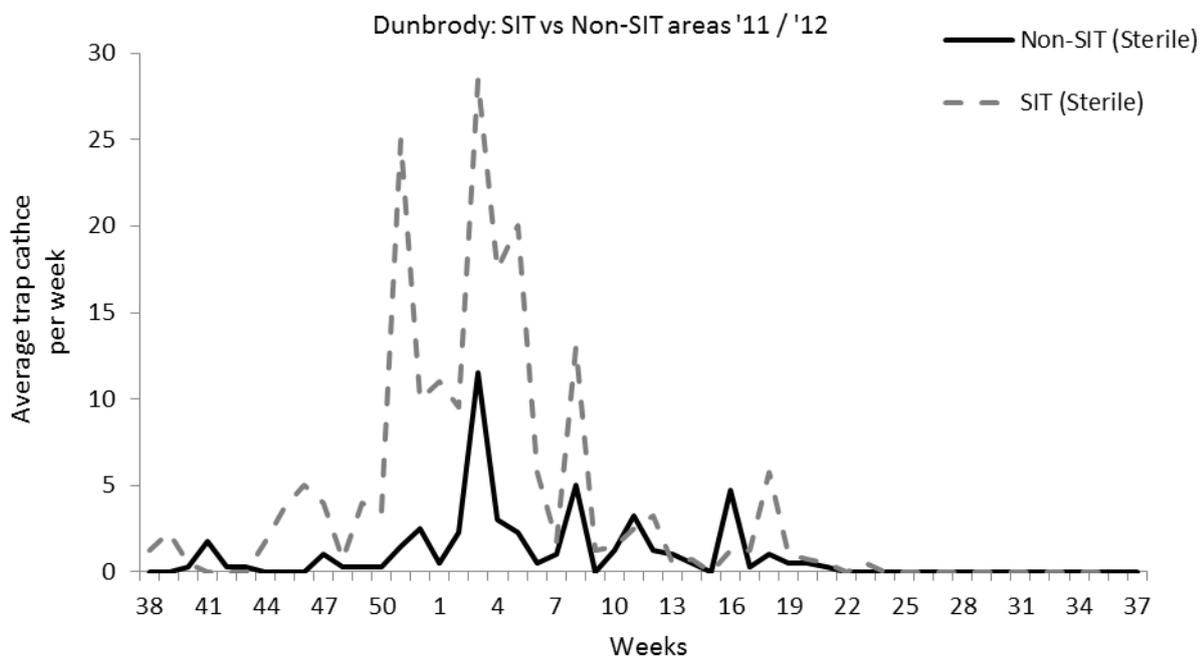


Figure 3.2.22.4 Average trap catches per week of sterile male false codling moth, comparing the SIT and Non-SIT areas of Dunbrody for the 2011 / 2012 season.

Figure 3.2.22.5 represents the same comparison as Figure 3.2.22.4, the only difference being Figure 3.2.22.5 represents the 2012 / 2013 season. The spill-over of sterile male FCM was minimal into the Non-SIT area, less so compared to the previous season (Figure 3.2.22.4). Figure 3.2.22.2 represents the same comparison as Figure 3.2.22.1, the only difference being Figure 3.2.22.2 represents the 2012 / 2013 season. When comparing the control of wild male FCM in the Non-SIT area with control in the SIT area it is clear that the control in the SIT area has been very effective. The control in the SIT area was less effective during the winter months. Figure 3.2.22.6 represents the same areas compared for Figure 3.2.22.2, 3.22.2.5 & 3.2.22.6. Figure 3.2.22.6 looks at fruit infestation as a percentage of the total fallen fruit. This data was collected from 10 data trees from each orchard. Control in the SIT area was again the most effective with a reduction in the effectiveness of control into the winter months, the same trend observed as in Figure 3.2.22.2.

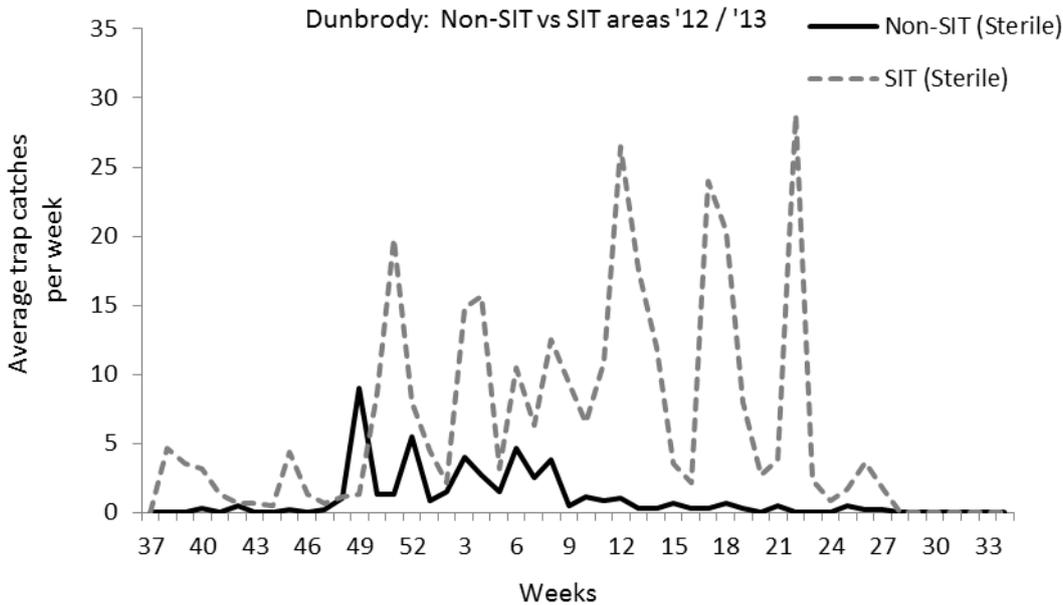


Figure 3.2.22.5 Average trap catches per week of sterile male false codling moth, comparing the SIT and Non-SIT areas of Dunbrody for the 2012 / 2013 season.

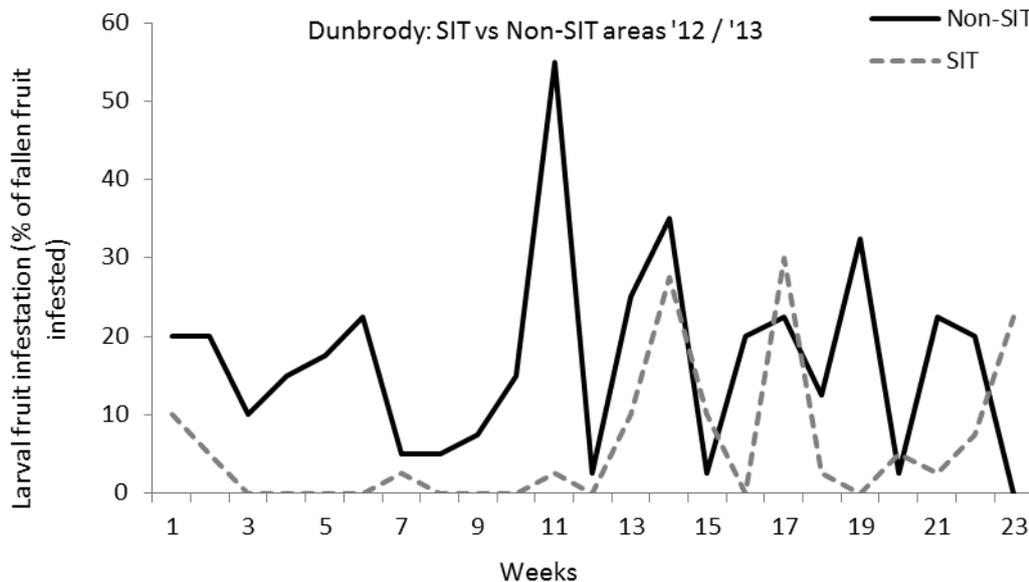


Figure 3.2.22.6 Average number of fruit infested with false codling moth larvae, comparing the SIT and Non-SIT areas of Dunbrody for the 2012 / 2013 season.

Figure 3.2.22.7 represents a comparison of a SIT farm with a Non-SIT farm both situated in the Penhill area for the 2012 / 2013 season. The orchards selected from both farms consisted of a mixture of navel orchards. The graph represents a similar comparison to the Dunbrody comparison (Figure 3.2.22.5) the only difference being the areas that were compared and the navel cultivars used. The trap catches of sterile male FCM was

very low throughout the season in the Non-SIT area, spill-over was minimal. The trap catches of sterile male FCM in the SIT area was high during the summer and very low during the winter a trend similar to what was observed in Dunbrody (Figure 3.2.22.5). Figure 3.2.22.3 represents the same comparison as Figure 3.2.22.7, however it represents the trap catches of wild male FCM. When comparing the control of wild male FCM in the Non-SIT area to the control in the SIT area it is clear that the control in the SIT area has been very effective. Control in the SIT area was very effective during the summer months and the control was less effective during the winter months, again a similar trend to the Dunbrody comparison (Figure 3.2.22.2).

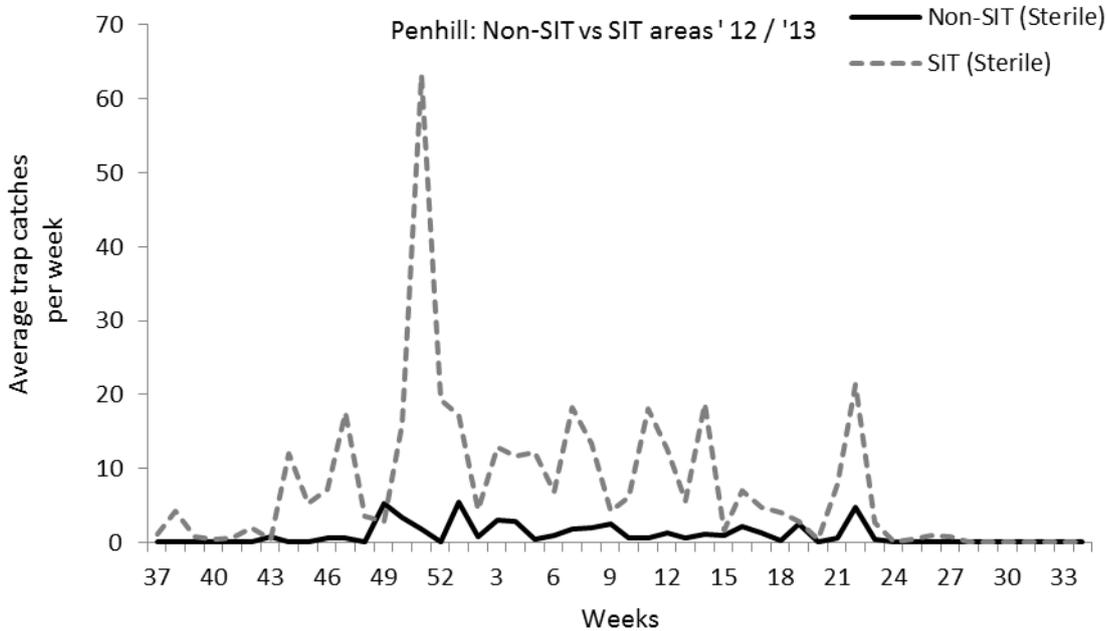


Figure 3.2.22.7 Average trap catches per week of sterile male false codling moth, comparing the sterile insect technique and Non-SIT areas of Penhill for the 2012 / 2013 season.

The temperature data was attained from the South African Weather Service to possibly link the decrease in the trap catches of sterile male FCM and the decrease in the efficacy of the SIT programme during the winter months to a drop in air temperature. FCM is nocturnal so only the temperatures are shown when the species is most active. Figure 3.2.22.8 represents the average air temperature per month for 2011-2013 at 18:00; this is when the moths start to become active. Figure 3.2.22.9 represents the average air temperature per month for 2011-2013 at 00:00; this is when the moths stop being active. The drop in the efficacy of the SIT programme during the winter months stretches from around March to August (Figure 3.2.22.2 and 3.2.22.3). This decrease in efficacy corresponds with the decrease in temperature from March to August, the onset and end of winter in the SRV (Figure 3.2.22.8 & 3.2.22.9).

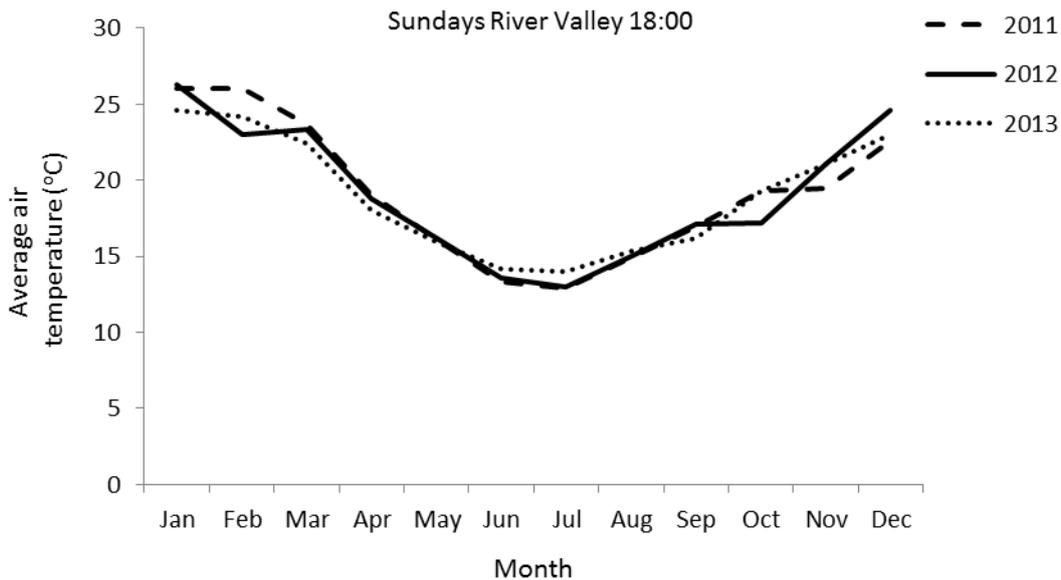


Figure 3.2.22.8 Average air temperature for each month taken at 18:00 from 2011-2013 for the Sundays River Valley.

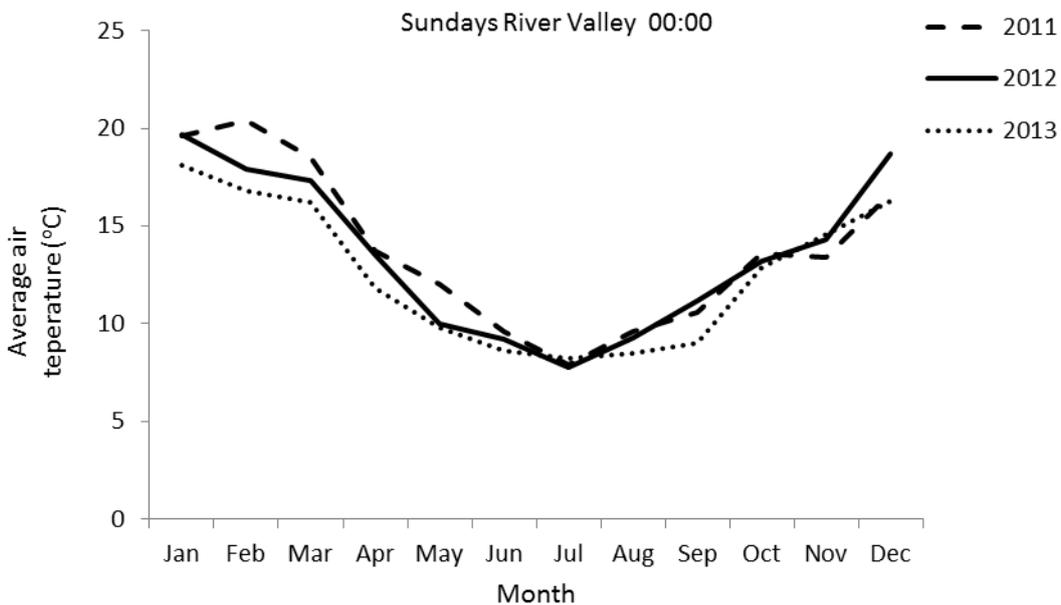


Figure 3.2.22.9. Average air temperature for each month taken at 00:00 from 2011-2013 for the Sundays River Valley.

Figure 3.2.22.10 represents the average number of sterile male FCM caught per wild male FCM caught (which represents the wild to sterile ratio) per week in the Dunbrody SIT area for the 2011 / 2012 and 2012 / 2013 season. The horizontal line represents the 1:10 target ratio; anything above this line exceeds the target ratio. The 1:10 ratio was achieved throughout the summer for the 2011 / 2012 and 2012 / 2013 season. The target ratio was met more regularly during the 2012 / 2013 season. The target ratio was not achieved during the colder winter months. Figure 3.2.22.11 represents the average number of sterile male FCM caught per wild male FCM caught (which represents the wild to sterile ratio) per week in the Penhill SIT area for the 2012 / 2013 season. The horizontal line also represents the 1:10 target ratio; anything above this line exceeds the target ratio. The 1:10 ratio was achieved throughout the summer for the 2012 / 2013 season. The target ratio was also not achieved during the colder winter months.

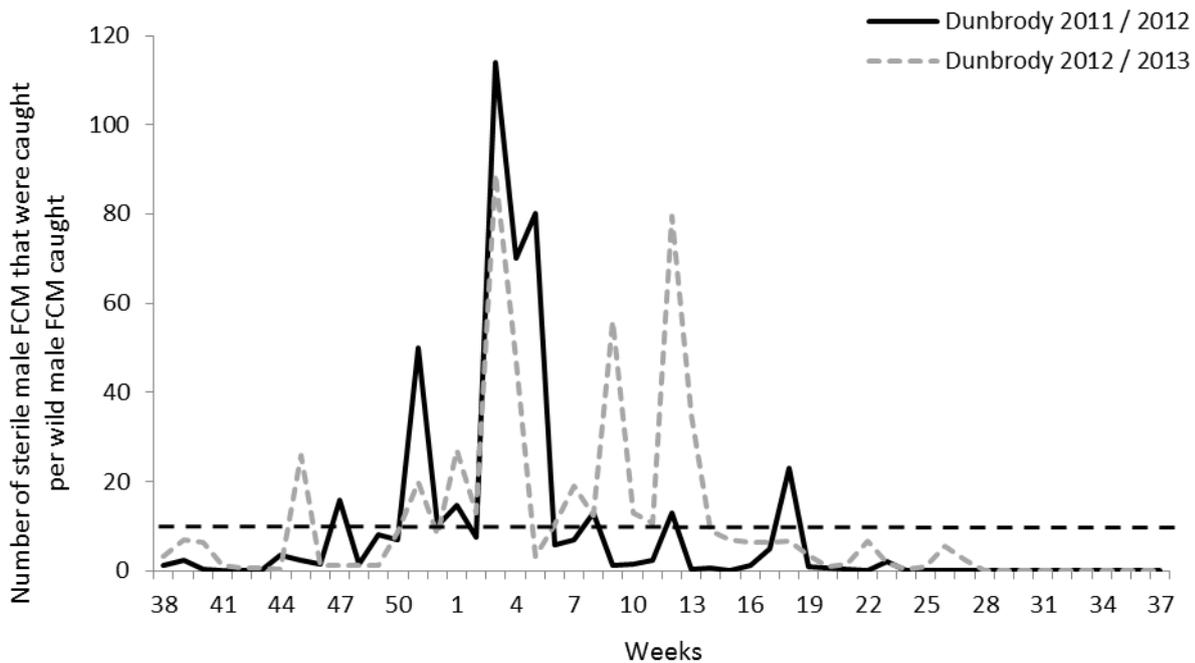


Figure 3.2.22.10 Average number of sterile male FCM caught per wild male FCM caught (representing wild to sterile ratio) on the Dunbrody SIT area for the 2011 / 2012 and 2012 / 2013 season. The horizontal line represents the 1:10 target ratio.

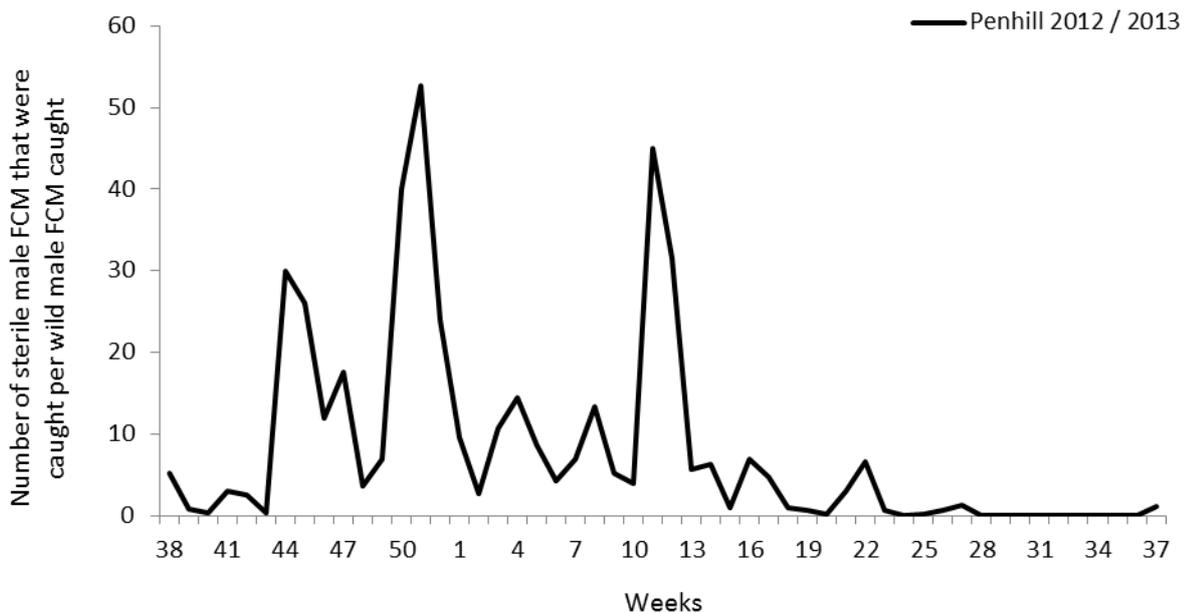


Figure 3.2.22.11 Average number of sterile male FCM caught per wild male FCM caught (representing wild to sterile ratio) on the Pehinll SIT area for the 2012 / 2013 season. The horizontal line represents the 1:10 target ratio.

Discussion

After a substantial literature review it appears that no published studies have looked at the success of the SIT programme since it has been implemented in the SRV in the Eastern Cape of South Africa. The results show that the SIT programme, which was implemented in the SRV during 2011, has been very successful in reducing the numbers of wild male FCM, when compared to the success of control on Non-SIT farms. This trend was also observed in the fruit infestation data. For all the comparisons the farms that implemented the SIT programme had lower trap catches of wild male FCM compared to farms implementing a more conventional. SIT programmes have been shown to be very effective against other insect pests including tsetse fly in sub-Saharan Africa (Feldman *et al.* 2007), codling moth (Bloem *et al.* 2005), pink bollworm (Bloem *et al.* 2005) and screwworm (Vargas-Teran, *et al.* 2005).

The comparisons made with the Penhill and Dunbrody data demonstrate the overall success of the SIT programme in the SRV compared to more conventional methods. What causes concern is the increase in the trap catches of wild male FCM throughout the autumn and winter months. The target ratio of 1:10 was also not attained during the winter months. The major threat that FCM poses to the South African citrus industry is the potential phytosanitary risk it has in store for international markets. The loss in fruit yield caused by fruit fall due to FCM larval infestation is insignificant. The high trap catches of wild male FCM coincides with the harvest period of the majority of the citrus varieties in the SRV citrus producing region. This is when the FCM population numbers should ideally be at their lowest, to reduce the possibility of fruit being harvested and exported with larvae in the fruit. This lapse in the programme's efficacy and the overall control of FCM in the SIT areas during the colder winter months, may be attributed to the species' inability to tolerate low temperatures (Stotter & Terblanche 2009). The majority of research on FCM has focused on taxonomy (Timm *et al.* 2008), mating disruption (Carpenter *et al.* 2007), biological control (Carpenter *et al.* 2004) and chemical resistance to pesticides (Hofmeyr & Pringle 1998).

Stotter & Terblanche (2009) were the first to look into the thermal biology of FCM and if it can be manipulated by temperature shock treatments. Shock treatments are carried out by exposing individuals to a temperature close to their thermal limit which subsequently improves their thermal limits. These shock treatments were unsuccessful in broadening the thermal limits of the treated FCM. The minimum temperature for flight in FCM ranges from 10-15°C, and this temperature range is higher for facility-reared moths (Stotter & Terblanche 2009). These temperatures were reached on a regular basis during the colder winter months in the SRV. The SRV experiences very cold conditions during the winter and this poses a risk to the success of the SIT programme in the area. It has been shown that altering the larval nutrition of insects may affect their response to thermal stress as adults (Andersen *et al.* 2010). This is a possibility that can be explored for FCM and may be achieved through the addition of additives to the diet of the larvae. A recently published study looked at experimentally inducing diapause in FCM (Terblanche *et al.* 2014). These attempts were unsuccessful and it seems that previous field studies were correct in concluding that FCM does not undergo diapause. Other Lepidoptera use diapause to survive periods of extreme cold (Terblanche *et al.* 2014).

The inability of FCM to fly at colder temperature (10-15°C) may shed some doubt on the late season efficacy of the SIT programme in the SRV, particularly due to the fact that facility-reared moths are less cold tolerant. There is a lot of work underway to improve and broaden the biological control options available against FCM. It is important to note that SIT will unlikely be implemented as a stand-alone control method against FCM. The current shift towards Integrated Pest Management (IPM) aims to combine multiple compatible control strategies to suppress insect pest populations. The granulovirus, *Cryptophlebia leucotreta*, is already being applied together with the SIT programme in the SRV. FCM is an important pest of citrus in South Africa and due to this fact, research into improving current control strategies (e.g. SIT) and developing new strategies will lead to better control of this pest in the near future. A recommendation to Xsit and other entities that conduct FCM population monitoring after conducting this study is that FCM monitoring should be intensified. Xsit focus their efforts on monitoring in SIT areas and less so in Non-SIT areas. This significantly decreased the comparisons between SIT and Non-SIT areas made for this study. It is also recommended that studies looking to increase the cold tolerance of facility-reared FCM should be prioritized.

References cited

- ANDERSON, L.H., KIRSTENSEN, T.N., LOESCHCKE, V., TOFT, S., MAYNTZ, D., 2010. Protein and carbohydrate composition of larval food affects tolerance to thermal stress and desiccation in adult *Drosophila melanogaster*. *Journal of Insect Physiology* **56**: 336-340.
- BLOEM, S., CARPENTER, E.J., HOFMEYER, J.H., 2003. Radiation biology and inherited sterility in false codling moth (Lepidoptera: Tortricidae). *Journal of Economical Entomology* **96(6)**: 1724-1731.
- BLOEM, K.A., BLOEM, S., CARPENTER, J.E., 2005. Impact of Moth Suppression/Eradication Programmes Using the Sterile Insect Technique or Inherited Sterility. pp 677-700. In: V.A. Dyck, J. Hendrichs and A.S. Robinson (eds.), *Sterile insect technique. Principles and practice in area-wide integrated pest management*. Springer, Dordrecht, Netherlands.
- CARPENTER, J.E., BLOEM, S., HOFMEYER, H., 2007. Area-wide control tactics for false codling moth *Thaumetotibia leucotreta* in South Africa: a potential invasive species. pp 351-360. In: Vreysen, M.J.B., Robinson, A.S., Hendrichs, J. (Eds.), *Area-wide Control of Insect Pests*. Springer, Berlin.
- Citrus Growers' Association (CGA), Key Industry Statistics. 2012.
- CITRUS RESEARCH INTERNATIONAL (CRI). 2013. *Integrated Production Guidelines, Volume III, Integrated Pest and Disease Management*, <http://www.citrusres.com>.

- DE VILLIERS, W.M., SCHOEMAN, A.S., 1988. *Tuin-plae en –siektes in Suid Afrika*. (1st edition). Struik publishers, Cape Town, South Africa.
- FELDMANN, U., DYCK, V.A., MATTIOLO, R.C., JANNIN, J., 2005. Potential impact of tsetse fly control involving the sterile insect technique. pp 701-723. In: V.A. Dyck, J. Hendrichs and A.S. Robinson (eds.), *Sterile insect technique. Principles and practice in area-wide integrated pest management*. Springer, Dordrecht, Netherlands.
- HILL, D.S., 1975. *Agricultural Insect Pests of the Tropics and their Control*. Cambridge University Press, Cambridge: pp 516.
- HOFMEYR, J.H., PRINGLE, K.L., 1998. Resistance of false codling moth, *Cryptophlebia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), to the chitin synthesis inhibitor, triflumuron. *African Entomology* **6**: 373-375.
- KIRKMAN, W., 2007. Understanding and improving the residual efficacy of the *Cryptophlebia leucotreta* granulovirus (CRYPTOGRAN). MSc thesis, Rhodes University, Grahamstown, Eastern Cape, South Africa.
- KIRKMAN, W., MOORE, S., 2007. A study of alternative hosts for the false codling moth, *Thaumatotibia* (= *Cryptophlebia*) *leucotreta* in the Eastern Cape. *SA Fruit Journal*, Apr/May: 33-38.
- KLASSEN, W., 2005. Area-Wide Integrated Pest Management and the Sterile Insect Technique, pp 39-68. In: V.A. Dyck, J. Hendrichs and A.S. Robinson (eds.), *Sterile insect technique. Principles and practice in area-wide integrated pest management*. Springer, Dordrecht, Netherlands.
- KOMAI, F., 1999. A taxonomic review of the genus *Grapholita* and allied genera (Lepidoptera: Tortricidae) in the Palearctic region. *Entomologica Scandinavica* **55**: 1-219.
- KRAFSUR, E.S., 1998. Sterile insect technique for suppressing and eradicating insect population: 55 years and counting. *Journal of Agricultural Entomology* **15(4)**: 303-317.
- MOORE, S.D., 2002. The development and evaluation of *Cryptophlebia leucotreta* granulovirus (CrleGV) as a biological control agent for the management of false codling moth, *Cryptophlebia leucotreta*, on citrus. PhD Thesis, Rhodes University.
- MOORE, S., KIRKMAN, W., 2007. A study of alternative hosts for false codling moth, *Thaumatotibia* (= *Cryptophlebia*) *leucotreta* in the Eastern Cape. *SA Fruit Journal*.
- MOORE, S., KIRKMAN, W., 2009. Citrus orchard sanitation with emphasis of false codling moth control. *SA Fruit Journal Dec/Jan*: 57- 60.
- MOORE, S.D., GROUT, T., HATTINGH, V., HOFMEYR, H., 2008. Threshold and guidelines for intervention against citrus pests. *SA Fruit Journal Aug/Sept*: 77-81.
- MOORE, S.D., 2011. Specific pests, false codling moth. In: Citrus Research International. *The Cutting Edge* **3(3)**: 1-9.
- MOORE, S.D., HENDRY, D.A., RICHARDS, G.I., 2011. Virulence of a South African isolate of the *Cryptophlebia leucotreta* granulovirus to *Thaumatotibia leucotreta* neonate larvae. *Biocontrol* **56**: 341 – 352.
- NEWTON, P.J., 1998. Lepidoptera: Butterflies and moths – In '*Citrus pests in the Republic of South Africa*'nd (2nd edition). *Institute for Tropical and Subtropical Crops*: 194-200.
- NEWTON, P.J., 1990. Ovipositional preferences amongst navel sweet orange types by the false codling moth, *Cryptophlebia leucotreta*. *Annual Journal of Applied Biology* **116**: 143-150.
- NEPGEN, E.S., 2014. A Study on the Application Technology of the Sterile Insect Technique, with Focus on False Codling Moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), a Pest of Citrus in South Africa. MSc thesis, Rhodes University, Grahamstown, Eastern Cape, South Africa.
- NICOLOSI, E., DENG, Z.N., GENTILE, A., LA MLFAFA, S., CONTINELLA, G., TRIBULATO, E., 2000. Citrus phylogeny and genetic origin of important species as investigated by molecular markers. *Theoretical Applied Genetics* **100**: 1155-1166.
- SCHWARTZ, A., 1981. *'n Bydrae tot die biologie en beheer van die valskodlingmot op nawels*. PhD Thesis, University of Stellenbosch, Western Cape, South Africa.
- STIBICK, J., 2007. New Pest Response Guidelines: False Codling Moth *Thaumatotibia leucotreta*. USDA–APHIS–PPQ–Emergency and Domestic Programs, Riverdale, Maryland. pp 110.
- STIBICK, J., 2008. New pest response guidelines: false codling moth *Thaumatotibia leucotreta*. USDA–APHIS–PPQ–Emergency and Domestic Programs, Riverdale, Maryland.
- STOTTER, R.L., TERBLANCHE, J.S., 2009. Low-temperature tolerance of false codling moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) in South Africa. *Journal of Thermal Biology* **34**: 320-325.
- STOTTER, R.L., SAMWAYS, M.J., HATTINGH, V., 2014. Preparing the way for sterile insect release: Determination of false codling moth distribution across a landscape mosaic. *Crop Protection* **60**: 1-4.
- TERBLANCHE, J.S., DE JAGER, Z., BOARDMAN, L., ADDISON, P., 2014. Physiological traits suggest limited diapause response in false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae). *Journal of Applied Entomology* **138**: 683-691.

- TIMM, A.E., WARNICH, L., GEERTSEMA, H., 2008. Morphological and molecular identification of economically important Tortricidae (Lepidoptera) on deciduous fruit tree crops in South Africa. *African Entomology* **16**: 209-219.
- VARGAS-TERAN, M., HOFMANN, H.C., TWEDDLE, N.E., 2005. Impact of screwworm eradication programmes using the sterile insect technique. pp 629-650. In: V.A. Dyck, J. Hendrichs and A.S. Robinson (eds.), *Sterile insect technique. Principles and practice in area-wide integrated pest management*. Springer, Dordrecht, Netherlands.
- VENETTE, R.C., DAVIS, E.E., DACOSTA, M., HEISLER, H. LARSON, M., 2003. Mini Risk Assessment: False codling moth, *Thaumatotibia* (= *Cryptophlebia*) *leucotreta* (Meyrick) [Lepidoptera: Tortricidae]. University of Minnesota, Department of Entomology, CAPS PRA. pp 1-30.
- VREYSEN, M.J.B., CARPENTER, J.E., MAREC, F., 2010. Improvement of the sterile insect technique for codling moth *Cydia pomonella* (Linnaeus) (Lepidoptera Tortricidae) to facilitate expansion of field application. *Journal of Applied Entomology* **134**: 168-181.
- WEBBER, H.J., 1967. History and development of the citrus industry. In: Reuther W, Webber HJ, Batchelor LD (eds) *The citrus industry*, vol 1. University of California Press, Berkeley, pp 1–39.

3.3 PROGRAMME: FRUIT FLY

Programme Coordinator: Aruna Manrakhan (CRI)

3.3.1 PROGRAMME SUMMARY

The fruit fly pests of citrus in South Africa and other citrus production areas in southern Africa are: *Ceratitis capitata* (Medfly), *Ceratitis rosa* (Natal fly) and *Bactrocera dorsalis* (Oriental fruit fly). Currently in South Africa, *Bactrocera dorsalis* is restricted to the northern and eastern parts of the country, being absent in the Northern Cape, Western Cape and Eastern Cape. *Bactrocera dorsalis* was previously recognized as *Bactrocera invadens*. The two species were recently synonymized following similarities found in their morphological characteristics and molecular structure. The three fruit fly species on citrus are all quarantine pests. There is a zero tolerance of fruit fly larvae in fruit consignments. The management of these fruit fly pests is mainly done at the pre-harvest stage with some export markets requiring additionally post-harvest cold disinfestation treatments to further reduce risk of introduction of these pests into the importing countries. The focus of the fruit fly programme in the year 2014-2015 was on optimising these pre-harvest control measures for the different fruit fly pests and gathering data on the biology and ecology of these pests.

A colony of the Oriental fruit fly was established at CRI Nelspruit and is being maintained together with colonies of fruit fly pests in the *Ceratitis* group (3.3.2). A new colony of the “cold” type Natal fly (*C. rosa* R2) was also established. The Natal fly is likely to be split in two species in the future – “hot” type *C. rosa* R1 and “cold” type *C. rosa* R2 based on scientific evidence of differences in their genetic structure and morphological characteristics. The developmental rates of the two *C. rosa* types were determined (3.3.5). The latter was completed in January 2015. Results from these studies showed that the *C. rosa* R2 was less heat tolerant than *C. rosa* R1. Both *C. rosa* types were however equally tolerant to low temperatures. Fruit fly colonies maintained at CRI were used in other CRI funded projects (3.3.3, 3.3.5 and 3.3.9) and also for research at universities with funding from other sources.

Experiments were conducted in an attempt to improve the performance of the paper-based bait station-Tephri cone (3.3.3). The efficacy of the Tephri cone to control Oriental fruit fly and Medfly is currently being tested in Star Ruby grapefruit orchards in Constantia. The project which aimed at determining the performance of yeast autolysates as replacements for protein hydrolysates such as HymLure was terminated (3.3.6). Yeast autolysates are believed to be safer for use with copper sprays leaving less phytotoxicity on fruit. The yeast autolysates were however found to be less attractive than the standard HymLure. As such, no further research was proposed on these yeast autolysates. An investigation was also carried out on the phytotoxic effect of the standard fruit fly bait: GF-120 on fruit of ‘Nadorcott’ mandarin (3.3.3). Phytotoxicity of GF-120 was recorded on ‘nadorcott’ mandarin fruit which were at the green and colour- break stages. No phytotoxicity of GF-120 was recorded when the fruit were at the full rind colour development. These results suggest that alternative methods of GF-120 application other than ground-based canopy sprays would therefore have to be sought for ‘nadorcott’ mandarin orchards, especially if baiting is to start early before full colour development. Use of other fruit fly management techniques such as M3 bait stations and other bait stations would also help avoid risk of phytotoxicity on fruit of ‘nadorcott’ mandarin at the green and colour break stages.

The surveillance monitoring of the Oriental fruit fly in citrus orchards in South Africa (3.3.4) was terminated in 2014 due to the change of status of the pest in the country and the establishment of the Production Unit

Code monitoring system. Trapping results obtained between 2012 and 2014 showed a peak in catches of the Oriental fruit fly in citrus orchards between February and May, a pattern similar to the local *Ceratitidis* pests. Another project aims to understand the Oriental fruit fly incursion patterns observed through genetic analysis of collected specimens (3.3.8). Fly samples from other African and Asian countries will also be included in the analysis. Genetic diversity estimates will be used to determine population relatedness, which should provide an indication of the incursion pattern of Oriental fruit fly in South Africa. Research on biology, ecology and control of the Oriental fruit fly was spread among four projects. The dispersal capacity of the Oriental fruit fly is currently being investigated using mark release recapture methods (3.3.7). The field studies are being conducted in three sites in Levubu. Previous laboratory studies have shown that fluorescent pigments at 2g/L were effective in marking the flies with marks remaining on the flies for at least 2 weeks under field conditions. The utilisation of hosts by the Oriental fruit fly is being studied in commercial fruit production areas and natural areas in Limpopo and Mpumalanga (3.3.9). The Oriental fruit fly was reared so far from mangoes, cashews and wild tobacco or bug weed. Fruit collected from the ground were good breeding sites for the pest. These results suggest that orchard sanitation is crucial to the management of the Oriental fruit fly in commercial citrus orchards. Different trap and attractant combinations were evaluated for monitoring of fruit fly pests under the ERAfrica fruit fly project (3.3.10), a project funded by the Department of Science and Technology. The Biolure 3 component lure was found to be more attractive to females of Oriental fruit fly than Questlure and other food-based attractants. The efficacy of different male annihilation treatments (MAT) for control of the Oriental fruit fly is also being investigated (3.3.11). In the first year of the field study, no significant differences were found between the different MAT products which are currently available in South Africa. MAT forms an important component in the management of the Oriental fruit fly since catches of the Oriental fruit fly were higher in orchards treated only with protein baits compared to orchards treated with a combination of protein baits and MAT.

PROGRAMOPSOMMING

Die vrugtevliegepeste van sitrus in Suid-Afrika en ander sitrusproduksie-areas in suidelike Afrika is: *Ceratitidis capitata* (Meditereense vrugtevlieg - Medfly), *Ceratitidis rosa* (Natalese vlieg) en *Bactrocera dorsalis* (Oosterse vrugtevlieg - Oriental fruit fly). Tans is *B. dorsalis* in Suid-Afrika slegs tot die noordelike en oostelike dele van die land beperk, en afwesig in die Noord-Kaap, Wes-Kaap en Oos-Kaap. *Bactrocera dorsalis* was voorheen as *B. invadens* bekend. Die twee spesies is onlangs sinoniem verklaar as gevolg van soortgelyke morfologiese kenmerke en molekuleêre struktuur. Die drie vrugtevliegespesies op sitrus is almal kwarantynplae. Daar is 'n nul toleransie vir vrugtevlieglarwes in vrugtebesendings. Die bestuur van hierdie vrugtevliegeplae geskied hoofsaaklik tydens die voor-oes stadium, met sommige uitvoermarkte wat addisionele na-oes koue-disinfestasië behandelings vereis ten einde verder die risiko vir die inbring van hierdie plae in die invoerlande te verminder. Die fokus van die vrugtevliegprogram in die jaar 2014-2015 was om hierdie voor-oes beheermaatreëls vir die verskillende vrugtevliegeplae te optimaliseer en data oor die biologie en ekologie van hierdie plae in te samel.

'n Kolonie van die Oosterse vrugtevlieg is by CRI Nelspruit gevestig en word saam met kolonies van vrugtevliegeplae in die *Ceratitidis* groep in stand gehou (3.3.2). 'n Nuwe kolonie van die "koue" tipe Natalese vlieg (*C. rosa* R2) is ook gevestig. Die Natalese vlieg sal moontlik in die toekoms in twee spesies verdeel word – "warm" tipe *C. rosa* R1 en "koue" tipe *C. rosa* R2, gebaseer op wetenskaplike bewyse van verskille in hul genetiese struktuur en morfologiese kenmerke. Die ontwikkelingstempos van die twee *C. rosa* tipes is by verskillende temperature bepaal (3.3.5). Laasgenoemde is in Januarie 2015 voltooi. Resultate vanuit hierdie studies het getoon dat *C. rosa* R2 minder hittebestand as *C. rosa* R1 was. Beide *C. rosa* tipes was egter ewe bestand teen lae temperature. Vrugtevliegekolonies wat by CRI in stand gehou word, is in ander CRI befondsde projekte gebruik (3.3.3, 3.3.5 and 3.3.9) en ook vir navorsing by universiteite met befonding vanaf ander bronne.

Eksperimente is uitgevoer in 'n poging om die werking van die papier-gebaseerde lokaasstasie, Tephri-cone, te verbeter (3.3.3). Die doeltreffendheid van die Tephri-cone in die beheer van die Oosterse vrugtevlieg en Meditereense vrugtevlieg word tans in Star Ruby pomelo boorde in Constantia getoets. Die wat ten doel gehad het om die werking van gis outolisate as plaasvervangers vir proteïen hidrolisate soos HymLure te ondersoek, is gestaak (3.3.6). Gis outolisate blyk veiliger te wees vir gebruik saam met koperspuitte, met minder fitotoksiteit op vrugte. Die gis outolisate wat onder Projek 1062 ondersoek is, is egter gevind om minder aantreklik te wees as die standaard HymLure. Geen verdere navorsing is dus op hierdie gis outolisate voorgestel nie. 'n Ondersoek is ook uitgevoer op die fitotoksiese effek van standaard vrugtevliege-lokaas, GF-120, op vrugte van 'Nadorcott' mandarin (3.3.3). Fitotoksiteit van GF-120 is op 'nadorcott' mandarynvrugte op die groen- en kleurbreuk-stadiums aangeteken. Geen fitotoksiteit van GF-120 is aangeteken wanneer die vrugte op volskil kleur-ontwikkeling was nie. Hierdie resultate het dus

voorgestel dat alternatiewe metodes van GF-120 toediening, anders as die grond-gebaseerde lowerdakspuite, gesoek moet word vir 'nadorcott' mandarynboorde, veral indien lokaas toediening vroeg moet begin, vóór volkleur-ontwikkeling. Gebruik van ander vrugtevlieg bestuurstegnieke, soos M3 lokaasstasies en ander lokaasstasies, sal ook help om die risiko van fitotoksiteit op vrugte van 'nadorcott' mandaryn by die groen- en kleurbreuk-stadiums, te vermy.

Die opname monitering van die Oosterse vrugtevlieg in sitrusboorde in Suid-Afrika (3.3.4) is in 2014 gestaak wees 'n verandering in die status van die plaag in die land, en die vestiging van die "Production Unit Code" moniteringssisteem. Lokvalresultate wat tussen 2012 en 2014 verkry is, het 'n piek in vangstes van die Oosterse vrugtevlieg in sitrusboorde tussen Februarie en Mei aangeteken, 'n patroon soortgelyk aan die plaaslike *Ceratitis* plaë. Nog 'n projek poog om die Oosterse vrugtevlieg inkursiepatrone deur genetiese analise van monsters te verstaan (3.3.8). Oosterse vrugtevlieg monsters van ander Afrika en Asiatiese lande sal ook in die analise ingesluit word. Genetiese diversiteitskattings sal gebruik word om die teenwoordigheid van populasie verwantskappe te bepaal, wat 'n aanduiding behoort te gee van die inkursiepatroon van Oosterse vrugtevlieg in Suid-Afrika. Navorsing op die biologie, ekologie en beheer van die Oosterse vrugtevlieg, is oor vier projekte versprei. Die verspreidingsvermoë van die Oosterse vrugtevlieg word tans ondersoek deur die gebruik van "merk-vrylaat-hervang" metodes (3.3.7). Die veldstudies word in drie plekke in Levubu uitgevoer. Vorige laboratoriumstudies het getoon dat fluoresserende pigmente teen 2g/L effektief was in die merk van vlieë met merke wat onder veldtoestande op die vlieë vir ten minste 2 weke bly. Die benutting van gashere deur die Oosterse vrugtevlieg word in kommersiële vrugte produksie-areas en natuurlike areas in Limpopo en Mpumalanga ondersoek (3.3.9). Die Oosterse vrugtevlieg is sover vanaf mango, kasjoeneute en wilde tabak geteel. Vrugte wat vanaf die grond versamel is, was goeie teel-areas vir die plaag. Hierdie resultate het getoon dat boordsanitasie noodsaaklik is vir die bestuur van die Oosterse vrugtevlieg in kommersiële sitrusboorde. Verskillende lokval en lokaas kombinasies is vir die monitering van vrugtevliegplaë onder die ERAfrica vrugtevliegprojek geëvalueer (3.3.10), 'n projek wat deur die Departement van Wetenskap en Tegnologie befonds word. Die Biolure 3 komponent lokaas is gevind om meer aantreklik vir wyfies van die Oosterse vrugtevlieg te wees as Questlure en ander voedingsgebaseerde lokase. Die doeltreffendheid van verskillende mannetjie uitwissingstegnieke ("male annihilation treatments" - MAT) vir die beheer van die Oosterse vrugtevlieg word ook ondersoek (3.3.11). In die eerste jaar van die veldstudie is geen betekenisvolle verskille tussen die verskillende MAT produkte wat tans in Suid-Afrika beskikbaar is, gevind nie. MAT vorm 'n belangrike komponent in die bestuur van die Oosterse vrugtevlieg aangesien vangstes van die Oosterse vrugtevlieg hoër was in boorde wat slegs met proteïen lokase behandel is, in vergelyking met boorde wat met 'n kombinasie van proteïen lokase en MAT behandel is.

3.3.2 PROGRESS REPORT: Fruit fly rearing

Project 407 (1999/2000 – 2015/16) by A Manrakhan, J-H Daneel, R Beck & G. Shongwe (CRI)

Summary

Colonies of *Ceratitis capitata* (Medfly), *Ceratitis rosa* (Natal fly- R1/hot type) and *Ceratitis cosyra* (Marula fly) continued to be maintained. Two new fruit fly colonies were established in the year 2014-2015: *Bactrocera dorsalis* (Oriental fruit fly) and *Ceratitis rosa* R2/cold type. The two new fruit fly colonies are currently maintained on a carrot-based diet. Fruit flies reared were used in different CRI projects: Project 915 (Evaluation of new bait), Project 1067 (Developmental rates of the two *C. rosa* types) and Project 1107 (Utilisation of citrus by *B. dorsalis*). Fruit fly and diet materials were also provided to University of Pretoria, University of Stellenbosch and University of KwaZulu Natal for fruit fly related research.

Opsomming

Kolonies van *Ceratitis capitata* (Medfly), *Ceratitis rosa* (Natalse vlieg - R1/warm tipe) en *Ceratitis cosyra* (Marula vlieg) word in stand gehou. Twee nuwe vrugtevliegkolonies is in die jaar 2014-2015 gevestig: *Bactrocera dorsalis* (Oosterse vrugtevlieg) en *Ceratitis rosa* R2/koue tipe. Die twee nuwe vrugtevliegkolonies word tans op 'n wortelgebaseerde dieet in stand gehou.

Geteelde vrugtevlieë is in verskillende CRI projekte gebruik: Projek 915 (Evaluasie van nuwe lokaas), Projek 1067 (Ontwikkelingstempo's van die twee *C. rosa* tipes) en Projek 1107 (Benutting van sitrus deur *B. dorsalis*). Vrugtevlieg- en dieetmateriale is ook aan die Universiteit van Pretoria, Universiteit van Stellenbosch en Universiteit van KwaZulu-Natal, vir vrugtevliegverwante navorsing, verskaf.

3.3.3 PROGRESS REPORT: A new bait for more effective control of all *Ceratitis* fruit flies Project 915 (2008/9 – 2015/16) by A Manrakhan, John-Henry Daneel & Rooikie Beck (CRI)

Summary

The focus of Project 915 was on improvement of the Tephri cone, a new bait station being developed at CRI. The new pulp paper from New Zealand was found to be an effective alternative to the SAPPI pulp paper which is no longer being produced in South Africa. Various new bait combinations with and without thickeners and humectants were tested in the Tephri cone holders in laboratory and field cage assays. None of the new bait combinations were as effective as the original bait mixture for the Tephri cone. The Tephri cone made of the New Zealand pulp paper and containing 10 ml of the original bait mixture is being tested and compared with M3 bait stations in Star Ruby grape fruit orchards in Constantia. The Tephri cone was placed at 160 stations per ha (every third tree) and the M3 bait stations were placed at 240 stations per ha (every second tree). Efficacy of the Tephri cone and the M3 bait stations for control of Medfly as well as Oriental fruit fly is being determined by fruit fly trapping and fruit sampling.

In order to properly quantify the control efficacy of M3 bait stations, further tests were carried out to determine the lethal and sub lethal effects of the bait station on *Ceratitis* flies (Medfly, Natal fly and marula fly). Under laboratory and semi field conditions, fly mortality rate within 1-2 days after exposure with M3 bait stations was found to be low. However, exposure to M3 bait stations led to reduced fecundity for Medfly and Natal fly but not marula fly.

An ad-hoc investigation under Project 915 was also carried out to determine the phytotoxic effect of GF-120 on 'Nadorcott' mandarin. This investigation followed reports from pack houses in 2012 and 2013 on phytotoxic burns observed on Nadorcott mandarin which were then associated with GF-120 sprays. Phytotoxicity of GF-120 was recorded on 'Nadorcott' mandarin fruit which were at the green and colour-break stages. Incidence of burn increased with increasing concentration of GF-120. Phytotoxicity of GF-120 was accentuated with increasing droplet size, droplet coverage and prolonged wetness of bait droplets. No phytotoxicity was observed when GF-120 was applied on Nadorcott mandarin at full rind colour development. Alternative methods of GF-120 application other than ground-based canopy sprays would therefore have to be sought for 'Nadorcott' mandarin orchards, especially if baiting is to start early before full colour development. Use of other fruit fly management techniques such as bait stations would also help avoid risk of phytotoxicity on fruit of 'Nadorcott' mandarin at the green and colour break stages.

Opsomming

Die fokus van Projek 915 was op die verbetering van die Tephri-cone, 'n nuwe lokaasstasie wat by CRI ontwikkel word. Daar is gevind dat die nuwe pulppapier vanaf New Zealand 'n effektiewe alternatief is vir die SAPPI pulppapier wat nie meer in Suid-Afrika geproduseer word nie. Verskeie nuwe lokaas kombinasies, met en sonder verdickers en bevochtigingsmiddel, is in die Tephri-cone houers in laboratorium- en veldhokproewe getoets. Geen van die nuwe lokaas kombinasies was so doeltreffend soos die oorspronklike lokaasmengsel vir die Tephri-cone nie. Die Tephri keël wat van die New Zealand pulppapier gemaak word, bevattende 10 ml van die oorspronklike lokaasmengsel, word getoets en met M3 lokaasstasies in Star Ruby pomelo vrugteboorde in Constantia vergelyk. Die Tephri-cone is by 160 stasies per ha geplaas (elke derde boom) en die M3 lokmiddelstasies is by 240 stasies per ha geplaas (elke tweede boom). Doeltreffendheid van die Tephri-cone en die M3 lokmiddelstasies vir beheer van die Mediterse vrugtevlug, asook die Oosterse vrugtevlug, word deur die lokval van vrugtevlug en vrugmonsterneming bepaal.

Ten einde beheer-doeltreffendheid van M3 lokaasstasies behoorlik te kwantifiseer, is verdere toetse uitgevoer ten einde die dodelike en sub-dodelike effekte van die lokaasstasie op *Ceratitis* vlieg te bepaal (Mediterse vlieg, Natalse vlieg en Marula vlieg). Onder laboratorium- en semi-veldtoestande, is gevind dat die vlieg mortaliteitstempo binne 1-2 dae ná blootstelling met M3 lokaasstasies, laag was. Blootstelling aan M3 lokaasstasies het egter tot verlaagde vrugbaarheid vir Mediterse vlieg en Natalse vlieg gelei, maar nie vir Marula vlieg nie.

'n Ad-hoc ondersoek onder Projek 915 is ook uitgevoer om die fitotoksiese effek van GF-120 op 'Nadorcott' mandaryn te bepaal. Hierdie ondersoek het gevolg op verslae vanaf pakhuse in 2012 en 2013 rakende fitotoksiese brande waargeneem op 'Nadorcott' mandaryn, wat toe met GF-120 spuite geassosieer is. Fitotoksieseiteit van GF-120 is op 'nadorcott' mandarynvrugte aangeteken wat op die groen- en kleurbreukstadiums was. Die voorkoms van brand het toegeneem met toename in konsentrasie van GF-120. Fitotoksieseiteit van GF-120 is verhoog met toename in druppelgrootte, druppelbedekking en verlengde

benatting van lokaasdruppels. Geen fitotoksisiteit is waargeneem wanneer GF-120 op 'nadorcott' mandaryn by volskil kleur-ontwikkeling toegedien is nie. Alternatiewe metodes van GF-120 toediening, behalwe vir grond-gebaseerde lowerdakspuite, moet dus vir 'Nadorcott' mandarynboorde gesoek word, veral indien lok vroeg, vóór volkleur-ontwikkeling, moet begin. Gebruik van ander vrugtevlieg bestuurstegnieke, soos lokaasstasies, sal ook help om die risiko van fitotoksisiteit op vrugte van 'Nadorcott' mandaryn by die groenen kleurbreuk-stadiums, te vermy.

3.3.4 FINAL REPORT: Surveillance of *Bactrocera dorsalis* (Hendel) in commercial citrus orchards in South Africa

Project 966 (December 2008- March 2014) by A Manrakhan, J Daneel, C Kotze, P Stephen, Rooikie Beck & H Le Roux (CRI)

Summary

The surveillance monitoring of *B. dorsalis* in commercial citrus orchards in South Africa started in December 2008 and was discontinued in 2014 following the change of the status of the pest in the country and the setting up of a Production Unit Code (PUC) based *B. dorsalis* monitoring system. Surveillance monitoring was conducted using methyl eugenol (ME) baited traps. At each monitoring site, 1-2 traps were placed. Traps were checked on a more or less monthly basis. Monitoring sites were additionally set up in selected citrus production areas in Southern Zimbabwe and in Swaziland in 2010.

The significant achievements of the surveillance project were the early detections of *B. dorsalis* in South Africa, Southern Zimbabwe and Swaziland between 2010 and 2013. The early detections of *B. dorsalis* in South Africa led to the implementation of appropriate actions following the national action plan on the pest and helped in delaying the establishment of *B. dorsalis* in different areas of the country. This delay allowed for registration of products for pre-harvest control of *B. dorsalis* in South Africa and completion of research on post-harvest treatment for the pest. In 2013, *B. dorsalis* was declared present in the Vhembe district, Limpopo Province in South Africa. In 2015, *B. dorsalis* was declared present in all districts of Limpopo Province and specified districts of Mpumalanga, North-West, Gauteng and KwaZulu-Natal. A peak in catches of *B. dorsalis* occurred between February and May in commercial citrus orchards in affected areas.

As a side investigation under the project, the non-target arthropods captured in ME traps were also collected and identified between 2009 and 2010. Captures of non-target arthropods in ME traps were relatively low in commercial citrus orchards and consisted mainly of *Leucocelis* spp. (Coleoptera: Scarabaeidae), *Carpophilus* spp. (Coleoptera: Scarabaeidae), Drosophilidae (Diptera), *Ceratitidis rosa* (Diptera: Tephritidae) and *Perilampus curta* (Diptera: Tephritidae). These findings indicate that ME used currently in control and monitoring of *B. dorsalis* will have little impact on non-target arthropods within commercial citrus orchards.

Opsomming

Die opname monitering van *B. dorsalis* in kommersiële sitrusboorde in Suid-Afrika het in Desember 2008 begin en is in 2014 gestaak weens die verandering in die status van die plaag in die land en die vestiging van 'n "Production Unit Code" (PUC) gebaseerde *B. dorsalis* moniteringssisteem. Opname monitering is uitgevoer deur die gebruik van lokvalle wat met metiel eugenol (ME) gelok word. Een tot twee lokvalle is by elke moniteringsplek geplaas. Lokvalle is min of meer elke maand nagegaan. Moniteringsplekke is addisioneel in geselekteerde sitrusproduksie-areas in Suidelike Zimbabwe en in Swaziland in 2010 opgestel.

Die betekenisvolle suksesse wat met die opname projek verkry is, was die vroeë waarnemings van *B. dorsalis* in Suid-Afrika, Suidelike Zimbabwe en Swaziland tussen 2010 en 2013. Die vroeë waarnemings van *B. dorsalis* in Suid-Afrika het gelei tot die instelling van geskikte aksies nadat die nasionale aksieplan teen die plaag in plet gesit is, en het gehelp om die vestiging van *B. dorsalis* in verskillende areas van die land te vertraag. Hierdie vertraging het die registrasie van produkte vir voor-oes beheer van *B. dorsalis* in Suid-Afrika toegelaat, en voltooiing van navorsing op na-oes behandeling vir die plaag. In 2013, is *B. dorsalis* teenwoordig verklaar in die Vhembe distrik, Limpopo Provinsie, in Suid-Afrika. In 2015, is *B. dorsalis* teenwoordig verklaar in al die distrikte van Limpopo Provinsie en gespesifiseerde distrikte van Mpumalanga, Noordwes, Gauteng en KwaZulu-Natal. 'n Piek in vangstes van *B. dorsalis* kom tussen Februarie en Mei in kommersiële sitrusboorde in geaffekteerde areas voor.

As 'n sub-ondersoek binne die projek, is die nie-teiken geleedpotiges wat in ME lokvalle gevang is, tussen 2009 en 2010 versamel en geïdentifiseer. Vangstes van nie-teiken geleedpotiges in ME lokvalle was relatief laag in kommersiële sitrusboorde en het hoofsaaklik uit die volgende bestaan: *Leucocelis* spp. (Coleoptera:

Scarabaeidae), *Carpophilus* spp. (Coleoptera: Scarabaeidae), *Drosophiliidae* (Diptera), *Ceratitis rosa* (Diptera: Tephritidae) en *Perilampus curta* (Diptera: Tephritidae). Hierdie bevindinge het aangetoon dat ME wat tans in beheer en monitoring van *B. dorsalis* gebruik word, min effek op nie-teiken geleedpotiges binne kommersiële sitrusboorde het.

Introduction

Bactrocera dorsalis (Hendel) (Diptera: Tephritidae) is a fruit fly pest of Asian origin which was found on the African continent, in Kenya, for the first time in 2003 (Lux et al. 2003, Drew et al. 2005). Thereafter, surveys conducted in several other countries in Africa and in the Indian Ocean islands revealed a wider distribution of the pest in the African and Indian Ocean region (De Meyer et al. 2014). *Bactrocera dorsalis* was first reported in Southern Africa in 2008 (Correia et al. 2008, De Meyer et al. 2014). This new pest was found to cause direct damage to commercially grown fruit including citrus in the countries where it was found (Mwatawala et al. 2006, Rwomushana et al. 2008, Goergen et al. 2011). *Bactrocera dorsalis* is also a quarantine pest in Europe and the US.

Bactrocera dorsalis from Africa was formerly recognized as *Bactrocera invadens* Drew Tsuruta and White. Similarities in morphological characteristics, molecular structure and chemoecology as well as sexual compatibility between *B. invadens* and *B. dorsalis* found in various studies have led the researchers involved in these studies to conclude that *B. invadens* is the same biological species as *B. dorsalis* and to recommend the synonymisation of *B. dorsalis* with *B. invadens* (Schutze et al. 2014b, Schutze et al. 2014a).

Reports of *B. dorsalis* in the Southern African region in 2008 prompted the setting up and maintenance of a monitoring programme for *B. dorsalis* covering citrus production areas across South Africa (Project 966), this in order to consolidate the surveillance network for early detection of invasive fruit flies which was established by the Department of Agriculture Forestry and Fisheries in 2006 (Barnes and Venter 2008).

Early detection is deemed to be one of the key requirements for successful eradication of an exotic insect pest (Myers et al. 2000). The other requirements for successful eradication are (1) sufficient resources for implementing eradication actions, (2) necessary authority to implement eradication actions, (3) susceptibility of the exotic insect pest to control measures and (4) prevention of re-invasion (Myers et al. 2000). *Bactrocera dorsalis* males are known to be highly attracted to the parapheromone – methyl eugenol (ME) (Metcalf et al. 1975). The setting up of an extensive network of ME baited traps with an adequate trapping density would thus ensure a high probability of detection of *B. dorsalis* (Shelly et al. 2010).

Stated objectives

- To monitor for early detection of *B. dorsalis*.
- To determine non-target arthropods which are attracted to methyl eugenol, the male attractant used for monitoring *B. dorsalis*.

Materials and methods

B. dorsalis monitoring sites in South Africa and Swaziland

The monitoring network for *B. dorsalis* covered seven provinces of South Africa. Monitoring sites were set up by Citrus Research International (CRI) and collaborators between December 2008 and December 2009 in the following provinces of South Africa: Western Cape (Paarl, Heidelberg, Riebeek Kasteel), Northern Cape (Vaalharts), Eastern Cape (Patensie, Fort Beaufort, Sundays River Valley), KwaZulu-Natal (Nkwalini, Pongola), North-West (Rustenburg, Britz, Zeerust), Limpopo (Letsitele, Hoedspruit, Tshipise, Beitbridge, Weipe, Pontdrif, Tom Burke, Baltimore, Limburg, Gillemburg) and Mpumalanga (Nelspruit, Hectorspruit, Komatipoort, Groblersdal). In October 2010, monitoring sites were additionally set up in two selected citrus farms (Ngonini Estates and Tambuti Estates) in Swaziland. The monitoring sites set up by CRI and collaborators are shown in Fig. 3.3.4.1.

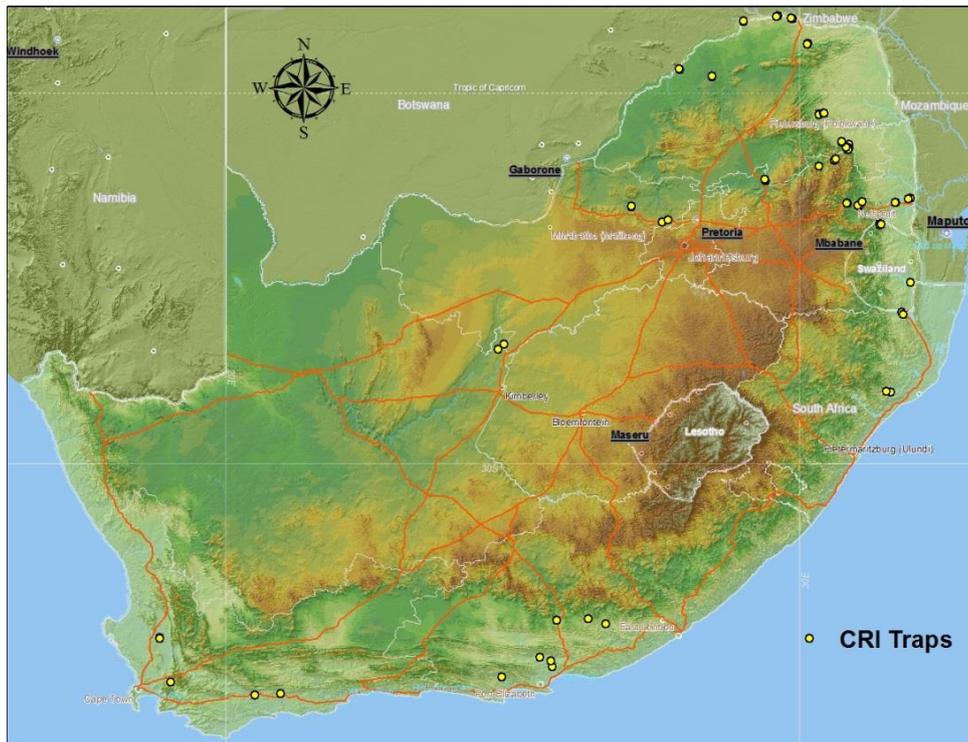


Figure 3.3.4.1. Location of *B. dorsalis* monitoring sites set up in South Africa and Swaziland between 2008 and 2010.

Between 2011 and 2012, monitoring sites maintained by collaborators in Western Cape, KwaZulu-Natal and most areas of the Eastern Cape were discontinued due to the set-up of the PUC surveillance system. Between 2013 and July 2014, traps which were maintained by CRI in the Northern Provinces: Limpopo, Mpumalanga and North-West, were discontinued to the change of status of *B. dorsalis* in South Africa. In July 2014, traps which were maintained by CRI in Swaziland were also discontinued due to the change of status of the pest in the country.

Trapping

At each site monitored by CRI and collaborators in South Africa and Swaziland, 2-5 bucket type traps (Moroccan or Lynfield) baited with methyl eugenol (ME) were placed on host trees (e.g. citrus, marula, mango). Traps were serviced more or less on a monthly basis throughout the year. During servicing, specimens in the traps were collected, old ME dispensers were removed and replaced by fresh ones. A strip (1 cm x 1 cm x 1 cm) of 19.5% dichlorvos placed inside each trap for killing attracted flies was also replaced during trap servicing. A datasheet to keep records of catches was filled in during each servicing.

Traps in Limpopo, Mpumalanga and North-West Provinces in South Africa were maintained by CRI. Traps in Swaziland were also maintained by CRI. In the other Provinces in South Africa, traps were maintained by collaborators.

Short term monitoring surveys in Zimbabwe by CRI

Between January 2010 and September 2011, 7 short term monitoring surveys using ME baited traps were carried out by CRI in Zimbabwe.

In January 2010, 27 traps were placed from Bubi river, 80 km north of Beitbridge through Runde river, Shuruwi, Gweru, Kwekwe, Kadoma, Chegutu, Harare, Ruwa, Bromley, Marondera, Macheke, Headlands, Rusape, Nyazura, Odzi to Mutare (Fig. 3.3.4.2). After Mutare, a day was spent to inspect Produzol citrus nursery in Chicamba in Mozambique. One to four traps were placed in each of the above locations. Traps were hung in mango trees wherever possible or near mango orchards. Five days after the start of the survey, on the way back from Chicamba to Bubi River, traps were inspected, emptied and removed. All traps were recovered. Trap exposure time was only a few hours in Chicamba and from there on trap exposure times were between 1-6 days.

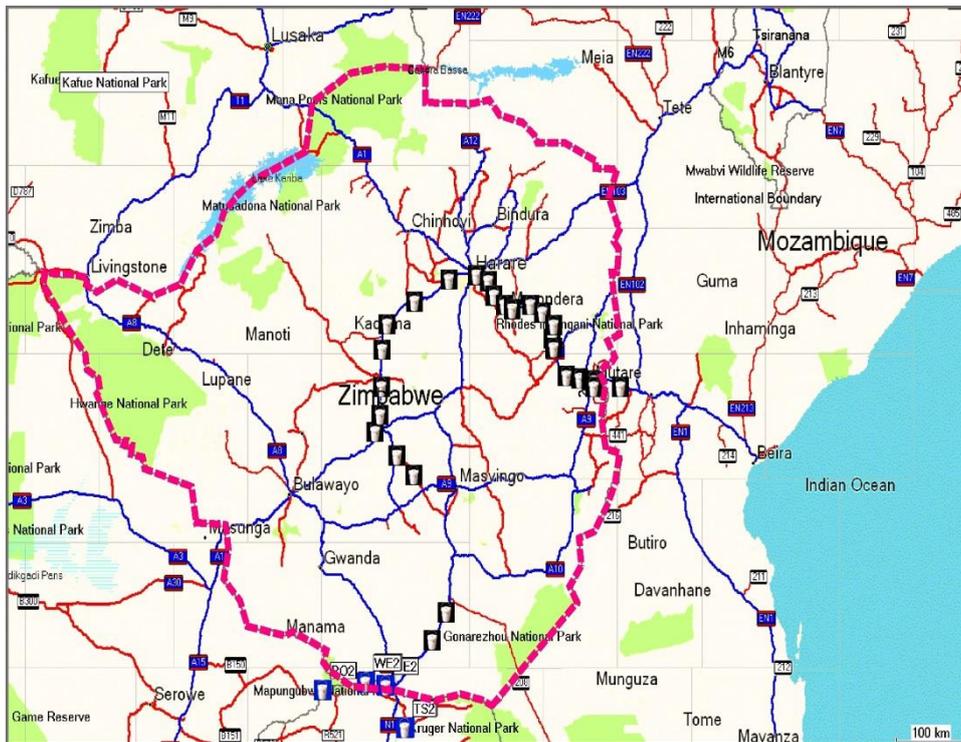


Figure 3.3.4.2: *B. dorsalis* detection trapping sites in Zimbabwe in January 2010. Trapping sites are indicated by the symbol .

On 15 May 2010, ME baited traps were set up by Nottingham Estates (on Zimbabwe/South Africa border, opposite of Weipe, South Africa) as part of a delimiting survey for *B. dorsalis*. On 22 May 2010, 38 ME baited traps were additionally set up by CRI in 5 citrus farms about 35 km from the Beitbridge border post (at Bishopstone, Cawood, Cunliffe, Benfur and Smith) (Fig. 3.3.4.3). Twenty traps were set up at Bishopstone (with a production area of about 700 ha). Eight traps were set up at Cawood (an area of about 150 ha). Ten traps were distributed among three other farms (Cunliffe, Benfur and Smith). Traps set up at Bishopstone, Cawood, Benfur, Cunliffe and Smith were checked the following day after placement.

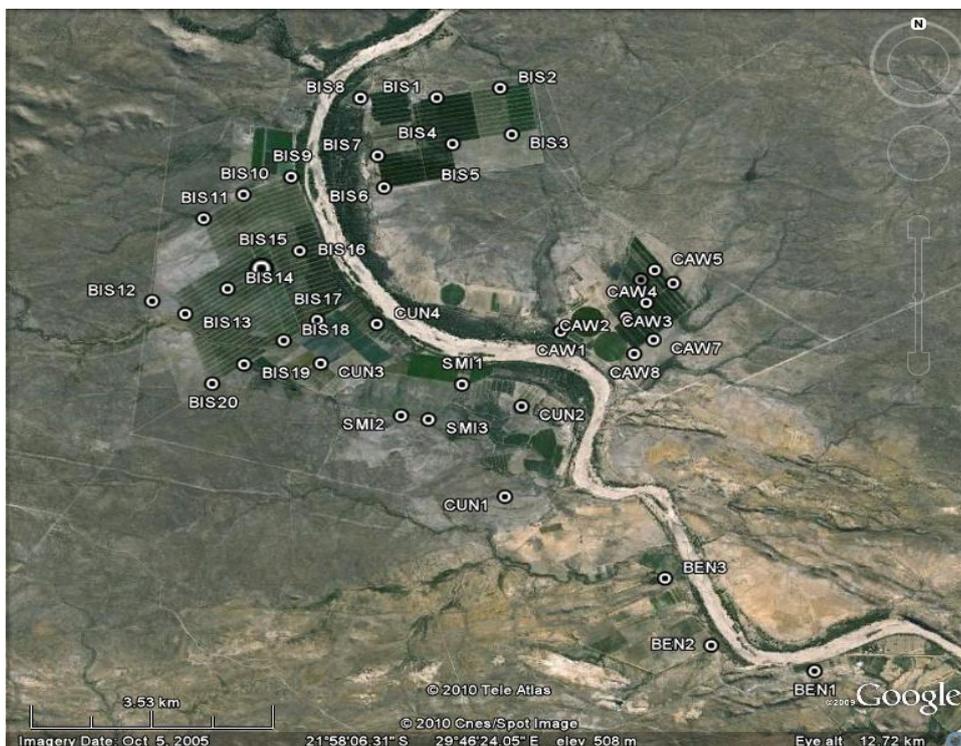


Figure 3.3.4.3. Network of ME baited traps set up at Bishopstone, Cawood, Benfur, Cunliffe and Smith farms, Zimbabwe in May 2010.

Between 11 and 19 June 2010 a short term survey for detection of *B. dorsalis* was conducted in Matabeleland South and in the western part of Zimbabwe. A total of 34 ME baited traps were placed from Thuli along the Shashe River through Bulawayo towards Victoria Falls (Fig. 3.3.4.4). Traps were checked after 1-6 days.

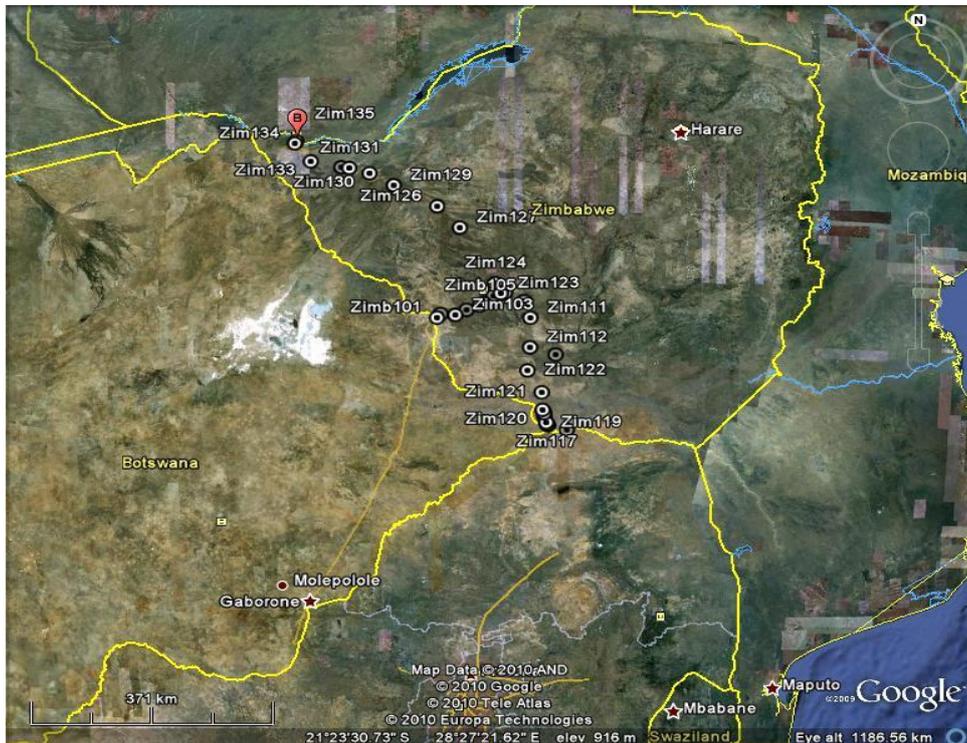


Figure 3.3.4.4. Network of ME baited traps placed in Matabeleland South and in the western part of Zimbabwe in June 2010.

In December 2010, another short term survey was carried out in Zimbabwe from Plumtree to Mvurwi. Three ME baited traps set since June 2010 between Plumtree and Bulawayo were inspected and rebaited. Eleven new ME traps were set from Plumtree to Mvurwi through Bulawayo, Harare and Mazoe (Fig. 3.3.4.5). All traps were inspected on the way back from Mazoe to Plumtree.

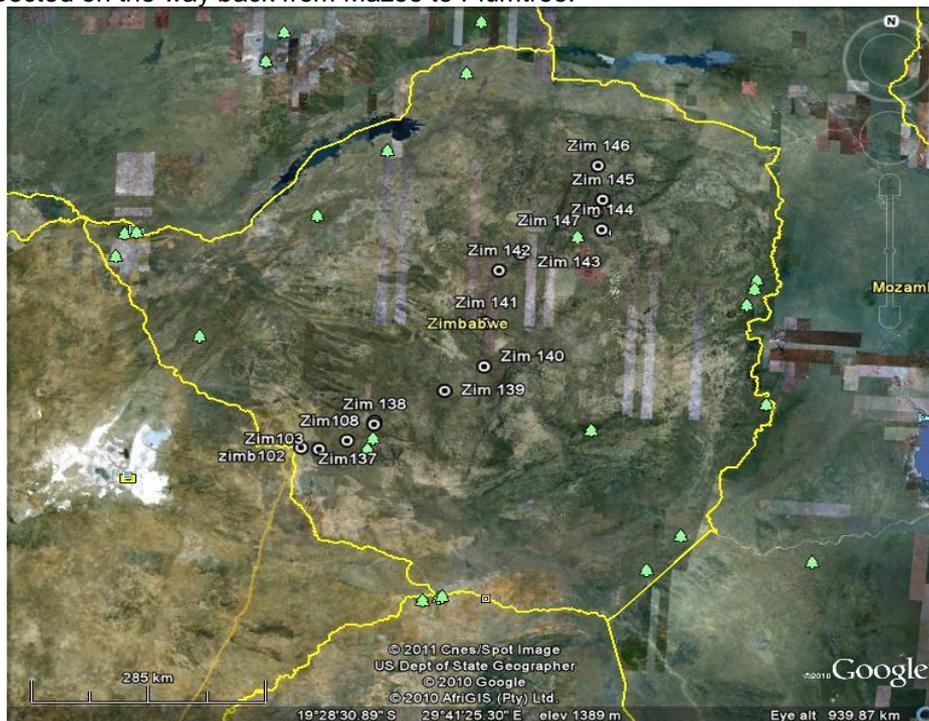


Figure 3.3.4.5. ME baited traps monitored in Zimbabwe between 7- 9 December 2010

In February 2011, traps set up in Harare, Mazowe and Mvurwi were checked during a visit in those areas by H. Le Roux and A. Manrakhan. Thereafter the traps at those three sites were rechecked by Louisa Makumbe, Department of Plant Quarantine and by John Perrott.

In April 2011 (11-15 April 2011), a survey for detection of *B. dorsalis* was conducted in Central Zimbabwe from Plumtree to Mutare through Masvingo following main road network. Ten ME baited traps inspected in June and/or December 2010 between Plumtree and Bulawayo were rechecked. All traps were missing and eight of them were replaced and rebaited. Seventeen new ME traps were set from Bulawayo to Mutare through Masvingo (Fig. 3.3.4.6). Traps were inspected on the way back.

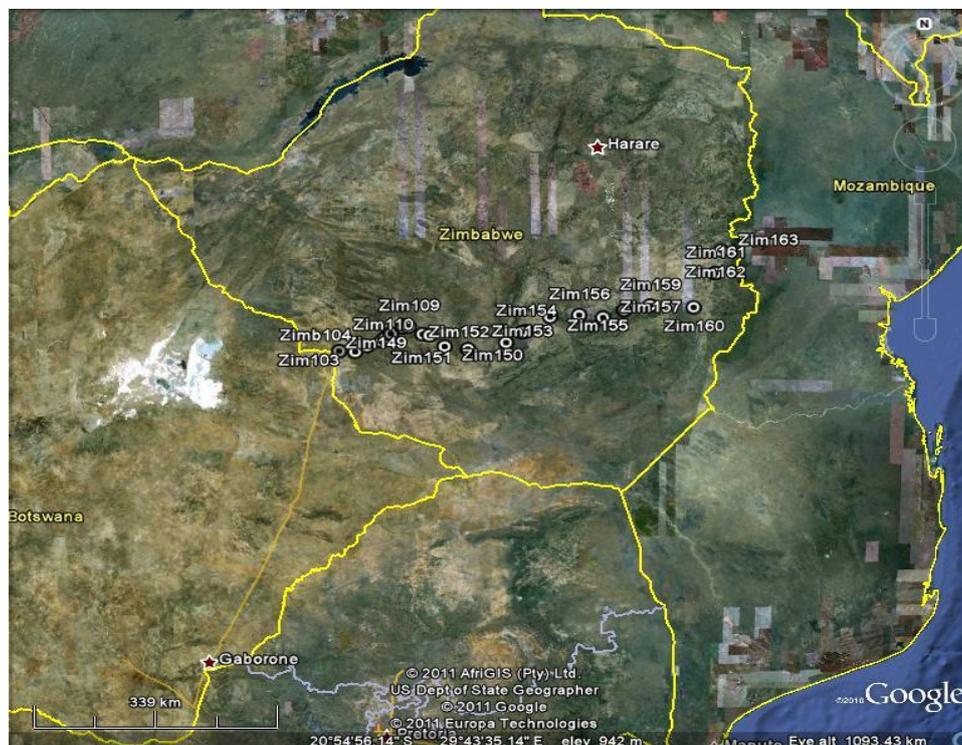


Figure 3.3.4.6. ME baited traps monitored in Zimbabwe between 11- 15 April 2011.

The last short term survey for *B. dorsalis* in Zimbabwe was carried out in September 2011. The survey was conducted in South-East and East of Zimbabwe from Bubi River (about 70 km north east of Beitbridge) to Marondera and surrounds through Mutare. Twenty new methyl eugenol baited traps (Lynfield traps baited with Invader Lure) were set from Bubi River to Marondera through Chiredzi and Mutare, between 12 and 13 September 2011 (Fig. 3.3.4.7). Traps were inspected on the way back (as from 14 September 2011). During inspection, specimens caught were collected and brought back to CRI for identification. Three traps set around Mutare on 12 April 2011 were also checked and rebaited.

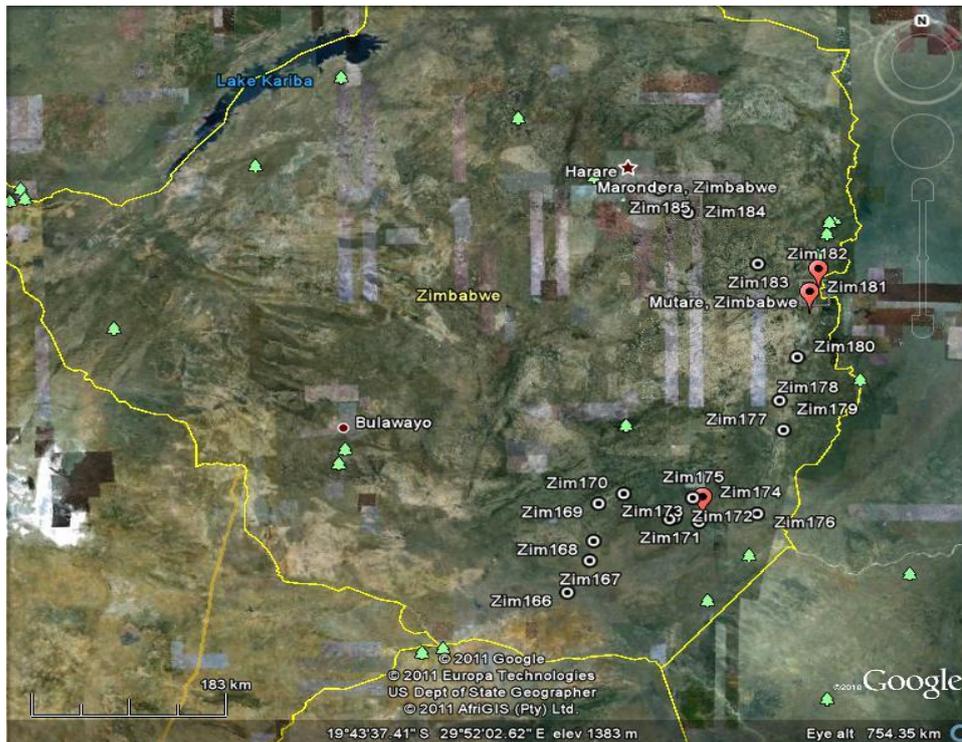


Figure 3.3.4.7. ME baited traps monitored in Zimbabwe between 12- 15 September 2011.

Short term monitoring surveys in Botswana by CRI

In Botswana, surveys for detection of *B. dorsalis* were carried by CRI between December 2009 and January 2013. Surveys were carried out using ME baited traps. One trap was set at Seleka farm (S 22° 56' 16.6" E 27° 58' 28.6"), a citrus farm, in Botswana on 2 December 2009.

Between 11 June and 19 June 2010 6 ME baited traps were placed along the Serule River, Moloutse River, Tholotsane River, Shashe River, in Francistown, at Tsheshebe, at Gatshawane, near and at Selebi Pikwe, in Sefophe, Mogapi and Lerala in Eastern Botswana (Fig. 3.3.4.8). Traps were checked either on the day of placement or after 6-8 days. Traps in Eastern Botswana were rechecked and rebaited on 22 July 2010.

In December 2010, a survey was carried out in the south east of Botswana where existing traps were rechecked and new traps were also placed further south in and around Gaborone. Eleven new ME traps were set from Gaborone to Letchane (Fig. 3.3.4.9). Six of the traps that were set since June 2010 were also inspected again and rebaited. All traps were inspected 4-5 days after setting and rebaiting.



Figure 3.3.4.8. ME baited traps set and monitored in Botswana between 11- 19 June 2010.



Figure 3.3.4.9. ME baited traps monitored in south eastern Botswana between 5- 10 December 2010.

In April 2011, another survey was carried out in the south-east of Botswana, from Gaborone to Francistown where existing traps were rechecked and rebaited. Nineteen ME traps set between June and December 2010 from Gaborone to Francistown were checked.

Finally on 17 January 2013, 3 ME baited traps set in Gaborone since December 2010 were checked.

Identification

Specimens collected during trapping by CRI personnel were brought back to CRI Nelspruit, identified and kept either as pinned specimens or in alcohol at CRI. Given that dipterans and coleopterans formed the majority of the insect specimens captured, they were sent for further identification and confirmation to family level. Non-Tephritid flies and coleopterans were sent to the Biosystematics Division of the ARC, Pretoria, for identification whilst Tephritid flies were sent to Dr Marc De Meyer, Royal Museum for Central Africa, Belgium for identification and confirmation to species level.

Database

A database of all trapping records was maintained in Microsoft Excel and Access (South African national *B. invadens* surveillance database).

Results and discussion

Bactrocera dorsalis catches

South Africa

The *B. dorsalis* surveillance project conducted by CRI enabled timely *B. dorsalis* detections between 2010 and 2013 in Limpopo, Mpumalanga and North West which led to successful eradication programmes of the pest within the affected areas in 2010 and 2011. The procedures used in the eradication programmes and the outcome of the first *B. dorsalis* eradication campaign in South Africa was described in (Manrakhan et al. 2011). These initial timely detections and successful control actions helped in delaying the eventual establishment of the pest in parts of South Africa. A progressive increase in catches of *B. dorsalis* however occurred across the years. Table 3.3.4.1 shows the annual occurrence of *B. dorsalis* detections in South Africa since 2009. A review on the progressive invasion of *B. dorsalis* in South Africa was published in the journal Biological Invasions (Manrakhan et al. 2015). In the review, the increase in catches of *B. dorsalis* over the years from 2006 to 2013 was discussed. In 2013, *B. dorsalis* was declared present in the Vhembe district, Limpopo Province in South Africa. In 2015, *B. dorsalis* was declared present in all districts of Limpopo Province and specified districts of Mpumalanga, North-West, Gauteng and KwaZulu-Natal.

Table 3.3.4.1. Annual occurrence of *B. dorsalis* detections in South Africa between 2009 and 2014. Numbers represent total catches in the South African surveillance network which includes traps set up and maintained by DAFF, CRI, Subtrop and Hortgro. Catches of *B. dorsalis* in the CRI surveillance network are indicated in brackets. Only provinces that were affected by *B. dorsalis* between 2009 and 2014 are included in the table.

Province	Total number of <i>B. dorsalis</i> adults trapped in the national surveillance network					
	2009	2010	2011	2012	2013	2014
Limpopo	0	23 (4)	26 (1)	1512 (12)	8690 (123)	28664 (373)
Mpumalanga	0	0	0	9 (1)	106 (99)	5807 (980)
North-West	0	0	0	5	61 (4)	456 (61)
Gauteng	0	1	4	2	9	171
KwaZulu-Natal	0	0	0	0	16	1600
Northern Cape	0	0	0	0	0	20

Catches of *B. dorsalis* in commercial citrus orchards in Limpopo and Mpumalanga between 2013 and 2014 were analysed to determine the seasonal population fluctuations of the new fruit fly pest. A peak in catches of *B. dorsalis* was found to occur between February and May in commercial citrus orchards in Limpopo and Mpumalanga (Fig. 3.3.4.10). The seasonal phenology of the new invasive fruit fly pest was similar to the phenology of three local fruit fly pests in the Nelspruit area (which has a temperate climate with dry winter and hot summer) (De Villiers et al. 2013).

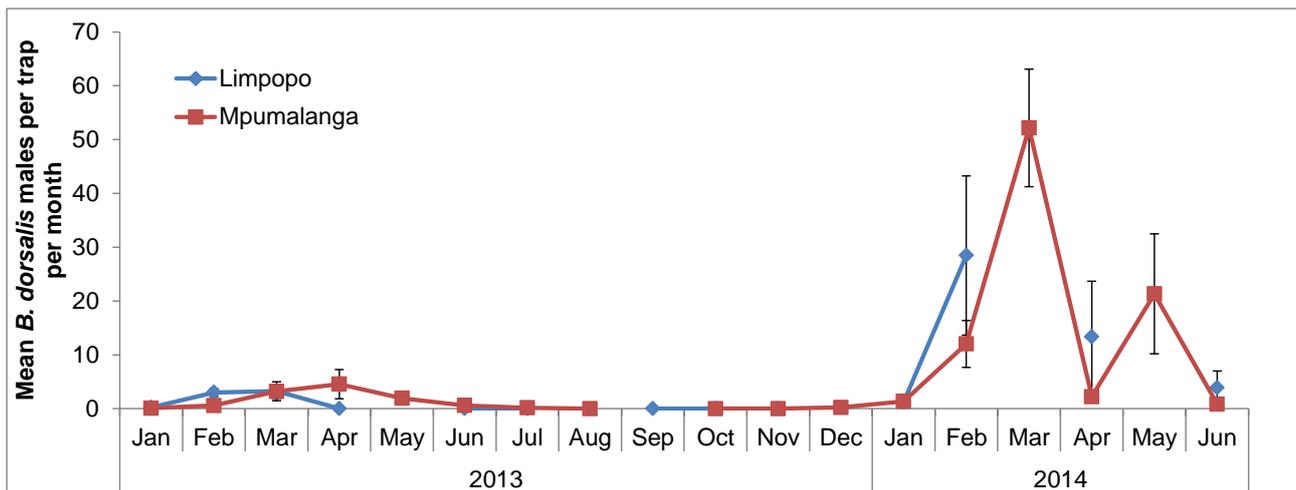


Figure 3.3.4.10. Monthly mean catches of *B. dorsalis* in commercial citrus orchards in Limpopo and Mpumalanga between January 2013 and June 2014. Missing data for Limpopo are as a result of no trapping for that particular month in the study sites in that province. Sampling was being carried out every 6 weeks in some of the study sites in Limpopo after the change of status of the pest in South Africa in 2013.

Swaziland

Four ME baited traps were set up at Ngonini Estates (North West of Swaziland) and Tambuti Estates (Central Swaziland) in October 2010 and were monitored on a more or less monthly basis until July 2014. The first detection of *B. dorsalis* occurred on 28 February 2013 in both farms. Despite the instigation of control measures including the male annihilation technique in the two farms following reports of the *B. dorsalis* finds, detections of the pest continued sporadically throughout the rest of 2013. In 2014, *B. dorsalis* was continuously detected in ME baited traps in both farms. Patterns of *B. dorsalis* catches in the commercial citrus orchards in Swaziland were similar to patterns observed in commercial citrus orchards in Limpopo and Mpumalanga (Fig. 3.3.4.11).

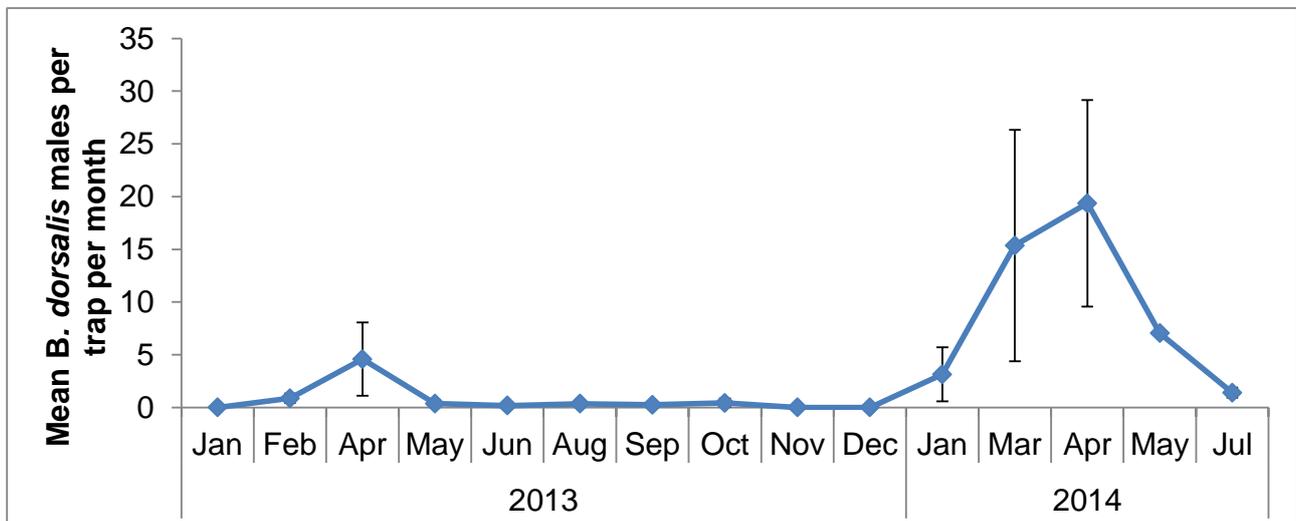


Figure 3.3.4.11. Monthly mean catches of *B. dorsalis* in commercial citrus orchards in Swaziland between January 2013 and July 2014.

Monitoring surveys in Zimbabwe

The first finds of *B. dorsalis* in Zimbabwe were at Nottingham Estates (near Beitbridge border post) during delimiting surveys carried out following the *B. dorsalis* finds in neighbouring Weipe, South Africa. Two *B. dorsalis* males were collected on 20.05.2010 and 21.05.2010 in two separate traps (Table 3.3.4.2). There were no further finds in that farm after eradication actions were implemented.

In June 2010, one *B. dorsalis* specimen was detected at Victoria Falls (at Zimbabwe- Zambia border) (Table 3.3.4.2). No *B. dorsalis* specimen was captured in the other short term monitoring surveys conducted in

January and December 2010, along the Beitbridge-Harare-Mutare and Plumtree-Mvurwi transects respectively. In 2011, *B. dorsalis* was found in different locations in northern Zimbabwe (Harare area, Nyika) and in the east and south eastern Zimbabwe (Mutare, Mvududu and Chiredzi) (Table 3.3.4.2).

Table 3.3.4.2. Catches of *B. dorsalis* during monitoring surveys in Zimbabwe in 2010 and 2011

Location	Trap Number	Latitude	Longitude	Date of capture of <i>B. dorsalis</i>	Number of <i>B. dorsalis</i> captured
Nottingham Estates, Beitbridge	4	S22° 08' 40.6"	E29° 36' 00.5"	20.05.2010	1
	26	S22° 07' 17.9"	E29° 38' 19.2"	21.05.2010	1
Victoria Falls	Zim 135	S17° 55' 48.4"	E25° 50' 30.7"	16.6.2010	1
Harare	Zim 144	S17° 48' 02.0"	E31° 02' 15.2"	24.02.2011	20
				8.3.2011	12
				14.3.2011	6
Mazowe (Mazowe citrus)	Zim 145	S17° 26' 27.9"	E31° 02' 04.8"	11.2.2011	9
				21.2.2011	3
Mazowe (Makumbe residence)	Zim 147	S17° 35' 37.7"	E30° 57' 56.0"	*NA	5
Mvurwi (Forrester Estate)	Zim 146	S17° 01' 56.0"	E30° 58' 06.9"	3.3.2011	2
				8.3.2011	12
				15.3.2011	6
Nyika	Zim 159	S19° 56' 08.0"	E31° 41' 45.4"	14.4.2011	1
Mutare	Zim 182	S18° 57' 23.9"	E32° 41' 05.5"	14.9.2011	1
Mvududu	Zim 181	S19° 10' 07.4"	E32° 36' 26.7"	15.9.2011	1
Chiredzi	Zim 174	S21° 03' 03.1"	E31° 40' 16.5"	15.9.2011	2

*NA Specimens collected from that trap were not dated. Trap servicing record sheet indicates a suspected *Bactrocera/Dacus* specimen on 16.12.2010 and 2 suspected *B. dorsalis* specimens on 17.2.2011

Monitoring surveys in Botswana

There were only 2 *B. dorsalis* specimens collected from trapping surveys carried out in Botswana. The first capture of *B. dorsalis* was in Seleka farm on 15.5.2010 (S22° 56' 16.5" E27° 58' 28.6") near the South African border post (Tom Burke). The second capture of *B. dorsalis* was in Francistown (S21° 11' 52.6" E27° 31' 54.6" Eastern Botswana) on 10.4.2011.

Catches of non- target arthropods in commercial citrus orchards

A total of 4192 non-target arthropod specimens were collected during 632 trap services in the north/north eastern parts of South Africa between 2009 and 2010. The non-target arthropods were categorized in 7 main orders (Fig. 3.3.4.12) with Coleoptera and Diptera representing the majority of the captures.

The most abundant non-target specimens captured in methyl eugenol traps were in the order Coleoptera (Table 3.3.4.3). Specimens of Coleoptera were represented in four major families: Scarabaeidae (*Leucocelis* species), Nitidulidae (*Carpophilus* species), Chrysomelidae and Scrtptiidae. The most dominant family of Coleopterans captured in ME baited traps were Scarabaeidae and *Leucocelis* species formed the majority of these specimens. *Leucocelis* species are most commonly found on a wide variety of flowers and may accidentally act as pollinators but are more damaging to flowers than beneficial (Riaan Stals, pers communication) *Carpophilus* species were the second most abundant group of Coleopterans in ME baited traps. *Carpophilus* species are usually associated with decaying fruits and decaying products of plant origin, and they include some stored-foodstuff pests (Riaan Stals, pers communication). *Carpophilus* species were also reported in methyl eugenol baited traps in Hawaii (Kido et al., 1996). The second most abundant non-target specimens were in the order Diptera (Table 3.3.4.3). The family Tephritidae was the most dominant group among the non-target Dipterans which were captured in ME baited traps. *Ceratitis rosa* and *Perilampus curta* were the main non-target Tephritids captured. *Perilampus curta* is a locally occurring fruit fly and usually occurs in fruits of the Loranthaceae family (commonly known as mistletoe).

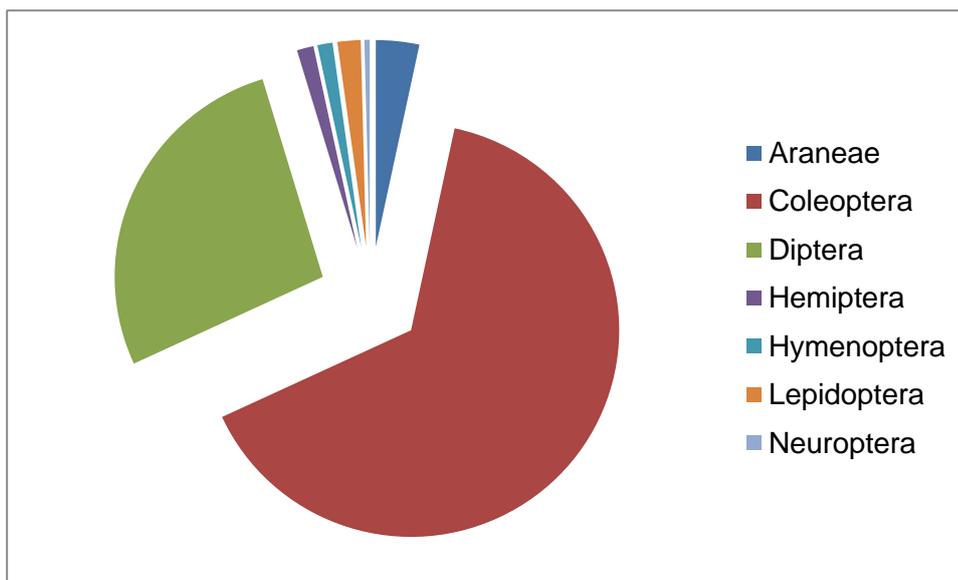


Figure 3.3.4.12. Composition of the orders of non-target arthropods captured in methyl eugenol baited traps in north and north-eastern parts of South Africa

Table 3.3.4.3. Captures of the most abundant non-target insects in methyl eugenol traps placed in citrus production areas in the north eastern parts of South Africa between 2009 and 2010.

Order	Family	Genus/species	Mean capture per trap per month
Coleoptera			3.30 ± 2.03
	Scarabaeidae	<i>Leucocelis</i> spp	2.38 ± 2.00
	Nitidulidae	<i>Carpophilus</i> spp.	0.46 ± 0.32
	Chrysomelidae		0.26 ± 0.07
	Scrtptiidae		0.08 ± 0.02
Diptera			1.82 ± 0.67
	Tephritidae	<i>Ceratitis rosa</i> and <i>Perilampus curta</i>	0.95 ± 0.54
	Drosophilidae		0.21 ± 0.08

Conclusion

The significant achievements of the *B. dorsalis* surveillance project were the early detections of *B. dorsalis* in South Africa, Southern Zimbabwe and Swaziland between 2010 and 2013. The early detections of *B. dorsalis* in South Africa led to the implementation of appropriate actions following the national action plan on the pest and helped in delaying the establishment of *B. dorsalis* in different areas of the country. This delay allowed for registration of products for pre-harvest control of *B. dorsalis* in South Africa and completion of

research on post-harvest treatment for the pest. In 2013, *B. dorsalis* was declared present in the Vhembe district, Limpopo Province in South Africa. In 2015, *B. dorsalis* was declared present in all districts of Limpopo Province and specified districts of Mpumalanga, North-West, Gauteng and KwaZulu-Natal.

It is highly likely that the distribution of *B. dorsalis* will further expand in South Africa. As such in the short term, it is important that surveillance for detection of the pest continue in the pest free areas. A proper and regular surveillance will enable timely detections and possible successful eradication in particular in isolated fruit production areas.

In the ME baited traps in commercial citrus, there were few non-target arthropods and the dominant ones such as *Leucocelis* species are not considered beneficial. As such, ME used for control of *B. dorsalis* is likely to have minimal impact on beneficial arthropods.

Technology transfer

Manrakhan A. & Grout T. 2008. Current status of the exotic fruit fly: *Bactrocera invadens* Drew, Tsuruta & White (Diptera: Tephritidae) in Africa, and future research perspectives. 5th Citrus Research Symposium, Drakensberg, 3-6 August 2008.

Manrakhan A., Daneel, J.-H., Kotze, C., Stephen, P. & Beck, R. 2009. Surveillance of the exotic fruit fly: *Bactrocera invadens* Drew, Tsuruta & White (Diptera: Tephritidae) in citrus production areas of South Africa. 6th Citrus Research Symposium, Drakensberg, 15-18 August 2010.

Manrakhan A., Daneel, J.-H., Kotze, C., Stephen, P. & Beck, R. 2009. Captures of native Tephritid species and non-target insects in methyl-eugenol baited traps in South Africa. 6th Citrus Research Symposium, Drakensberg, 15-18 August 2010.

Manrakhan, A., Grout, T. & Hattingh, V. 2009. Combating the African Invader fly *Bactrocera invadens*. South African Fruit Journal 8 (1): 57-61

Manrakhan, A., L. Brown, J. H. Venter, W. Stones, and J.-H. Daneel. 2011. The *Bactrocera invadens* surveillance programme in South Africa. SA Fruit Journal 10: 78-80.

Manrakhan A. 2012. Fruit flies: Are we winning the war?. 7th Citrus Research Symposium, Drakensberg, 19-22 August 2012.

[A number of grower talks were also given on *Bactrocera dorsalis* at various study group meetings between 2008 and 2014]

References cited

Barnes, B. N., and J.-H. Venter. 2008. The South African fruit fly action plan- area-wide suppression and exotic species surveillance, pp. 271-283. In R. L. Sugayama, R. A. Zucchi, S. M. Ovruski and J. Sivinski [eds.], 7th International Symposium on Fruit Flies of Economic Importance Biofabrica Moscomed Brasil, Salvador, Bahia, Brazil.

Correia, A. R. I., J. M. Rego, and M. Olmi. 2008. A pest of significant economic importance detected for the first time in Mozambique: *Bactrocera invadens* Drew, Tsuruta & White (Diptera: Tephritidae: Dacinae). Bollettino di Zoologia agraria e di Bachicoltura 40: 9-13.

De Meyer, M., S. Mohamed, and I. M. White. 2014. Invasive fruit fly pests in Africa.

De Villiers, M., A. Manrakhan, P. Addison, and V. Hattingh. 2013. The distribution, relative abundance, and seasonal phenology of *Ceratitis capitata*, *Ceratitis rosa*, and *Ceratitis cosyra* (Diptera: Tephritidae) in South Africa. Environmental Entomology 42: 831-840.

Drew, R. A. I., K. Tsuruta, and I. M. White. 2005. A new species of pest fruit fly (Diptera: Tephritidae) from Sri Lanka and Africa. African Entomology 13: 149-154.

Goergen, G., J.-F. Vayssieres, D. Gnanvossou, and M. Tindo. 2011. *Bactrocera invadens* (Diptera: Tephritidae), a new invasive fruit fly pest for the Afrotropical region: Host Plant Range and Distribution in West and Central Africa. Environmental Entomology 40: 844-854.

Lux, S. A., R. Copeland, I. M. White, A. Manrakhan, and M. Billah. 2003. A new invasive fruit fly species from *Bactrocera dorsalis* group detected in East Africa. Insect science and its applications 23: 355-361.

Manrakhan, A., J. H. Venter, and V. Hattingh. 2015. The progressive invasion of *Bactrocera dorsalis* (Diptera: Tephritidae) in South Africa. Biological Invasions.

Manrakhan, A., V. Hattingh, J.-H. Venter, and M. Holtzhausen. 2011. Eradication of *Bactrocera invadens* (Diptera: Tephritidae) in Limpopo Province, South Africa. African Entomology 19: 650-659.

Metcalf, R. L., W. C. Mitchell, T. R. Fukuto, and E. R. Metcalf. 1975. Attraction of the oriental fruit fly, *Dacus dorsalis*, to methyl eugenol and related olfactory stimulants. Proceedings of the National Academy of Sciences, USA 72: 2501-2505.

- Mwatawala, M. W., M. De Meyer, R. H. Makundi, and A. P. Maerere. 2006. Seasonality and host utilization of the invasive fruit fly, *Bactrocera invadens* (Dipt., Tephritidae) in central Tanzania. *Journal of Applied Entomology* 130: 530-537.
- Myers, J. H., D. Simberloff, A. M. Kuris, and J. R. Carey. 2000. Eradication revisited: dealing with exotic species. *Trends in Ecology and Evolution* 15: 316-320.
- Rwomushana, I., S. Ekesi, I. Gordon, and C. K. P. O. Ogol. 2008. Host plants and host plant preference studies for *Bactrocera invadens* (Diptera: Tephritidae) in Kenya, a new invasive fruit fly species in Africa. *Annals of the Entomological Society of America* 101: 331-340.
- Schutze, M. K., K. Mahmood, A. Pavasovic, W. Bo, J. Newman, A. R. Clarke, M. N. Krosch, and S. L. Cameron. 2014a. One and the same: integrative taxonomic evidence that *Bactrocera invadens* (Diptera: Tephritidae) is the same species as the Oriental fruit fly *Bactrocera dorsalis*. *Systematic Entomology*.
- Schutze, M. K., N. Aketarawong, W. Amornsak, K. F. Armstrong, A. A. Augustinos, N. Barr, W. Bo, K. Bourtzis, L. M. Boykin, C. CACeres, S. L. Cameron, T. A. Chapman, S. Chinvinijkul, A. Chomič, M. De Meyer, E. Drosopoulou, A. Englezou, S. Ekesi, A. Gariou-Papalexiou, S. M. Geib, D. Hailstones, M. Hasanuzzaman, D. Haymer, A. K. W. Hee, J. Hendrichs, A. Jessup, Q. Ji, F. M. Khamis, M. N. Krosch, L. U. C. Leblanc, K. Mahmood, A. R. Malacrida, P. Mavragani-Tsipidou, M. Mwatawala, R. Nishida, H. Ono, J. Reyes, D. Rubinoff, M. San Jose, T. E. Shelly, S. Srikachar, K. H. Tan, S. Thanaphum, I. Haq, S. Vijaysegaran, S. L. Wee, F. Yesmin, A. Zacharopoulou, and A. R. Clarke. 2014b. Synonymization of key pest species within the *Bactrocera dorsalis* species complex (Diptera: Tephritidae): taxonomic changes based on a review of 20 years of integrative morphological, molecular, cytogenetic, behavioural and chemoecological data. *Systematic Entomology*: n/a-n/a.
- Shelly, T., J. Nishimoto, A. Diaz, J. Leathers, M. War, R. Shoemaker, M. Al-Zubaidy, and D. Joseph. 2010. Capture probability of released males of two *Bactrocera* species (Diptera: Tephritidae) in detection traps in California. *Journal of Economic Entomology* 103: 2042-2051.

3.3.5 FINAL REPORT: Developmental threshold and critical thermal limits for two *C. rosa* types in South Africa

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

Summary

There is currently mounting scientific evidence that what was known as Natal fly could actually be two distinct species. Recent studies revealed the occurrence of two genotypes and morphotypes of Natal fly. The two types of Natal fly were categorised as *C. rosa* R1 and *C. rosa* R2. There were some contradicting findings from previous studies on the ecological requirements of Natal fly. Some studies revealed that Natal fly was more tolerant to lower temperatures compared to Medfly. Other studies done in South Africa found the reverse. These studies already then indicated that there were possibly two different types of Natal fly with different ecological requirements. When the two types of Natal fly were later confirmed in genetic studies, the hypothesis was that one type was possibly more cold tolerant than the other. As such in this project, the aim was to determine whether there were indeed any differences in developmental rates between the two *C. rosa* types. Comparative analysis of development and survivorship of immature stages of *C. rosa* R1 and *C. rosa* R2 were studied at seven constant temperatures (10, 15, 20, 25, 30, 33, 35°C). The *C. rosa* R1 originated from Nelspruit and the *C. rosa* R2 originated from Pretoria. There was no development of immature stages for both *C. rosa* types at 10°C. The developmental duration was found to significantly decrease with increasing temperature for *C. rosa* R1 and *C. rosa* R2. Survivorship of larvae of *C. rosa* R1 and *C. rosa* R2 was lowest at 35°C (22%) and 33°C (0.33%) respectively. Results from temperature summation models showed that *C. rosa* R1 tolerated more heat compared to *C. rosa* R2. There was no major difference between the two types of *C. rosa* in their tolerance to low temperatures. These results demonstrate that *C. rosa* R1 and *C. rosa* R2 were physiologically distinct in their response to different temperature regimes, particularly at the higher temperatures.

Opsomming

Daar is tans 'n toename in wetenskaplike bewyse dat, wat bekend was as die Natalse vlieg, eintlik twee aparte spesies kan wees. Onlangse studies het die voorkoms van twee genotipes en morfotipes van die Natalse vlieg bekend gemaak. Die twee tipes Natalse vlieg is as *C. rosa* R1 en *C. rosa* R2 gekategoriseer. Daar was 'n mate van teenstrydige bevindinge vanuit vorige studies rakende die ekologiese vereistes van die Natalse vlieg. Sommige studies het getoon dat die Natalse vlieg meer bestand teen laer temperature in vergelyking met die Mediterreense vlieg was. Ander studies wat in Suid-Afrika uitgevoer is, het die teenoorgestelde gevind. Hierdie studies het reeds toe aangedui dat daar moontlik twee verskillende tipes

van die Natalse vlieg is, met verskillende ekologiese vereistes. Nadat die twee tipes Natalse vlieg later in genetiese studies bevestig is, was die hipotese dat een tipe moontlik meer kouebestand as die ander een was. Soos in hierdie projek, was die doel om vas te stel of daar wel enige verskille in ontwikkelingstempas tussen die twee *C. rosa* tipes was. Vergelykende analise van ontwikkeling en oorlewing van onvolwasse stadiums van *C. rosa* R1 en *C. rosa* R2 is by sewe konstante temperature (10, 15, 20, 25, 30, 33, 35°C) bestudeer. Die *C. rosa* R1 kom vanaf Nelspruit en die *C. rosa* R2 kom van Pretoria. Daar was geen ontwikkeling van onvolwasse stadiums vir beide *C. rosa* tipes by 10°C nie. Daar is gevind dat die duurte van ontwikkeling betekenisvol afneem met toename in temperatuur vir *C. rosa* R1 en *C. rosa* R2. Oorlewing van larwes van *C. rosa* R1 en *C. rosa* R2 was die laagste by 35°C (22%) en 33°C (0.33%) onderskeidelik. Resultate van temperatuur opsommingsmodelle het getoon dat *C. rosa* R1 meer hitte verdra het in vergelyking met *C. rosa* R2. Daar was geen groot verskil tussen die twee tipes van *C. rosa* in hul bestandheid teen lae temperature nie. Hierdie resultate het gedemonstreer dat *C. rosa* R1 en *C. rosa* R2 fisiologies verskillend is in hul reaksie op verskillende temperatuur regimes, veral by die hoër temperature.

The final report is adapted from a manuscript "C.M. Tanga, A. Manrakhan, J.H. Daneel, S.A. Mohamed, F.M. Khamis, S. Ekesi. Is it just all about legs or do the two Natal fruit fly groups also differ in their developmental rates?" submitted for publication in the peer reviewed journal Zookeys

Introduction

Ceratitis rosa Karsch commonly known as the Natal fly is a pest of phytosanitary concern in South Africa. Worldwide, Natal fly is restricted to East and Southern Africa and some Indian Ocean islands. In South Africa, *C. rosa* is widely distributed across the country but is either scarce or absent in the drier inland regions (De Meyer, 2001; De Villiers et al. 2013).

Ceratitis rosa is morphologically very similar to two other species within the same subgenus *Pterandrus*: *C. fasciventris* (Bezzi) and *C. anonae* Graham (De Meyer and Freidberg, 2006). The 3 species form a complex known as the FAR complex (Virgilio et al. 2013) and are sexually dimorphic (De Meyer and Freidberg, 2006). The males within the FAR complex can be readily separated based on differences on their leg and anepisternal pilosity patterns (De Meyer and Freidberg, 2006) whilst the females within the FAR complex cannot be separate using morphological characters. Recent genetic analysis has shown that the FAR complex is probably five entities, rather than the three taxonomic species (Virgilio et al. 2013). A neighbor joining tree from these studies showed that morphospecies of *C. rosa* and *C. fasciventris* was represented by two well-supported clusters of populations depicted as R1 and R2 (for *C. rosa*), and F1 and F2 (for *C. fasciventris*). Morphological comparisons of the two *C. rosa* clusters: *C. rosa* R1 and *C. rosa* R2 showed differences in the shape and ornamentation of the mid-tibia of the males (Virgilio et al. 2013). The males of the two *C. rosa* groups can be distinguished from each other as follows: The black area of the mid tibia of *C. rosa* R1 reaches the lateral margins while the black area of the mid tibia of *C. rosa* R2 does not reach the lateral margins (De Meyer, personal communication).

The possibility of two forms of *C. rosa* was earlier suggested in molecular studies by Barr et al. (2006) who associated the forms with geographical distribution of the pest (South Africa and La Reunion form versus Kenyan form). Moreover, differences in thermal developmental rates between *C. rosa* from La Reunion and South Africa were found in studies conducted separately in the respective countries (Duyck and Quilici, 2002; Grout and Stoltz, 2007) leading Grout and Stoltz (2007) to suggest the possibility of existence of two biotypes of *C. rosa*, one being more cold tolerant than the other.

Temperature is the single most important environmental factor determining development and survival of tephritid fruit flies (Fletcher 1989). Various tephritid species have specific optimal temperature range for development limited by lower and upper thresholds (base temperature and upper limit). Below and above these temperature limits, development does not occur and this can vary both with developmental stage and geographical origin (Honék and Kocourek 1990). Information on the thermal requirements of insect groups forms an important basis in understanding and predicting the geographical distribution of the different insect groups.

Given the recent evidence of existence of the two types of *C. rosa*, studies were undertaken to determine the thermal developmental rates and thresholds of each of the *C. rosa* types. This is in order to see whether one of the types would be more of a risk (in terms of cold tolerance) to export markets than the other.

Stated objectives

1. To evaluate the temperature-dependent development of the two *C. rosa* types.
2. To determine the critical thermal limits of the two *C. rosa* types.

Materials and methods

Temperature-dependent development of the two C. rosa types

Founder flies for the colonies of two types of *C. rosa* were obtained from samples of infested fruit from two locations: Pretoria (S25 45' 13.7" E28 13' 45") and Nelspruit (S 25 27' 08.19" E30 58' 11.27"). The flies originating from Pretoria were identified as R2 and those from Nelspruit as R1 by M. De Meyer (Royal Museum for Central Africa). In the colonies, eggs of the two types were collected from apples (*Malus domestica* Borkh c.v Granny Smith) pricked on the sides with a row of pins. The two types of *C. rosa* were reared on a carrot based artificial diet in laboratory at Citrus Research International for 5-8 generations before the start of the study.

The methods used were adapted from Grout and Stoltz (2007). All tests were carried out in two modified Conviron CMP3023 environmental chambers (Controlled Environments, Manitoba, Canada) under a photoperiod Light:Dark of 12:12 hours. Development of the immature stages of the two *C. rosa* types were determined concurrently at seven constant temperatures: 10, 15, 20, 25, 30, 33 and 35 °C (± 0.03). The relative humidity ranging in the environmental chambers varied from 42% to 76%. Temperature and relative humidity were monitored daily using a Lascar data logger EL-USB-2 (with an accuracy of $\pm 0.5^\circ\text{C}$). Since there were only two chambers for the study, only 2 temperatures could be evaluated at a time. For each temperature tested, there were 3 replicates of the immature and adult stages of each *C. rosa* type except for the egg stages where there were up to 6 replicates. There were more replicates of the egg stages in order to obtain sufficient numbers of individuals for the successive developmental stages.

Egg development

Eggs of each *C. rosa* type which were approximately 12 h old were collected from adult flies which were 14 days and above, using Granny Smith apples pricked four times with a row of eight pins (0.8 mm diameter and 2.5 mm apart). Each row of pins was dissected and eggs were removed by rinsing the dissected area with distilled water. Using a fine brush, 100 eggs were transferred onto a moist black cloth, placed inside a Petri dish as a replicate for each *C. rosa* type. Petri dishes containing eggs were moved to the environmental chamber immediately following egg placement onto the black cloth. The duration and survival of the egg stages were determined by checking the Petri dishes every 2 hours daily for hatched eggs using a stereomicroscope. The start time was taken as the time when the eggs were collected from the apple. The observations on egg hatch were stopped when there were no further increases in numbers of hatched eggs after three consecutive observations. The developmental time of the egg stages for each replicate was calculated by the sum of the products of the duration in days (from start time to observation time) and the eggs hatched at each observation divided by the total number of eggs hatched for that particular replicate. Percentage egg survival for each replicate was determined as the total number of hatched eggs divided by the total number of eggs placed per Petri dish.

Larval development

For each replicate of each *C. rosa* type, 100 neonate larvae (less than 12 h old) were placed on 2-5 squares (1cm x 1 cm) of filter paper which were then placed on top of 150 g of carrot-based larval diet inside a 11.5 cm diameter plastic container to determine larval development. The container with the larval diet was then placed in an aerated plastic container (28.5 cm x 28.5 cm x 14 cm height) containing a thin layer of sand spread at the bottom. These containers were checked five times per day to count the number of larvae jumping and the number of pupae forming. At each observation, the larvae or pupae were picked from the sand using a spoon and placed in a 9 cm Petri dish. The Petri dish was labelled according to *C. rosa* type, replicate, date and time. The start time of the larval stage was taken as the time that the neonate larvae were placed on the diet. The observations on larval development ended when there was no further larval jumping for 5 consecutive observations. The duration of the larval stage for each replicate of each *C. rosa* type was determined by the sum of the products of the duration in days (from start time to observation time) and the number of larvae jumping at each count divided by the total number of larvae that jumped. Percentage larval survival for each replicate was determined as the number of larvae jumping divided by the number of neonate larvae inoculated per diet container.

Pupal development

For each replicate of each *C. rosa* type, pupae (approximately 24 h old) formed at the peak day of larval jumping were placed using a soft pair of forceps in a 9 cm Petri dish lined with a moist filter paper. The number of pupae used per replicate varied depending on the number of pupae formed on the peak day of larval jumping for a particular replicate of a particular *C. rosa* type. The Petri dish containing the pupae were placed in a 28.5 cm x 28.5 cm x 14 cm aerated plastic container to allow for adult emergence. The Petri dishes containing pupae were checked daily for adult eclosion. The daily observation was carried out under a stereomicroscope in order to capture the first stage of adult emergence when the adult fly would break open the pupal case. The start time of the pupal stage was taken as the peak day of larval jumping. The observations ended when there was no further adult emergence for three consecutive days. The duration of the pupal stage for each replicate was determined by the sum of the products of the duration in days (from start time to observation time) and the number of adults eclosing at each observation divided by the total number of adults that eclosed. Percentage pupal survival for each replicate was determined as the total number of adults eclosed divided by the number of pupae placed per Petri dish.

Data analysis and determination of thermal requirements

The developmental times and percentage survival of each life stage of each *C. rosa* type at different constant temperatures were compared using ANOVA (XLSTAT, version 2014.1.01, Addinsoft, New York, USA). The developmental times and percentage survival between the *C. rosa* types at each temperature and life stage were compared using two-sample t test (XLSTAT, version 2014.1.01, Addinsoft, New York, USA). The developmental times and percentage survival of immature times were $\log(x+1)$ and Arcsin square root $(x+1)$ transformed respectively in order to meet the assumptions of the parametric tests. Means were compared using the Student Newman-Keuls (SNK) ($P=0.05$).

A linear regression model and the non-linear Briere regression model (Briere et al. 1999) were used to calculate the thermal requirements of the different life stages of each *C. rosa* type. For each model, the developmental rate taken as $1/\text{developmental time (d)}$ was plotted against the study temperatures. For the egg stage, developmental rates at temperatures ranging between 15 °C and 30 °C were used since conditions below and above the latter range were suboptimal depending on *C. rosa* type. For the larval and pupal stages, the developmental rates at temperatures ranging between 15 °C and 25 °C were used since conditions below and above the latter range were suboptimal for those two life stages of the two *C. rosa* types. All regression analyses were carried out in XLSTAT (version 2014.1.01, Addinsoft, New York, USA). For the linear regression, the equation of the model was $1/d = a + bT$, where d is the mean developmental time, T is the rearing temperature, a is the developmental rate at $T=0^\circ\text{C}$ and b is the slope of the line. The T_{\min} or lower developmental threshold (below which no development occurs) was calculated as $-a/b$. The thermal constant K which is the number of heat units or day-degrees above T_{\min} required to complete development (Fletcher 1989) was calculated as $1/b$ (Campbell et al. 1974).

For the non-linear Briere regression, the equation of the model was $1/d = aT(T-T_{\min})(T_{\max}-T)^{1/2}$ (Briere et al. 1999) where d is the mean developmental time, a is an empirical constant and was chosen as 0.0003 for the egg stage and 0.0001 for the larval and pupal stages based on constants calculated for other insect species (Briere et al. 1999), T is the rearing temperature, T_{\min} is the lower developmental threshold and T_{\max} is the upper developmental threshold above which no development occurs. The T_{\min} and T_{\max} values obtained from the non-linear model was used to calculate the optimum temperature (T_{opt}) as follows: $T_{\text{opt}} = 4T_{\max} + 3T_{\min} + [(16T_{\max}^2 + 9T_{\min}^2 - 16T_{\min}T_{\max})^{1/2}]/10$ (Briere et al. 1999).

Critical thermal limits of the two *C. rosa* types

Studies on the critical thermal limits were to be undertaken by Dr Chris Weldon at the University of Pretoria. These studies could however not be completed at the time of the submission of this final report due to problems experienced in the temperature acclimation of the flies sent to University of Pretoria prior to determination of their critical thermal limits. The studies will still be conducted but at a later stage and will be reported separately.

Results

Developmental duration of immature stages of the two *C. rosa* types

The time required for eggs to hatch ranged from 7.53 ± 0.10 d at 15°C to 1.69 ± 0.01 d at 33°C for *C. rosa* R1 (Table 3.3.5.1). The egg developmental time of *C. rosa* R2 was longest at 15°C and shortest at 30°C (Table 3.3.5.1). However, no significant differences in egg developmental duration were observed between the two groups of *C. rosa* at 15, 20, 25, and 30°C (Table 3.3.5.1). The eggs of both *C. rosa* groups failed to develop at 10°C .

Table 3.3.5.1. Mean \pm SE developmental time (days) of immature stages of *C. rosa* R1 and *C. rosa* R2 from South Africa at different constant temperatures

Temperature (°C)	Egg		Larva		Pupa		Total (days)	
	<i>C. rosa</i> R1	<i>C. rosa</i> R2	<i>C. rosa</i> R1	<i>C. rosa</i> R2	<i>C. rosa</i> R1	<i>C. rosa</i> R2	<i>C. rosa</i> R1	<i>C. rosa</i> R2
10	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	-	-	-	-
15	7.53 \pm 0.10 ^a A	7.40 \pm 0.06 ^a A	17.73 \pm 0.19 ^a B	20.74 \pm 0.40 ^a A	36.56 \pm 0.28 ^a A	36.18 \pm 0.29 ^a A	61.80 \pm 0.24 ^a A	64.72 \pm 0.45 ^b B
20	3.22 \pm 0.00 ^b A	3.06 \pm 0.00 ^b A	11.38 \pm 0.12 ^b B	13.63 \pm 0.36 ^b A	17.14 \pm 0.36 ^b A	19.02 \pm 0.87 ^b A	31.75 \pm 0.43 ^b A	35.71 \pm 1.06 ^b B
25	2.14 \pm 0.01 ^c A	2.11 \pm 0.03 ^d A	7.59 \pm 0.11 ^d B	8.44 \pm 0.12 ^d A	11.81 \pm 0.22 ^c A	12.09 \pm 0.24 ^c A	21.54 \pm 0.20 ^c A	22.67 \pm 0.23 ^c B
30	1.61 \pm 0.00 ^e A	1.60 \pm 0.02 ^e A	7.90 \pm 0.03 ^c B	9.92 \pm 0.61 ^c A	7.53 \pm 0.02 ^d	-	17.04 \pm 0.05 ^d	-
33	1.69 \pm 0.01 ^e B	1.99 \pm 0.08 ^d A	5.74 \pm 0.06 ^e	6.73 [*]	-	-	-	-
35	1.88 \pm 0.03 ^d A	2.36 \pm 0.03 ^c B	6.32 \pm 0.05 ^f	7.85 \pm 0.00 ^{**d}	-	-	-	-

Means in the same column followed by the same lower case letter are not significantly different (ANOVA and Student-Newman-Keul's (SNK) test, $P < 0.05$) and means within the same row of each developmental stage followed by the same upper case letter are not significantly different (Student's t test)

* Data obtained from only one replicate; no larva jumping in the other replicates

** Data obtained from two replicates; no larvae jumping in the third replicate

At larval stage, developmental duration was generally shorter for *C. rosa* R1 compared to *C. rosa* R2 at temperatures ranging from 15°C to 35°C (Table 3.3.5.1). The developmental duration of *C. rosa* R1 decreased from 17.73 \pm 0.19 d at 15°C to 5.74 \pm 0.06 d at 33°C while that of *C. rosa* R2 decreased from 20.74 \pm 0.40 d at 15°C to 6.73 d at 33°C. The larvae of both *C. rosa* groups did not develop at 10°C (Table 3.3.5.1).

For *C. rosa* R1, no eclosion was observed at 10, 33 and 35°C while for *C. rosa* R2, no eclosion was recorded at 10, 30, 33 and 35°C. Pupal developmental duration of both *C. rosa* R1 and *C. rosa* R2 varied significantly when compared across the test temperatures. Between the two *C. rosa* groups, again no significant differences in pupal development were observed at 15, 20 and 25°C (Table 3.3.5.1). The longest pupal developmental duration for *C. rosa* R1 was 36.56 \pm 0.28 d at 15°C and that of *C. rosa* R2 was 36.18 \pm 0.29 d at the same temperature.

Total developmental duration from egg to adult for *C. rosa* R1 was longest at 15°C (61.80 \pm 0.24 d) and shortest at 30°C (17.04 \pm 0.05). For *C. rosa* R2, in contrast, there was no complete development of the immature life stages at 30°C. Total developmental duration from egg to adult for *C. rosa* R2 was longest at 15°C (64.72 \pm 0.45 d) and shortest at 25°C (22.67 \pm 0.23). Significant differences were found between the two *C. rosa* groups when egg to adult developmental durations were compared across all the temperatures (Table 3.3.5.1). The egg-adult development of *C. rosa* R1 was significantly faster than that of *C. rosa* R2 at temperatures ranging from 15°C to 25°C (Table 3.3.5.1).

Estimated parameter values of the linear and nonlinear models are presented in Table 3.3.5.2. A positive linear relationship was observed between temperature and development rates for egg, larval and pupal stages for both *C. rosa* groups.

Table 3.3.5.2. Parameter estimates and their approximate standard errors for linear and *Brière-1 nonlinear models describing the relationship between temperature and development rate (1/D) of C. rosa R1 and C. rosa R2 from South Africa*

Model	Parameters	<i>C. rosa R1</i>			<i>C. rosa R2</i>		
		Egg	Larva	Pupa	Egg	Larva	Pupa
Linear	T _{min}	10.72	7.77	9.80	10.45	8.61	8.27
	K	30.79	132.94	178.34	30.89	142.28	207.31
	R ²	0.999	0.991	0.999	0.996	0.974	0.979
	P	0.001	0.06	0.023	0.002	0.103	0.09
<i>Brière-1</i>	T _{min}	16.37	14.96	14.48	16.13	15.28	14.35
	T _{opt}	30.48	27.71	24.30	30.18	27.38	24.13
	T _{max}	35.31	32.07	27.79	34.97	31.58	27.61
	R ²	0.689	0.657	0.640	0.757	0.677	0.403

For the egg, the lowest developmental threshold was estimated to be 10.72°C for *C. rosa R1* and 10.45°C for *C. rosa R2*. The egg stage required 30.79 DD to complete development in the *C. rosa R1* and 30.89 DD in *C. rosa R2*. The *C. rosa R1* group required 132.94 DD to develop above a threshold of 7.77°C from larval stage to the pupal stage while *C. rosa R2* required 142.28 DD to develop above a threshold of 8.61°C (Table 3.3.5.2). The lower developmental thresholds for the pupal stages of *C. rosa R1* and *R2* were estimated at 9.80 and 8.27°C, while the corresponding thermal constants were 178.34 DD and 207.31 DD, respectively.

The low developmental threshold values generated by the *Brière-1* model for egg, larva and pupal stages for both *C. rosa* groups were found to be higher compared to values estimated by the linear regression model (Table 3.3.5.2). For *C. rosa R1* (16.37, 14.96 and 14.48°C, respectively) low developmental thresholds of egg, larva and pupa were slightly different compared to that of *C. rosa R2* (16.13, 15.28 and 14.35°C, respectively) (Figure 3.3.5.1 and Table 3.3.5.2). An optimum temperature range of 24.30 – 30.48°C was estimated for *C. rosa R1* and 24.13 – 30.18°C for *C. rosa R2* for the various developmental stages. The lethal temperatures for *C. rosa R1* and *C. rosa R2* were estimated to range from 27.80 - 35.31°C and 27.61 - 34.97°C, respectively, for the various developmental stages (Table 3.3.5.2).

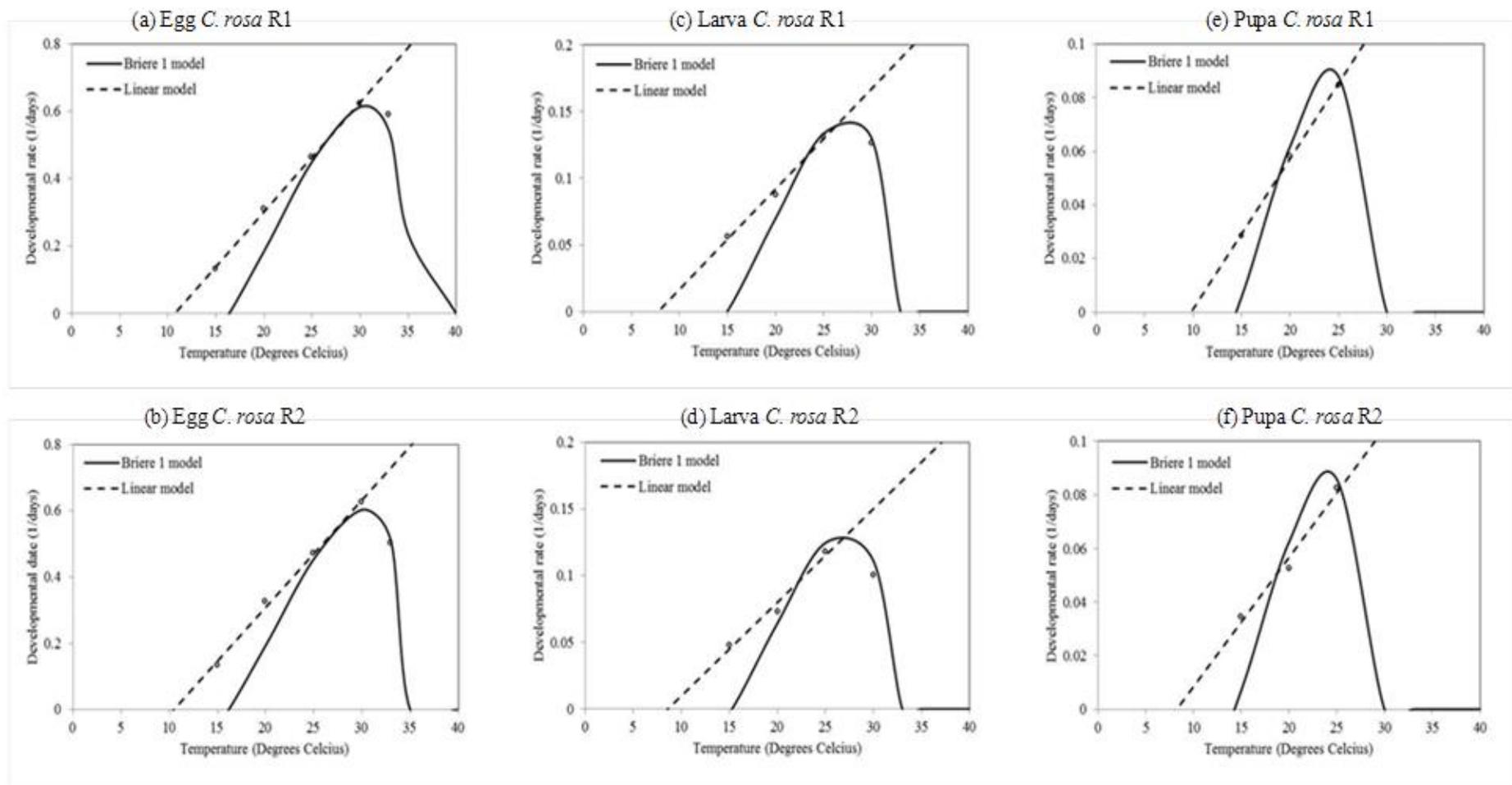


Figure 3.3.5.1: Linear and non-linear regressions of temperature related developmental rates of immature stages of two groups of *C. rosa*

Survival of immature life stages

At the egg stage, the percentage survival of *C. rosa* R1 ($F = 92.63$; $d.f = 6, 47$; $P < 0.0050$) was significantly higher compared to that *C. rosa* R2 ($F = 22.94$; $d.f = 6, 49$; $P = 0.0070$) across the temperature range of 15°C to 35°C (Table 3.3.5.3). The highest survival rate of *C. rosa* R1 was recorded at 20°C ($96.0 \pm 2.08\%$), while that of *C. rosa* R2 was recorded at 30°C ($75.11 \pm 2.23\%$).

Table 3.5.5.3. Mean \pm SE survivorship (%) of immature stages of *C. rosa* R1 and *C. rosa* R2 from South Africa at different constant temperatures.

Temperature (°C)	Egg		Larva		Pupa	
	<i>C. rosa</i> R1	<i>C. rosa</i> R2	<i>C. rosa</i> R1	<i>C. rosa</i> R2	<i>C. rosa</i> R1	<i>C. rosa</i> R2
10	0.00 ± 0.00^c	0.00 ± 0.00^c	0.00 ± 0.00^e	0.00 ± 0.00^d	-	-
15	77.00 ± 4.99^bA	54.67 ± 8.74^bB	61.33 ± 4.41^bA	73.67 ± 2.91^aA	84.10 ± 3.91^aA	90.06 ± 1.94^aA
20	96.00 ± 2.08^aA	$58.00 \pm 2.31^{ab}B$	61.67 ± 4.06^bA	41.67 ± 1.86^bB	62.71 ± 5.15^bA	69.37 ± 6.47^bA
25	90.67 ± 0.99^aA	$61.33 \pm 1.20^{ab}B$	72.67 ± 1.45^bA	23.33 ± 2.85^cB	68.18 ± 8.67^bA	52.38 ± 9.52^cA
30	93.22 ± 0.89^aA	75.11 ± 2.23^aB	87.00 ± 2.65^aA	39.33 ± 8.76^bB	68.33 ± 2.73^b	0.00 ± 0.00^d
33	79.50 ± 3.48^bA	$66.80 \pm 3.12^{ab}B$	36.33 ± 4.10^cA	0.33 ± 0.33^dB	0.00 ± 0.00^c	0.00 ± 0.00^d
35	74.00 ± 1.00^bA	57.33 ± 2.92^bB	22.25 ± 3.22^dA	0.80 ± 0.58^dB	0.00 ± 0.00^c	0.00 ± 0.00^d

For *C. rosa* R1, percentage survival of the larval stage ranged between $22.25 \pm 3.22\%$ at 35°C to $87.0 \pm 2.65\%$ at 30°C, while that for *C. rosa* R2 ranged between $0.33 \pm 0.33\%$ at 33°C to $73.67 \pm 2.91\%$ at 15°C. For both *C. rosa* groups, no significant difference in larval survival was observed at 15°C. However at temperatures ranging from 20°C to 35°C, percentage larval survival of *C. rosa* R1 was significantly higher compared to *C. rosa* R2 (Table 3.3.5.3).

For *C. rosa* R1, no eclosion was recorded at 10, 33, 35°C while for *C. rosa* R2, no pupa survived at 10, 30, 33 and 35°C. The highest pupal survival rate for both *C. rosa* R1 and R2 was recorded at 15°C ($84.10 \pm 3.91\%$ and $90.06 \pm 1.94\%$, respectively). For *C. rosa* R1, the lowest survival rate was recorded at 20°C ($62.71 \pm 5.15\%$), while that of *C. rosa* R2 was $52.38 \pm 9.52\%$ at 25°C. Percentage pupal survival of *C. rosa* R1 and *C. rosa* R2 were significantly different when compared across the test temperatures.

Discussion

In this study, we found that *C. rosa* R1 was more heat tolerant but not less cold tolerant than *C. rosa* R2. As such *C. rosa* R2, often termed as the “cold” type, does not present more of a risk to export markets than *C. rosa* R1.

Developmental duration of the immature stages of both *C. rosa* types were found to decrease with increasing temperature up to 30°C, followed by a slight increase in developmental duration beyond 30°C. Immature larval stages of *C. rosa* R1 tended to develop faster across all temperatures than those of *C. rosa* R2. The larval survivorship of *C. rosa* R1 on the artificial diet at all temperatures were significantly higher than that of *C. rosa* R2 and could have led to higher metabolic heat within the diet and therefore faster development.

Although the observed developmental times of the immature stages of the two *C. rosa* types in this study were similar to those recorded in previous studies (Grout and Stoltz 2007; Duyck and Quilici 2002), the

developmental threshold values for both *C. rosa* types estimated in this study were different to the estimates derived from these previous studies. It is likely that Grout and Stoltz (2007) did their studies on *C. rosa* R1 as more recently the *C. rosa* colonies kept at CRI were identified as *C. rosa* R1. As such values estimated by Grout and Stoltz (2007) were possibly for *C. rosa* R1. Based on the findings by Virgilio et al. 2013, populations of *C. rosa* in Reunion island belong to *C. rosa* R2 and therefore values estimated by Duyck and Quilici (2002) would be for *C. rosa* R2. The T_{min} values estimated by linear model in this study for *C. rosa* R1 were higher for the egg stage and lower for the larval and pupal stages than those estimated by Grout and Stoltz (2007) also using a linear model. The T_{min} values estimated in this study for *C. rosa* R2 were in contrast higher for the egg and larval stages and lower for the pupal stages than those estimated by Duyck and Quilici (2002). Grout and Stoltz (2007) also used the Briere-1 non-linear model for estimation of developmental thresholds and the values estimated from their model were different to the estimates derived in this study using the same model. The slight differences in developmental times of the immature stages at the different temperatures between this study and the previous studies could have possibly led to bigger differences in estimated values of their developmental thresholds.

Fletcher (1989) noted that large differences in thermal requirements among various species of tephritids can be attributed to difference in experimental methodologies and geographic variation of populations. Besides geographic variation, factors such as food quantity and quality and larval density in the rearing chambers have been reported to influence the thermal requirements of larval stages of tephritids (Vargas et al. 1996; Duyck and Quilici 2002). The laboratory adaptation of the flies could also possibly have led to the differences in the estimated developmental threshold values between the different studies. Here we used flies which were reared for up to 8 generations before the start of the studies. Duyck and Quilici 2002 used flies which were reared for 25 generations or more. This might also have been the case with Grout and Stoltz (2007).

Conclusion

Our results support the existence of two genetically distinct populations of *C. rosa* that are divergent in their physiological response to temperature, in particular at the higher temperatures.

Future research

No further research is recommended on the developmental physiology of the two *C. rosa* types except for the completion of the studies on the critical thermal limits of the two types.

Given that Natal fly might be split in two species, colonies of the two *C. rosa* types will continue to be maintained and differences in the attraction of the two types to protein baits and male lures will be determined. Moreover, all future trapping records for male *C. rosa* will be categorised according to types.

Technology transfer

Not applicable.

References cited

- Barr NB, Copeland RS, De Meyer M, Masiga D, Kibogo HG, Billah M, Osir E, Wharton RA, McPherson BA (2006) Molecular diagnostics of economically important *Ceratitis* fruit fly species (Diptera: Tephritidae) in Africa using PCR and RFLP analyses. *Bulletin of Entomological Research* 96: 505-521.
- Briere JF, Pracros P, Le Roux AY, Pierre JS (1999) A novel rate model of temperature-dependent development for arthropods. *Environmental Entomology* 28: 22-29.
- Campbell A, Frazer BD, Gilbert NE, Gutierrez AP, Mackauer M (1974) Temperature requirements of some aphids and their parasites. *Journal of Applied Entomology* 11: 431-438.
- De Meyer M (2001) Distribution patterns and host-plant relationships within the genus *Ceratitis* MacLeay (Diptera: Tephritidae) in Africa. *Cimbebasia* 17: 219-228.
- De Meyer M, Freidberg A (2006) Revision of the Subgenus *Ceratitis* (Pterandrus) Bezzi (Diptera: Tephritidae). *Israel Journal of Entomology* 36: 197-315.
- De Villiers M, Manrakhan A, Addison P, Hattingh V (2013) The distribution, relative abundance, and seasonal phenology of *Ceratitis capitata*, *Ceratitis rosa*, and *Ceratitis cosyra* (Diptera: Tephritidae) in South Africa. *Environmental Entomology* 42: 831-840.
- Duyck PF, Quilici S (2002) Survival and development of different life stages of three *Ceratitis* spp. (Diptera: Tephritidae) reared at five constant temperatures. *Bulletin of Entomological Research* 92: 461-469.

- Fletcher BS (1989). Temperature – development rate relationships of the immature stages and adults of tephritid fruit flies. In: Fruit flies their biology, natural enemies and control, Vol. 3A. Ed. by Robinson AS, Hooper G, Elsevier, Amsterdam
- Grout TG, Stoltz KC (2007) Developmental rates at constant temperature of three economically important *Ceratit*s spp. (Diptera: Tephritidae) from Southern Africa. Environmental Entomology 36: 1310-1317.
- Honék A, Kocourek F (1990). Temperature and development time in insects: a general relationship between thermal constants. Zoologische Jahrbücher für Systematik 117: 401-439.
- Virgilio M, Delatte H, Quilici S, Backeljau T, De Meyer M (2013) Cryptic diversity and gene flow among three African agricultural pests: *Ceratit*s *rosa*, *Ceratit*s *fasciventris* and *Ceratit*s *anonae* (Diptera, Tephritidae). Molecular Ecology 22: 2526–2539

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

Project 1062 (Apr 2013 – Mar 2014) by T.G. Grout and P.R. Stephen (CRI)

Summary

Due to increasing usage of copper fungicides shortly before the application of protein baits for fruit fly control and the risk of this combination causing fruit phytotoxicity, an investigation of attractants containing higher amounts of yeast autolysate rather than yeast hydrolysate was conducted. A commercial yeast autolysate from Australia was less than a third as attractive as HymLure to *Ceratit*s *capitata* and *C. rosa* and locally-formulated autolysates were also inferior. Consumption of autolysates was higher after 3 h of feeding than for HymLure, but within the first hour of feeding there was no difference and adequate toxicant would have been consumed during this time. The autolysates also provided no benefit over HymLure for the control of *C. cosyra*. This research was therefore terminated without investigating whether the autolysates were safer when sprayed on copper residues because the inferior attraction of fruit flies to autolysates would compromise our control of these quarantine pests.

Opsomming

Weens die toenemende gebruik van koper swamdoders kort voor die aanvang van proteïen lokaas toediening en die gepaardgaande fitotoksisiteitsrisiko van die kombinasie, is daar tydens hierdie studie gefokus op die evaluering van lokase met 'n hoër gis outolisaat-inhoud in plaas van die erkende gis hidrolisaat. 'n Kommersiële gis outolisaat van Australiese afkoms, sowel as 'n Suid Afrikaanse geformuleerde gis outolisaat was onderskeidelik een derde en tot 'n redelike mate minder aanloklik vir *Ceratit*s *capitata* en *C. cosyra* as HymLure. Alhoewel die vlieë na 3 ure meer op die gis outolisaat gevoed het, was daar na 1 uur voedingstyd geen verskil nie. Daar sou dus genoegsame gifstof ingeneem kon word binne die eerste uur ongeag die lokaas. Verder was die outolisaat nie meer effektief as HymLure in die beheer van *C. cosyra* nie. Daarom, weens die oneffektiwiteit van gis outolisaat asook die kwarantynstatus van vrugtevlieë, is hierdie navorsingsprojek gestaak en is outolisaat nie verder ondersoek as 'n veiliger produk vir aanwending saam met koper nie.

Introduction

There is increasing pressure to stop the use of mancozeb on citrus or reduce the currently accepted residue levels. The only alternative contact fungicide for citrus black spot is copper, in various formulations, and this would need to be sprayed at least twice a season, depending on what other fungicides are used in the spray programme. When copper fungicides are sprayed during high temperatures or when protein hydrolysate fruit fly bait is applied to fruit with copper residues, permanent dark marks often result and cause downgrading of the fruit (Georgala 1969, Schutte et al. 1997). This makes the use of copper late in the season hazardous, particularly if protein bait sprays are being used for fruit fly control. Citrus growers in Queensland, Australia say they have never noticed this form of phytotoxicity after copper sprays with the yeast autolysate that they use for control of *Bactrocera tryoni* (D. Papacek, pers. comm.). If yeast autolysate is more palatable than our salty protein hydrolysates and the flies consume larger quantities we may be able to lower the amount of toxicant used in our fruit fly baits. We therefore wanted to evaluate yeast autolysates to determine how effective they are for our fruit flies and if equivalent or better than our protein hydrolysates to check their phytotoxicity on copper residues and evaluate combinations with toxicants against *B. dorsalis*, *Ceratit*s *capitata* and *C. rosa*.

Stated objectives

1. Find a yeast autolysate formulation that is at least as attractive as HymLure for Medfly and Natal fly, but more palatable.
2. Test this product for copper phytotoxicity relative to HymLure.
3. Test the best combination with malathion and spinosad against Medfly, Natal fly and *B. dorsalis*.

Materials and methods

HymLure as manufactured by Pioneer Foods contains an approximate 50:50 mixture of yeast hydrolysate and yeast autolysate. This product has successfully been used for the control of *Ceratitis capitata* and *C. rosa* in South Africa since 1987. Pioneer Foods provided us with two altered HymLure formulations containing 80% autolysate and 100% autolysate. We also obtained Bugs for Bugs' Fruit Fly Lure from Queensland, Australia which is a yeast autolysate (Meats and Kelly 2008) that contains no salt. Although *C. cosyra* is not a citrus pest we included this fly in the trials because it is known to be less attracted to protein baits than the other two *Ceratitis* species and we thought it may respond more to yeast autolysate.

Attraction

Relative attraction to products was determined by placing diluted products in McPhail bucket traps that had been painted black in two field cages and releasing protein-starved flies. Water only was included as a control with the other four treatments. The concentrations of the yeast extracts used were all 2% and approximately 250 ml mixture was poured into the bottom of each trap. All three species of flies, aged approximately 10 d, were released simultaneously from a central point in each cage at 09h30 and left to disperse for an hour before the five treated traps were hung in the cage. One hundred flies of each sex and each species were released in each cage. The traps were randomly placed in the cage initially but they were rotated one position every 2 h during daylight and finally removed after 24 h exposure. Two replicates were conducted simultaneously using both cages and the trial was conducted three times on 19 March, 18 April and 25 April 2013. Numbers of flies captured per trap were expressed as a percentage of the total number caught in that cage on that day to reduce variability between days. These percentages were square root arc sine transformed before being statistically analysed.

Palatability

The products were evaluated for palatability by comparing actual amounts of bait solution consumed by flies using J-tubes (Nigg et al. 2004, Nigg et al. 2007). The J-tubes were made from Marienfeld capillary tubes with an internal diameter of 1.1mm such that 1 mm length of tube contained 1 μ l of liquid. A filling mark was etched into the long arm approximately 20 mm above the mouth of the short arm. The tube was fixed into a closed cell plastic foam stopper so that the short arm could be inserted into a test cage while the long arm remained on the outside (Figure 3.3.6.1). The female flies used were 10-14 d old adults that had been fed only sugar and water with no protein. All three *Ceratitis* species were used. Bait mixtures were prepared at 2% and green food colouring (1% Moir's double concentrated green food colour) was added to enable identification of flies that had fed by the green colour of their abdomens. Small, transparent test cages were constructed from 600 ml disposable food containers (10.4 cm diameter x 7 cm high). A hole (22.5 mm diameter) was cut in the side for insertion of the J-tube plug and a large hole was cut in the lid and covered with coarse mesh (Figure 3.3.6.2). Sufficient flies were first taken from the rearing cage and placed in a glass-topped box. Then 10 females per replicate were aspirated, cooled and placed in each test cage at least 1 h before each test. A small amount of granulated sugar was placed in each test cage and coarse drops of distilled water were sprayed into the cage every hour. For every treatment, 5 cages were prepared with flies each and 5 cages with no flies for assessment of evaporation loss. The bait mixtures with colourant were injected into the J-tubes using a small syringe fitted with a needle. Care was taken to fill the J-tubes exactly to the calibration mark and they were then placed in the test cages. The exact time of introduction was noted for each replicate.

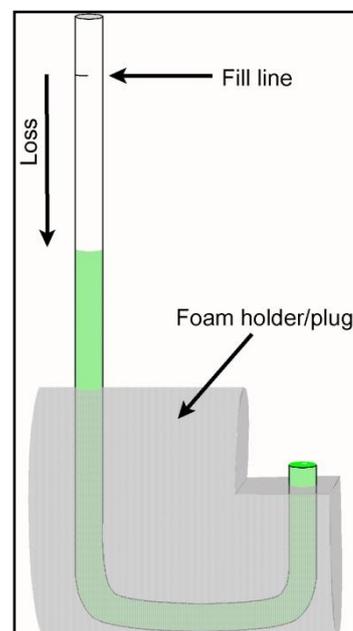


Figure 3.3.6.1. J-tube.

A digital caliper was used to measure the amount of bait mixture eaten/evaporated and these measurements plus assessments of the number of flies fed (green) were conducted after 1 and 3 h. The mean evaporation rate in cages with no flies was used to correct the ingested bait volume. Trials were conducted on 3 May (*C.*

capitata), 7 May (*C. rosa*) and 9 May 2013 (*C. cosyra*). A combined trial was conducted on 20 January 2014 where all three species were evaluated simultaneously on standard HymLure and Fruit Fly Lure.



Figure 3.3.6.2. Test cages containing fruit flies with J-tubes in place

Results and discussion

Attraction

When comparing treatments across all species and sexes, HymLure attracted the highest proportion of flies and this was significantly more than any other treatment ($P < 0.05$) (Table 3.3.6.1). There was no significant difference between the two HymLure formulations with higher concentrations of autolysate, although they both attracted significantly more flies than the Australian Fruit Fly Lure. The Fruit Fly Lure did attract significantly more flies than water alone which attracted no flies. When the sexes were compared over all species and all treatments, significantly more females were attracted to the baits than males ($P < 0.05$). This is understandable because the females need the protein for egg maturation. When the attraction of the species was compared for all sexes and all treatments, Medfly responded the most to the attractants and significantly more than the other two species. Trap catches of *C. rosa* were significantly higher than *C. cosyra* and the lowest numbers of *C. cosyra* were caught with Fruit Fly Lure, so the yeast autolysate certainly did not improve efficacy against *C. cosyra*. Overall, 632 flies were captured from 3 600 released, or 17.6%.

Table 3.3.6.1. Total numbers of flies caught per treatment and category over 6 replicates

Treatments	Medfly <i>C. capitata</i>		Natal fly <i>C. rosa</i>		Marula fly <i>C. cosyra</i>		Totals
	Male	Female	Male	Female	Male	Female	
Water only	0	0	0	0	0	0	0 d
HymLure standard	67	102	14	27	9	16	235 a
HymLure 80% autolysate	24	49	19	25	2	12	131 b
HymLure 100% autolysate	58	80	14	27	7	9	195 b
Fruit Fly Lure (Bugs for Bugs)	15	41	4	4	1	6	71 c
Grouped factors:	All males	All females		All Med	All Natal	All Marula	
Total nos. caught & stat diffs	234 q	398 p		436 x	134 y	62 z	

Statistical differences were based on transformed proportions of flies caught in each replicate with $\alpha = 0.05$

Palatability

When all four attractants were compared using female *C. capitata*, the only significant difference in consumption was the total amount consumed in 5 replicates in 3 h of Fruit Fly Lure autolysate compared to HymLure 100% autolysate (Table 3.3.6.2), with the former being more palatable than the latter. With the other two *Ceratitis* species there were no significant differences between any of the amounts consumed. With *C. capitata* and *C. rosa* there was a trend for HymLure to be consumed the most within the first hour after exposure and for Fruit Fly Lure autolysate to be consumed later in the 3 h period. The autolysates did not appear to be any more palatable to *C. cosyra* than HymLure. When the three species were compared simultaneously using HymLure and Fruit Fly Lure there was no significant difference in the total amounts of each attractant consumed by all flies in the first hour of feeding when considered as a volume per treatment or volume per fly (Table 3.3.6.3). However, in the second and third hour of feeding, significantly more Fruit Fly Lure was consumed overall per treatment than HymLure ($P < 0.05$) but this difference was not significant when expressed per fly. This did confirm the trend found in the previous palatability studies where consumption of autolysates by *C. capitata* and *C. rosa* sometimes increased considerably in hours 2 and 3 after exposure. This may be due to lower salt content in the autolysates. In the simultaneous species comparison, *C. cosyra* consumed significantly less of either attractant than the other two species at both 1 h and 3 h after initial exposure. This confirmed that yeast autolysate did not offer any advantage for the control of *C. cosyra*.

Table 3.3.6.2. Quantities of attractant consumed by female flies per treatment and per fly via J-tubes (corrected for evaporation) in two different periods after exposure

Trts.	<i>C. capitata</i> Medfly (3 May)				<i>C. rosa</i> Natal fly (7 May)				<i>C. cosyra</i> Marula fly (9 May)			
	1 h		3 h		1 h		3 h		1 h		3 h	
	µL/trt	µL/fly	µL/trt	µL/fly	µL/trt	µL/fly	µL/trt	µL/fly	µL/trt	µL/fly	µL/trt	µL/fly
HymL	63.4a	1.88a	57.0ab	1.41a	40.5a	1.40a	49.7a	1.31a	18.9a	1.18a	28.0a	0.89a
H80	34.5a	1.68a	51.4ab	1.57a	42.4a	1.80a	52.9a	1.66a	10.6a	0.90a	10.2a	0.26a
H100	40.9a	1.23a	49.6b	1.40a	7.8a	0.71a	35.2a	1.25a	6.4a	0.70a	6.7a	0.01a
FFL	59.0a	2.01a	64.2a	1.43a	24.6a	1.65a	56.7a	1.70a	3.6a	0.69a	8.5a	1.08a

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

Table 3.3.6.3. Quantities of attractant consumed by female flies of all three *Ceratitis* species compared simultaneously

Treatments	Fly species	1 h		3 h	
		µL/trt	µL/fly	µL/trt	µL/fly
HymLure standard	<i>C. capitata</i>	47.1	1.48	49.0	1.21
	<i>C. rosa</i>	51.9	1.71	55.6	1.56
	<i>C. cosyra</i>	7.2	0.32	25.5	0.64
Fruit Fly Lure autolysate	<i>C. capitata</i>	69.5	1.65	74.6	0.55
	<i>C. rosa</i>	60.9	1.96	72.9	0.54
	<i>C. cosyra</i>	2.4	0.01	15.1	0.59
Contrasts:	HymLure vs. Fruit Fly Lure	P=0.151 NS	P=0.821 NS	P=0.040 Signif	P=0.248 NS
Species consumption (per replicate)	<i>C. capitata</i>	11.66 a	1.57 a	12.36 a	1.44 a
	<i>C. rosa</i>	11.28 a	1.84 a	12.85 a	1.58 a
	<i>C. cosyra</i>	0.96 b	0.29 b	4.05 b	0.62 b

Conclusion

The total number of all three species of *Ceratitis* attracted to the standard HymLure was significantly higher than any of the other attractants containing more yeast autolysate. HymLure was more than three times more attractive than the Australian Fruit Fly Lure to these species. In the first hour after exposure to the attractants, *C. capitata* and *C. rosa* consumed similar quantities of all attractants, but more than *C. cosyra*. In the second and third hour of feeding, consumption of HymLure tapered off but feeding on the autolysates continued. Due to the inferior attraction of the autolysates the research was terminated because any possible benefit from less phytotoxicity with copper fungicides would be compromised by poorer fruit fly control.

Future research

No future research on yeast autolysates is planned unless they prove to be much more effective against *Bactrocera dorsalis* than protein hydrolysates.

Technology transfer

It will be mentioned at grower meetings that autolysates do not offer a solution to phytotoxicity from copper and if time allows, this information may be published.

References cited

- Georgala, M. B. 1969. Control of false codling moth and fruit flies in citrus orchards. The S.A. Citrus Journal 421: 3, 5, 7.
- Meats, A., and G. L. Kelly. 2008. Relation of constant, daily fluctuating, and ambient feeding temperature to daily and accumulated consumption of yeast autolysate and sucrose by female Queensland fruit fly. Entomologia Experimentalis et Applicata 129: 87-95.
- Nigg, H. N., R. A. Schumann, J. Yand, and S. Fraser. 2007. Consumption of bait solutions by *Anastrepha suspensa*. Florida Entomologist 90: 370-377.
- Nigg, H. N., R. A. Schumann, J. J. Yang, L. K. Yang, S. E. Simpson, E. Etxeberria, R. E. Burns, D. L. Harris, and S. Fraser. 2004. Quantifying Individual Fruit Fly Consumption with *Anastrepha suspensa* (Diptera: Tephritidae). Journal of Economic Entomology 97: 1850-1860.
- Schutte, G. C., K. V. Beeton, and J. M. Kotze. 1997. Rind Stippling on Valencia Oranges by Copper Fungicides Used for Control of Citrus Black Spot in South Africa. Plant Disease 81: 851-854.

3.3.7 PROGRESS REPORT: Dispersal capacity of *Bactrocera dorsalis*

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

Summary

The key outcome of this project is to establish the dispersal capacity of the invasive fruit fly, *Bactrocera dorsalis*, with regard to environmental and physiological variables. Now that contracts have been signed and funds are available, all effort is being made to work towards reaching the objectives of the project. The PhD student assigned to the project, Mrs Louisa Makumbe, is making reasonable progress towards two research objectives: to determine the best pigment dose and colours for marking *B. dorsalis* in dispersal experiments, and to establish the dispersal capacity of *B. dorsalis* in relation to sex, maturity and environmental variables. Research to date indicates that a pigment dose of at least 2 g/L pupae is suitable for marking *B. dorsalis* for at least two weeks before beginning to fade. The first of four releases for the dispersal study is ongoing. One hundred traps (50 methyl eugenol, 50 BioLure), have been installed at each of three study sites near Louis Trichardt in Limpopo province, and are supplemented by traps installed by the Department of Agriculture, Forestry and Fisheries. Two shipments of 30,000 sterile *B. dorsalis* pupae of African origin have been received from the International Atomic Energy Agency, marked with fluorescent pigments, and released at the sites. Flight mills are currently being built to address how flight of *B. dorsalis* is affected by temperature to aid in understanding field dispersal results. An associated project completed by an Honours student, Mr Sandiso Mnguni, found that fruit flies reared in a low-protein larval diet (such as fruit) led to adults that were more desiccation resistant than when they developed in a high protein larval diet.

Opsomming

Die hoofdoel van die projek is om die verspreidingsvermoë van die indringer vrugtevlug, *Bactrocera dorsalis*, te bepaal met betrekking tot die omgewings – en fisiologiese veranderlikes. Weens die suksesvolle betekening van alle kontrakte, en volle toegang tot beskikbare bevondsing, word alle hulpbronne tans aangewend ten einde die projek doelwitte te bereik. Mev Louisa Makumbe, die Doktorale student aan wie die projek toegeken is, vorder redelik ten opsigte van twee projek doelwitte: om eerstens die mees geskikte pigment dosisse en kleure vir die merk van *B. dorsalis* te bevestig, en tweedens om die verspreidingsvermoë van *B. dorsalis* te bepaal in verhouding tot geslag, volwassenheid en omgewingsveranderlikes. Navorsing tot op hede dui aan dat 'n pigment dosis van minstens 2 g/L papies geskik is vir die merk van *B. dorsalis* vir 'n tydperk van twee weke, voordat kleur begin kwyn. Die eerste van vier vrylatings ten opsigte van die verspreidingsstudie word tans onderneem. Dit het die installasie van 100 lokvalle (50 metiel eugenol, 50 BioLure) by drie studie areas naby Louis Trichardt in Limpopo behels, met ondersteunende lokvalle wat geïnstalleer is deur die Departement van Landbou, Bosbou en Visserye (DAFF). Twee vragte van 30,000

sterielle *B. dorsalis* papies, oorspronklik vanuit Afrika, is ontvang vanaf die Internasionale Atomiese Energie Agentskap (IAEA), elk gemerk met fluoresserende pigmente, en by die studie areas vrygelaat. Vlugwaarnemende harnasse word tans gebou om die uitwerking van temperatuur op die vlug van *B. dorsalis* te bestudeer, ten einde verspreidingspatrone beter te interpreteer. Verwante navorsing wat deur Mnr. Sandiso Mnguni, 'n hoonersstudent onder die toesig van Dr. Weldon, voltooi is, dui aan dat vrugtevlieglarwes op 'n lae-proteïen dieet (vrugte as voorbeeld) lei tot meer waterstres bestandheid in volwasse vlieë, as vir larwes op 'n relatief hoër proteïendieet.

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

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

Summary

There have been a number of incursions of *Bactrocera dorsalis*, previously recognized as *B. invadens*, in the northern parts of South Africa since 2010. Incursions of *B. dorsalis* were eradicated successfully in all areas between 2010 and 2011. Incursions of *B. dorsalis* however recurred in a number of areas in consecutive years between 2010 and 2012. In 2013, the pest was declared present in the Vhembe district, Limpopo province, and reported in other districts in Limpopo, Mpumalanga, North-West, Gauteng and KwaZulu Natal provinces. This project aims to understand the *B. dorsalis* incursion patterns observed in the northern parts of South Africa through genetic analysis of the specimens captured in the various areas across the years. *Bactrocera dorsalis* samples collected from locations in Provinces which were affected will be used for the study. Sample size will vary between 2 and 10 flies per location per year. *Bactrocera dorsalis* samples from other African countries and some Asian countries, will also be included in the analysis to allow comparison with a population where the pest is established. Individual flies will be genotyped at eleven previously characterized polymorphic microsatellite loci. Genetic diversity estimates will be derived for each sample. These estimates will then be used to determine the presence of population relatedness, which should provide an indication of the incursion pattern of *B. dorsalis* in South Africa.

Opsomming

Daar was sedert 2010 'n aantal inkursies van *Bactrocera dorsalis*, voorheen bekend as *B. invadens*, in die noordelike dele van Suid-Afrika. Inkursies van *B. dorsalis* is suksesvol in alle areas tussen 2010 en 2011 uitgewis. Inkursies van *B. dorsalis* het egter weer in 'n aantal areas in opeenvolgende jare tussen 2010 en 2012 voorgekom. In 2013 is verklaar dat die plaag teenwoordig was in die Vhembe distrik, Limpopo provinsie, en aangeteken in ander distrikte in Limpopo, Mpumalanga, Noordwes, Gauteng en KwaZulu-Natal provinsies. Hierdie projek poog om die *B. dorsalis* inkursiepatrone wat in die noordelike dele van Suid-Afrika waargeneem is, deur genetiese analise van monsters wat in die verskeie areas deur die jare versamel is, te verstaan. *Bactrocera dorsalis* monsters wat in areas geneem is in provinsies wat geaffekteer is, sal vir die studie gebruik word. Monstergrootte sal tussen 2 en 10 vlieë per area per jaar varieer. *Bactrocera dorsalis* monsters van ander Afrika lande en sommige Asiatiese lande, sal ook in die analise ingesluit word, ten einde vergelyking met 'n populasie waar die pes al gevestig is, toe te laat. Individuele vlieë sal by elf voorheen gekarakteriseerde polimorfiese mikrosatelliet loci gegenotipeer word. Genetiese diversiteitskattings sal vir elke monster afgelei word. Hierdie skattings sal dan gebruik word om die teenwoordigheid van populasie verwantskappe te bepaal, wat 'n aanduiding behoort te gee van die inkursiepatroon van *B. dorsalis* in Suid-Afrika.

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

Project 1107 (2014/15 – 2016/17) by Christopher Weldon (UP) & Aruna Manrakhan (CRI)

Summary

The key outcomes of this project are to determine the fruit species and varieties used by the invasive fruit fly, *Bactrocera dorsalis*, with a particular emphasis on citrus, and the properties of citrus varieties that make them more or less susceptible to attack. The PhD student assigned to the project, Ms Charmaine Theron, has achieved excellent progress towards determining the host range of *B. dorsalis* within its current distribution in South Africa. Since July 2014, Ms Theron has been conducting monthly fruit sampling in tandem with adult trapping in agricultural and untransformed areas of Limpopo and Mpumalanga. Host use

patterns in South Africa are currently coinciding with records from central and northern Africa, with *B. dorsalis* emerging from mango (*Mangifera indica*), cashew (*Anacardium occidentale*) and wild tobacco (*Solanum mauritianum*). However, previous studies did not separate fruit collected from the ground and the tree, and so far, *B. dorsalis* have only been found to emerge from fruit that had fallen to the ground and in areas where high numbers of adults are caught in traps. *Bactrocera dorsalis* has not yet been recovered from any sampled citrus varieties. Adult captures at all sites were low or absent from July to November 2014 before increasing dramatically from December 2014. This increase was most pronounced in cultivated areas where little or no fruit fly management was practiced. Traps in untransformed habitats caught relatively few adults. These results suggest that orchard sanitation and removal of the invasive *S. mauritianum* should complement management of *B. dorsalis* with bait sprays and mass trapping. A *B. dorsalis* culture has also been established at CRI in Nelspruit to permit assessments of egg laying in grapefruit, lemon, orange and soft citrus varieties.

Opsomming

Die projek se hoofdoel is om die vrugte spesies en variëteite wat benut word deur die indringer-vrugtevlieg, *Bactrocera dorsalis*, vas te stel, met klem op sitrus soorte en die eienskappe van sitrus variëteite wat die spesie vatbaar maak tot aanval. Die PhD student aan wie die projek toegeken is, Me. Charmaine Theron, het reeds uitstekende vordering gemaak ten einde die verskillende draers van *B. dorsalis* vas te stel, inaggenome die huidige verspreiding in Suid-Afrika. Me. Theron het vanaf Julie 2014 maandeliks monsters van vrugte geneem, asook 'n lokval skema vir volwasse vrugtevlieë op landbou en onbewerkte areas in Limpopo en Mpumalanga onderneem. Draer-gebruik patrone in Suid Afrika stem tans ooreen met patrone in Sentraal- en Noord-Afrika, waar *B. dorsalis* vanuit mango (*Mangifera indica*), (*Anacardium occidentale*) en wilde tabak (*Solanum mauritianum*) ontsnap. Vorige studies het egter nie vrugte wat reeds op die grond was geskei van die wat steeds in die boom was nie, en dit is tot op hede bevestig dat *B. dorsalis* slegs vanuit vrugte op die grond ontsnap, in areas waar hoë getalle volwassenes in lokvalle gevang word. *Bactrocera dorsalis* is tot op hede nie waargeneem in sitrus variëteite nie. Getalle vir volwasse vangstes by alle studie areas was laag tot afwesig tussen Julie en November 2014, waarna dit drasties toegeneem het vanaf Desember 2014. Die toename was meer sigbaar in bewerkte liggings waar min tot geen vrugtevlieg beheer in plek was nie. Lovalle in onbewerkte liggings het betreklik min volwassenes tot gevolg gehad. Die getalle dui aan dat opgeruimde boorde waar die indringer, *S. mauritianum*, verwyder is, die beheer van *B. dorsalis* met lokaas bespuitings en massa-lokval stelsels sal komplimenteer. 'n Kultuur vir *B. dorsalis* is ook by die CRI in Nelspruit gevestig, weens die behoefte om eierplasing in pomelo, suurlemoen, lemoen en sagtesitrus te assesseer.

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

Project ERAfrica (project funded by Department of Science and Technology: Jan 2014- Jan 2017) by Aruna Manrakhan, John-Henry Daneel, Rooikie Beck (CRI), Charmaine Theron, Christopher Weldon, Louisa Makumbe (UP), Caroline Knox (RU), Marc De Meyer (Royal Museum for Central Africa), François Hala N'Klo (Centre National de Recherche Agronomique), Serge Quilici, Helene Delatte, Pierre Francois Duyck (CIRAD)

Summary

The ERAfrica fruit fly project aims to develop effective and accurate detection methods for fruit fly pests. The efficacy of different trap and attractant combinations for fruit fly monitoring were determined. Six study sites were selected in commercial and non-commercial fruit production areas in Limpopo and Mpumalanga. A total of 10 fruit fly attractants were selected for evaluation. Out of the 10 attractants, 4 were considered as new attractants in Africa. In each site, 30 traps were set up representing three replicates of each attractant. At each study site, the traps are checked and emptied on a fortnightly to monthly basis. The attractants inside the traps were replaced on a monthly basis. Trap samples collected were brought back to the laboratory at CRI for identification. Biolure 3 component seems to be the most effective food-based attractant for monitoring Oriental fruit fly and other *Ceratitidis* pest species. EGO Pherolure was more effective in monitoring *Ceratitidis* males other than Medfly. Methyl Eugenol was specific to Oriental fruit fly. Number of Oriental fruit fly increased significantly in January and February 2015. Catches of Oriental fruit fly were 6 times higher in natural unsprayed areas than in commercial citrus areas.

Opsomming

Die ERAfrica vrugtevliegprojek het ten doel om effektiewe en akkurate waarnemingsmetodes vir vrugtevliegplae te ontwikkel. Die effektiwiteit van verskillende lokval en lokaas kombinasies vir vrugtevliegmonitering, is bepaal. Ses studiepersele is in kommersiële en nie-kommersiële vrugte produksie-areas in Limpopo en Mpumalanga geselekteer. 'n Totaal van 10 vrugtevlieg lokase is vir evaluasie geselekteer. Uit die 10 lokase, is 4 as nuwe lokase in Afrika beskou. In elke perseel, is 30 lokvalle opgestel wat drie herhalings van elke lokaas verteenwoordig. By elke studieperseel, is die lokvalle op 'n twee-weeklikse tot maandelikse basis nagegaan en leeggemaak. Die lokase binne die lokvalle is op 'n maandelikse basis vervang. Lokvalmonsters wat versamel is, is na die laboratorium by CRI vir identifikasie teruggebring. Biolure 3 komponent blyk die mees effektiewe voedingsgebaseerde lokaas vir monitering van Oosterse vrugtevlieg en ander *Ceratitis* plaagspesies te wees. EGO Pherolure was meer effektief in monitering van *Ceratitis* mannetjies anders as die Mediterreense vlieg. Metiel Eugenol was spesifiek vir die Oosterse vrugtevlieg. Die aantal Oosterse vrugtevlieë het betekenisvol in Januarie en Februarie 2015 toegeneem. Vangste van Oosterse vrugtevlieë was 6 keer hoër in natuurlike ongespuite areas as in kommersiële sitrus-areas.

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

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

Summary

A field trial to determine the performance of different male annihilation methods for *B. dorsalis* was conducted in Star Ruby grapefruit orchards in Constantia, Limpopo Province for a period of 14 weeks including a pre-treatment and a post-treatment week. Six male annihilation treatments (MAT) were compared. Two different densities of Invader-b-lok were also tested. A control with no male annihilation treatment was also included in the trial. All treatments were combined with the M3 bait stations. The efficacy of the treatments was determined by adult fly trapping and fruit damage assessment. There were no differences in catches of *B. dorsalis* between the different treatments. *Bactrocera dorsalis* catches were higher in blocks treated with only M3 bait stations compared to blocks treated with a combination of male annihilation treatment and M3 bait stations. No fruit fly infestation was recorded in any of the treatments. A repeat of the trial is being carried out in the year 2015-2016.

Opsomming

'n Boordproef om te bepaal hoe verskillende mannetjie uitwissingstegnieke vir *B. dorsalis* presteer, was in 'n Star Ruby pomeloboord te Constantia, Limpopo Provinsie oor 'n tydperk van 14 weke, insluitende 'n voor- en nabehandeling week, uitgevoer. Ses mannetjie uitwissingstegnieke is vergelyk. Twee verskillende digthede van Invader-b-lok is ook getoets. 'n Kontrole met geen mannetjie uitwissingstegniek is ook in die proef ingesluit. Alle behandelings is met M3-lokaasstasies gekombineer. Die behandelings se effektiwiteit is deur die vangste van volwasse vlieë en vrugskadeopnames bepaal. Daar was geen verskil in die vangstes van *B. dorsalis* tussen die verskillende behandelings nie. *Bactrocera dorsalis* vangstes was hoër in blokke wat slegs met M3-lokaasstasies behandel is, in vergelyking met blokke wat met 'n kombinasie van die mannetjie uitwissingstegniek en M3-lokaasstasies behandel is. Geen vrugtevlieg besmetting is in enige van die behandelings waargeneem nie. 'n Herhaling van die proef word in jaar 2015-2016 uitgevoer.

3.4 **PROGRAMME: MEALYBUG AND OTHER MARKET ACCESS PESTS**

Programme coordinator: Sean D Moore (CRI)

3.4.1 **PROGRAMME SUMMARY**

Although mealybug is the main pest mentioned in the name of this programme, only one of the four projects within the programme focussed on mealybug during the last research cycle, and neither did this project focus exclusively on mealybug. The remaining three projects focussed on carob moth, which is often closely associated with mealybug. Carob moth is not a phytosanitary pest for most markets. However, there are exceptions, such as China. Carob moth has always been considered as a secondary and sporadic pest with negligible pest status. However, its occurrence on citrus now appears to be higher than originally observed, at least in some production regions. Additionally, the morphological similarity of its larvae to those of false codling (FCM) moth have made its recognition and management more important.

The only project that involved mealybug, also included other pests such as grain chinch bug and FCM. The aim of the project was to evaluate GRAS fumigants for post-harvest disinfestation of fruit from phytosanitary pests in general (3.4.3). Vapormate was highly effective in controlling grain chinch bug, but not as effective against mealybug and FCM. Carbon dioxide was more effective against FCM, particularly when followed by a short cold treatment, inducing 100% mortality.

The first carob moth project examined the morphology and ecology of carob moth in the Western Cape (3.4.2). A user-friendly identification key for all life stages of carob moth was developed, particularly pointing out morphological differences with FCM. In a field study, a clear seasonal cycle history of the carob moth was shown, which closely followed the phenology of the citrus tree in orchards in the Western Cape. The second carob moth study, investigated the relationship between carob moth and FCM and their two major cultivated hosts, pecan nuts and citrus, in the Vaalharts region (3.4.4). Indications are that carob moth infestation of both pecans and citrus is higher than for FCM, and that both carob moth and FCM shuttle between the two crop types. The final carob moth study was initiated ad hoc due to the unexpected finding that up to 60% of larvae infesting citrus fruit in one region were carob moth (3.4.5). Consequently it was considered of great urgency to quantify the pest status of carob moth on citrus in all production regions in the country and to develop reliable monitoring and control strategies for carob moth.

PROGRAMPSOMMING

Alhoewel witluis die hoofplaag is wat in die naam van dié projek genoem word, het net een van die vier projekte in die program gedurende die laaste navorsingssiklus op witluis gefokus, en die fokus was ook nie eksklusief op witluis nie. Die oorblywende drie projekte het op karobmot gefokus, wat dikwels nou verband hou met witluis. Vir meeste markte is karobmot nie 'n fitosanitêre plaag nie, maar daar bestaan sekere uitsonderings soos China. Karobmot is altyd as 'n sekondêre en sporadiese plaag beskou met 'n besondere lae plaagstatus. Sy voorkoms op sitrus blyk nou egter hoër as wat voorheen waargeneem is. Nog 'n faktor wat sy herkenning en bestuur meer belangrik maak is die morfologiese ooreenkoms van sy larwes met dié van die valskoldingmot (VKM).

Die enigste projek wat witluis ingesluit het, het ook ander plae soos graanstinkbesie en VKM ingeluit. Die doel van die projek was om GRAS na-oes berokingsmiddels vir disinfestasië van vrugte van fitosanitêre plae in die algemeen te evalueer (3.4.3). Vapormate was hoogs doeltreffend in sy beheer van die graanstinkbesie, maar nie so doeltreffend teen witluis en VKM nie. Koolstofdiksied was meer doeltreffend teen VKM, veral waar 'n kort koue behandeling gevolg het, en het 100% mortaliteit veroorsaak.

Die eerste karobmot projek het die morfologie en ekologie van karobmot in die Wes-Kaap ondersoek (3.4.2). 'n Verbruiker-vriendelike identifikasie sleutel vir alle lewensstadiums van karobmot is ontwikkel en het veral morfologiese verskille tussen karobmot en VKM uitgewys. In 'n veldstudie is 'n duidelike seisoenale siklus geskiedenis van die karobmot opgeleë, wat die fenologie van die sitrusboom in boorde in die Wes-Kaap baie nou gevolg het. Die tweede karobmot studie het die verhouding tussen karobmot en VKM en hulle twee hoof gekweekte gashere in die Vaalharts streek, sitrus en pekanneute, ondersoek (3.4.4). Aanduidings is dat karobmot besmetting van albei pekanneute en sitrus hoër is as vir VKM, en dat albei karobmot en VKM tussen die twee gewasse beweeg. Die finale karobmot studie is ad hoc begin as gevolg van die onverwagte bevinding dat tot 60% van larwes wat sitrusvrugte in een streek besmet het wel karobmot was (3.4.5). As gevolg hiervan is dit as baie dringend beskou om die plaagstatus van karobmot op sitrus in alle produksie streke in die land te bepaal en om betroubare moniterings stelsels en beheer strategieë vir karobmot te ontwikkel.

3.4.2 FINAL REPORT: The morphology and ecology of the Carob moth in citrus orchards US/ENT-11-A3 (2012/7-2014/12) by P Addison, G Morland, and H Geertsema (SU)

Summary

The Carob moth, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae: Phycitinae) became known initially as a Mediterranean pest of stored commodities such as pods of the Carob tree (*Ceratonia siliqua*) and dates, but became a pest of phytosanitary concern in South Africa when recorded in 1974 as a pest of citrus in the Citrusdal area in the Western Cape. Since then it has been a pest of questionable concern to the citrus industry. In its larval stage the Carob moth is often confused with that of the False Codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), presenting a problem when contaminated fruit exports are intercepted at ports of entry. The aim of this study was thus to establish some guidelines for the

development of an integrated pest management programme, which will enable growers to more effectively manage Carob moth infestations as well as to present morphological detail to facilitate definite identification of the Carob moth in all of its life stages. This was achieved by collating and screening all available literature, ranging from obscure historical to modern texts, to arrive at a clear understanding of key morphological features of use to classify the Carob moth from ordinal to the species level. These features were then used and supplemented to produce a detailed morphological study of the Carob moth's life cycle. Morphological detail was then condensed into a user-friendly key based on and restricted to the most distinguishing characteristics to aid the identification of the Carob moth and the False Codling moth and to point out morphological characteristics separating the two species. A field study was also carried out in the Western Cape to determine the Carob moth's seasonal cycle within local citrus orchards. This was determined by using a pheromone based trapping system and a set protocol for damage assessment by actively monitoring for two growing seasons. A pheromone lure preference trial was conducted in all areas of study to assess two commercially available lures. The outcomes of this study aim towards a better understanding of the nomenclatorial and morphological history of the Carob moth, as well as serving as a user friendly morphological identification key. The field results showed a clear seasonal cycle history of the Carob moth within citrus orchards of the Western Cape, closely following the phenology of the citrus tree. A lure preference was recorded for only one of the study areas. The Carob moth was found to be a minor pest, compared to False Codling moth, and presented more of an economic threat in certain areas with suitable hosts. A longer study should be undertaken to ascertain factors affecting the sporadic nature of the pest.

Opsomming

Die karobmot, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae: Phycitinae), was aanvanklik bekend as 'n Mediterreense plaag van gestoorde produkte, soos byvoorbeeld die peule van die Johannesbroodboom (*Ceratonia siliqua*) en dadels, maar het 'n plaag van fitosanitêre belang in Suid-Afrika geword toe dit in 1974 as 'n plaag van sitrus in die Citrusdal gebied in die Wes-Kaap bekend geword het. Sedertdien is dit 'n plaag van groot belang vir die sitrusbedryf. In sy larwale stadium word die karobmot dikwels verwar met dié van die valskodlingmot, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), wat op sy beurt 'n probleem veroorsaak wanneer besmette uitvoervrugte by invoerhawens onderskep word. Die doel van hierdie studie was dus gemik om sekere riglyne daar te stel vir die ontwikkeling van 'n geïntegreerde plaagbestuurprogram wat produsente in staat sal stel om besmettings van die karobmot beter te bestuur, asook om morfologiese besonderhede wat die akkurate identifikasie van die karobmot in al sy stadia bevorder beskikbaar te stel. Dit is bereik deur die samestelling en nagaan van alle beskikbare literatuur, wisselend van skaars histories tot modern, om sodoende 'n duidelike begrip van sleutel morfologiese kenmerke om die karobmot van ordinale tot spesiesvlak te klassifiseer, te verkry. Hierdie kenmerke is dan gebruik en aangevul om 'n gedetailleerde morfologiese studie van die lewenssiklus van die karobmot te bewerkstellig. Morfologiese besonderhede is dan gekondenseer tot 'n gebruikersvriendelike sleutel gebaseer op, en beperk tot, die mees onderskeidende kenmerke as hulp by die identifikasie van die karobmot en die valskodlingmot om morfologiese kenmerke wat die twee spesies skei, uit te wys. 'n Veldstudie is in die Wes-Kaap uitgevoer om die karobmot se seisoenale siklus in sitrusboorde te bepaal. Dit is bereik deur die gebruik van 'n feromoon-gebaseerde lokvalsisteem en 'n gestelde protokol vir skadebepaling deur aktiewe monitoring gedurende twee groeiseisoene. 'n Feromoon voorkeurproef is ook in alle studiegebiede uitgevoer. Die uitkomst van hierdie studie poog om 'n beter begrip van die nomenklatoriese en morfologiese geskiedenis van die karobmot te voorsien, en ook om as 'n gebruikersvriendelike morfologiese identifikasie sleutel te dien. Die veldresultate toon 'n duidelike seisoenale siklus geskiedenis van die karobmot, in ooreenstemming met die fenologie van sitrus in sitrusboorde van die Wes-Kaap. 'n Lokaasvoorkeur is vir net een van die studiegebiede aangeteken. Die karobmot was gevind as 'n plaag van minder belang as die valskodlingmot. Dit was ook gevind dat die karobmot meer ekonomies skadelik is in areas waar daar meer toepaslike gasheer is. 'n Langer studie is nodig om al die faktore in te reken.

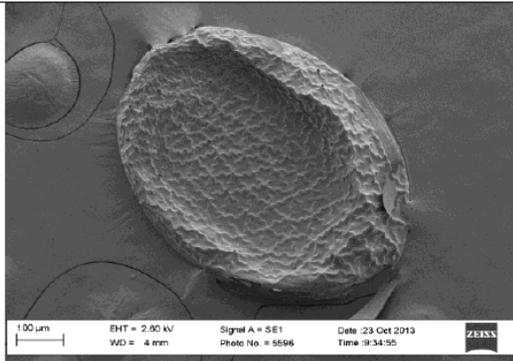
Results and discussion

By virtue of the Carob moths' morphology, the following key was designed to assist advisors and researchers in accurate separation of the two species, Carob moth and False Codling moth (Table 3.4.2.1).

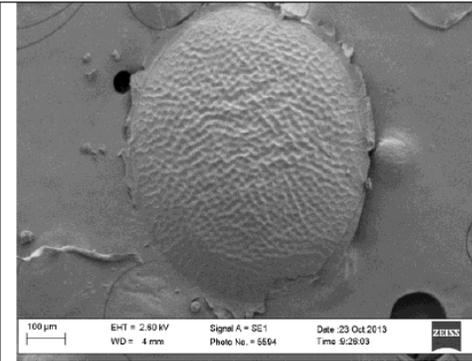
Table 3.4.2.1. A comparison of the most important difference between the carob moth and FCM in the adult, egg, larval and pupal stages of the respective life cycles.

Carob moth		False Codling moth	
Adult			
<ul style="list-style-type: none"> Wings held flat across the abdomen when in a restive position 	 <p style="text-align: right; font-size: small;">Dr. P. Addison</p>	<ul style="list-style-type: none"> Wings held roof-like across the abdomen when in a restive position ♂ with fluff on hind legs 	 <p style="text-align: right; font-size: small;">Dr. P. Addison</p>
Egg			

- Oval in shape
- Lattice pattern

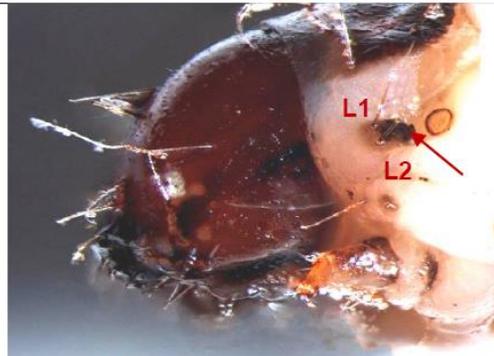


- Slightly oval in shape
- Stippled pattern



Larva

- A dark sclerotized patch on the T1 segment encompassing the L-group of seta
- Only two seta in the L-group on the T1 segment



Monique Rentel 2013

- No sclerotized patch encompassing the L-group of seta
- Three seta in the L-group on the T1 segment



Monique Rentel 2013

- No anal comb on segment A10



Monique Rentel 2013

- An anal comb is present segment A10



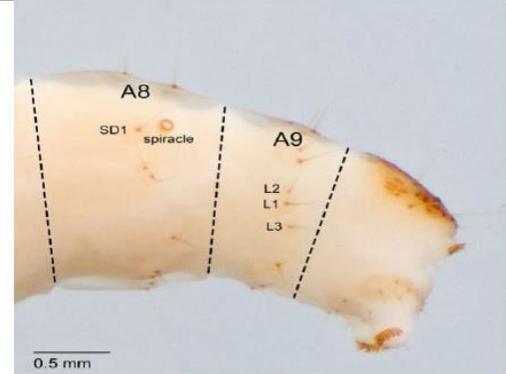
Monique Rentel 2013

- There is a sclerotized ring around the SD1 seta on the A8 segment pinaculum
- The SD1 seta is situated dorsal to spiracle segment A8



<http://idtools.org/id/leps/lepintercept/index.html>

- There is no sclerotized ring around the SD1 seta on the A8 segment
- The SD1 seta is situated in line with and anterior to spiracle segment A8



<http://idtools.org/id/leps/lepintercept/index.html>

- Crochets in the form of an irregular biordinal circle



- Crochets in the form of an irregularly triordinal circle



- Four mandibular teeth



- Five mandibular teeth

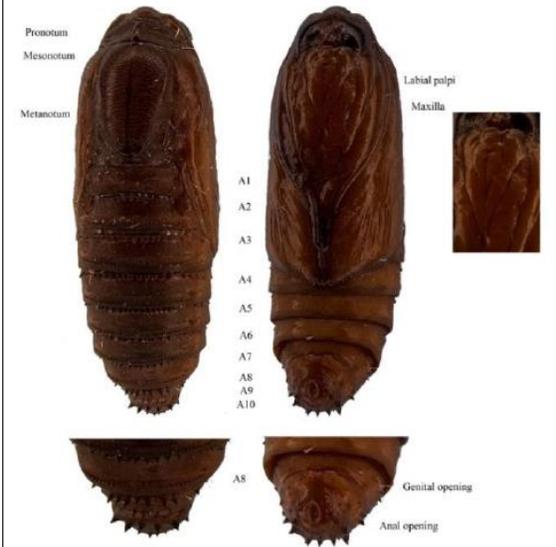


Pupa

- Thoracic spiracles present
- Gibba absent
- Cremastral setae slender and hooked with a slightly curved conical protuberance
- Lateral projections on the dorsal side of the notum



- Cremaster indistinct



Monique Rentel 2013

In the laboratory, carob moth only emerged in the first sampling season from damaged citrus fruit from the Citrusdal study sites. From a total of 80 damaged fruit collected, only three carob moths emerged in the first season (Table 3.4.2.2). FCM was more prolific in its emergence from the damaged fruit from both study sites. From the same 80 fruits collected in the Citrusdal area, only four FCM emerged from the damaged fruit. In the Robertson/Bonnievale area in the first season 69 damaged fruits were collected yielding five FCM adults from the damaged fruit. In the second sampling season, no FCM adults were collected in the Citrusdal area, whereas from the 89 damaged fruits collected in the Robertson/Bonnievale area 35 FCM emerged.

Table 3.4.2.2. Percentage of moth emergence from damaged citrus fruit collected in Citrusdal and Robertson/Bonnievale areas for the period of February 2013 to April 2014.

	Citrusdal			Robertson/Bonnievale		
	Nr of fruits collected	<i>Thaumatotibia leucotreta</i> %	<i>Ectomyelois ceratoniae</i> %	Nr of fruits collected	<i>Thaumatotibia leucotreta</i> %	<i>Ectomyelois ceratoniae</i> %
Feb-13	42	2.38	2.38	61	3.29	0
Mar-13	16	6.25	6.25	8	12.50	0
Apr-13	22	13.64	4.55	0	25.00	0
May-13	26	0	0	0	0	0
Jun-13	10	0	0	0	0	0
Feb-14	18	0	0	28	42.86	0
Mar-14	11	0	0	54	40.74	0
Apr-14	10	0	0	7	14.29	0

Technology Transfer

The results of this study were presented at the 2013 Congress of the Entomological Society of southern Africa and at the 2014 Citrus Research Symposium.

3.4.3 PROGRESS REPORT: Evaluating GRAS post-harvest fumigants for phytosanitary pests Project 913 (2011/2 – 2015/6) by T G Grout, K C Stoltz and P R Stephen (CRI)

Summary

Further research was conducted with Vapormate and carbon dioxide. Vapormate at 250 g/m³ for 4 h at 15°C killed 38 861 grain chinch bug adults without any survivors and this treatment was shown to be safe for citrus, pears and stone fruit, although damaged pears showed an increase in waste. Vapormate at 250 g/m³ for 24 h at 25°C did not provide complete control of mealybug eggs and gave similar results to a 4 h treatment so combination with a short cold treatment would be required to guarantee freedom from mealybug. Vapormate continued to prove inconsistent in controlling false codling moth (FCM) larvae inside citrus fruit with wide fluctuations in mortality between different cultivars. Carbon dioxide at 60%, which is not as effective against external insects as Vapormate, was more consistently effective against FCM larvae in the fruit than Vapormate, and fumigation for 24 h results in approximately 65% mortality. Short cold treatments of 7-9 days at 1-2°C immediately after CO₂ fumigation are providing 100% mortality of FCM so

further research will be conducted on this with different citrus cultivars and a short cold treatment at 2°C. The effect of Vapormate on Fullers rose beetle and scale insects will be investigated next year.

Opsomming

Verdere navorsing is gedoen met Vapormate en koolstofdioksied. Vapormate teen 250 g/m³ vir 4 h by 15°C het 38 861 volwasse graanstinkbesies doodgemaak sonder enige oorlewendes. Hierdie behandeling is getoon om veilig te wees vir sitrus, pere en steenvrugte, alhoewel beskadigde pere 'n toename in bederf getoon het. Vapormate teen 250 g/m³ vir 24 h teen 25°C het nie volkome beheer van witluis eiers verskaf nie en het resultate gelewer wat dieselfde was as 'n 4 h behandeling. 'n Gekombineerde behandeling met 'n kort koue behandeling sal benodig word om afwesigheid van witluis te waarborg. Vapormate het voorentoe wisselvallige resultate in die beheer van valskodlingmot (VKM) larwes binne sitrus vrugte gelewer met groot variasie in mortaliteit tussen verskillende kultivars. Koolstofdioksied teen 60%, wat nie so effektief soos Vapormate teen eksterne insekte is nie, was meer konstant effektief teen VKM larwes binne die vrugte as Vapormate. Beroking vir 24 h lei tot ongeveer 65% mortaliteit. Kort koue behandelings van 7-9 dae teen 1-2°C onmiddelik na CO₂ beroking het gelei tot 100% VKM mortaliteit. Verdere navorsing sal op hierdie behandeling gedoen word met verskillende sitrus kultivars en 'n kort koue behandeling van 2°C. Die effek van Vapormate op Fuller se rooskewer en dopluis sal volgende jaar ondersoek word.

3.4.4 **PROGRESS REPORT: The association of a lepidopteran borer complex between pecan nuts (*Carya illinoensis*) and citrus (*Citrus sinensis*) in the Vaalharts region** Project 1051-UFS (2013-14) by Andre van Rooyen, Vaughn Swart (UFS) and Sean Moore (CRI)

Summary

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

This project is now complete and the student is expected to hand in his thesis in July 2015. The final report will therefore be published in the next annual report.

Opsomming

Karobmot, *Ectomyelois ceratoniae*, word gewoonlik as 'n sekondêre plaag van sitrus beskou dus besmet dit gewoonlik slegs vrugte wat reeds besmet is met witluis of wat nalaatsels van heuning dou en roetskimmel bevat. Hierdie hou verband met witluis en ander suigende insekte of vrugte wat reeds beskadig is (bv. nawelentbars). Dit is egter ook opgemerk dat die vrugte in die nabyheid van meer voordelige gashere (bv. eikebome, granate, pekanneute) ook besmet kan raak. In die Vaalharts streek, word sitrus en pekanneute

naby aan mekaar verbou. Dit bied die ideale geleentheid om die voglende te bepaal: a) die samestelling van die Lepidoptera stronkboorder kompleks op sitrus en pekanneute (dit word vermoed dat dit hoofsaaklik valskodlingmot (*Thaumatotibia leucotreta*) (VKM) en karobmot is); b) die relatiewe proporsies van die verskillende spesies op die twee gewasse; c) seisoenale fluktuasies in spesifieke plaag vlakke en d) die impak van Lepidoptera pes migrasie tussen die twee gewasse. Dit sal dus moontlik wees om a) die potensiële erns van karobmot as 'n plaag vir sitrus, en b) die bedreiging wat 'n alternatiewe voedselbron kan inhou vir sitrus aangaande karobmot en VKM risiko. 'n Opname van pekanneute in die winter van 2010 het getoon dat 29% van die neute was besmet was. Alle geïdentifiseerde motte was karobmot gewees. Daarna, het verdere klein opnames deur die Universiteite van die Vrystaat en Stellenbosch dit tot lig gebring dat beide VKM en *Ephestia* sp. as plaeg van pekanneute optree. Nog 'n potensiële voordeel van die bepaling van die plaagstatus van VKM op pekanneute is indien dit bewys kan word dat 'n beduidende gedeelte van die kompleks inderdaad VKM is, kan 'n SIT program in die Vaalharts-besproeiingskema opgestel word. Hierdie studie het begin in 2012 alhoewel slegs deur CRI befonds sedert 2013, en sal voortgaan tot Julie 2014. Ongeveer 100 pekanneute en 50 sitrus vrugte (van die bome en grond) is elke maand van aangrensende boorde, 10 en 50 m van die rand af ingesamel van beide pekan- en sitrusboorde. Feromoonlokvalle vir beide VKM en karobmot is ontplooi by alle studie areas, ten einde om 'n verhouding tussen lokval data en besmetting van versamelde materiaal te bepaal. Sewe persele met hierdie omstandighede is as studie persele gekies. Pekanneut monsters is in uitbroei bokse geplaas en toegelaat om tot volwassenheid te ontwikkel. Sitrusvrugte is intern ondersoek vir larwes; versamelde larwes is op 'n voedings medium geplaas en toegelaat om papies te vorm en tot volwassenheid te ontwikkel. Monsters is na Dr M Krüger (Transvaalse Museum) gestuur vir identifikasie. Aanduidings tot dusver is dat karobmot besmetting van beide pekanneute en sitrus is hoër as vir VKM; en albei karob mot en VKM migreer tussen albie boorde.

Hierdie projek is nou voltooi en dit word verwag dat die student sy tesis in Julie 2015 sal inhandig. Die finale verslag sal dus in die volgende jaarverslag verskyn.

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

Project 1110 (ad hoc) by Sean Moore (CRI), Sean Thackeray (RU), Martin Gilbert, Wayne Kirkman, Peter Stephen (CRI), Daniel Victor (Rosle Farm), Arjan de Jongh (Schoeman Boerdery), Johanna Mathewson (Saamfarm), Michael van Niekerk (Lemoenkop)

Summary

During May and June, large samples of infested Navel oranges were collected from orchards in a number of different regions throughout the country. This was for use in FCM cold treatment trials under project 1039. However, the opportunity was taken to identify the species of every larva dissected from all fruit both before cold treatment (untreated control samples) and after cold treatment, using the reliable diagnostic tool developed by Stellenbosch University. Initial identifications were molecularly verified by Alicia Timm (Rhodes University). It was consequently determined that up to 60% of fruit infestation in the Loskop production region was carob moth, up to 40% in Nelspruit and over 10% in the Eastern Cape. These figures were way above any expectation. Additionally, it has been determined by Van Rooyen *et al* in project 1051 that carob moth infestation of citrus in the Vaalharts region is consistently higher than for FCM. Due to this, it was considered of great importance to ascertain the real pest status of carob moth on citrus throughout the country's growing regions and throughout the production cycle. Development of a reliable monitoring system, which will provide both a picture of carob moth cycles in the field and a relationship between trap catches and fruit infestation, are essential in developing effective control measures and to investigate the likelihood of carob moth infestation in fruit packed for export. This project was therefore initiated on ad hoc funding. It has subsequently been taken over by Rhodes University as an MSc study.

Opsomming

Gedurende Mei en Junie is groot monsters besmette Nawellemoene van boorde in verskeie streke deur die land versamel. Hierdie was vir gebruik in VKM koue behandelings proewe onder projek 1039. Die geleentheid is egter gebruik om die spesie van elke larwe wat uit alle vrugte gesny is te identifiseer, albei voor koue behandeling (onbehandelde kontrole) en na koue behandeling, met behulp van die betroubare diagnostiese sleutel wat deur Stellenbosch Universiteit ontwikkel is. Aanvanklike identifikasies is deur Alicia Timm (Rhodes Universiteit) molekulêr geverifieer. Gevolglik is dit bepaal dat tot 60% van vrugbesmetting in die Loskop produksie streek karobmot was, tot 40% van Nelspruit en meer as 10% van die Oos-Kaap. Hierdie syfers is ver bo verwagting. Boonop is dit deur Van Rooyen *et al* in projek 1051 bepaal dat karobmot besmetting van sitrus in die Vaalharts streek konstant hoër as VKM besmetting is. As gevolg hiervan is dit besluit dat dit uiters belangrik is om die ware plaagstatus van karobmot in al die land se produksie streke en

deur die produksie siklus te bepaal. Ontwikkeling van 'n betroubare moniterings stelsel, wat albei 'n prentjie van karobmot siklusse in die veld sal skep en ook 'n verhouding tussen lokvalvangstes en vrugbesmetting sal bepaal, is uiters belangrik om doeltreffende beheermaatreels te ontwikkel en om die waarskynlikheid van karobmot besmetting in kartonne gepak vir uitvoer te bepaal. Hierdie projek is dus met ad hoc bevonding geïnisieer. Dit is onlangs deur Rhodes Universiteit oorgeneem as 'n MSc studie.

3.5 **PROGRAMME: NON-PHYTOSANITARY KEY PESTS** Programme Coordinator: Tim G Grout (CRI)

3.5.1 **PROGRAMME SUMMARY**

Research continued in the quest for IPM-compatible treatments for late season populations of citrus thrips and mealybug (3.5.2). Entomopathogenic fungi have at times caused some suppression of citrus thrips and mealybug populations but on the whole their impact has been short-lived and disappointing. This research has now been terminated although some products will be evaluated further in other projects. Due to difficulties in maintaining a woolly whitefly culture and having to prioritise the rearing of its parasitoid *Cales noacki*, only two series of bioassays were conducted with short-residual chemicals (3.5.3). These showed that Xterminator and Ecossearch Extract were effective but Runner had no effect. Horticultural mineral oil at 0.5% was consistently effective against woolly whitefly and even Teepol 0.1% caused 70% mortality. However, in the field it is always difficult to get optimal coverage of this pest. With the negative publicity around imidacloprid, a product causing mating disruption in red scale was evaluated as an alternative (3.5.4). Application three weeks prior to petal fall gave similar results to generic imidacloprid drench treatments, but later applications at petal fall were less effective. This project also confirmed how effective a winter oil spray can be in keeping red scale under control. Further research will be conducted on the mating disruptant because it is a perfect fit for IPM.

PROGRAMOPSOMMING

Navorsing in die soektog vir IPM-verenigbare behandelings vir laat seisoen bevolkings van sitrusblaaspootjie en witluis is voortgesit (3.5.2). Entomopatogeniese swamme het soms 'n mate van onderdrukking van sitrusblaaspootjie en witluis bevolkings veroorsaak maar oor die algemeen was hul impak van korte duur en dus teleurstellend. Hierdie navorsing is nou beëindig, hoewel sekere produkte verder in ander projekte geëvalueer sal word. As gevolg van probleme in die instandhouding van 'n wollerige witvlieg kultuur en die prioritisering van die aanteling van sy parasitoïed *Cales noacki*, is slegs twee reekse biotoetse met kort nawerkende chemikalieë uitgevoer (3.5.3). Hierdie het gewys dat Xterminator en Ecossearch Extract doeltreffend was, maar Runner het geen effek gehad nie. Hortologiese minerale olie teen 0.5% was deurgaans doeltreffend teen wollerige witvlieg en selfs Teepol 0.1% het 70% mortaliteit veroorsaak. In die veld is dit egter altyd moeilik om optimale dekking van hierdie plaag te kry. Met die negatiewe publisiteit rondom imidacloprid, was 'n produk wat paringsontwrigting van rooidopluis veroorsaak, as 'n alternatief geëvalueer (3.5.4). Toedienings drie weke voor blomblaarval het soortgelyke resultate as generiese imidacloprid grondtoedienings veroorsaak, maar later toedienings by blomblaarval was minder doeltreffend. Hierdie projek het ook die doeltreffendheid van 'n winter olie bespuiting bevestig om rooidopluis onder beheer te hou. Verdere navorsing sal op die paringsontwrigter gedoen word want dit pas perfek by 'n IPM program in.

3.5.2 **PROGRESS REPORT: Evaluation of entomopathogenic fungi against thrips and mealybug** Project 1029 (2011/2-2014/5) by Tim G Grout, Sean D Moore, Peter R Stephen and Wayne Kirkman (CRI)

Summary

There is an urgent need for plant protection products that can be used in summer for the control of citrus thrips and mealybug without disrupting natural enemies of key pests such as false codling moth. In 2011/2 the evaluation of two dosages of a commercially formulated entomopathogenic fungus (EPF) in different parts of the country gave disappointing results so in the 2013/4 season two experimental EPF isolates were evaluated at two dosages in northern and southern citrus regions. The results were again disappointing, although some thrips suppression was recorded for a week in the south. In the 2014/5 season, results from Limpopo Province showed that a *Metarhizium anisopliae* isolate caused an initial reduction in larval thrips numbers with a corresponding reduction in early scarring that was similar to that achieved with abamectin plus oil, but it was short-lived. A commercial biological product TripStop + BFA gave similar results against thrips. Neither of these products showed significant efficacy against mealybug. A new chemical product

DPX8723, with a good IPM profile, gave excellent control of thrips for a month but also had no impact on mealybug.

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

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

Opsomming

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

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

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

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

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

Summary

There is a shortage of registered control options that can be used for late season control of thrips, citrus psylla, leafhoppers and woolly whitefly. The objective of this research is to evaluate unregistered products, and products that have been recently registered on citrus against other pests, which are likely to have short preharvest intervals. Only two bioassays with woolly whitefly (WWF) were possible this year because difficulties were experienced in maintaining the WWF culture and priority had to be given to providing enough host material for the rearing of the Spanish parasitoid of WWF *Cales noacki*. Medium grade horticultural mineral oil at 0.5% was kept as a standard in the bioassays and consistently caused 99%+ mortality. Methoxyfenozide caused no mortality but Teepol soap at 1 ml/L water caused 70% mortality which shows why soap can be effective in controlling this pest in home gardens. Abamectin 20 ml/hL water plus medium oil 0.25%, chlorpyrifos EC at 75 ml/hL, Xterminator (natural pyrethrins) 500 ml/hL, and Ecosearch Extract 1% all caused more than 90% mortality. The latter two products, as well as Requiem and Pygar-Super which

were earlier shown to have similar efficacy, require further investigation and comparison with horticultural mineral oil from a cost and efficacy viewpoint, because the required concentrations are similar.

Opsomming

Daar is 'n tekort aan geregistreerde beheermaatreëls om blaaspotjie, sitrusbladvlooi, bladspringer en wollerige-witvlieg (WWV) laat in die seisoen te beheer. Die doel van hierdie navorsing is om ongeregisterde middels en middels wat onlangs teen ander sitrusplae geregistreer is, met moontlike kort vooroes intervalle, te evalueer. Slegs twee laboratoriumproewe met WWV is hierdie jaar voltooi as gevolg van probleme wat ondervind is met die instandhouding van die WWV-kultuur en dit was ook 'n prioriteit om genoeg gasheermateriaal te voorsien vir die teel van die Spaanse parasitoïed *Cales noacki*. Medium graad hortologiese minerale olie teen 0.5% is gebruik as 'n standaardbehandeling in die laboratoriumproewe en het konstant tot 99%+ mortaliteit gelei. Methoxyfenozide het geen mortaliteit veroorsaak nie, maar Teepol seep teen 1 ml/L water het 70% mortaliteit veroorsaak wat wys hoekom seep doeltreffend kan wees vir die beheer van WWV in tuine. Abamectin 20 ml/hL water plus medium olie teen 0.25%, chlorpyrifos EK teen 75 ml/hL, Xterminator (natuurlike pieretriene) 500 ml/hL en Ecosearch Extrak 1% het almal meer as 90% mortaliteit veroorsaak. Die laasgenoemde twee middels sowel as Requiem en Pygar-Super, wat vroeër soortgelyke doeltreffendheid getoon het, moet verder ondersoek en vergelyk word met hortologiese minerale olie veral vanuit 'n koste en doeltreffendheid oogpunt, aangesien die verlangde konsentrasies dieselfde is.

3.5.4 PROGRESS REPORT: Mating disruption for red scale control Project 1076 (2014/5 – 2015/6) by T G Grout and P R Stephen (CRI)

Summary

Mating disruption for red scale control was proposed more than 25 years ago but has not been cost effective until the recent development of a mesoporous dispenser used in a system called Saturel CRS. We wanted to evaluate this system at three sites in the northern production regions but lost one site after applying treatments because the grower top-worked the orchard. The remaining two sites were in Mpumalanga and Limpopo provinces and included an imidacloprid soil drench and winter medium grade horticultural oil spray at 1% as standards. In Mpumalanga, Saturel CRS treatments starting 3 weeks prior to 100% petal fall or at 100% petal fall were both superior to no treatment and equivalent in efficacy to imidacloprid drench at 9 ml/tree. However, the winter oil spray treatment was significantly less infested than these other treatments in March. In Limpopo, the later initiation of the Saturel CRS treatment at petal fall was significantly inferior to other treatments and similar to the untreated control, while the earlier Saturel CRS treatment was again equivalent to an imidacloprid soil drench. When this site was evaluated in January the winter oil treatment followed by the earlier Saturel CRS treatment was significantly less infested than the earlier Saturel CRS treatment alone, but five weeks later the infestations in these two treatments were similar. It therefore appears that an application of Saturel CRS three or four weeks before 100% petal fall can serve the same purpose as an imidacloprid drench in suppressing red scale populations. These trials will be repeated in 2015/6 with similar treatments.

Opsomming

Paringsontwrigting vir die beheer van rooidopluis is meer as 25 jaar gelede al voorgestel. Dit was egter tot onlangs nie koste-effektief nie. Dit het egter verander met die ontwikkeling van 'n medium-poreuse vrysteller wat in 'n sisteem, bekend as Saturel CRS, gebruik word. Ons wou die sisteem by drie persele in die noordelike produksie streke evalueer, maar het een perseel verloor na die behandelings toegedien is omdat die produsent die boord oorgewerk het. Die oorblywende twee plekke was in Mpumalanga en Limpopo provinsies, en het 'n imidacloprid grond-toediening en 'n winter medium graad hortologiese olie bespuiting teen 1% as standarde behandelings ingesluit. In Mpumalanga, het Saturel CRS behandelings 3 weke voor 100% blomblaarval of teen 100% blomblaarval begin waar beide beter as geen behandeling gevaar het, en gelyk in doeltreffendheid aan imidaclopridgrond-toediening teen 9 ml/boom was. Die winter oliebespuiting was in Maart egter betekenisvol minder besmet as die ander behandelings. In Limpopo, was die later begin van die Saturel CRS behandeling teen blomblaarval betekenisvol minder doeltreffend as die ander behandelings en soortgelyk aan die onbehandelde kontrole, terwyl die vroeër Saturel CRS behandeling weereens gelyk aan die imidacloprid grond-toediening was. Met die evaluering van hierdie perseel in Januarie was die winter oliebehandeling gevolg deur die vroeër Saturel CRS behandeling betekenisvol minder besmet as die vroeër Saturel CRS behandeling alleen, maar vyf weke later was die besmettings in hierdie twee behandelings dieselfde. Dit blyk dus dat 'n toediening van Saturel CRS drie tot vier weke voor 100% blomblaarval ook dieselfde doel kan dien as 'n imidacloprid grond-toediening in die onderdrukking van rooidopluis populasies. Hierdie proewe sal in 2015/6 met soortgelyke behandelings herhaal word.

3.6 PROGRAMME: MINOR PESTS AND MITES

Programme Coordinator: Tim G Grout (CRI)

3.6.1 PROGRAMME SUMMARY

Some pests may be considered of minor importance by most growers but in certain situations or climates they can be extremely problematic. This is the case for woolly whitefly (WWF) which is becoming a serious pest where growers apply few pesticides for sucking insects. The WWF parasitoid *Cales noacki* was successfully imported from Spain and released at a few sites in North-West Province and Mpumalanga where WWF was present (3.6.2). A few of these parasitoids have subsequently been recovered from release sites but their impact on WWF populations is not yet evident. Maximising the benefit we receive from natural enemies between December and harvest is important for quarantine pests like false codling moth and mealybugs and also for cosmetic pests for which there are few plant protection products with short pre-harvest intervals. For this reason, bioassays were conducted to determine the non-target effects of some recently registered pesticides (3.6.3). Pynex CS was found to be very harmful to parasitoids for a long period. Delegate was very harmful to false codling moth parasitoids, slightly harmful to others and repelled predatory mites. Closer had little effect on insect natural enemies but did reduce numbers of predatory mites. Natural banana is proving a useful attractant for monitoring fruit piercing moths (3.6.4) and both *Serrodus partita* and *Eudocima* sp. have been caught in traps, but the relationship between trap catches and fruit damage is not significant. This research will now be written up and terminated.

3.6.1 PROGRAMOPSOMMING

Sekere plae word beskou as van geringe belang deur meeste produsente maar in sekere situasies of klimaat kan hulle baie problematies wees. Dit is wel die geval vir wollerige witvlieg (WWF) wat besig is om as 'n ernstige plaag te ontwikkel waar produsente minder plaagdoders vir suigende insekte toedien. Die WWF parasitoïed *Cales noacki* is suksesvol van Spanje ingevoer en vrygestel by 'n paar persele in die Noordwes-Provinsie en Mpumalanga waar WWF teenwoordig is (3.6.2). 'n Paar van hierdie parasitoïedes is tot dusver teruggekry, maar die impak daarvan op die WWF bevolking is nog nie duidelik nie. Maksimalisering van die voordeel van natuurlike vyande tussen Desember en oestyd is belangrik vir kwarantyn plae soos valskodlingmot en witluis en ook vir kosmetiese peste waarvoor daar min plantbeskermingsprodukte met kort onthoudings tydperke is. Om hierdie rede, is biotoetse gedoen om die nie-teiken effekte van sommige onlangs geregistreerde plaagdoders (3.6.3) te bepaal. Pynex CS was baie skadelik vir parasitoïedes vir 'n geruime tydperk. Delegate was baie skadelik vir valskodlingmot parasitoïedes, effens skadelik vir ander parasitoïedes en het roofmyte afgeweer. Closer het min effek op natuurlike vyande gehad, maar het roofmyte getalle verminder. Natuurlike piesang blyk 'n effektiewe lokmiddel vir die monitering van vrugte-steekmotte (3.6.4) en albei *Serrodus partita* en *Eudocima* sp. is in lokvalle gevang, maar die verhouding tussen lokval vangstes en vrug skade was nie betekenisvol nie. Hierdie navorsing sal nou beëindig en opgeskryf word.

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

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

Summary

After 5 years, permission was granted to import and release the most effective parasitoid for woolly whitefly (WWF) in the world, *Cales noacki*. The first package we received from Spain was a failure due perhaps to anaerobic conditions and mould but we received live specimens on 18 November 2014 and we started a laboratory culture on WWF in Nelspruit. The first release was made in an infested Empress mandarin orchard near Mooiwooi, North-West Province on 22 January 2015 by placing an infested potted plant with emerging *C. noacki* between the trees. On 10 March, 10 *C. noacki* were recovered from infested leaves on immediately adjacent trees to the potted plant but not from trees further away. Other releases were made in infested trees in the Nelspruit environs but only two *C. noacki* have been recovered so far. Problems have been experienced in maintaining a large culture of WWF due to few eggs being laid on potted plants with apparently suitable foliage. This has reduced the numbers of *C. noacki* available for release and slowed plans for distribution to other areas. Once the parasitoid has become the dominant natural enemy of WWF in an area we hope to be able to transfer large amounts of parasitoids from there to other parts of the country.

Opsomming

Na 5 jaar, is toestemming verleen vir die invoer en die vrylating van *Cales noacki*, die mees doeltreffende parasitoïed in die wêreld vir wollerige witvlieg (WWV). Die eerste versending wat van Spanje ontvang is was

'n mislukking, moontlik as gevolg van anaërobiese toestande en skimmel, maar lewendige monsters is op 18 November 2014 ontvang en hiermee is 'n laboratoriumkultuur op Nelspruit begin. Die eerste vrylating is gedoen in 'n WWV besmette Empress mandaryn boord naby Mooinooi, Noordwes-provinsie op 22 Januarie 2015 deur 'n potplant besmet met WWV en *C. noacki* volwassenes, wat besig was om uit te broei, tussen die bome te plant. Op 10 Maart is 10 *C. noacki* herwin uit WWV besmette blare van bome aangrensend aan die potplant, maar geen *C. noacki* is verkry vanuit bome wat verder weg was nie. Ander vrylatings is gedoen in besmette bome in die Nelspruit-omgewing, maar slegs twee *C. noacki* is tot dusver herwin. Probleme is ondervind met die instandhouding van 'n groot kultuur WWV omdat te min eiers gelê is op potplante met oënskynlik geskikte blare. Dit het die getalle van *C. noacki* wat beskikbaar was vir vrylating verminder en planne vir verspreiding na ander gebiede is vertraag. Sodra die parasitoïed die dominante natuurlike vyand van die WWF in 'n gebied is, is die hoop om in staat te wees om groot hoeveelhede oor te dra na ander dele van die land.

3.6.3 FINAL REPORT: Non-target effect updates

Project 1095 (Apr 2014 – Mar 2015) by T.G. Grout and K.C. Stoltz (CRI)

Summary

Semi-field bioassays were conducted to determine the non-target effects of some recently-registered pesticides on important natural enemies in citrus orchards. Methoxyfenozide (Runner) had no acute negative effects, although we were not testing for insect growth regulator effects. Spinetoram (Delegate) repelled predatory mites and was very harmful to them. It was also very harmful to *Trichogrammatoidea cryptophlebiae* and slightly harmful to *Coccidoxenoides perminutus*, so could have a negative impact late in the season when these natural enemies are needed. The recently registered product sulfoxaflor (Closer) was softer than spinetoram but did reduce predatory mite numbers considerably. The micro-encapsulated chlorpyrifos formulation (Pyrinex CS) was extremely persistent and very harmful to parasitoids so should preferably be used soon after petal fall. No further non-target bioassays are planned because of the difficulties experienced in acquiring good quality insects when needed.

Opsomming

Semi-veld biooetse is gedoen om die nie-teiken effekte van sommige onlangs geregistreerde plaagdoders op belangrike natuurlike vyande in sitrusboorde te bepaal. Methoxyfenozide (Runner) het geen akute negatiewe gevolge nie, maar ons het nie vir insekgroeireguleerder effekte getoets nie. Spinetoram (Delegate) het roofmyte afgeweer en was vir hulle baie skadelik. Dit was ook baie skadelik vir *Trichogrammatoidea cryptophlebiae* en effens skadelik vir *Coccidoxenoides perminutus*, dus kan dit 'n negatiewe impak laat in die seisoen hê wanneer hierdie natuurlike vyande belangrik is. Die onlangs geregistreerde produk sulfoxaflor (Closer) was sagter as spinetoram maar het aansienlik roofmyt getalle verminder. Die mikrogeënkapseleerde chlorpirifos formulاسie (Pyrinex CS) was uiters nawerkend en baie skadelik vir parasitoïedes en moet dus verkieslik kort na blomblaarval gebruik word. Geen verdere nie-teiken biooetse word beplan nie as gevolg van probleme in die verkryging van goeie gehalte insekte wanneer dit nodig is.

Introduction

Semi-field bioassay techniques were developed for key natural enemies of citrus pests in the late 1990s (Hattingh et al. 2000) and for several years research was conducted to develop a database of non-target effects from pesticides commonly used in citrus (Grout et al. 2011). When this research was terminated, companies wanting to register new plant protection products in citrus were supposed to contract a research organisation to conduct the bioassays as part of the registration requirement. A few companies like Bayer and DuPont have maintained this approach but other companies ignore it. We therefore have several chemicals or new formulations of old chemicals that are being used in citrus orchards without knowledge of their impact on beneficial insects. We conducted one bioassay series against the 4 key natural enemies that are readily available from commercial insectaries to obtain information about recently-registered products that are now being used widely in citrus. The impact categories used in these bioassays are similar those in the IOBC semi-field, persistence tests (Hassan et al. 1988): Harmless (<25%), Slightly harmful (25-50%), Harmful (51-75%) and Very harmful (>75%).

Stated objective

Conduct non-target effect bioassays against *Euseius citri*, *Coccidoxenoides perminutus*, *Trichogrammatoidea cryptophlebiae* and *Cryptolaemus montrouzieri* for Delegate, Runner, Pynex CS and Dursban WG.

Materials and methods

Neither *Aphytis* spp. nor *Chilocorus nigritus* are available from commercial insectaries anymore and oleander scale to rear *C. nigritus* on butternuts is no longer available in the country. We therefore used the mealybug predator *Cryptolaemus montrouzieri* as the beetle natural enemy.

1 *Cryptolaemus montrouzieri*

This research was conducted from 28 May to 31 July 2014.

1.1 Source of beetles

Beetles used in the bioassays were from a commercial insectary culture (Du Roi IPM) maintained on mealybug on butternut squash.

1.2 Effect on adult and larval mortality

Ten modified Munger cells were set up per treatment group. Each cell consisted of a petri dish lid which provided a closed test arena of 30 mm diameter and 10 mm high. The inside of each test arena was coated with Fluon [Polytetrafluoroethylene (PTFE) dispersion, grade GP1]. Ventilation holes were made on opposite sides of the test arena. Thin plastic tubing was tightly fitted through the precut holes, with fine mesh material over the ends. Each leaf was placed on top of a damp filter paper, on top of a 100 mm x 60 mm x 3 mm glass plate. The test arena was placed over the leaf surface and secured onto the glass plate with a "bulldog" clamp. One side of each cell was connected to a pressurised plenum via the plastic tubing, so that air was passed through each cell at a rate of one volume exchange per minute.

In the adult beetle assessment, five beetles were inserted into each cell. To assess the effects on larvae, five first instar larvae were transferred onto the dorsal leaf surface in each cell, using a fine paint brush.

The cells were held for 48 hours under laboratory conditions of 22°C before the number of live insects was determined. Treatment mortalities were corrected for control mortality, using Abbott's (1925) correction.

$$MA \text{ or } ML = (MT - Mc)/(100 - Mc) \times 100$$

Where: MA = % corrected mortality of adults
ML = % corrected mortality of larvae
MT = mortality in the treated group
MC = mortality in the control group

Bioassays were conducted with progressively older residues (1, 7, 14, 28 and 42 days) until the corrected mortality dropped below 25 %.

1.3 Persistence

A persistence factor (P) was incorporated into the impact rating. The relevant factor was determined by the residue age at which the corrected effect dropped below 25 %. The factors used for the residue age categories 1, 7, 14, 28 and 42 days were 1.03, 1.07, 1.1, 1.2 and 1.4 respectively. A factor of 2.0 was used if the corrected effect was still greater than 25% at 42 days. The persistence factor was combined with the maximum corrected adult (MA) and larval (ML) mortalities as follows:

$$IA = MA \times 0.5P \text{ and } IL = ML \times 0.5P$$

1.4 Overall impact rating

The adult and larval mortalities, both adjusted for persistence, were combined to obtain a single impact rating (I):

$$I = 0.5IA + 0.5IL$$

2 Coccidoxenoides perminutus

This research was conducted from 29 August to 8 November 2014.

2.1 Source of parasitoids

Parasitoids were obtained from the commercial insectary, Du Roi IPM in Letsitele.

2.2 Effect on mortality

Ten modified Munger cells were set up per treatment group. Each cell consisted of a Petri dish lid (34 mm diameter and 10 mm high) which was placed upside-down on the leaf with residue and held in place with two spring-loaded clamps against a piece of glass (100 mm x 65 mm x 3 mm). A piece of moist blotting paper was placed between the leaf and the glass to maintain the condition of the leaf. A 6 mm diameter hole was made in the side of the cell and another 6 mm diameter hole was made on top of the cell. Minute streaks of diluted honey were provided as food on opposite walls of the cell. Polyethylene tubing (5.6 mm external diameter and 4.3 mm internal diameter) was tightly fitted through the hole in the cell wall with fine polyester mesh fabric over the end. This tube was later connected to a pressurised plenum that ventilated the cell at a rate of one volume exchange per minute. A 1.5 mm diameter capillary tube was attached to an adaptor so that it fitted tightly in the hole on top of the test arena and 12 pre-fed, 24 hour-old adults were sucked into each cell using a portable vacuum pump. This hole was then plugged with a short piece of polyethylene tubing covered with mesh. Each cell was then attached to the plenum via the tubing in the wall of the cell. Adult mortality was recorded after 24 hours of exposure. Bioassays were conducted with progressively older residues (1, 7, 14, 28 and 42 days) until the corrected mortality dropped below 25%.

Treatment mortalities were corrected for control mortalities (Abbott, 1925):

$$MA = (MT - MC)/(100 - MC) \times 100$$

Where: MA = % corrected adult mortality
MT = mortality in the treated group
MC = mortality in the control group

2.3 Persistence

The relevant persistence factor (P) for adult mortality was determined by the residue age at which the corrected adult mortality dropped below 25%. The factors used for the residue ages were categorised as before for *Cryptolaemus montrouzieri*. The persistence factor was combined with the maximum corrected adult mortality (MA) as well as an additional factor of 0.5 to prevent the maximum possible combination of persistence factor and corrected mortality from exceeding 100%. Thus:

$$IA = MA \times 0.5P$$

Where: IA = maximum adult mortality adjusted for persistence

2.4 Overall impact rating

The product's impact (I) was equivalent to IA.

3 Trichogrammatoidea cryptophlebiae

This research was conducted from 23 May to 6 December 2014.

3.1 Source of egg parasitoids

Parasitised FCM eggs on wax paper were obtained from Vital Bugs near Tzaneen and the parasitoids normally commenced eclosion one day after receiving the shipment.

3.2 Effect on mortality

Ten test cells were set up per treatment group. Each cell consisted of a small Petri dish lid which provided a test arena of 34 mm diameter and 10 mm high. Ventilation holes were made on opposite sides of the test arena. Polyethylene tubing (5.6 mm external diameter) was tightly fitted through the pre-cut holes, with fine mesh fabric over the ends. Residue-bearing leaves were placed on top of glass plates (100 mm x 65 mm x 3 mm). Fine streaks of diluted honey were applied to the walls of the cell as food. Each test arena was tightly clamped over the leaf surface using two spring-loaded clamps.

Wax paper containing parasitised FCM eggs was placed inside a plastic container (227 mm x 178 mm x 74 mm) that had been painted black. Twenty holes were punched in the lid of the container. Small glass polytop vials were inserted into the holes to collect emerging parasitoids. Each vial was replaced as soon as 20 - 50 parasitoids had been collected. The parasitoids were randomly allocated to treatments. Each cell was attached to a pressurised plenum via the polyethylene tubing and air was passed through each test arena at approximately one volume exchange per minute.

The number of live adults was determined after 24 hours of exposure. Bioassays were conducted with progressively older residues (1, 7, 14, 28 and 42 days) until the corrected mortality dropped below 25%.

The treatment mortalities were corrected for control mortality (Abbott, 1925):

$$MA = (MT - MC)/(100 - MC) \times 100$$

Where: MA = % corrected adult mortality
 MT = mortality in the treated group
 MC = mortality in the control group

3.3 Overall impact rating

The corrected mortality figures were adjusted for persistence using the summation method. The corrected mortality figures (including zeroes for those after tests were stopped), obtained from different residue ages were summed as follows to obtain an overall impact rating (I):

$$I = \frac{\sum((Yx = -1.429x + 100)(M_A))}{\sum(Yx = -1.429x + 100)} \quad (\text{Where } x = 1, 7, 14, 28, 42)$$

The product's impact (I) was categorised as before.

4 *Euseius citri*

This research was conducted from 28 November 2014 to 16 January 2015.

4.1 Source of predatory mites

Mites were collected from a citrus orchard at Crocodile Valley Citrus, 24 hours before the setup of the bioassays. The substrate for the colony consisted of large inverted citrus leaves placed on floating sponge rafts. Each raft consisted of a 135 mm x 190 mm sponge, floating on a polystyrene raft (135 mm x 190 mm) in a water-filled container. Strips of cotton wool were placed on the edges of the leaf to ensure a continuous wet barrier. The mites were fed with pollen from *Typha capensis* by sprinkling the pollen on the leaf surface.

4.2 Effect on adult survival

Treated and untreated leaves were collected one and three days after spraying, and thereafter at weekly intervals until corrected mortality dropped below 5% for a second time. Disks, 22 mm in diameter, were punched out of the leaves. The flattest disks were placed on water-saturated filter paper (Whatman 90) disks (25 mm diameter) on top of a floating sponge raft. Gaps between the filter paper disks on the sponge prevented movement of mites between disks. Care was taken to ensure continued contact between the filter paper and the entire ventral surface of the leaf disk to prevent the mites from going underneath the leaves.

Twenty leaf disks were used per treatment. Two disks of filter paper (6 mm diameter) were placed on each leaf disk to provide shelter for the mites and a fibre substrate for the attachment of eggs. Pollen (*Typha capensis*) was lightly dusted onto the leaf disks.

Five adult female mites were transferred to each leaf disk from the bioassay colony. Each disk from each treatment received one mite before a second mite was placed on each disk. The bioassay trays were left for 48 h under laboratory conditions before the number of live, adult females was determined. Missing mites or mites found drowned in the sponge were considered dead. Treatment mortalities were corrected for control mortality using Abbott's correction (1925):

$$MA = (MT - MC)/(100 - MC) \times 100$$

Where: MA = corrected mortality
 MT = mortality in treatment
 MC = mortality in control

4.3 Persistence

A persistence factor (P) was determined by the residue age at which the adult mortality dropped below 5 % for a second time. The factors used for the categories 0-15 days, 16-30 days, 31-45 days and more than 45 days were 1.1, 1.2, 1.4, and 2.0, respectively. The persistence factor was combined with the maximum corrected adult mortality (MA) as well as an additional factor of 0.5 (to prevent the maximum possible combination of persistence factor and corrected mortality from exceeding 100%) to determine the impact of the product on adults (IA), i.e.

$$IA = (\max MA) \times 0.5P$$

4.4 Effect on adult fecundity, egg hatch and immature mortality (population increase)

Once the adult mortality remained below 5% for a second test, a “once-off” procedure was used to determine the effects on egg production, egg hatch and survival of immature life stages. The adult mites were removed from the last bioassay. The eggs were left to hatch and develop to adults and were fed every second day. The number of adults (offspring) surviving was determined nine days after the original females were placed on the disks. This number was expressed as a percentage (S_{F1}) of the number of offspring in the untreated control

$$S_{F1} = [(\text{number of offspring in treatment}) / (\text{number of offspring in control})] \times 100$$

Where: S_{F1} = survival percentage (including any effect on fecundity, egg hatch or immature mortality relative to the control)

The detrimental effect on reproduction or mortality of the F1 generation (M_{F1}) is therefore $100 - S_{F1}$. This negative effect on reproduction (M_{F1}) was then adjusted according to the potential contribution to the overall impact rating, giving:

$$I_{F1} = M_{F1}[(100 - IA)/100]$$

Where: I_{F1} = Impact on reproduction adjusted for potential to contribute to the overall impact rating.

4.5 Overall impact rating

The overall impact rating (I) was obtained as follows:

$$I = IA + I_{F1}$$

Results and discussion

Under normal circumstances, based on past experience, this research should have been completed within 4 months but it took 9 months to complete because of the poor quality of natural enemies received and sometimes the failure of courier companies to deliver at all. We ordered 1.4 million *T. cryptophlebiae* in order to get enough for the bioassays and 540 000 *C. perminutus*. Several bioassays had to be repeated because of failures 2 or 4 weeks into the 6-week series.

1 *Cryptolaemus montrouzieri*

Beetles are generally more tolerant of pesticides than hymenopterous parasitoids, with the exception of pyrethroids, carbamates, neonicotinoids and insect growth regulators. The mortality caused by Lannate was higher with the larvae than adults (Table 3.6.3.1) and the overall impact rating of 73.6 categorised the product as Harmful (Table 3.6.3.2). This was slightly lower than the 80 previously obtained for *Chilocorus nigritus* (Grout et al. 2011). All other products caused little or no mortality to *C. montrouzieri* and were categorised as Harmless (Table 3.6.3.2). However, these bioassays were not designed to show chronic effects of insect growth regulators (IGRs) so any such properties of methoxyfenozide would not have been detected.

Table 3.6.3.1. Effect of aged residues on *C. montrouzieri* adult and larval mortality after exposure for 48 hours

Formulation	Dosage	Corrected % larval mortality ML after exposure to aged residues (days)					Corrected % adult mortality MA after exposure to aged residues (days)				
		1	7	14	28	42	1	7	14	28	42
Methoxyfenozide 240 SC	60 ml/hl	0.0	-	-	-	-	2.2	-	-	-	-
Spinetoram 250 WG	20 g/hl	0.0	-	-	-	-	2.2	-	-	-	-
Sulfoxaflor 240 SC	12 ml/hl	0.0	-	-	-	-	4.1	-	-	-	-
Methomyl 900 SP	100 g/hl	98.0	84.0	100.0	31.7	38.3	65.3	67.3	62.9	26.5	20.0
Chlorpyrifos 750 WG	64 g/kg	8.3	-	-	-	-	18.4	-	-	-	-
Chlorpyrifos 250 CS	200 ml/hl	2.0	-	-	-	-	2.0	-	-	-	-

Table 3.6.3.2. The overall impact of aged residues on *C. montrouzieri*

Formulation	Dosage	Impact on larval mortality adjusted for persistence (I _L)	Impact on adult mortality adjusted for persistence (I _A)	Overall impact rating (I)	Resultant category
Methoxyfenozide 240 SC	60 ml/hl	0.0	1.1	0.6	Harmless*
Spinetoram 250 WG	20 g/hl	0.0	1.1	0.6	Harmless
Sulfoxaflor 240 SC	12 ml/hl	0.0	2.1	1.1	Harmless
Methomyl 900 SP	100 g/hl	100.0	47.1	73.6	Harmful
Chlorpyrifos 750 WG	64 g/kg	4.3	9.5	6.9	Harmless
Chlorpyrifos 250 CS	200 ml/hl	1.0	1.1	1.0	Harmless

*Bioassays were not designed to test all properties of insect growth regulators

2 *Coccidoxenoides perminutus*

Methoxyfenozide caused no mortality to adult *C. perminutus*, but this test would not have exposed detrimental IGR effects (Table 3.6.3.3). All other products caused high levels of mortality on 7-day-old residues (Table 3.6.3.3) and methomyl and chlorpyrifos CS (micro-encapsulated) were highly persistent. Chlorpyrifos CS was much more persistent and detrimental than the WG formulation which with an impact factor of 60.0 (Table 3.6.3.4) was more detrimental than the EC formulation used in a previous bioassay ($I=45.4$) (Grout et al. 2011). Extreme persistence of a micro-encapsulated formulation was previously found to be the case with Pennacp-M, a CS formulation of methyl parathion which gave impact factors of 100 for *Aphytis* and *Trichogrammatoidea cryptophlebiae*. With chlorpyrifos being one of the few organophosphates that can still be used after petal fall, growers need to be aware of the long residual effect against this mealybug parasitoid. Methomyl fell into the Harmful category in this bioassay whereas in the past it was considered Very harmful for this natural enemy (Grout et al. 2011). Spinosad (Tracer) had also previously been more toxic to *C. perminutus* than spinetoram in these bioassays.

Table 3.6.3.3. Effect of aged residues on *C. perminutus* adult survival after exposure for 24 hours

Formulation	Dosage	Corrected % adult mortality after exposure to aged residues (days)				
		1	7	14	28	42
Methoxyfenozide 240 SC	60 ml/hl	0.0	-	-	-	-
Spinetoram 250 WG	20 g/hl	84.0	90.8	20.6	10.9	-
Sulfoxaflor 240 SC	12 ml/hl	51.0	56.0	25.0	4.7	-
Methomyl 900 SP	100 g/hl	84.5	97.9	66.7	21.3	11.8
Chlorpyrifos 750 WG	64 g/kg	100.0	93.8	43.3	5.7	-
Chlorpyrifos 250 CS	200 ml/hl	91.0	100.0	100.0	100.0	86.9

Table 3.6.3.4. The overall impact of aged residues on *C. perminutus*

Formulation	Dosage	Overall impact rating adjusted for persistence ($I = I_A$)	Resultant category
Methoxyfenozide 240 SC	60 ml/hl	0.0	Harmless
Spinetoram 250 WG	20 g/hl	50.0	Slightly harmful
Sulfoxaflor 240 SC	12 ml/hl	33.6	Slightly harmful
Methomyl 900 SP	100 g/hl	58.8	Harmful
Chlorpyrifos 750 WG	64 g/kg	60.0	Harmful
Chlorpyrifos 250 CS	200 ml/hl	100.0	Very harmful

3 *Trichogrammatoidea cryptophlebiae*

Methoxyfenozide again appeared to have a negligible effect on *T. cryptophlebiae* and sulfoxaflor only caused some mortality on 1-day-old residues (Table 3.6.3.5). Spinetoram gave similar results to Lannate and both chlorpyrifos formulations were extremely detrimental for 6 weeks.

Table 3.6.3.5. Effect of aged residues on the mortality of *T. cryptophlebiae* after exposure for 24 hours

Formulation	Dosage	Residue age (days)					Impact rating (I)	Resultant category
		1	9	14	28	42		
Methoxyfenozide 240 SC	60 ml/hl	4.2	-	-	-	-	1.1	Harmless
Spinetoram 250 WG	20 g/hl	95.4	90.9	92.2	68.0	93.9	89.0	Very harmful
Sulfoxaflor 240 SC	12 ml/hl	18.0	-	-	-	-	4.8	Harmless
Methomyl 900 SP	100 g/hl	100.0	100.0	96.7	88.1	95.0	96.8	Very harmful
Chlorpyrifos 750 WG	64 g/kg	96.1	100.0	100.0	99.2	95.8	98.3	Very harmful
Chlorpyrifos 250 CS	200 ml/hl	100.0	100.0	100.0	100.0	100.0	100.0	Very harmful

4 *Euseius citri*

The adult predatory mites collected from Crocodile Valley Citrus outside Nelspruit are still resistant to organophosphates and only a low level of mortality was caused by chlorpyrifos WG with one-day-old residues (Table 3.6.3.6). Sulfoxaflor had no effect on adult *E. citri* and the effect of methoxyfenozide was negligible. Spinetoram and methomyl had a similar impact (I_A) on adults but the mortality caused by methomyl was initially higher it did not last as long as for spinetoram (Table 3.6.3.6). An unusual but very obvious effect of spinetoram was that it repelled the mites from the leaf surface and even beyond the wet cotton wool barrier. This resulted in a significantly lower ($P < 0.05$) proportion of mites relative to the numbers in the control treatment being present at the end of the exposure period then for spinetoram or methomyl (Table 3.6.3.7). In the field this may drive the predatory mites off the foliage or at least reduce their predation. As this product is registered for the control of citrus thrips on which this mite preys, this repellency is counter-productive.

Table 3.6.3.6. Percentage corrected mortality of adult *E. citri* on aged residues

Formulation	Dosage	Residue age (days)								Adult impact adjusted for persistence (I _A)
		1	3	7	14	21	28	35	42	
Methoxyfenozide 240 SC	60 ml/hl	1.1	0.0	-	-	-	-	-	-	0.6
Spinetoram 250 WG	20 g/hl	41.7	87.3	63.8	58.0	20.1	18.9	6.9	0.0	61.1
Sulfoxaflor 240 SC	12 ml/hl	0.0	0.0	0.0	-	-	-	-	-	0.0
Methomyl 900 SP	100 g/hl	85.3	96.0	0.3	0.2	0.0	-	-	-	52.8
Chlorpyrifos 750 WG	64 g/kg	13.4	0.0	0.0	-	-	-	-	-	7.4
Chlorpyrifos 250 CS	200 ml/hl	0.0	0.0	-	-	-	-	-	-	0.0

Table 3.6.3.7. Mean proportion of adult *E. citri* numbers recovered dead or alive after exposure to residues, relative to the numbers recovered in the control

Formulation	Dosage	Proportion recovered
Spinetoram 250 WG	20 g/hl	0.21 a
Sulfoxaflor 240 SC	12 ml/hl	1.07 b
Methomyl 900 SP	100 g/hl	0.89 b

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

The number of offspring recovered was expressed as a percentage of the number of adults in the untreated control. Although sulfoxaflor appeared to have no effect on adult mites (Tables 3.6.3.6, 3.6.3.7) it caused a 76% reduction in progeny which resulted in the final impact being Very harmful (Table 3.6.3.9). The micro-encapsulated formulation of chlorpyrifos caused dramatic hormoligosis and doubled the numbers of progeny (Table 3.6.3.8).

Table 3.6.3.8. Effect of aged residues on the population increase of *E. citri*

Formulation	Dosage	Survival % (S _{F1})	% progeny decrease (M _{F1})	I _{F1}
Methoxyfenozide 240 SC	60 ml/hl	113.3	-13.3	-13.3*
Spinetoram 250 WG	20 g/hl	21.2	78.8	30.7
Sulfoxaflor 240 SC	12 ml/hl	23.8	76.2	76.2
Methomyl 900 SP	100 g/hl	39.5	60.5	28.6
Chlorpyrifos 750 WG	64 g/kg	38.1	61.9	57.3
Chlorpyrifos 250 CS	200 ml/hl	215.0	-115.0	-115.0*

*Negative values indicate hormoligosis where sublethal stress increases fecundity

Table 3.6.3.9. The overall impact of aged residues on *E. citri*

Formulation	Dosage	Impact on adult mortality adjusted for persistence (I _A)	Further impact on progeny (I _{F1})	Overall impact rating (I)	Resultant category
Methoxyfenozide 240 SC	60 ml/hl	0.6	-13.3	-12.6	Harmless
Spinetoram 250 WG	20 g/hl	61.1	30.7	91.8	Very harmful
Sulfoxaflor 240 SC	12 ml/hl	0.0	76.2	76.2	Very harmful
Methomyl 900 SP	100 g/hl	52.8	28.6	81.4	Very harmful
Chlorpyrifos 750 WG	64 g/kg	7.4	57.3	64.7	Harmful
Chlorpyrifos 250 CS	200 ml/hl	0.0	-115.0	-115.0	Harmless

Conclusion

For all natural enemies, methoxyfenozide appeared Harmless, although it may have IGR effects that were not detected here. Sulfoxaflor was Harmless or Slightly harmful for 3 natural enemies but Very harmful for predatory mites, so depending on the requirement for predatory mites it could be IPM-compatible. Spinetoram repelled predatory mites and was Very harmful to them. It was also Very harmful to one parasitoid and Slightly harmful to another, so cannot really be considered IPM compatible. The micro-encapsulated chlorpyrifos (Pyrinex) should be used with caution and preferably soon after petal fall because of its extremely persistent negative effect on parasitoids.

Future research

No future research is planned on non-target effects of important natural enemies in citrus because of the poor quality of natural enemies received via courier from the insectaries.

Technology transfer

These results will be presented at the CRI Citrus Research Symposium in 2016 and if possible, published in a refereed journal.

References cited

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18: 265-267.
- Grout, T.G., K.C. Stoltz, and B.A. Tate. 2011. Database of non-target impact ratings (similar to percentage mortality but taking persistence into account) against five key natural enemies in citrus. <http://www.citrusres.com/category/miscellaneous-information>
- Hassan, S. A., F. Bigler, H. Bogenschutz, E. Boller, J. Brun, P. Chiverton, P. Edwards, F. Mansour, E. Naton, P. A. Oomen, W. Overmeer, L. Polgar, W. Rieckmann, Samsøe-Petersen, A. Stäubli, G. Sterk, K. Tavares, J. V. Tuset, G. and A. Vivas. 1988. Results of the fourth joint pesticide testing programme carried out by the IOBC/WPRS-Working Group 'Pesticides and Beneficial Organisms'. *Journal Appl. Entomol.* 105: 321-329.
- Hattingh, V., A. B. Ware, and T. G. Grout. 2000. The Development of a Non-Target Evaluation System for Southern African Citrus. *Proceedings of the International Society of Citriculture*: 795-797.

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

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

Summary

Serrododes partita (Fabricius, 1775) (Lepidoptera: Noctuidae), *Achaea lienardi* (Boiduval, 1833) (Lepidoptera: Noctuidae) and other fruit-feeding moths are injurious in citrus orchards. However, there is no method to monitor their populations. In this study we aimed to quantify the attractiveness of banana against a range of artificial banana odours, to fruit-feeding moths in Satsuma mandarin. Furthermore, we aimed to establish a relationship between trap catches and the level of damage caused to fruit. From 2013 to 2015, banana was compared with various artificial banana odours, in the Kat River Valley, Sundays River Valley and Grahamstown growing regions. Ten traps were used to establish a relationship between trap catches of fruit-feeding moths in the orchards and the level of damage they caused to fruit. Traps were deployed and emptied weekly over a three month period. Four to 10% of moths caught weekly were the fruit-piercing moth, *S. partita*. Two to three weeks after a peak in *S. partita* catches, damage was observed on the fruit, but this relationship was not significant. Fruit-piercing moth damage to fruit was higher than any other cause, both within the orchard and on the northern edge of the orchard next to the windbreak (where moths would enter the orchard). A trial was also conducted in 2014 and 2015 on Alicedale Packers Farm in Tshipise, Limpopo, using frozen banana. The trial ran from the end of January for 7 weeks, where 10 traps were deployed and emptied weekly. The only fruit-piercing moths caught in the traps were of the genus, *Eudocima* and damage to fruit was low, with less than 1% of the fruit being damaged from this cause. After three years of field work, this project will be terminated and fully analysed in 2015.

Opsomming

Serrododes partita (Fabricius, 1775) (Lepidoptera: Noctuidae), *Achaea lienardi* (Boiduval, 1833) (Lepidoptera: Noctuidae) en ander vrugte-voedende motte is skadelik in sitrusboorde. Daar is tans geen metode om hul bevolking te monitor nie. In hierdie studie is gepoog om die aanloklikheid van piesang te kwantifiseer teen 'n reeks kunsmatige piesang reuke, teenoor vrugte-voedende motte in Satsuma mandaryne. Verder het ons daarop gemik om 'n verhouding tussen lokval vangste en vlak van skade aan vrugte vas te stel. Van 2013 tot 2015, is piesang en verskeie kunsmatige piesang reuke vergelyk, in die Katriviervallei, Sondagsriviervallei en Grahamstad produksie streke. Tien lokvalle is gebruik om 'n verhouding tussen lokval vangste van vrugte-voedende motte in die boorde en die vlak van die skade wat hulle veroorsaak het om in vrugte te vestig. Lokvalle is ontplooi en weekliks leeggemaak oor 'n tydperk van drie maande. Vier tot 10% van die motte wat weekliks gevang is, was die vrugte-steekmot, *S. partita*. Twee tot drie weke ná 'n piek in *S. partita* vangste, was die skade waargeneem op die vrugte, maar hierdie verhouding was nie betekenisvol nie. Vrugte-steekmot skade aan vrugte was hoër as enige ander oorsaak, albei binne die boord, sowel as op die noordelike rand van die boord langs die windbreek (waar motte die boord sou betree). Veldproewe is in 2014 en 2015 op Alicedale Packers plaas in Tshipise, Limpopo gedoen, met behulp van bevrore piesang. Die veldproewe het vanaf die einde van Januarie vir 7 weke geduur, waar 10 lokvalle ontplooi is en weeklikse leeggemaak is. Die enigste vrugte-steekmotte wat in die lokvalle gevang is, was van die genus, *Eudocima*. Minder as 1% van die vrugte is deur vrugte-steekmot beskadig. Na drie jaar se veldwerk sal hierdie projek in 2015 tot einde kom en volledig geanaliseer word.

4 PORTFOLIO: DISEASE MANAGEMENT

4.1 PORTFOLIO SUMMARY

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

The Disease Management portfolio is continuing to serve the southern African citrus industry. Most grower priorities are addressed in projects designed to meet certain short-, medium- and long-term strategic objectives. These service and research objectives/strategies and highlights from the various programmes are briefly summarised below. Progress of the 2014-15 reporting period is summarised in the programme summaries.

Service objectives in Graft Transmissible Diseases programme are to provide diagnostic services for the Citrus Improvement Scheme (CIS) through re-indexing of mother block trees, pathogen elimination and pre-immunisation of new entries. Diagnostic services are ongoing and are continually reviewed in order to improve wherever possible (see CIS report). Since the performance of certain 'cleaned-up' CIS material has been criticised, a project was started to evaluate the horticultural performance of old-clone material with CIS material (1074); infected and CIS trees were made and will be planted out shortly. Research objectives are largely focussed on sustainable control of *Citrus Tristeza virus* (CTV), which is based on cross-protection. On a more fundamental level, the mechanisms involved in mild strain cross protection is research (885B, 1056, 1100), while applied research projects evaluate the suitability of candidate cross-protection sources for various climate regions, citrus types and cultivars (738, 739, 742, 789, 968). The epidemiology of African Citrus Greening, and specifically the alternative hosts of the bacterium, is studied. Importantly, this project also collaborates with international research on the feared Asiatic form of this disease (886B). Two from three embryo-rescue clones that proved to be greening tolerant in pre-screening have developed low levels of greening symptoms; evaluation has been intensified and will be conducted for another season (815).

The Soilborne Diseases programme researches sustainable options (alternatives to harsh chemicals) for root rot and citrus nematode control, and certain promising options have been identified (762, 1030). A new project was also initiated on the etiology and control of Armillaria root rot (1068); however, dieback that was previously thought to be caused by Armillaria appears to be caused by a complex of pathogens. Early diagnosis of citrus tree decline is essential to improve the chances of remedial actions. Multiple parameters have been evaluated as potential decline indicators prior to severe (visual) symptom development (910); the most suitable parameters are being studied further in ongoing research. A new project was initiated on the preventative and curative management of soilborne diseases in citrus nurseries (1101), which also supports the CIS's goal of supply of disease free citrus trees to growers in southern Africa.

In the Citrus Black Spot programme, a considerable amount of time has been spent on technical market access support. A collaborative project with USA, Brazilian and Argentinian researchers to develop a probabilistic model to quantitatively predict the risk of fruit as a pathway for CBS has also successfully been concluded and a scientific article is being prepared (1026). CBS epidemiology is being studied in this project, as well as in the Eastern Cape, Limpopo and Mpumalanga provinces through spore trapping and weather monitoring (919, 1026). The global population structure of the CBS pathogen is being studied, which will further elucidate CBS epidemiology and global movement of the pathogen (977). Spray programmes are continuously studied to improve our understanding of CBS control, our ability to cost-effectively control CBS, to manage fungicide resistance, improve formulations, and to register new active ingredients (970, 1012). Highlights from these trials are the imminent registration of novel compounds and combinations that should save growers up to two spray rounds. Six new projects were initiated on CBS. Three projects will study knowledge gaps in CBS epidemiology; one at CRI (1128), and two contracted to third parties. On control measures, one project will investigate the potential of reduced volume fungicide application to control CBS, while another will study the effects of postharvest treatments on the viability and reproduction potential of CBS fruit lesions. A web-based information system is being developed to improve CBS management; this system aims to package weather and CBS epidemiological model output to improve general understanding of CBS epidemiology and to enable better informed CBS management.

In the Fruit and Foliar Diseases programme, new control options for Alternaria brown spot (ABS) is continuously being studied (750). Research also focuses on improving spray application through optimal use of spray machines or adjuvants (891). This project was written up as a final report, whilst the research is continued in three projects aiming to characterize citrus tree canopies with radar technology to improve calibration (1089), and the use of adjuvants to improve fungicide foliar spray deposition and control of Alternaria brown spot (1096). The study of Botrytis blossom blight and fruit drop in lemons was concluded. Suitable fungicides were identified, but the economic impact of Botrytis blossom infection and optimal timing of application needs to be determined (1015).

The Postharvest Diseases programme remains a very high priority and several projects were directly aimed at improving postharvest disease management in packhouses. Potential alternative fungicides and sanitisers are continuously screened in pilot trials (123), before further trials are recommended. Imazalil, thiabendazole and pyrimethanil drench and in-line (fungicide bath or JBT heated flooder application) application and subsequent control of green mould were studied, giving valuable insight into the optimal use of these postharvest fungicides (1050, 1103, 1104). The study of integration of preharvest silicon, and postharvest heat and biocontrol against chilling injury and green mould has been concluded (UKZN1). Potential pre-harvest risk indicators for postharvest decay were studied in an attempt to identify those indicators that will enable growers or packhouses to classify fruit consignments in risk categories, but due to limited capacity the project was terminated after one season (1073).

The Diagnostic Centre (DC) continues to perform a sterling service to the Citrus Improvement Scheme through routine soil and water analyses for *Phytophthora* and nematodes, as well as through these analyses in research experiments in the Soilborne Diseases project. In total, 6870 samples were analysed by one diagnostician, a technician and assistant.

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

PORTFEULJEOPSOMMING

Die siektebestuurportefeulje gaan voort om Suidelike Afrika se sitrus-industrie te dien. Die meeste produsente-prioriteite word aangespreek in projekte wat ontwerp word om sekere kort-, medium- en langtermyn strategiese doelwitte te bereik. Hierdie diens- en navorsingsdoelwitte/-strategieë en hoogtepunte van die verskeie programme word kortliks hieronder opgesom. Die vordering vir die 2014-15 verslagperiode word in die program-opsommings opgesom.

Diensdoelwitte in die ent-oordraagbare siekte program is om diagnostiese dienste aan die Sitrusverbeteringskema (SVS) te verskaf, deur her-indeksering van moederblokbome, groeipunt-enting en preïmmunisasie van nuwe kultivars. Diagnostiese dienste is deurlopend en word deurgaans geëvalueer ten einde te verbeter waar nodig (sien SVS verslag). Aangesien die prestasie van sekere "skoongemaakte" SVS materiaal gekritiseer is, is 'n projek begin om die hortologiese prestasie van voor-Skema materiaal, met SVS materiaal, te evalueer (1074); geïnfecteerde en SVS bome is gemaak en sal binnekort uitgeplant word. Navorsingsdoelwitte fokus grootliks op volhoubare beheer van *Sitrus Tristeza virus* (CTV), wat op kruisbeskerming gebaseer is. Op 'n meer basiese vlak, word die meganismes betrokke in kruisbeskerming nagevors (885B, 1056, 1100), terwyl toegepaste navorsingsprojekte die volhoubaarheid van potensiële kruisbeskermingsbronne vir verskeie klimaatstreke, sitrustipes en kultivars evalueer (738, 739, 742, 789, 968). Die epidemiologie van Afrika Sitrusvergroening, en veral die alternatiewe gashere van die bakterie, word bestudeer. Van belang is dat hierdie projek ook in samewerking met internasionale navorsing op die gevreesde Asiatiese vorm van hierdie siekte plaasvind (886B). Twee van drie kultivars wat in vooraf-evaluasies belofte getoon het om vergroeningsbestand te wees, het lae vlakke van vergroeningsimptome getoon; evaluasie gaan meer intensief voort en sal vir 'n verdere seisoen gedoen word (815).

Die grondgedraagde siekte program doen navorsing op volhoubare opsies (alternatiewe vir harde chemikalieë) vir beheer van wortelvrot en die sitrusnematode, en sekere belowende opsies is geïdentifiseer (762, 1030). 'n Nuwe projek is ook op die etiologie en beheer van *Armillaria* wortelvrot geïnisieer (1068). Terugsterwing wat egter voorheen vermoedelik deur *Armillaria* veroorsaak is, blyk deur 'n kompleks van patogene veroorsaak te word. Vroeë diagnose van sitrusboom-agteruitgang is noodsaaklik om die kans vir herstellende aksies te verbeter. Veelvuldige parameters is as potensiële agteruitgang-indikatoren, vóór ernstige (sigbare) simptome-ontwikkeling, geëvalueer (910); die mees geskikte parameters word verder in voortgaande navorsing bestudeer. 'n Nuwe projek is op die voorkomende en genesende bestuur van grondgedraagde siektes in sitruskwekerie (1101) geïnisieer, wat ook die SVS se doelwit ondersteun om siektevrye sitrusbome aan produsente in suidelike Afrika te verskaf.

In die Sitrus Swartvlek (SSV) program is 'n aansienlike hoeveelheid tyd op tegniese marktoegang-ondersteuning spandeer. 'n Gesamentlike projek met die VSA, Brasiliaanse en Argentynse navorsers, ten einde 'n waarskynlikheidsmodel te ontwikkel om kwantitatief die risiko van vrugte as 'n verspreidingsweg vir SSV te voorspel, is suksesvol afgehandel en 'n wetenskaplike artikel word voorberei (1026). SSV-epidemiologie word in hierdie projek deur spoorvangstudies en weermonitering in die Oos-Kaap, Limpopo en Mpumalanga provinsies bestudeer (919, 1026). Die globale populasie-struktuur van die SSV-patogeen word bestudeer, wat verder SSV-epidemiologie en globale beweging van die patogeen sal uitlê (977). Spuitprogramme word voortdurend bestudeer ten einde ons kennis van SSV-beheer en ons vermoë om SSV

koste-effektief te beheer te verbeter, om fungisiedweerstand te bestuur, formulasies te verbeter, en om nuwe aktiewe bestanddele te registreer (970, 1012). Hoogtepunte vanuit hierdie proewe, is die naderende registrasie van nuwe verbindings en kombinasies wat produsente tot soveel as twee spuitronndes behoort te spaar. Ses nuwe projekte is op CBS geïnisieer. Drie projekte sal kennisgapinge in CBS epidemiologie bestudeer, een by CRI (1128), en twee wat aan derdepartye gekontrakteer is. Op die gebied van beheermaatreëls, gaan een projek die potensiaal van verminderde volume fungisiedtoediening ten einde CBS te beheer, ondersoek, terwyl 'n ander een die effekte van na-oesbehandelings op die lewensvatbaarheid en voortplantingspotensiaal van CBS vrugletsels ondersoek. 'n Web-gebaseerde inligtingsisteem word ontwikkel om CBS bestuur te verbeter; hierdie sisteem het ten doel om weer- en CBS epidemiologiese model-uitsette te verpak, om die algemene kennis van CBS epidemiologie te verbeter, en om beter ingeligte CBS bestuur moontlik te maak.

In die vrug- en blaarsiekte program, word nuwe beheer-opsies vir *Alternaria* bruinvlek (ABV) voortdurend bestudeer (750). Navorsing fokus ook op die verbetering van spuittoediening deur optimale gebruik van spuitmasjiene of byvoegmiddels (891). Hierdie projek is as 'n finale verslag opgeskryf, terwyl die navorsing in drie projekte voortgesit word, wat ten doel het om sitrusboom lowerdakke met radartegnologie te karakteriseer ten einde kalibrasie te verbeter (1089), en die gebruik van benatters om swamdoder toediening en beheer van *Alternaria* bruinvlek te verbeter (1096). Die studie oor *Botrytis* bloeiselsversenging en vrugval in suurlemoene is voltooi. Geskikte fungisiedes is geïdentifiseer, maar die ekonomiese impak van *Botrytis* bloeisel-infeksie en optimale tyd vir toediening moet egter nog bepaal word (1015).

Die na-oes siekte program bly 'n baie hoë prioriteit en verskeie projekte is direk gerig op die verbetering van na-oes siektebestuur in pakhuse. Moontlike alternatiewe fungisiedes en saniteerders word deurlopend in loodsproeve geëvalueer (123) voordat verdere proewe aanbeveel word. Imazalil, thiabendazole en pyrimethanil stort ('drench') en in-lyn (fungisiedbad of JBT verhitte vloedtoediening) toediening, en gevolglike beheer van groenskimmel, is bestudeer, en het waardevolle insig in die optimale gebruik van hierdie na-oes fungisiede gegee (1050, 1103, 1104). Die studie van integrasie van voor-oes silikon, en na-oes hitte en biobeheer teen koue-skade en groenskimmel, is voltooi (UKZN1). Potensiële voor-oes risiko-indikatore vir na-oes verval is bestudeer in 'n poging om daardie indikatore te identifiseer wat produsente of pakhuse in staat sal stel om vrugbesendings in risiko-kategorieë te klassifiseer, maar weens beperkte kapasiteit, is die projek ná een seisoen gestaak (1073).

Die Diagnostiese Sentrum (DS) lewer steeds 'n uitstekende diens aan die Sitrusverbeteringskema deur roetine grond- en waterontledings vir *Phytophthora* en aalwurms, asook deur ontledings vir navorsers in die Grondgedraagde Siekte projek. In totaal het die DS 6870 monsters geanaliseer, en dit met slegs een diagnostikus, een tegnikus en een assistent.

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

4.2 PROGRAMME: GRAFT TRANSMISSIBLE DISEASES

Programme koördinator: G. Cook (CRI)

4.2.1 PROGRAMME SUMMARY

Research in the Graft Transmissible Disease programme is inherently linked to the requirements of the Citrus Improvement Scheme (CIS) to ensure supply of healthy propagation material to the industry. Supply of good quality material since the inception of the CIS has limited the number of graft-transmissible diseases that challenge production. CTV and African Greening are the two pathogens that require management strategies for control as they are also transmitted by insect vectors. A strong research emphasis is therefore placed on the CTV cross-protection programme to mitigate the effects of this virus especially in grapefruit. This virus is extremely complex and consists of a number of strains and variants. Diagnostics to identify these strains and variants has progressed significantly over the last couple of years, but still remains very challenging due to various recombination events between strains. Since the virus naturally occurs as mixtures of strains, it is difficult to understand which strains are involved in the disease expression and which are required to mitigate the disease. The only approach to understand this disease is to identify single-strain sources and to apply them in glasshouse trials for specific analyses. Two projects have focussed on characterisation of CTV sources to elucidate the cross-protection mechanism(s) (Projects 885B & 1056) and are continued in Project 1100. Field trials have been ongoing to assess the field performance of promising pre-immunisation sources in grapefruit, sweet orange and soft citrus (Projects 738, 739, 742, 789 & 968). As the diagnostic capabilities and understanding of strain interactions advance, it is expected that we will gain a

clearer understanding of the field results. The biological assessment of the effect of CTV is a lengthy process of numerous years, but is a vital to understanding the complexity of the interaction of this virus with its host and the environment.

Claims of better fruit quality and yields using old clone (pre-scheme) material containing viroids and CTV compared to that obtained using the viroid-free and CTV pre-immunised material supplied by the Citrus Improvement Scheme (CIS), is being investigated in a comparative study (Project 1074). Field evaluation of sweet orange, embryo-rescued clones, selected for resistance/tolerance to greening is ongoing as resistant varieties are seen as an important means of disease control (Project 815). Greening symptoms were observed on three trees of two clones after 7 years and the presence of the Liberibacter in these trees was confirmed by PCR. Trees of one promising clone were still greening free. Production of these clones compared well with the commercial control. Indigenous plants of the citrus family (*Rutaceae*) are evaluated for their ability to host "*Candidatus Liberibacter africanus*" (Laf), the African greening pathogen (Project 886B). Laf has not been detected in any indigenous host yet. Some Rutaceous genera have now been shown to harbour Liberibacters similar, but differing from Laf and include *Zanthoxylum*, *Vepris*, *Clausena*, *Teclea*, *Orcia* and *Calodendrum*. These genera were shown to be graft compatible with sweet orange and this will enable transmission studies of the Liberibacters found in these genera to *Citrus* to investigate whether they can infect citrus.

PROGRAMOPSOMMING

Navorsing in die Ent-oordraagbare Siekte program is inherent gekoppel aan die vereistes van die Sitrus Verbeteringskema (SVS) om die verskaffing van gesonde voortplanting materiaal aan die bedryf te verseker. Verskaffing van goeie gehalte materiaal sedert die aanvang van die SVS het die getal van ent-oordraagbare siektes wat produksie beïnvloed, aansienlik beperk. CTV en Afrika Vergroening is die twee patogene wat beheer strategieë vereis omdat hulle ook deur insekte vektore oorgedra word. 'n Navorsingsklem is dus op die CTV kruis-beskerming geplaas om die gevolge van die virus te beperk, veral in pomelos. Hierdie virus is uiters kompleks en bestaan uit 'n aantal rasse en variante. Diagnostiese identifikasie metodes om hierdie rasse en variante te kan onderskei, het oor die laaste paar jaar aansienlik gevorder, maar is nog steeds 'n groot uitdaging as gevolg van rekombinasie tussen rasse. Omdat die virus natuurlik voorkom as ras-mengsels, is dit moeilik om te bepaal watter rasse betrokke is by die siekte uitdrukking en watter benodig word om die siekte uitdrukking te beperk. Die beste navorsingsbenadering om die siekte te ondersoek, is om enkel-ras bronne te identifiseer en in glashuis proewe spesifiek te ontleed. Twee projekte is gefokus op karakterisering van CTV bronne om sodoende die kruis-beskerming-meganisme(s) te ondersoek (Projekte 885B & 1056) en word voortgesit in Projek 1100. Veldproewe word deurlopend geëvalueer om te bepaal watter CTV preïmmuniseringsbronne die beste beskerming bied en produksie lewer in pomelos, soet lemoen en sagte sitrus (Projekte 738, 739, 742, 789 & 968). Soos wat diagnostiese vermoëns en begrip van CTV ras interaksies verbeter, is die verwagting dat daar 'n beter begrip van die veld resultate sal ontstaan. Die biologiese evaluasie van die effek van CTV is 'n lang proses wat oor jare strek, maar is 'n noodsaaklikheid om die kompleksiteit van die interaksie van hierdie virus met sy gasheer en die omgewing te verstaan.

Stellings wat gemaak word dat beter gehalte vrugte en beter opbrengste verkry word met die gebruik van ou kloon (voor-skema) materiaal, wat viroïede en CTV bevat, as dié verkry met viroïed-vrye materiaal van die SVS, word ondersoek in 'n vergelykende studie. Proefbome is voorberei en sal in die lente van 2015 geplant word (Projek 1074).

Gasheer weerstand vir vergroening word as 'n belangrike beheer metode beskou en veld evaluasie van embryo-herwinningsklone vanaf soetlemoen wat potensiële weerstand / toleransie teen vergroening toon, is voortgesit (Projek 815). Vergroening simptome is waargeneem in drie bome van twee klone na 7 jaar in die veld en die teenwoordigheid van Liberibacter in hierdie bome is bevestig deur PCR. Bome van een belowende kloon wys nog geen vergroening nie. Produksie van hierdie klone vergelyk goed met die kommersiële kontrole.

Inheemse plante van die sitrus familie (*Rutaceae*) word geëvalueer vir hul vermoë om as alternatiewe gasheer vir "*Candidatus Liberibacter africanus*" (Laf), die Afrika-vergroening patogeen, te dien (Projek 886B). Laf is nog nie in enige inheemse gasheer gevind nie. Sommige genera in die *Rutaceae* familie, *Zanthoxylum*, *Vepris*, *Clausena*, *Teclea*, *Orcia* en *Calodendrum*, het wel soortgelyke Liberibacters wat van Laf verskil, gehad. Dit is bepaal dat hierdie boom-genera entversoenbaar met soetlemoen is en dit sal oordrag studies van hierdie Liberibacter subspesies vergemaklik.

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

Project 738 (2004 - 2016) by S.P. van Vuuren, J.H.J. Breytenbach and G. Cook (CRI)

Summary

Indications of a possible severe *Citrus tristeza virus* component in the Nartia (GFMS 12) cross-protecting source necessitated the separation of the strain populations into sub-isolates by single aphid transmissions. These sub-isolates were derived from two Nartia sources (A = GFMS 12, C = GFMS 14) and a Mouton source derived from sweet orange. The GFMS 14 and Mouton sub-isolates were done at Beltsville, USA, and imported back to South Africa. After biological indexing, four sub-isolates showed potential for further evaluation (GFMS 14: B389-1, B389-4; Mouton: B390-3, B390-5). Two sub-isolates from the ARC-ITSC sources (GFMS 12/7, GFMS 12/9) were included in the trial as well as GFMS 12 (previous standard cross-protector for white grapefruit) and GFMS 35 (standard cross-protector for red grapefruit). Virus-free Star Ruby trees were prepared in a glasshouse and were pre-immunised with the various sources. A virus-free treatment was included as a control and field-infection indicator. After confirming pre-immunisation by ELISA, trees were planted in the Kakamas area in September 2004. This is a duplicate experiment of Project 679, planted in 2003 in Swaziland, and the two experiments are aimed at assessing the CTV expression in different climatic conditions. In 2007 similar trials were also planted in the Malelane and Letsitele areas (Project 742). During this report period, tree sizes were measured at the Kakamas trial, 10 years after planting. Trees grew much slower than in other grapefruit production areas. Stem pitting evaluations were done and trees containing sub-isolate B389/1 developed moderate stem pitting and those with GFMS 12, 12/7 and B390/5, light pitting. Fruit were also harvested and graded into export sizes. The average yield per tree of all the treatments did not differ significantly. Trees with B389/1 yielded the least fruit and GFMS35 the most but with significantly more small fruit than all the other treatments. Trees with GFMS 12 yielded the most large-sized fruit but were equal to the two sub-isolates (GFMS 12/7 and GFMS 12/9) and that of the trees that were planted virus-free. The virus-free trees still remain symptomless after 9 years indicating low natural pressure by aphids and/or CTV strains in the area.

Opsomming

Weens aanduidings van 'n strawwe *Citrus tristeza virus* (CTV) komponent in die Nartia (GFMS 12) kruisbeskermingsbron was dit nodig om die virus populasie in sub-isolate deur middel van enkel plantluis oordragings te skei. Sub-isolate is vanaf twee Nartia bronne (A=GFMS 12, C=GFMS 14) en 'n Mouton bron verkry. Die GFMS 14 en Mouton sub-isolate is by die kwarantyn fasiliteit in Beltsville, VSA, voorberei en terug na Suid Afrika ingevoer. Nadat die sub-isolate deur biologiese indeksing ge-evalueer is, is gevind dat slegs vier potensiaal toon vir verdere evaluasie (GFMS 14: B389-1, B389-4; Mouton: B390-3, B390-5). Twee belowende Nartia sub-isolate afkomstig van die LNR-ITSG (GFMS 12/7, GFMS 12/9) is by die proef ingesluit. GFMS 12 (vorige kruisbeskermingsbron) en GFMS 35 (huidige kruisbeskermingsbron) is as kontrole verwysings gebruik. Virusvrye Star Ruby boompies is in 'n glashuis voorberei en met die verskeie bronne gepreïmmuniseer. 'n Virusvrye behandeling is as kontrole ingesluit wat natuurlike besmettings sal aandui. Hierdie proef is 'n herhaling van Projek 679 wat in Swaziland aangeplant is, asook gedeetelike herhaling van proewe aangeplant in Malelane en Letsitele (Projek 742). Die verskeie proewe dien om CTV in die verskillende sitrus produserende streke te evalueer. Nadat preïmunisering deur middel van ELISA bevestig is, is die boompies in die Kakamas omgewing gedurende September 2004 uitgeplant en word jaarliks vir boomgrootte, stamgleuf, oes opbrengs en vruggrootte ge-evalueer. Die bome se groottes is 10 jaar na uitplant gemeet. Die bome groei heelwat stadiger as bome in die ander pomelo produserende streke. Stamgleuf evaluasies is gedoen en sub-isolaat B389/1 het matige stamgleuf ontwikkel en GFMS 12, 12/7 en B390/5 ligte stamgleuf. Vrugte is geoes en gegradeer volgens uitvoer groottes. Die gemiddelde opbrengs per boom van al die behandelings het nie betekenisvol verskil nie. Bome met B389/1 het die minste vrugte geproduseer en GFMS35 die meeste maar betekenisvol meer kleiner vrugte as die ander behandelings. Bome met GFMS 12 het betekenisvol die meeste groot vrugte geproduseer maar was nie beter as die twee sub-isolate (GFMS12/7 en GFMS12/9) en die van die bome wat virusvry geplant is nie. Die virusvrye bome is na 10 jaar nog steeds simptomeeloes wat aandui dat daar 'n lae druk van plantluis en/of CTV in die omgewing is.

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

Project 739 (2004 - 2017) by S.P. van Vuuren, J.H.J. Breytenbach and G. Cook (CRI)

Summary

Disease expression of *Citrus tristeza virus* (CTV) is influenced by citrus cultivar and climatic conditions. It is therefore necessary to evaluate the various cross-protecting CTV sources in various citrus production areas. Mild CTV sources derived from sweet orange trees (SM 46, SM 47, SM 48, SM 49) were used to pre-immunise virus-free Delta -, Midnight -, and Turkey Valencia on C35 citrange rootstocks. These sources will be compared to LMS 6 (standard pre-immunisation source for sweet oranges) and virus-free controls. Pre-immunisation was confirmed by means of ELISA. The trees were planted at Karsten Boerdery in the Kakamas area in September 2007. Tree size was measured 7 years after planting. Trees with SM 48 were significantly smaller in all three cultivars. Trees were harvested for the first time in 2014. Although there are significant differences in growth and yield between trees with the different CTV treatments, the results should be seen only as trends at this stage. Trees will be evaluated annually for growth, production, fruit size distribution and tree health. The influence of pre-immunisation sources will only be apparent after evaluation over a number of seasons.

Opsomming

Siekte uitdrukking van *Citrus tristeza virus* (CTV) verskil tussen sitrus kultivars en onder verskillende klimaatstoestande. Dit is dus nodig om verskillende CTV bronne in verskillende sitrus produserende streke te evalueer. Pontensiële CTV preïmmuniseringsbronne wat oorspronklik vanaf soetlemoenbome versamel is (SM 46, SM 47, SM 48, SM 49), is gebruik om virusvrye Delta -, Midnight -, en Turkey Valencia op C35 citrange onderstam te preïmmuniseer. Hierdie bronne word met LMS 6 (die standaard preïmmuniseringsbron vir soetlemoene) vergelyk, asook met bome wat virusvry geplant is. Preïmmunisering is deur middel van ELISA bevestig, waarna die boompies gedurende September 2007 by Karsten Boerdery in die Kakamas omgewing geplant is. Die boomgroottes is 7 jaar na uitplant gemeet. Bome met SM 48 was in al drie kultivars betekenisvol die kleinste. Bome is vir die eerste keer in 2014 geoes. Alhoewel daar betekenisvolle verskille in boomgrootte en opbrengs tussen die verskillende behandelings by elke kultivar was, is dit nodig om data van produksie en boomgroei oor 'n aantal jare te versamel vir 'n volledige evaluasie.

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

Project 742 (2004 - 2017) by S.P. van Vuuren, J.H.J. Breytenbach and G. Cook (CRI)

Summary

Budwood was collected from 108 superior grapefruit trees from the different grapefruit production areas of southern Africa that harbour possible mild CTV sources. After the CTV sources were established in the glasshouse at CRI, material was inoculated to virus-free Mexican lime indicator plants to evaluate the severity of the CTV sources. After the first biological test, 19 sources were selected for further evaluation. These 19 sources were inoculated again to virus-free Mexican lime plants and compared to GFMS 12, GFMS 35, GFMS 12/7, GFMS 12/9, and the four best Beltsville sub-isolates (GFMS 14: B389-1, B389-4; Mouton: B390-3, B390-5). The Mexican lime plants were evaluated for growth and stem pitting. Virus titre was determined by ELISA. The four most promising of the 19 field sources (Tabankulu 1 – derived from Star Ruby in Swaziland; New Venture 41/2 – derived from Star Ruby in the Nkwale Valley; ORE 8 – derived from Marsh in the Hoedspruit area; Tshipise 19/5 – derived from Marsh in Tshipise), indexed free for citrus viroids and are being evaluated as pre-immunising agents for Marsh and Star Ruby trees. These sources are compared to GFMS 12 (standard for white grapefruit at the time), GFMS 35 (standard for red grapefruit), as well as the four best Beltsville sub-isolates (B389-1, B389-4, B390-3, B390-5) and the ITSC sub-isolates (GFMS 12/7, GFMS 12/9). Pre-immunisation was confirmed by means of ELISA and the Star Ruby trees were planted at Bosveld Citrus Farm in the Letsitele area in February 2007, while the Marsh trees were planted at Riverside in the Malelane area in March 2007. The trees were evaluated for growth and health 7 years after planting. Both the Marsh and Star Ruby trees containing GFMS 12 had developed unacceptably high stem pitting and resulted in suppressed tree growth. Although differences were observed, conclusions as to the preferred pre-immunisation source can only be made after production and tree health rating data is acquired over a number of production seasons. The indication is that trees with the single aphid transferred sub-isolate sources produce better and higher quality fruit than trees with the field sources.

Opsomming

Enthout is vanaf 108 uitstaande pomelo bome, wat gesondheid en produksie betref, in die verskillende pomelo gebiede in suider Afrika versamel. Die bronne is op virusvrye onderstamme in die glashuis by CRI gevestig. Hierna is die verskillende bronne afsonderlik op Meksikaanse lemmetjie geïnkuleer (biologiese indeksering) om te bepaal of die bome moontlik ligte rasse van *Citrus tristeza virus* (CTV) huisves wat as kruisbeskermingsbronne kan dien. Na die eerste biologiese indeksering van 6 maande het slegs 19 bronne potensiaal getoon en is vir verdere evaluasie gebruik. Hierdie 19 bronne is 'n tweede keer op Meksikaanse lemmetjie geïnkuleer en met bekende bronne GFMS 12, GFMS 35, GFMS 12/7, GFMS 12/9 en die vier beste Beltsville sub-isolate (GFMS 14: B389-1, B389-4; Mouton: B390-3, B390-5) vergelyk. Na 'n tydperk van 6 maande is die geïnkuleerde plante vir groei en voorkoms van stamgleuf asook die virus titer d.m.v. ELISA ge-evalueer. Die 4 mees belowende bronne, wat vry is van viroïede, is Tabankulu 1 (versamel vanaf Star Ruby in Swaziland), New Venture 41/2 (versamel vanaf Star Ruby in die Nkwaleni Vallei), ORE 8 (versamel vanaf Marsh in die Hoedspruit gebied) en Tshipise 19/5 (versamel vanaf Marsh in Tshipise). Hierdie bronne is verder gebruik om virus-vrye Marsh en Star Ruby boompies vir boord evaluasie te preïmmuniseer. Die bronne word met GFMS 12 (vorige standaard vir wit pomelos), GFMS 35 (huidige standaard vir pomelos), asook die vier beste Beltsville sub-isolate (B389-1, B389-4, B390-3, B390-5) en LNR-ITSG sub-isolate (GFMS 12/7, GFMS 12/9) vergelyk. Preïmmunisering is deur middel van ELISA bevestig voordat bome geplant is. Die Star Ruby boompies is gedurende Februarie 2007 op Bosveld Sitrus Plaas in die Letsitele omgewing geplant en die Marsh boompies is gedurende Maart 2007 by Riverside in die Malelane omgewing geplant. Die boompies is die sewende jaar na uitplant vir groei en stamgleuf ge-evalueer. Tot op hede het bome met GFMS 12 in beide Marsh en Star Ruby onaanvaarbare hoë stamgleuf ontwikkeling getoon wat ook sodoende die groei belemmer het. Alhoewel daar verskille voorkom tussen die verskillende bronne ten opsigte van vrug en boom groottes, kan gevolgtrekkings eers gemaak word na 'n aantal jare se oes en boomgesondheid opnames. Daar is aanduidings dat bome met die enkel-plantluis oorgedraagde sub-isolaat bronne beter produseer met hoër gehalte vrugte as die bome met die veldbronne.

4.2.5 PROGRESS REPORT: Identification of suitable *Citrus tristeza virus* sources for pre-immunising Turkey Valencia

Project 789 (2005 - 2017) by S.P. van Vuuren, J.H.J. Breytenbach and G. Cook (CRI)

Summary

Turkey Valencia appears to be more sensitive to CTV than other Valencia types. Since Turkey Valencia is an early Valencia type, it is an important component of the local citrus export portfolio and it is therefore important to identify a suitable CTV pre-immunising source for this cultivar. Virus-free Turkey Valencia on Troyer citrange rootstocks were prepared in the glasshouse and inoculated with different CTV sources; LMS 6 (standard), SM 46, SM 47, SM 48, SM 49 (all obtained from sweet orange) to identify the best source for cross-protection purposes. Trees inoculated with GFMS 12 and virus-free trees serve as positive and negative controls, respectively. Pre-immunisation was confirmed by means of ELISA. The trees were planted at Riverside in the Malelane area in March 2007. Tree growth and yield were measured 7 years after planting and differences were observed. Trees which were planted virus-free had the highest production but were not significantly better than trees with SM 46, SM 47, GFMS 12 and LMS 6 (standard) CTV sources. The production of fruit larger than count 105 were also very similar, 63% (virus-free), 61%, 68%, 60% and 70% respectively. Trees with SM 48 were significantly smaller than the rest and only 31% of the crop was larger than count 105. Conclusions as to the preferred pre-immunisation source can only be made after production and tree health rating data is acquired over a number of production seasons.

Opsomming

Daar is gevind dat Turkey Valencia meer gevoelig vir *Citrus tristeza virus* (CTV) as ander Valencia tipes is. Aangesien Turkey Valencia 'n vroeë Valencia is, is dit 'n belangrike kultivar in die bedryf en daarom is dit 'n hoë prioriteit om 'n geskikte CTV preïmmunisasie bron vir Turkey Valencia te vind. Virusvrye Turkey Valencia op Troyer citrange onderstam is in 'n glashuis voorberei en met verskeie CTV bronne, LMS 6 (standaard), SM 46, SM 47, SM 48, SM 49 (almal vanaf soetlemoene versamel), geïnkuleer om die beste ligte CTV bron vir kruisbeskermingsdoeleindes te identifiseer. Bome wat met die GFMS 12 bron geïnkuleer is en bome wat virusvry gelaat is, dien as positiewe en negatiewe kontroles onderskeidelik. Preïmmunisasie is deur middel van ELISA bevestig en die bome is gedurende Maart 2007 by Riverside in die Malelane omgewing geplant. Die boompies is 7 jaar na uitplant, vir groei en produksie ge-evalueer. Bome wat virusvry geplant was het die hoogste produksie gehad maar was nie betekenisvol beter as bome met SM 46, SM 47, GFMS 12 en LMS 6 (standaard) CTV bronne nie. Die produksie van vrugte groter as telling 105 was ook baie dieselfde, onderskeidelik 63% (virusvry), 61%, 68%, 60% en 70%. Bome met SM 48 was betekenisvol kleiner as die res en slegs 31% van die produksie was groter as telling 105. Alhoewel daar verskille voorkom tussen die

verskillende bronne ten opsigte van vrug en boom groottes, kan gevolgtrekkings eers gemaak word na 'n aantal jaar se oes en boomgesondheid opnames.

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

Project 968 (2004 - 2020) by S.P. van Vuuren, J.H.J. Breytenbach and G. Cook (CRI)

Summary

During re-indexing of the Citrus Foundation Block mother trees in 2003 it was found that many Clementine and mandarin trees did not contain CTV despite pre-immunisation with LMS 6 CTV source. This caused concern as the budwood that was multiplied from these mother trees and supplied to the commercial nurseries, were virus-free, rendering the trees unprotected against natural CTV infection with severe strains introduced by aphids. A change to another CTV source compatible with mandarin types was required. The GFMS 12 CTV source was approved for pre-immunisation in the interim until a suitable CTV pre-immunising source for soft citrus is identified. A glasshouse trial was conducted in 2006 to evaluate additional CTV sources in four different soft citrus cultivars. The current field trials are extensions of the glasshouse trial. Two Clementine selections (Clemenluz, Esbal) and two mandarin selections (Valley Gold, Morr 22) on Troyer citrange rootstock have been grown and pre-immunized with different CTV sources; i.e. CTVSC, SM 47, SM 48 and SM 49. Trees with these sources will be compared to trees that were pre-immunised with GFMS 12 (standard) and trees planted virus-free. Pre-immunisation was confirmed by means of ELISA and the trees were planted during 2010/11 at two localities in different climatic regions suitable for the production of soft citrus, i.e. Groblersdal in Mpumalanga and Citrusdal in the Western Cape. Trees at both trials sites were lost due to frost and poor drainage one year after planting. New trees had to be prepared and the trials re-planted at two new sites. The first trial was planted in the Citrusdal area during December 2012 and the second was planted during spring 2014 at Burgersfort. Growth data were collected at the Citrusdal trial but not at Burgersfort. The CTV sources had no effect on the growth of Clemenluz, Valley Gold and Morr 22; however, the growth of Esbal was negatively affected by some CTV sources.

Opsomming

Tydens die her-indeksering van die Grondvesblok se moederbome gedurende 2003 is gevind dat 'n groot aantal Clementine en mandaryn bome geen CTV bevat het nie ten spyte van pre-immunisering met die LMS 6 bron. Dit het kommer gewek as gevolg van die feit dat enthout wat aan die kommersiële kwekerye verskaf word, virusvry is en nie beskerming bied teen natuurlike CTV rasse wat in die veld deur plantluise oorgedra word nie. Die GFMS 12 CTV bron is goedgekeur om tydelik gebruik te word tot 'n geskikte bron vir sagte sitrus gevind is. 'n Glashuis proef is gedurende 2006 gedoen om CTV bronne in vier verskillende kultivars te evalueer. Die veldproewe is 'n uitbreiding van die glashuis proef. Twee Clementine seleksies (Clemenluz, Esbal) en twee mandaryn hibried seleksies (Valley Gold, Morr 22) is op Troyer citrange onderstamme gekuleer en gepreïmmuniseer met verskillende CTV bronne, nl. CTVSC, SM 47, SM 48 en SM 49. Bome met hierdie bronne word met GFMS 12 (standaard) en bome wat virusvry geplant is, vergelyk. Nadat preïmmunisering deur middel van ELISA bevestig is, is die bome gedurende 2010/11 in twee verskillende klimaatstreke wat geskik vir sagte sitrus geplant (Groblersdal in Mpumalanga en Citrusdal in die Wes Kaap). As gevolg van dreineringsprobleme en koue skade is proefbome in beide persele verloor. Nuwe bome is voorberei en gedurende Desember 2012 in die Citrusdal omgewing geplant en gedurende die lente van 2014 is die proef te Burgersfort geplant. Groei data is by die Citrusdal proefbome geneem maar nie by Burgersfort nie. Na een jaar het die CTV bronne geen effek op die Clemenluz, Valley Gold en Morr 22 boompies gehad nie. Die groei van die Esbal boompies is egter negatief beïnvloed deur sommige CTV bronne.

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

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

Summary

During the current report period we reassessed the usefulness of immunocapture of *citrus tristeza virus* (CTV) particles for use as templates in next generation sequencing runs. The technique, while theoretically viable, once again yielded low numbers of CTV specific reads. We also determined that a PCR system, targeted at the p33 gene (modified from one developed in 2013) does have some bias in amplification of different CTV genotypes, with Taiwan-Pum/SP/T1, RB, CTZA and T36 being detected at underrepresented levels given the defined template prepared. The p33 gene is an essential gene to use in the characterisation of CTV populations in plants for cross protection because of its association with super-infection exclusion

specificity in CTV. Therefore, as the PCR was still capable of detecting all the tested genotypes, we used it for a survey of Star Ruby grapefruit trees, where 96 samples were analysed. Interestingly, in spite of the technique bias against RB amplification, this genotype proved to be the dominant component of most trees analysed (especially younger trees), and was present in all samples. Numerous other genotypes were also observed. Large numbers of replicates of single aphid transmissions and dilution end point inoculations using bark slash and bark flap methods were conducted to isolate pure CTV genotypes. While a total of 5 sub-isolates were detected at either 3 or 6 months post-inoculation, none of these sub-isolates could be detected any longer following a very hot period in the tunnel. We suspect virus titers were suppressed or virus was eliminated from the young seedlings at this stage. Single aphid transmission from field-collected aphids was successful in transferring CTV to six sub-isolates. All of these were, however, still mixed infections of genotypes. We were unable to find any T36 sources amongst grapefruit grafted Mexican lime samples or from original grapefruit material; however, one sample was observed that appeared to be a homogenous source of NZRB-TH30, following genotype specific PCRs, multiple cloning and Illumina NGS analysis of the p33 gene. The B390/3 source was confirmed to be of mild virulence and appears to be a homogeneous source of an NZRB2-like genotype, although in one test B165 was also found in two different plants.

Opsomming

Tydens die huidige verslag periode het ons die bruikbaarheid van “immunocapture” van sitrus tristeza virus (CTV) virus partikels as 'n templaar in “next generation sequencing” (NGS) bepaal. Al is die tegniek teoreties van groot waarde, is daar weereens lae CTV spesifieke volgordes gekry. 'n PCR toets wat ontwikkel is en gerig is teen die p33 geen van CTV, is bevind om Taiwan-Pum/SP/T1, RB, CTZA en T36 swakker te amplifiseer as verwag. Ten spyte hiervan is die tegniek gebruik binne 'n opname van CTV genotipes van Star Ruby pomelos aangesien die p33 geen 'n besonder relevante geen is om te gebruik in die karakterisering van CTV populasies in kruisbeskerming aangesien hierdie geen geassosieer is met super-infeksie uitsluiting. Ses-en-negentig monsters van GFMS12 ge-immuniseerde Star Ruby pomelos is met PCR getoets. Ten spyte van die laer amplifikasie doeltreffendheid van RB in die PCR, is hierdie genotipe bevind as die dominante komponent in veral jong bome, terwyl dit ook in alle monsters voorgekom het. Verskeie ander genotipes is ook gevind. Groot getalle enkel plantluis oordragings, sowel as verdunnings-eindpunt meganiese oordragings met bas vlap en bas sny tegnieke, is gedoen. Terwyl 'n totaal van 5 sub-isolate verkry is na 6 maande, het al die plante later negatief getoets na 'n ongewoon warm periode in die glashuis. Ons vermoed dat die virus titers onderdruk is of dat die virus selfs moontlik ge-elimineer is hierdeur. Ses sub-isolate van CTV is verkry na enkel plantluis oordragings vanaf plantluis versamel van sitrus in kommersiële boorde. Al ses was egter nog steeds gemengde infeksies van genotipes. Ons het geen bronne van T36 verkry in Meksikaanse lemmetjie plante geënt met pomelos nie. Een bron was egter 'n homogene bron van NZRB-TH30 soos bepaal met behulp van genotype spesifieke PCR, volgorde bepaling van verskeie klone van amplikone, of NGS. Die B390/3 bron is bevestig om 'n matige isolaat te wees en blyk homogeen te wees vir n NZ-RB2 tipe genotype, alhoewel daar twee plante gevind is wat in enkele toets ook postief was vir B165.

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

Project 1056 (2012/13 – 2013-14) by G. Cook, J.H.J. Breytenbach and S.P. van Vuuren (CRI)

Summary

Citrus tristeza virus (CTV) is a complex of strains. Genotypic strain characterisation of CTV has progressed significantly, but the phenotypic expression of strains is poorly established due to CTV naturally occurring as mixed strain populations. A screening system for analysis of mixed CTV populations was required and a published CTV strain-specific detection assay was expanded and improved to facilitate detection of currently known strains. Using this system, it was possible to identify single-strain sources. Several citrus varieties were inoculated with four single-strain CTV isolates to monitor transmission and evaluate symptom expression of the different strains in various citrus hosts. This is ultimately to understand which strains are potentially problematic and important for cross-protection. ELISA was used to detect CTV in each trial plant at 3 specific intervals post inoculation. The influence of the CTV strains on early plant growth and stem pitting development were investigated. Variation in the titre of these strains in the different citrus hosts was observed. Significant reduction in plant growth was only obtained with one commercial cultivar, 'Nules' Clementine, and the susceptible indicator host, 'Mexican' lime. Stem pitting was detected in the four grapefruit varieties tested as well as 'Mexican' lime. A relative quantitative RT-PCR test for CTV was developed and optimised using 3 plant reference genes. This was done to determine the relative titres of single CTV strains in specific hosts to investigate whether viral titre is correlated to symptom severity. The quantitative RT-PCR was not applied in this trial as symptom expression was less than expected, but will be applied in Project 1100 where the effect of strains and strain mixtures in grapefruit will be studied.

Additionally various pre-immunising and maintenance sources were characterised using the strain-specific RT-PCR assays. Segregation of strains was detected as various maintenance sources lacked strains contained within the original sources. These results demonstrate that the maintenance of mixed strain populations is challenging as various factors can facilitate strain segregation. This is an important aspect in the propagation of budwood for industry supply where the continued effectiveness of the cross-protection program is required. This emphasises the need to understand which strains are required for cross-protection.

Opsomming

Sitrus tristeza virus (CTV) bestaan uit 'n kompleks van rasse. Genotipiese ras karakterisering het aansienlik gevorder, maar die fenotipiese uitdrukking van rasse is onseker omdat CTV natuurlik voorkom as gemengde rasbevolkings en nie as enkel isolate nie. Ons het uitgebrei en verbeter op 'n gepubliseerde opsporingstelsel vir CTV rasse om huidig bekende CTV rasse te kan opspoor. Met die gebruik van hierdie stelsel, was dit moontlik om enkelras bronne te identifiseer. Verskeie sitrus kultivars is ge-inokuleer met vier enkelras isolate om die oordrag, translokasie en simptome uitdrukking van die rasse in verskillende sitrus kultivars te evalueer. Die uiteindelige doel hiermee is om te verstaan watter rasse potensieel problematies en belangrik is vir kruisbeskerming. ELISA toetse is op 3 spesifieke tussenposes na inokulasie gedoen. Die invloed van die rasse op vroeë groei van die plant en stamgleuf ontwikkeling is ondersoek. Variasie in die virus titer van hierdie rasse in die verskillende sitrus kultivars is wel waargeneem. Beduidende vermindering in die groei van plante is slegs waargeneem in een kommersiële kultivar, 'Nules' Clementine en die CTV-vatbare gasheer, "Meksikaanse" lemmetjie. Stamgleuf ontwikkeling is waargeneem in die vier pomelo variëteite wat getoets is asook by 'Meksikaanse' lemmetjie. 'n Kwantitatiewe PKR toets is ontwikkel om die relatiewe titers van die verskillende rasse van CTV in sekere kultivars te kan bepaal om sodoende ondersoek in te stel of virus titer met simptome uitdrukking gekorreleer kan word. Die toets is nie in hierdie proef toegepas nie weens minder as verwagte simptome uitdrukking, maar sal in Projek 1100 toegepas word waar die effek van ras en ras mengsels in pomelo ondersoek sal word. Bykomend is verskeie pre-immuniserings- en instandhoudingsbronne met behulp van die ras-spesifieke PKR toetse gekarakteriseer. Skeiding van rasse is waargeneem deurdat sekere instandhoudingsbronne nie meer die volle komponent van rasse besit wat in die ouer pre-immuniseringsbronne teenwoordig is nie. Hierdie bevindinge toon dat die instandhouding van gemengde rasbevolkings problematies kan wees. Verskeie faktore sal segregasie kan fasiliteer en hierdie aspek is van belang by die vermeerdering van okuleerhout vir die bedryf waar die voortgesette doeltreffendheid van die kruisbeskermingsprogram vereis word. Die bevindinge lig ook die belangrikheid uit om te bepaal watter rasse 'n rol speel in kruisbeskerming.

Introduction

Citrus tristeza virus (CTV) is endemic in southern Africa and has been responsible for losses within the local citrus industry and also worldwide (4, 5). To minimize losses within the local citrus industry, the South African Citrus Improvement Scheme (CIS) implemented cross-protection using mild CTV sources in shoot-tip grafted material to reduce the effect of challenges by endemic severe CTV strains, introduced by aphids in the field. The strategy has been successful, but cases of cross-protection breakdown have occurred. The mechanism of cross-protection is complex and not fully elucidated although recent research indicates a genotype specific exclusion principle (2). The CTV complex consists of various strain groups (3). Differences in CTV susceptibility and strain selection by citrus hosts have been observed within citrus types and even between cultivars (1). Research and experience gained locally within the CIS has also demonstrated host selection of CTV sources (6). To implement cross-protection, it is important to understand which strains are effective in this process. All current pre-immunising sources of the CIS comprise strain mixtures and currently strain transmission during cross-protection is not monitored.

This project aims to serve as a biological screening of important industry cultivars to investigate the potential host selectivity of strains and to monitoring strain pathogenicity and translocation.

Objectives

1. To investigate host selection of various CTV strains with the aim to assist in the selection of pre-immunising CTV sources.
2. To monitor symptom expression and to assess the influence of each strain on early plant growth in the various citrus cultivars.
3. This project is a pilot project for a PhD study.

Materials and methods

Variety selection for trial:

The 8 main industry varieties, based on budwood supply from the CFB from 2006 to 2011, were selected for use in the trial and the rest were chosen to ensure inclusion of 3 cultivars of each main citrus type. Other considerations for inclusion were previously observed differences in CTV reaction such as Turkey Valencia and mid-season cultivars which show bud union crease on rough lemon or Carrizo rootstocks. The variety selection is listed using cultivar accession numbers and names (CIS importance categories are indicated in brackets):

<i>Citrus aurantifolia</i> (Lime):		'Mexican lime' (biological indicator host)
<i>C. paradisi</i> :	1179 -	'Star Ruby' (cat 1)
(Grapefruit)	1183 -	'Nel Ruby' (cat 2) ^{ARC}
	1057 -	'Marsh' (cat 2)
		'Duncan' (biological indicator host)
<i>C. limon</i> :	1073 -	'Eureka' (cat 1)
(Lemon)	1536 -	'Limoneira' 8A (cat 2)
	1537 -	'Genoa' (cat 3)
<i>C. reticulata</i> :	1562 -	'Nadorcott 1' (cat 1) ^{Citrogold}
(Mandarin Hybrids)	0803 -	'Nova' (cat 2)
	1730 -	'Mor 26' (cat 2) ^{Citrogold}
<i>C. sinensis</i> :	1061 -	'Bahianinha' (cat 1)
(Navel)	1051 -	'Palmer' (cat 1)
	1277 -	'Washington' (cat 1)
<i>C. sinensis</i> :	1043 -	'Delta' (cat 1)
(Valencia)	1052 -	'Late' (cat 1)
	1044 -	'Midnight' (cat 1)
<i>C. sinensis</i> :	1285 -	'Turkey' (cat 2) ^{Citrogold/CGACC}
(Mid season)	5009 -	'Premier' (cat 3) ^{CGACC}
	5045 -	'Hamlin' (cat 4)
<i>C. clementina</i> :	1094 -	'Nules' (cat 2)
(Clementine)	1262 -	'Esbal' (cat 3)
	1048 -	'SRA 63' (<i>uncertainty regarding accession identity</i>)
<i>C. unshiu</i> :	0983 -	'Miho Wase' (cat 1)
(Satsuma)	1469 -	'Ohtsu' (cat 5)
	1270 -	'Okitso Wase' (cat 5)

CTV 'single strain' sources used in the trial:

1. 'Maxi': VT-like strain, derived from Valencia orange;
2. GFMS 12-8: T68-like strain obtained by single aphid transfer (SAT) from the 'Nartia' grapefruit source GFMS 12;
3. LMS 6-6: HA16.5-like strain obtained by SAT from the Mexican lime source LMS 6;
4. B390-5: RB strain obtained by SAT from a 'Mouton' sweet orange source.

Development of strain-specific RT-PCR tests

See Addendum A: Expanded strain-specific RT-PCR assay for differential detection of currently known *Citrus tristeza virus* strains: a useful screening tool.

Preliminary identification of the CTV sources on a host range, using strain specific RT-PCRs and direct sequencing.

Each source was placed on a citrus host range including Madam Vinous sweet orange (*Citrus sinensis* (L.) Osb.), Sour orange (*C. aurantium* L.), Mexican lime (*C. aurantifolia* (Christm.) Swing.) and Duncan grapefruit (*C. paradisi* Macf.). The host range was used to eliminate the possible host selection of strains to confirm single strain status on various citrus species. Each host was tested with 8 strain-specific RT-PCRs (Addendum A) and PCR products were sequenced for confirmation. Additionally a mid-genome region of each isolate was amplified using degenerate primers shown below. This was done to enable detection of possible mixtures, with the expectation of obtaining single sequences for single-strain isolates.

Mid-genome degenerate primers:

CTV9480F: GAACCGGCTCGYGTTTCGGCGT

CTV11013R: GCAAACATCYGACTCAACTACC

Plant preparation:

Virus-free Rough lemon (*C. jambhiri* L.) seedlings were planted singly in 3L planting bags and maintained in an aphid-free environment. Virus-free scions of the varieties were budded on rootstocks according to normal nursery practices. After 6 months, the four single-strain isolates were inoculated separately to five plants of each variety and four to five plants were left as un-inoculated controls where possible. Inoculation was done by budding 2 bark chips of the source plant to the scion. All plants were inoculated at the same height and after inoculation the scions were cut back approximately 10cm above the inoculation points and one shoot of the new growth was allowed to grow from the top bud. Survival of the inoculum was confirmed after 3 weeks. Plants were removed where rootstock shoots were mistaken for scion buds. Plants were maintained in an aphid-free tunnel. Temperature and humidity readings were taken every hour using a data logger. The data was processed by dividing day and night temperatures. Day periods were from 06h00 to 18h00 and night periods from 18h00 to 06h00.

Trial Treatments:

(5) Treatments = [(4) genotypes + (1) un-inoculated control] x [(26) varieties = (24) industry varieties + (2) indicator host] x [(5) repetitions] = 650 plants.

The infection and translocation of the CTV in each plant was monitored by quantitative ELISA by sampling leaves of each plant as indicated below. The CTV ELISA reagent set (SRA 78900) from AGDIA Inc (Elkhart, Indiana, USA) was used as per supplier's protocol. Results were recorded by measuring absorbance values at 405nm after 30 min incubation.

ELISA 1 = 7 weeks post inoculation, 15 cm above inoculation point;

ELISA 2 = 13 weeks post inoculation, 30 cm above inoculation point;

ELISA 3 = 24 weeks post inoculation, at the shoot tip.

Pathogenicity assessment

Growth measurement of each plant was done after the third ELISA. All plants were cut back 20cm above inoculation site and plants allowed a second regrowth cycle. Final analysis included plant regrowth measurements after a further 10 months (16 months post inoculation).

Minimal to no stem pitting was observed at both the 6 and 16 month evaluations, even on Mexican lime, the indicator host. Plants were left for a further 11 months to allow for symptom expression. A rating scale of 0 to 5 was used where 0 is no stem pitting and 5 represents severe stem pitting [0 = no stem pitting, 1 = mild (few shallow pits), 2 = mild - moderate, 3 = moderate (regular, deeper pits), 4 = moderate - severe and 5 = severe (many deep coalescing pits)].

Confirmation of negative ELISA results by cross-budding and RT-PCR.

Single-strain isolate, LMS6-6, was not detected by ELISA in a number of cultivars despite take of the inoculated bark. To test whether the negative results were possibly due to erratic distribution of the CTV isolate within the original source material, a cross-budding test was done with a number of cultivars. A cultivar that tested negative for the strain was budded onto another cultivar that was positive for this strain. The reverse was also done by budding a positive cultivar onto a plant that tested negative. The buds were allowed to grow and both the original scion and the budded cultivar were tested by RT-PCR for the CTV strain after 3 months.

Strain profiling of all CTV sources.

CTV sources maintained at CRI were characterised for their strain profile using the strain-specific RT-PCRs. GFMS12 and GFMS35 sources maintained at CRI, ARC-ITSC and University of Pretoria were tested as well as all grapefruit mother trees maintained at the CFB (see Addendum A) .

Quantitative RT-PCR testing of CTV titres in selected varieties.

A relative quantitative RT-PCR test for CTV was developed and optimised using 3 plant reference genes. Details of the methodology are presented in Project 1100.

Results and discussion

Objective / Milestone	Achievement
A. Plant Preparation: 1. Propagate virus-free rootstocks, budding of each cultivar onto rootstocks 2. Inoculation with the Single strain CTV isolates	Achieved
B. Acquisition and confirmation of single strain status of CTV sources	Achieved: This facet was not initially proposed as the experiment was based on prior characterisation of sources which were incorrect. Sources were tested with strain-specific RT-PCRs on a differential host range to determine single isolate status.
C. Perform ELISA tests and record growth	Achieved: ELISA tests on each plant completed. Plant growth recorded and plants cut back for second and third regrowth cycles.
D. Develop a relative quantitative RT-PCR	Achieved
E. Relative RT-PCR quantification of CTV in selected varieties	Not achieved: Objective moved to project 1100 as symptom expression was poor overall and association of symptoms with titre would be difficult.
Additional	Achieved: Genotype profile determination of various CTV sources using RT-PCR

ELISA

CTV ELISA tests on each trial plant was done at 7, 13 and 24 weeks after inoculation and sampled at 15 cm, 30 cm and at the shoot tip respectively. The absorbance readings taken after 30min for each trial plant are presented in Figures 4.2.8.1-9. Various trends were noted within the different citrus types and are discussed separately.

Results obtained with isolate LMS6-6 showed no transmission to a number of cultivars, erratic transmission in some and good transmission in only a few cultivars. This indicated a potentially limited host range. To test whether the negative results were possibly due to erratic distribution of the CTV isolate within the original source material, a cross-budding test was done with a number of cultivars. Results of these tests showed that the source material was problematic as LMS6-6 was transmitted to 'Delta' Valencia, 'Bahianinha' navel as well as 'Limoneira' and 'Genoa' lemon in this manner and confirmed them as hosts for this isolate. All trial plants that were inoculated with LMS6-6 and tested negative in the 3 ELISA tests, were again tested with both ELISA and RT-PCR. The 'Nadorcott 1' plants tested negative again in the follow-up ELISA test, but positive in the RT-PCR test, indicating titres below the detection limit of the ELISA for LMS6-6 in this cultivar.

The host susceptibility of 'SRA63' (uncertainty regarding this accession identity) and 'Nules' Clementine, 'Mor26' mandarin and 'Washington' navel to LMS6-6 remain undetermined as they tested negative in both ELISA and RT-PCR. Cross-budding tests to positive plants are required to confirm their putative resistance to the strain. This will continue within Project 1100.

Eureka lemon tested negative in the ELISAs for isolate B390-5 and similarly cross-budding tests need to be done to confirm this possible 'resistance' to the strain.

An observation that titres of sub-isolate GFMS12-8, in certain cultivars, were consistently lower in the third ELISA test compared to the prior 2 ELISAs, requires further investigation. This could either indicate a poor upward translocation ability of the strain within the cultivars or potentially a plant defence mechanism limiting virus build-up. This was noted with 'Nova', 'Mor26' and 'Nadorcott 1' mandarins, 'Washington' navel, 'Late' Valencia, 'Genoa' lemon and 'Mexican' lime. Similarly the virus titres of the 'Maxi' isolate was lower on the third ELISA in 'Turkey' mid-season and 'Mexican' lime and, with the exception of one plant, also in 'Delta' Valencia.

Grapefruit

CTV ELISA of the four grapefruit cultivars inoculated with isolate B390-5 yielded similar results. Isolate B390-5 reached higher titres than the other three single strain isolates, in all four grapefruit cultivars, at 7 and 13 weeks at the specific sampling points. The strain was efficiently transmitted, reached high titres in all four cultivars within the first two testing periods and the same high titre levels were detected at the 24 week test period. The results suggest efficient translocation and propagation of this strain within the grapefruit cultivars tested.

The two red grapefruit cultivars, 'Star Ruby' and 'Nel Ruby', displayed similar trends with all four CTV strains inoculated. Similar results were obtained with isolates LMS6-6 and 'Maxi' in these two cultivars with relative low titres detected at 7 and 13 weeks, but higher virus titres detected at the shoot tip at 24 weeks.

Sub-isolate GFMS12-8 was detected in all grapefruit varieties at each testing period, but titres were lower than B390-5 in all cases.

Erratic translocation of LMS6-6 and 'Maxi' isolates was obtained with 'Marsh', a white grapefruit variety. The erratic transmission obtained can, however, be ascribed to the inoculation source, as previously discussed.

'Duncan' grapefruit was the most receptive cultivar for all four strains tested, confirming this to be a good CTV indicator host.

Soft citrus types

Poor transmission of isolate LMS6-6 to soft citrus cultivars belonging to the Mandarin-hybrid -, Satsuma - and Clementine groups can in part be ascribed to the inoculation source, however all positive ELISA reactions gave weak signals. Titres of LMS6-6 in 'Nadorcott1' plants were below the detection limit of the ELISA and detection was only possible with RT-PCR. The host susceptibility of 'SRA63', 'Nules' and 'Mor26' for LMS6-6 are unconfirmed. The confirmation will be continued in project 1100.

Overall, soft citrus was not a good host for the CTV isolates tested compared to other citrus types, with very erratic detection of the various isolates.

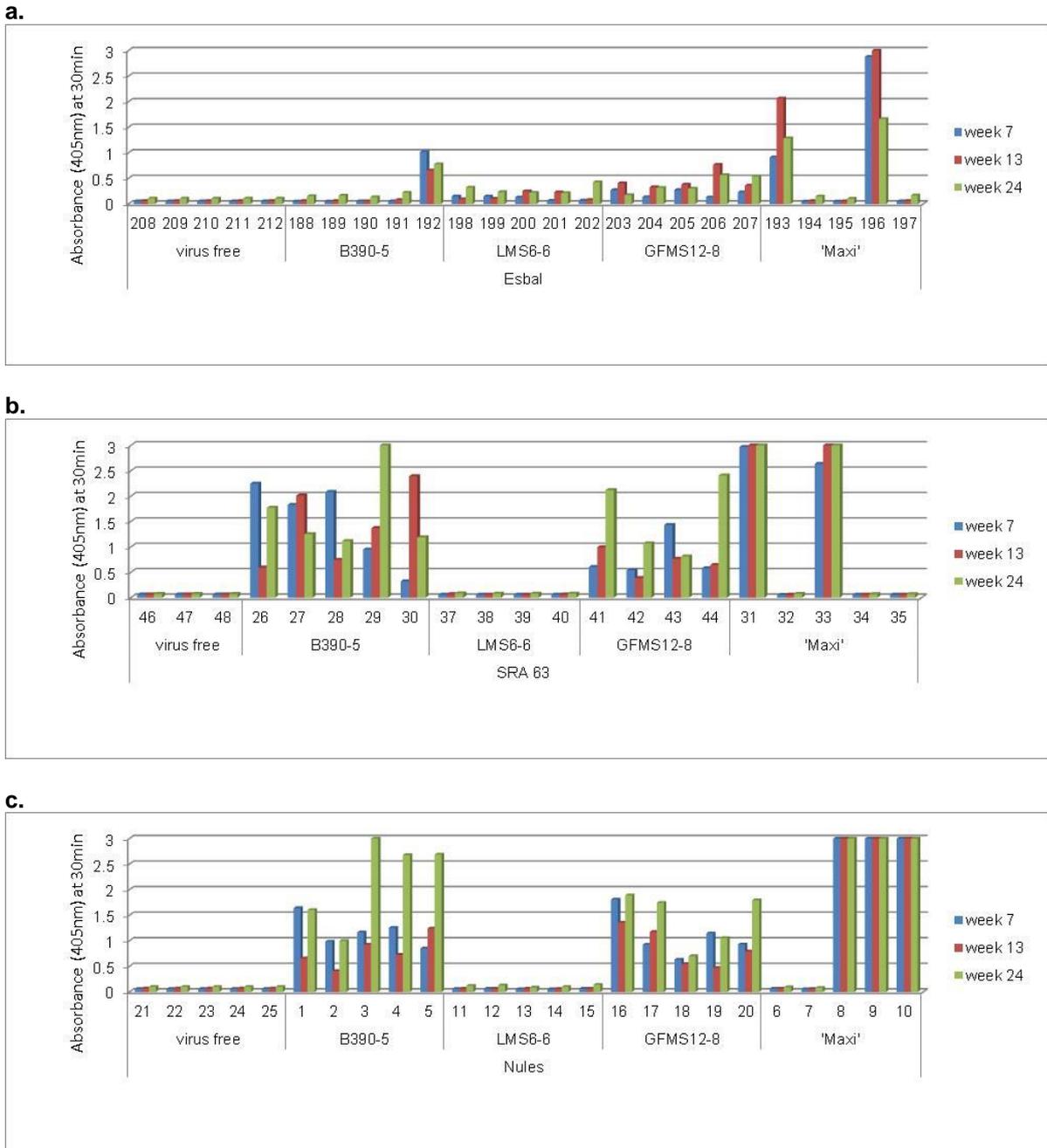


Figure 4.2.8.2. ELISA results for each Clementine trial plant at 7, 13 and 24 weeks post inoculation (a) 'Esbal' (b) 'SRA 63' and (c) 'Nules'.

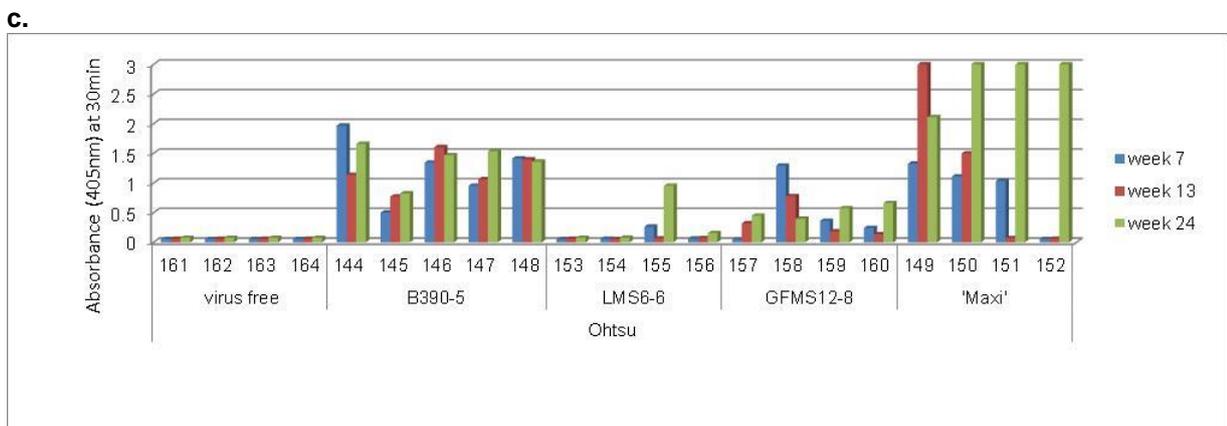
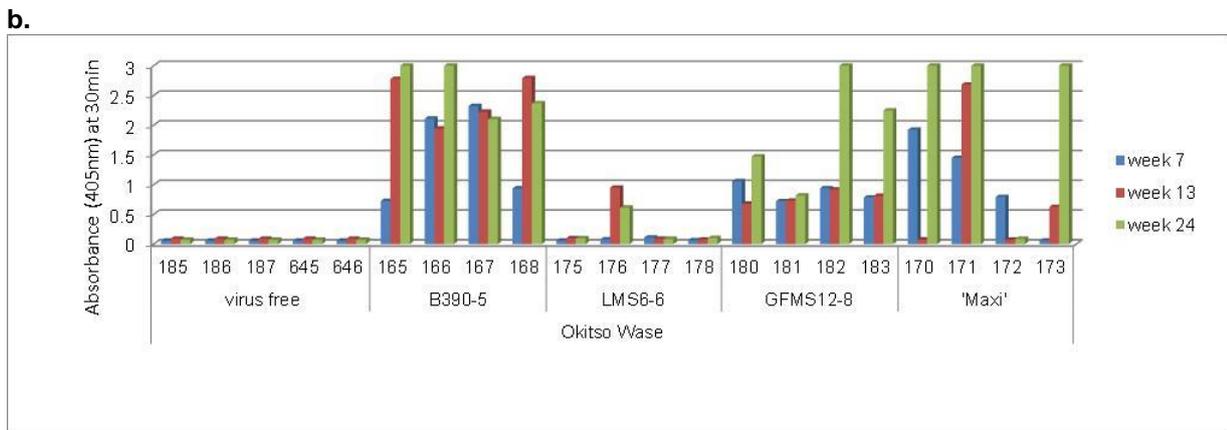
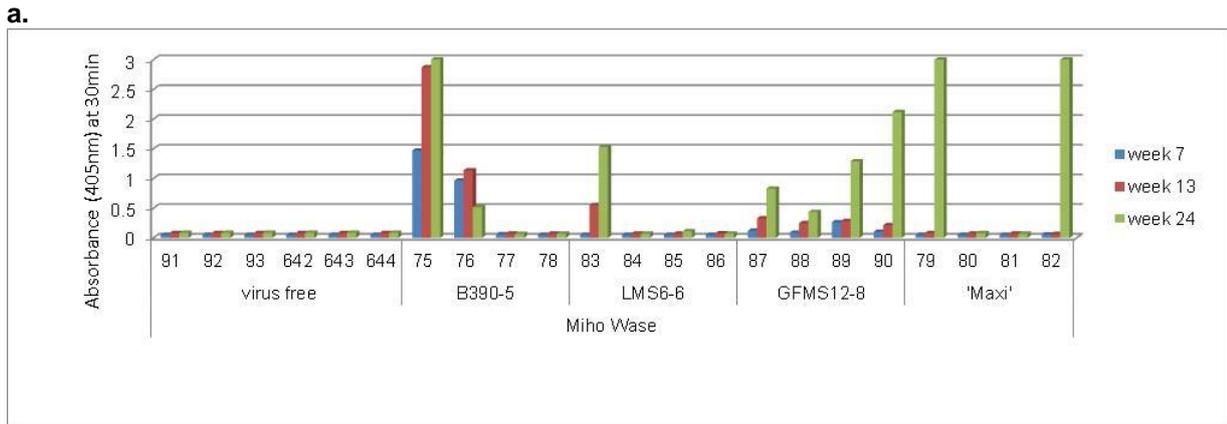


Figure 4.2.8.3. ELISA results for each Satsuma trial plant at 7, 13 and 24 weeks post inoculation (a) 'Miho Wase' (b) 'Okitsu Wase' and (c) 'Ohtsu'.

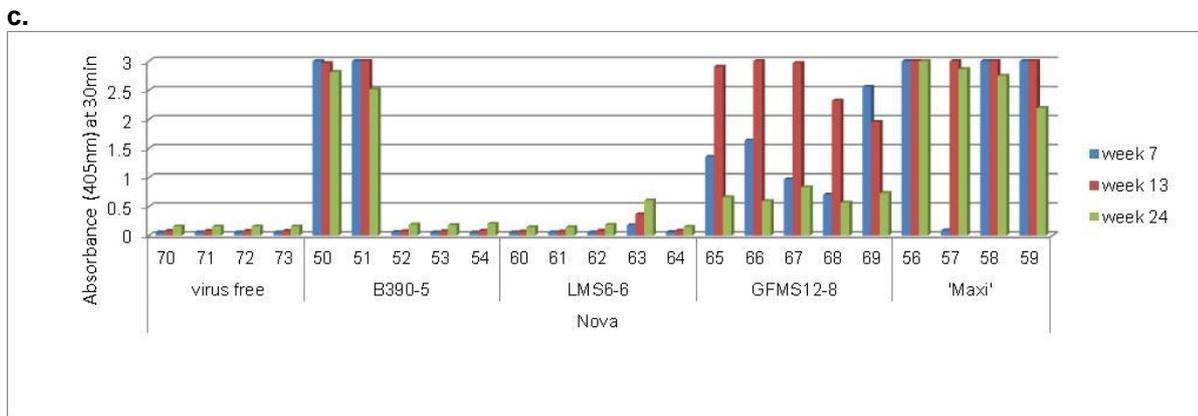
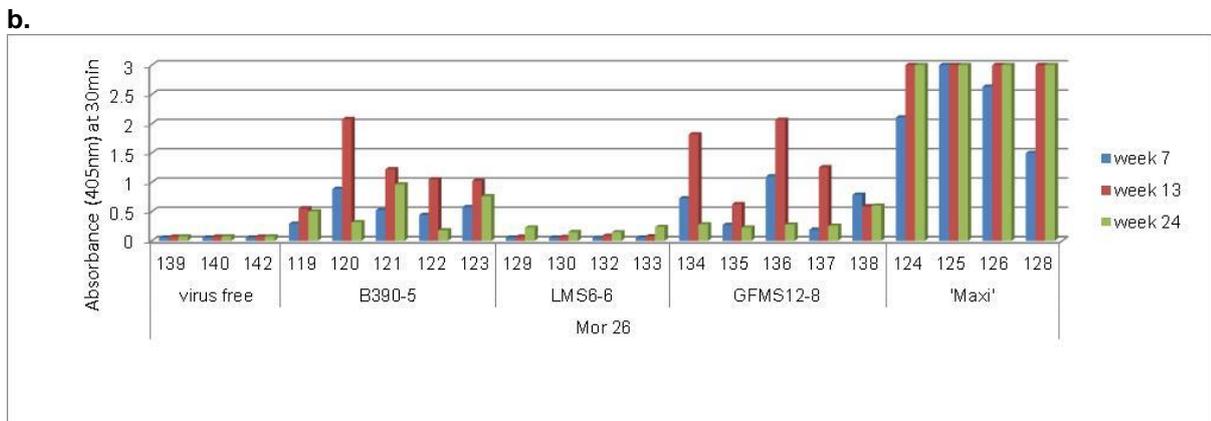
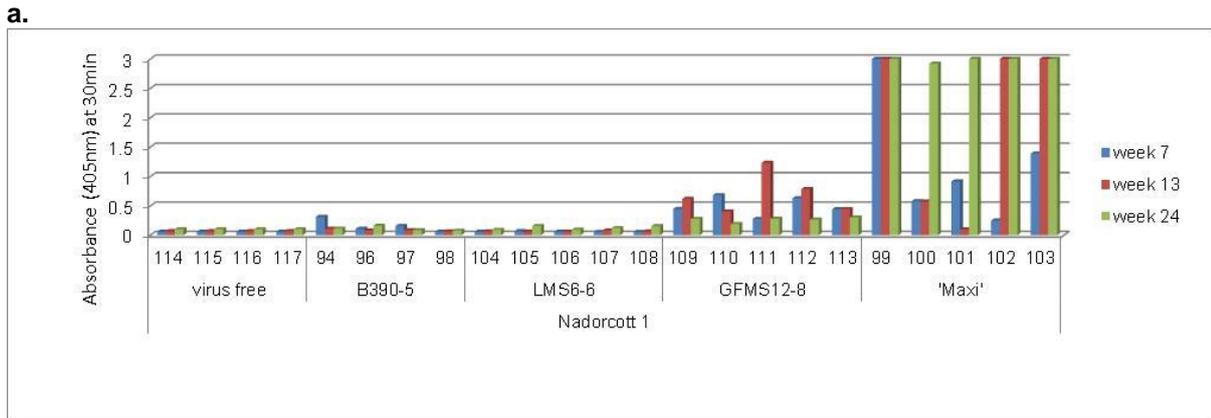


Figure 4.2.8.4. ELISA results for each Mandarin hybrid trial plant at 7, 13 and 24 weeks post inoculation (a) 'Nadorcott' 1 (b) 'Mor 26' and (c) 'Nova'.

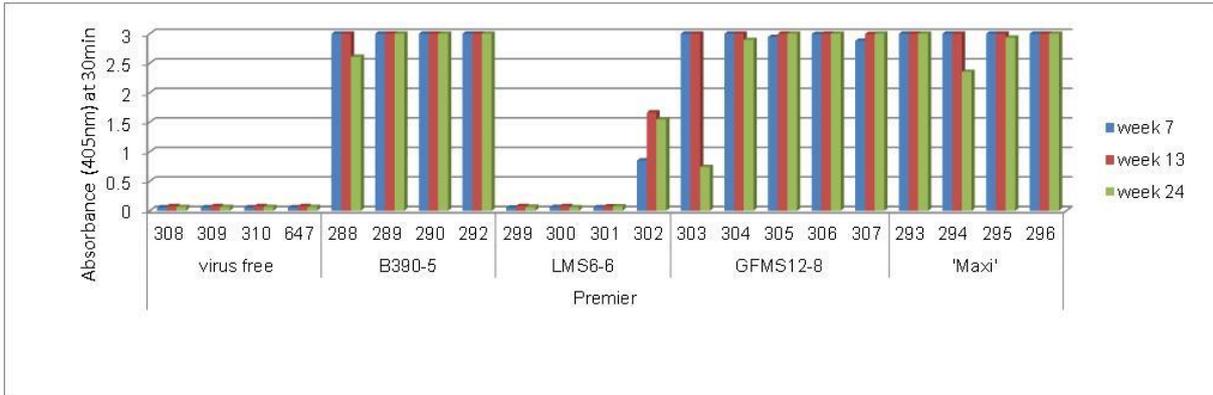
Sweet oranges

'Turkey' midseason was the only sweet orange variety where isolate GFMS12-8 was not detected, indicating possible resistance to this strain. Confirmation by cross-budding tests will be done in project 1100 to confirm this result.

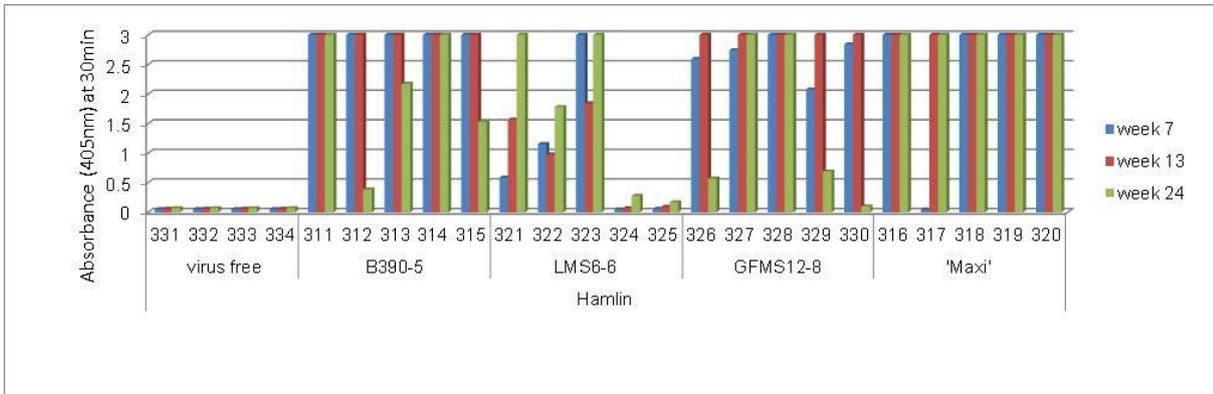
Sub-isolate LMS6-6 was not detected in cultivars; 'Delta' Valencia, 'Washington' and 'Bahianinha' navels in the ELISA tests, but 'Delta' and 'Bahianinha' were shown to be hosts by the cross-budding tests. This will also be done for 'Washington' navel.

The 'Maxi' isolate (VT) was consistently detected in all sweet orange varieties tested indicating that sweet orange varieties are good hosts for this strain.

a.



b.



c.

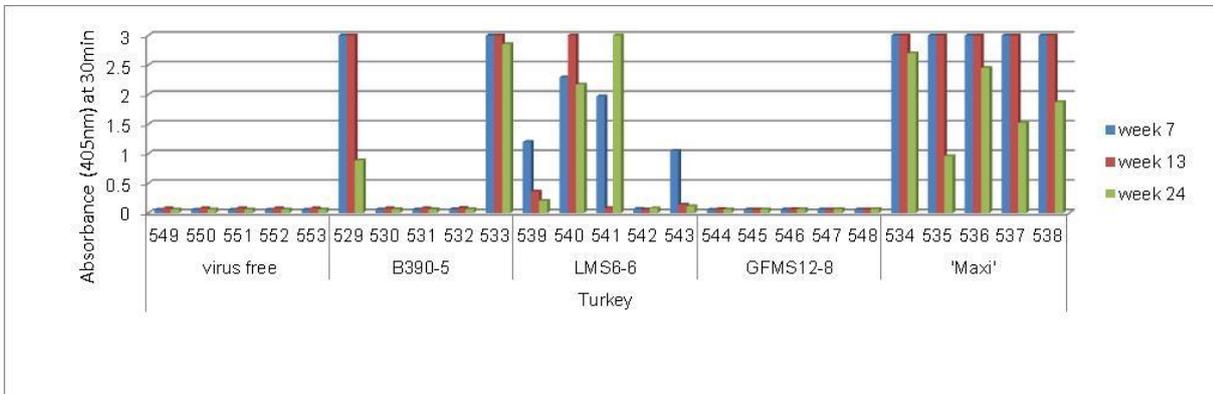


Figure 4.2.8.5. ELISA results for each mid-season trial plant at 7, 13 and 24 weeks post inoculation (a) 'Premier' (b) 'Hamlin' and (c) 'Turkey'.

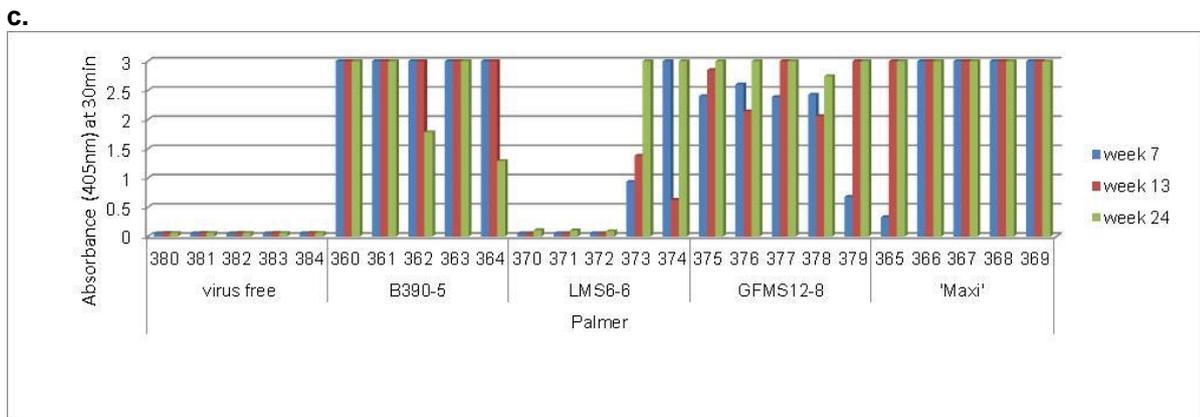
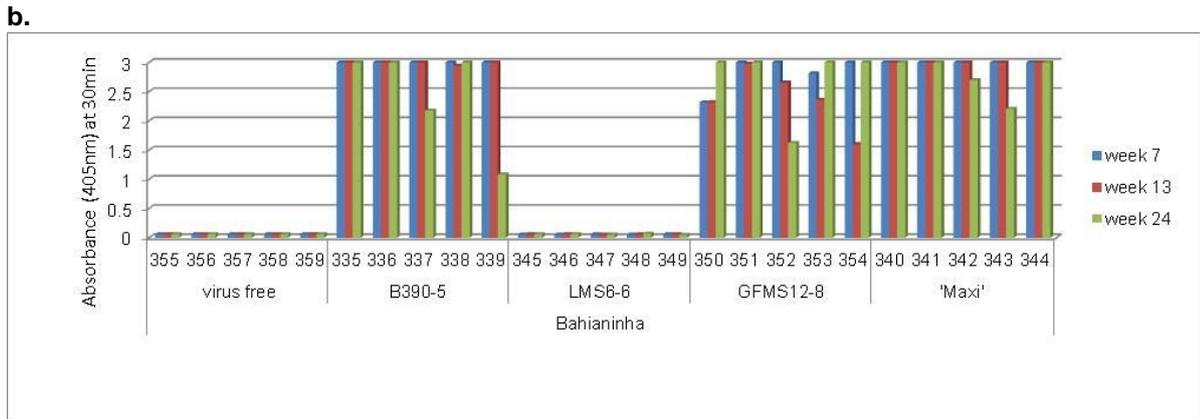
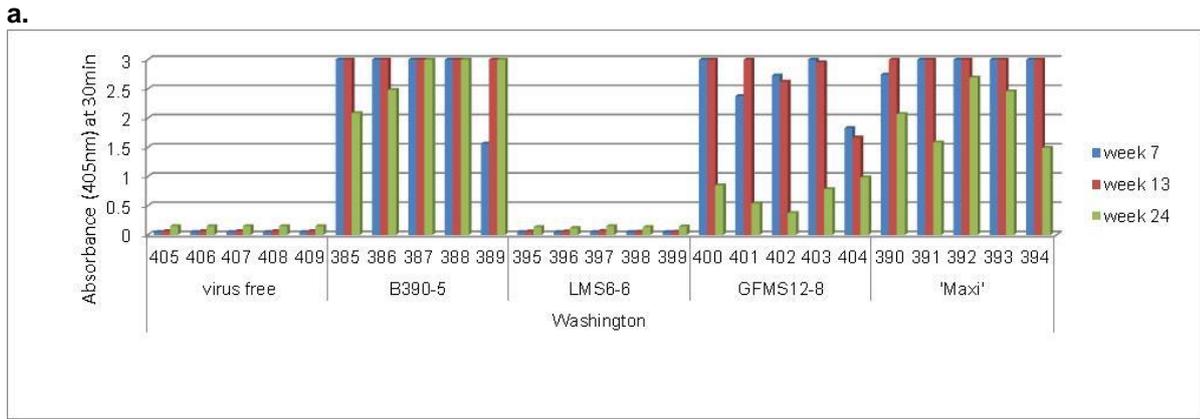


Figure 4.2.8.6. ELISA results for each Navel trial plant at 7, 13 and 24 weeks post inoculation (a) 'Washington' (b) 'Bahianinha' and (c) 'Palmer'.

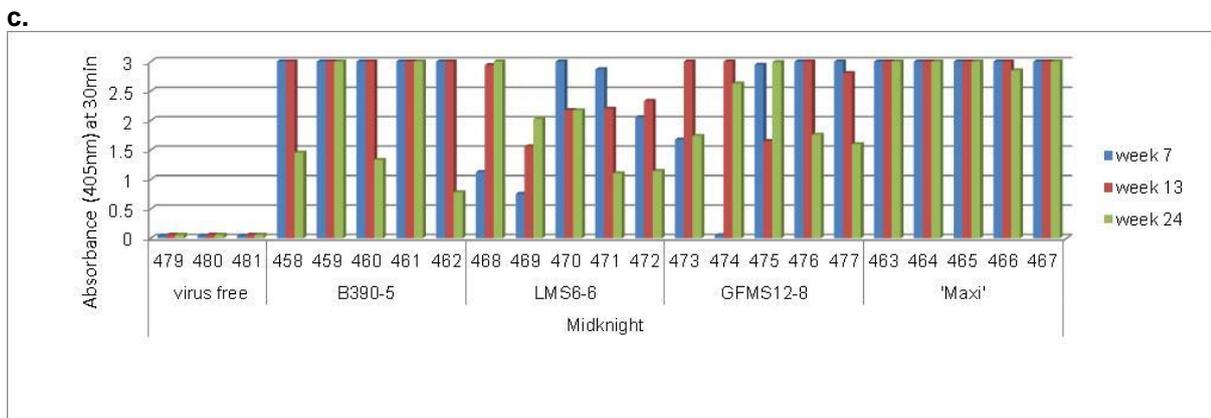
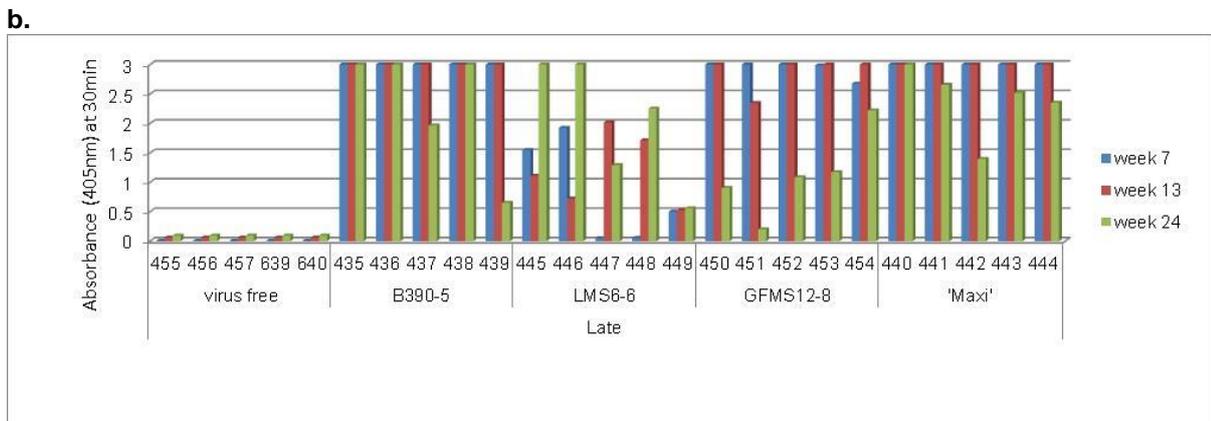
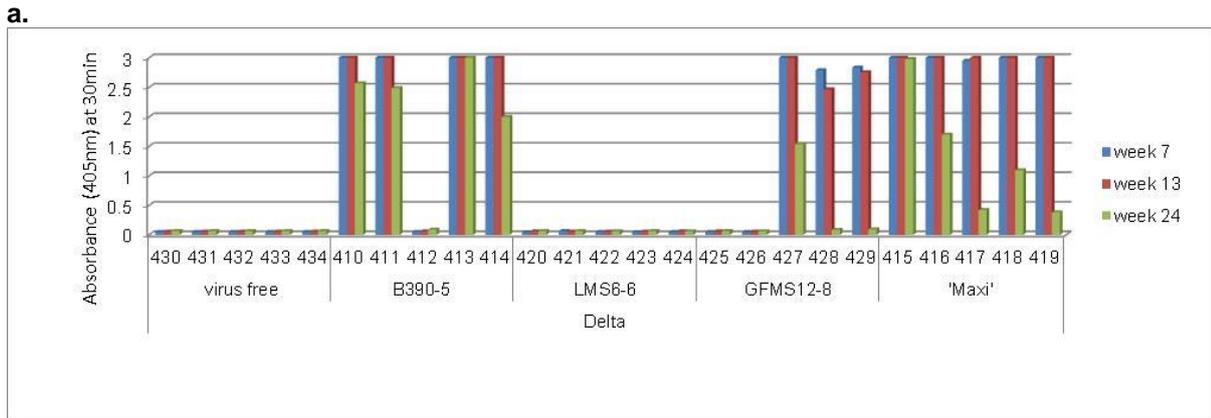
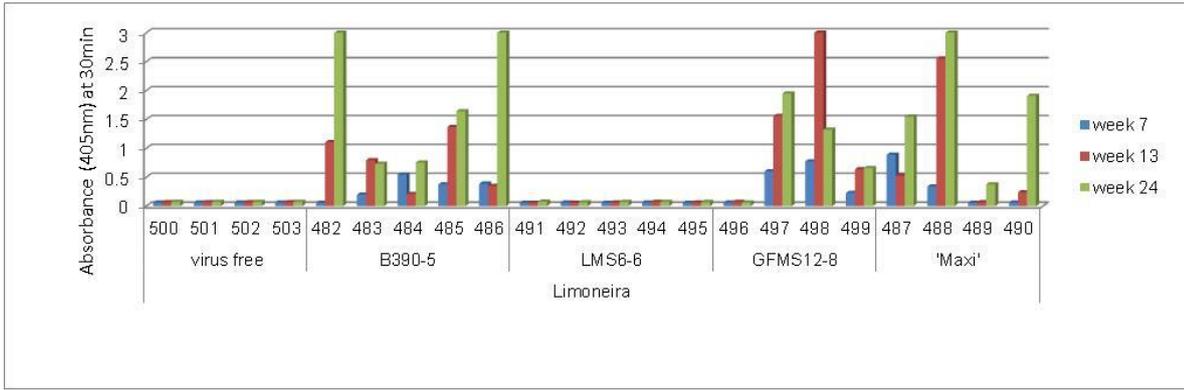
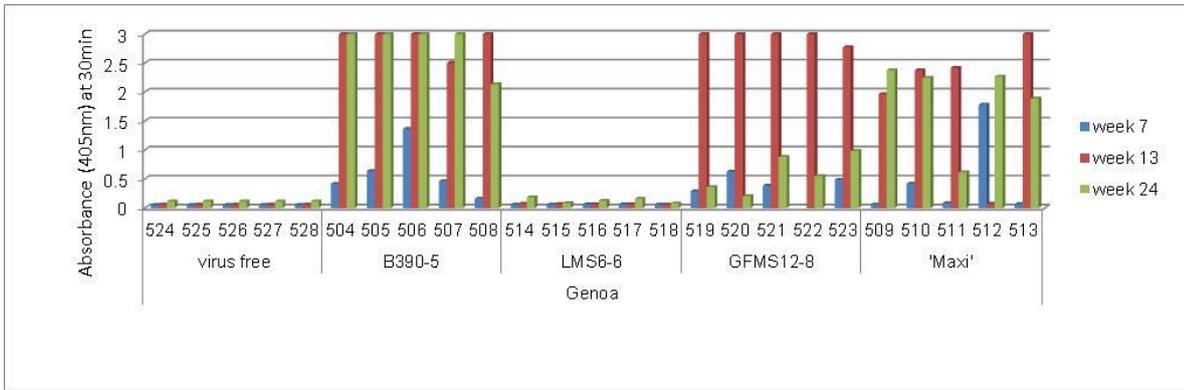


Figure 4.2.8.7. ELISA results for each Valencia trial plant at 7, 13 and 24 weeks post inoculation (a) 'Delta' (b) 'Late' and (c) 'Midnight'.

a.



b.



c.

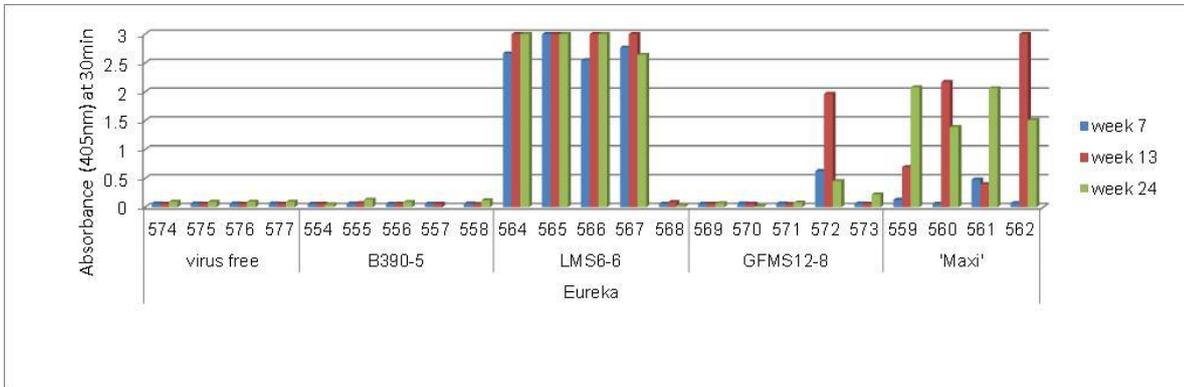


Figure 4.2.8.8. ELISA results for each Lemon trial plant at 7, 13 and 24 weeks post inoculation (a) 'Limoniera' (b) 'Genoa' and (c) 'Eureka'.

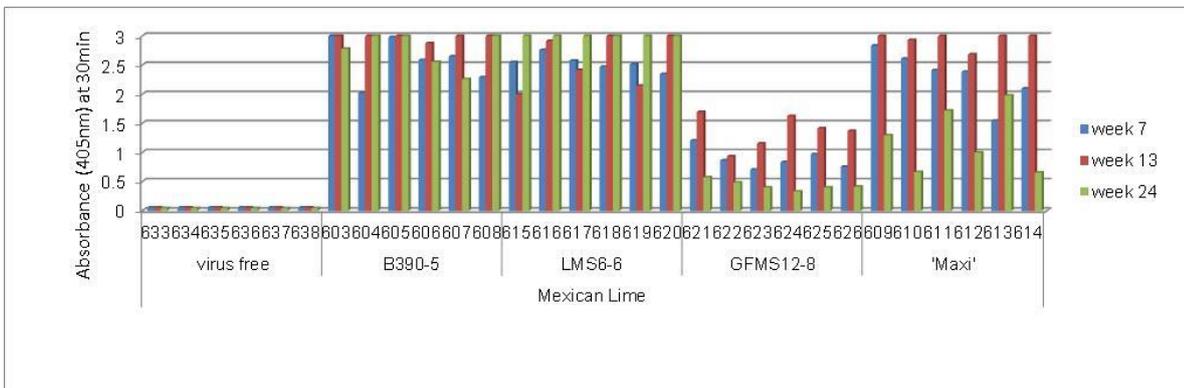


Figure 4.2.8.9. ELISA results for each 'Mexican' lime trial plant at 7, 13 and 24 weeks post inoculation.

Pathogenicity assessment

Plant growth measurements were done after 6 months, cut back and growth measured again after a further 10 months. The two plant length measurements were added to determine total growth over the trial period. Significant reduction in plant growth was only observed in one commercial cultivar, 'Nules' Clementine, and the susceptible indicator host, 'Mexican' lime. Isolate B390-5 (RB strain) reduced plant growth in 'Nules' Clementine compared to the other treatments (Table 4.2.8.1), whereas both B390-5 and 'Maxi' (VT strain) reduced plant growth in 'Mexican' lime (Table 4.2.8.2). Severe stunting of Mexican lime inoculated with the 'Maxi' isolate was observed in the first evaluation, but growth was less restricted in the second regrowth period. The inoculation seemed to induce an initial shock reaction from which the plant was able to recover.

Table 4.2.8.1. Mean plant length (cm) of 'Nules' Clementine plants over the total 16 months.

Isolate	Plant length (cm)	*
GFMS12-8	222	a
Control	216	a
LMS6-6	207	a
'Maxi'	200	a b
B390-5	174	b

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

Table 4.2.8.2. Mean plant length (cm) of 'Mexican' lime plants over the total 16 months.

Isolate	Plant length (cm)	*
Control	172	a
GFMS12-8	163	a
LMS6-6	156	a
B390-5	131	b
'Maxi'	115	b

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

Minimal to no stem pitting was observed at both the 6 and 16 month evaluation periods even on the 'Mexican' lime indicator host and plants were left for a further 11 months to allow for further symptom expression. Stem pitting was observed after the third growth period on 'Mexican' lime and on all four grapefruit varieties. Isolates B390-5 and GFMS12-8 induced significantly more stem pitting on the 'Mexican' lime host than the 'Maxi' and LMS6-6 (Table 4). Isolate B390-5 also induced the most stem pitting on the grapefruit hosts (Table 4.2.8.5).

It is most likely that the temperatures over the various evaluation periods influenced stem-pitting expression. A summary of the temperature data for each growth period is presented in Table 3 and average temperatures recorded for the 3rd growth period where stem-pitting symptoms were observed, were lower than those for first two growth periods. Generally temperatures below 28°C are conducive to stem pitting expression and if maximum day temperatures exceed this value for prolonged periods, stem pitting expression is lower (see progress report Project 796).

In the spring months boat-shaped leaves were noted on the Satsuma varieties. With 'Ohtsu' this was seen with plants inoculated with B390-5, 'Maxi' and GFMS12-8 whereas the symptom was associated on 'Okitsso Wase' with GFMS12-8 and on 'Miho Wase' with B390-5.

Table 4.2.8.3. Summary of average temperature values for the three growth periods prior to stem pitting evaluations.

Stem pitting evaluation period	Duration (months)	Stem pitting	Avg temp (°C) over period	Avg day temp (°C)	Avg night temp (°C)	Max day temp (°C)
1 Oct 2012 - Apr 2013	6	minimal	24	28	20	43
2 May 2013 - Feb 2014	10	minimal	21	25	17	40

Table 4.2.8.4. Average stem-pitting (SP) ratings induced by the different CTV isolates on 'Mexican' lime.

<i>CTV Isolate</i>	<i>SP means*</i>	
B390-5	4.000	a
GFMS12-8	3.833	a
'Maxi'	2.333	b
LMS6-6	1.833	b
Control	0.000	c

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

Table 4.2.8.5. Average stem-pitting (SP) ratings induced by the different CTV isolates on four grapefruit hosts.

Grapefruit host	CTV isolate	<i>SP means*</i>	
'Marsh'	B390-5	3.600	a
'Duncan'	B390-5	3.400	a b
'Nel Ruby'	B390-5	2.600	a b
'Star Ruby'	B390-5	2.200	b c
'Nel Ruby'	'Maxi'	1.200	c d
'Star Ruby'	GFMS12-8	1.000	c d
'Marsh'	GFMS12-8	0.800	d
'Duncan'	GFMS12-8	0.800	d
'Duncan'	'Maxi'	0.800	d
'Star Ruby'	'Maxi'	0.600	d
'Nel Ruby'	GFMS12-8	0.600	d
'Marsh'	'Maxi'	0.500	d
'Duncan'	LMS 6-6	0.000	d
'Marsh'	LMS 6-6	0.000	d
'Nel Ruby'	LMS 6-6	0.000	d
'Star Ruby'	LMS 6-6	0.000	d
'Duncan'	'Duncan' control	0.000	d
'Marsh'	'Marsh' control	0.000	d
'Nel Ruby'	'Nel Ruby' control	0.000	d
'Star Ruby'	'Star Ruby' control	0.000	d

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

Strain profiles of various CTV sources

Various CTV sources maintained at CRI were also tested for their strain composition. Findings are summarised in Table 4.2.8.6. The strain composition of GFMS12 sources maintained at CRI, ARC-ITSC and University of Pretoria differed. Addendum A also records the strain profile found in the grapefruit mother trees which differ from the pre-immunising source. It was shown that segregation of the strains within GFMS12 and GFMS35 has occurred and that various sources lack different strains that were detected as components of the older sources.

Table 4.2.8.6. CTV strains profiles of various CTV sources determined using the strain-specific RT-PCR assay.

CTV Source	CTV strain							
	T30	VT	T68	T36	T3	RB1	RB2	HA16-5
Nartia A (GFMS12) and sub-isolates								
Nartia A (CRI)			√			√	√	
GFMS12 ITSC			√					
GFMS12 CRI			√					
GFMS12 UP (08-0001)			√			√		
GFMS12-7 (UP)			√					
GFMS12-8 (UP)			√					
GFMS12-9 (UP)			√					
GFMS35								
GFMS 35			√			√	√	
LMS6 and sub-isolates								
LMS6			√			√		√
LMS 6/1							√	
LMS 6/4						√		√?
LMS 6/6								√
LMS 6/8						√		√
LMS 6/11							√	
LMS 6/14							√	
GFMS 14 = Nartia C and sub-isolates								
B389-1								√
B389-4							√	
Mouton sub-isolates								
B390-3								√
B390-5								√
SM49 and sub-isolates								
SM49		√	√				√	√
SM49/5/1								√
SM49/5/2								√
SM49/6/3								√
SM49/7/4								√
SM49/7/5								√
SM49/8/1								√
SM49/10/3								√
SM49/10/6								√
Various other								
'Maxi'		√						
VT ex ARC		√						
Tambankulu 1		√	√		√?			√
New Venture 41.2		√	√		√?	√	√	
CTVSC1	√	√	√		√	√	√	

Conclusions

This study was conducted to investigate whether certain citrus hosts select or suppress certain CTV strains in order to understand which strains impact negatively on the various citrus types and to ultimately gain an understanding of which strains are of importance for cross-protection. Although it initially appeared that isolate LMS6-6, a HA16-5-like strain, might have a more limited host range than the other strains used in the trial, it was demonstrated that erratic distribution of the CTV isolate within the original source material might account for the lack of transmission to certain of the citrus hosts. Confirmation of some putative resistance is still outstanding and will be continued in Project 1100. Similarly the lack of transmission of isolate B390-5 to 'Eureka' lemon and GFMS12-8 to 'Turkey' mid-season sweet orange will also be verified. These findings are detailed in the results. Soft citrus varieties generally demonstrated lower titres than the other citrus types. In the commercial cultivars, only 'Nules' Clementine showed a growth reduction associated with the RB strain, B390-5, and stem-pitting was only observed in the grapefruit cultivars. In the spring months boat-shaped leaves were noted on the Satsuma varieties. No negative effects were noted on any of the sweet orange or lemon varieties tested, but this trial did not investigate the impact that CTV strains might have on production.

Diagnostic tools were developed to enable the characterisation of CTV populations in terms of their strain composition and to identify single strain isolates. A relative quantitative RT-PCR test for CTV was also developed and optimised. This was done to determine the relative titres of single CTV strains in specific hosts to investigate whether viral titre is correlated to symptom severity. The quantitative RT-PCR was not applied in this trial as symptom expression was less than expected, but will be applied in Project 1100 where the effect of strains and strain mixtures in grapefruit will be studied. Both these tools are useful for application in future work. The strain-specific assays are currently being implemented for diagnostic purposes within the improvement scheme and for general diagnostic purposes.

Technology transfer

- G. Cook, V.Z. Maqutu, J.H.J. Breytenbach and S.P. van Vuuren 2013. South African *Citrus tristeza virus* cross-protection and source characterisation. Nineteenth Conference of the International Organisation of Citrus Virologists., Skukuza, Kruger Park, 28th July – 2nd Aug 2013. (oral presentation).
- G. Cook, J.H.J. Breytenbach and S.P. van Vuuren 2014. *Citrus tristeza virus* (CTV) characterisation of cross-protection and sources and CTV strain analysis in grapefruit field trials. 8th Citrus Research Symposium. Central Drakensberg. (oral presentation).
- Cook, G, van Vuuren, S.P., Breytenbach, J.H.J, Burger, J.T. and Maree, H.J. (2015) Expanded strain-specific RT-PCR assay for differential detection of currently known *Citrus tristeza virus* strains: a useful screening tool. Journal of Phytopathology (submitted for review).

Further objectives and work plan

A new project was initiated (project 1100) and is the basis of a PhD study. This project comprises biological trials focussed on monitoring the symptom expression and interactions of CTV strains within 2 grapefruit varieties. Full genome characterisation of the single strains used in this trial and others identified will be done.

Literature cited

1. Folimonova, S. Y., Folimonov, A. S., Tatineni, S., and Dawson, W. O. 2008. Citrus Tristeza Virus: Survival at the Edge of the Movement Continuum. Journal of Virology 82 (13):6546-6556.
2. Folimonova, S. Y., Robertson, C. J., Shilts, T., Folimonov, A. S., Hilf, M. E., Garnsey, S. M., and Dawson, W. O. 2010. Infection with Strains of *Citrus Tristeza Virus* Does Not Exclude Superinfection by Other Strains of the Virus. Journal of Virology 84 (3):1314-1325.
3. Hilf, M. E., Mavrodieva, V. A., and Garnsey, S. M. 2005. Genetic Marker Analysis of a Global Collection of Isolates of *Citrus tristeza virus*: Characterization and Distribution of CTV Genotypes and Association with Symptoms. Phytopathology 95:909-917.
4. Moreno, P., Ambros, S., Albiach-Marti, M. R., Guerri, J., and Peña, E. 2008. *Citrus tristeza virus*: a pathogen that changed the course of the citrus industry. Molecular Plant Pathology 9 (2):251-268.
5. Roistacher, C. N., and Moreno, P. 1991. The Worldwide Threat from Destructive Isolates of Citrus Tristeza Virus-A Review. Pages 7-19 in: 11th Conf. Int. Organ. Citrus Virol. IOCV Riverside, CA.
6. van Vuuren, S. P., and Breytenbach, J. H. J. 2011. Transmission and movement of potential *Citrus tristeza virus* cross-protection sources in four soft citrus cultivars under greenhouse conditions. S. Afr. J. Plant & Soil 28 (1):43-48.

ADDENDUM A

(Submitted for review at the Journal of Phytopathology)

Expanded strain-specific RT-PCR assay for differential detection of currently known *Citrus tristeza virus* strains: a useful screening tool

G. Cook¹, S.P. van Vuuren¹, J.H.J. Breytenbach¹, J. T. Burger² and H.J. Maree^{2,3}

¹ Citrus Research International, P.O. Box 28, Nelspruit, 1200, South Africa

² Department of Genetics, Stellenbosch University, Private Bag X1 Matieland, 7602, South Africa

³ Agricultural Research Council, Infruitec-Nietvoorbij (The Fruit, Vine and Wine Institute), Private Bag X5026, Stellenbosch, 7599, South Africa

Abstract

Genotypic characterisation of *Citrus tristeza virus* (CTV) strains has progressed significantly, but their phenotypic expression is poorly established as CTV naturally occurs as mixed strain populations. A screening system for analysis of mixed strain populations is required for population studies and the correlation to symptom expression. In this study a published CTV strain-specific detection assay was expanded and improved to facilitate detection of currently known CTV strains. Supplementary RT-PCR assays were developed for two variant groups of the RB strain and the HA16-5 strain and assays for the T36 strain and generic CTV detection were improved. The strain components of two CTV cross-protecting sources, GFMS35 and LMS6, used in the South African budwood certification scheme were determined and the segregation of strains in budwood source trees detected.

Introduction

Citrus tristeza virus (CTV), a member of the family *Closteroviridae*, has been responsible for significant losses worldwide (Moreno et al., 2008). At least seven clades or strain groups of CTV have been identified

(Harper, 2013), however, the interactions of strains and their effect on host symptom expression is poorly understood as they mostly occur as mixed populations in addition to citrus being a genetically diverse crop. CTV was implicated in the failure of sour orange as a rootstock for citrus production in South Africa and despite the subsequent use of tolerant rootstocks; stem-pitting and resulting tree and production decline of grapefruit varieties remained problematic. A cross-protection strategy was introduced to reduce the effects of CTV on grapefruit. This approach has significantly extended the productive life of grapefruit varieties from about 10 and 15 years for pigmented and white varieties respectively, to approximately 25 years. CTV cross-protection is a management strategy using mild strain sources of the virus to reduce the deleterious effects of secondary infections, introduced by aphid vectors. Brazil (Salibe et al., 2002), Peru (Bederski et al., 2005) and Australia (Broadbent et al., 1991) also apply cross-protection for CTV and report diminished expression of disease symptoms and improved production.

To enable population studies and to characterise sources, comprehensive strain-specific assays are needed. The genotype specific RT-PCR system (Roy et al., 2010) did not allow for differentiation between the T36 and RB strains and also lacked a detection assay for the HA16-5 strain. In this study the genotype specific strain assay was improved to enable the differential detection of all currently known CTV strains. The genotype specific RT-PCR assays were used to identify the CTV strains in the South African cross-protecting sources, GFMS35, used for cross-protection of all grapefruit varieties and LMS6, used for cross-protection of limes and sweet orange. Strain segregation in grapefruit budwood source trees was also detected.

Materials and Methods

The South African CTV cross-protection sources are maintained at two facilities in secure glasshouses and the primary grapefruit budwood source trees are maintained in insect-proof tunnels. The budwood source trees were all pre-inoculated with the GFMS35 CTV source and comprise four trees of Star Ruby (red variety), five of Marsh (white variety) and one Flame (red variety).

RNA was isolated using an acid-phenol extraction buffer comprising 38% sodium acetate-saturated phenol (pH 5.0), 0.8 M guanidine isothiocyanate, 0.4 M ammonium thiocyanate, 0.1 M sodium acetate (pH 5.0), and 5% (v/v) glycerol. Bark shavings and/or leaf midribs (500 mg) were placed in maceration bags (Agdia Inc., USA) and macerated in 5ml of the extraction buffer using a power homogenizer. Samples were incubated for 5 min on ice where after 2ml of each homogenate was transferred to a micro-centrifuge tube and centrifuged at 12,000 × *g* for 5 min at 4°C. The aqueous phase was transferred to a new tube and extracted twice with chloroform. From the final aqueous phase, 800 µl was precipitated at room temperature with the addition of 200 µl iso-propanol and 200µl 4M LiCl for 10 min, and centrifuged at 12,000 × *g* for 15 min. The pellet was rinsed in 75% ethanol and re-suspended in 100 µl nuclease-free water.

Strain-specific primers for detection of strains T68, T3, VT and T30 (Roy et al., 2010) were used in this study and detailed in Table 1. Alternate generic CTV primers were developed which do not contain degenerate bases and target conserved regions in the 3' non-coding region. The T36 primers were replaced with primers which do not cross-amplify the closely related RB variants and bind within the inter-domain region (IDR) of open reading frame (ORF)1a. Two other primer sets were added that differentially amplify variants within the RB clade. The RB group-1 primer sequences match genotypes NZRB-TH28 [FJ525433], NZRB-M12 [FJ525431], NZRB-G90 [FJ525432] and HA18-9 [GQ454869], whereas the RB group-2 primer sequences match genotypes NZRB-TH30 [FJ525434], NZRB-M17 [FJ525435] and Taiwan-Pum/SP/T1 [JX266712]. Primers to detect strain HA16-5 [GQ454870] and an additional sense primer that will detect both HA16-5 and Taiwan-Pum/M/T5 [JX266713] were developed. Primers for RB group-1, RB group-2 and HA16-5 all amplify portions of the LProII domain of ORF1a. Details of the replacement and additional primers used are provided in Table 1.

Two-step RT-PCR reactions were performed. Synthesis of cDNA was done using RevertAid H Minus Reverse Transcriptase (Thermo Fisher Scientific Inc. NY, USA) with modifications to the manufacturer's instructions. RNA template (0.5-1µg total RNA) and anti-sense primer were incubated together at 65°C for 3 min and chilled on ice prior to adding the other reaction components. Forty units of RT enzyme and 10 units of Ribolock RNase Inhibitor (Thermo Fisher Scientific) were used per reaction. Reverse transcription was performed at 50°C for 60 min followed by inactivation at 85°C for 5 min. PCRs were performed in a total reaction volume of 20 µl using the GoTaq® Hot Start Green Master Mix (Promega Corp., Madison, WI, USA) and 2 µl cDNA. Cycling parameters were 95°C for 3 min followed by 35 cycles of 95°C for 20 s, 30 s at specific annealing temperature (indicated in Table 1), 72°C for 20 s and a final extension of 72°C for 5 min. Positive controls for each genotype, apart from T36, were from various plant sources which tested positive and for which the amplifications were confirmed by sequencing. The T36 clone, SP6-CTV 947-2 (Tatineni et al., 2003), was used as a positive control in the T36 assay. PCR products were gel purified using the Zymoclean™ Gel DNA recovery kit (Zymo Research, CA, USA). Direct sequencing was performed with each strain-specific primer pair in both orientations. Overlapping sequences were aligned and low quality bases and primer sequences removed using BioEdit (Hall, 1999). Closest sequence identity was determined using BLAST (Altschul et al., 1990).

Results and discussion

The oldest maintenance plants of the GFMS35 and LMS6 CTV cross-protection sources, maintained at the 2 different facilities, tested positive for the same CTV strains. Both contained strain T68 and two variants of the RB strain group, but in addition, LMS6 also contained strain HA16-5. Strain identification was validated by sequencing of the amplicons and the sequence deposited in GenBank under the accession numbers KP721477 to KP721483. Table 2 indicates the strains detected in the two sources and their respective accession numbers. The additional RB strain-specific tests were able to differentially amplify two RB strain variants from the mixed CTV populations of GFMS35 and LMS6. The RB group-1 amplicon sequences of both the CTV sources showed closest identity to the RB isolate NZRB-TH28 [FJ525433] in a BLAST search. The RB group-1 amplicon sequence obtained from the GFMS35 source was 99% homologous to NZRB-TH28, while LMS6 had 98% homology. The RB group-2 amplicon sequence obtained from the LMS6 CTV source was 100% homologous to RB isolates Crete 1825 [KF908013] and Taiwan-Pum/SP/T1 [JX266712], while GFMS35 was 99% homologous with the same isolates. The sequences for the T68 amplicons from both GFMS35 and LMS6 were 99% homologous to CT-ZA3 [KC333868] and CT-ZA2 [KC333869]. Sequences of the HA16-5 amplification from LMS6 showed closest homology (99%) to HA16-5 [GQ454870]. The T36 primer set described in this report did not cross amplify any of the RB strains in the sources tested, indicating greater specificity than those previously published (Roy et al., 2010).

The primary grapefruit budwood source trees, pre-inoculated with GFMS35, all tested positive for the two RB strain variants and negative for strain T68 which is a component of the original source. These findings are summarised in Table 3. The strains detected in these budwood source trees differed from the original source plants and show segregation of a strain in the three grapefruit varieties tested. These results demonstrate that the maintenance of mixed strain populations is challenging since various host transfers, host selection of strains and varied strain distribution in the host can all facilitate strain segregation. This is further complicated in the propagation of budwood for industry supply where the continued effectiveness of the cross-protection program is required. It has not been determined which strain(s) are important for CTV cross-protection or if mechanisms other than super-infection exclusion (Folimonova et al., 2010) contribute to cross-protection. No severe stem-pitting has been reported from orchards planted with trees derived from the tested budwood source trees without the T68 strain component which was present in the original source plants. The enhanced CTV strain-specific assay presented in this study was shown to be useful for screening purposes to investigate strain profiles of mixed infections and will also enable the monitoring of CTV strain transmission within the budwood multiplication scheme.

Acknowledgements

This work was funded by Citrus Research International, South Africa.

References cited

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) Basic local alignment search tool. *Journal of molecular biology*, **215**:403-410.
- Bederski, K., Roistacher, C. N. & Müller, G. W. Cross protection against the severe *Citrus tristeza virus* stem pitting in Peru. In: Hilf, M. E., Duran Vila, N. & Rocha-Peña, M. A. (eds), Proceedings of the Sixteenth Conference of the IOCV Texas, USA, IOCV, 2005, pp. 117-125.
- Broadbent, P., Bevington, K. B. & B. G. Coote, B. G. Control of stem pitting of grapefruit in Australia by mild strain protection. In: Brlansky, R. H., Lee, R. F. & Timmer, L. W. (eds), Proceedings of the Eleventh Conference of the IOCV Orlando, USA, IOCV, 1991, pp. 64-70.
- Folimonova, S. Y., Robertson, C. J., Shilts, T., Folimonov, A. S., Hilf, M. E., Garnsey, S. M. & Dawson, W. O. (2010) Infection with Strains of *Citrus Tristeza Virus* Does Not Exclude Superinfection by Other Strains of the Virus. *Journal of Virology*, **84**:1314-1325.
- Hall, T. A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**:95-98.
- Harper, S. J. (2013) Citrus tristeza virus: Evolution of Complex and Varied Genotypic Groups. *Front Microbiol*, **4**:93.
- Moreno, P., Ambros, S., Albiach-Marti, M. R., Guerri, J. & Peñna, E. (2008) *Citrus tristeza virus*: a pathogen that changed the course of the citrus industry. *Molecular Plant Pathology*, **9**:251-268.
- Roy, A., Ananthkrishnan, G., Hartung, J. S. & Brlansky, R. H. (2010) Development and Application of a Multiplex Reverse-Transcription Polymerase Chain Reaction Assay for Screening a Global Collection of *Citrus tristeza virus* Isolates. *Phytopathology*, **100**:1077-1088.
- Salibe, A. A., Souza, A. A., Targon, M. L. P. N. & Müller, G. W. Selection of a mild sub-isolate of *Citrus tristeza virus* for preimmunization of Pera sweet orange. In: duran Vila, N., Milne, R. G. & da Graca, J. V. (eds), Proceedings of the Fifteenth Conference of the IOCV Paphos, Cyprus, IOCV, 2002, pp. 348-351.

Tatineni, S., Gowda, S., Ayllon, M. A. & Dawson, W. O. (2003) Frameshift mutations in infectious cDNA clones of *Citrus tristeza virus*: a strategy to minimize the toxicity of viral sequences to *Escherichia coli*. *Virology*, **313**:481-491.

Table 1. Species and strain-specific primers sequences used in a two-step RT-PCR to amplify *Citrus tristeza virus* (CTV) RNA.

CTV strain	Polarity	Primer sequences from 5' to 3'	Annealing temp. (°C)	Accession no. for nucleotide position	Nucleotide positions of primer	Product size (bp)
Primers of this study:						
CTV generic	Sense	TCT GAT TGA AGT GGA CGG AAT AAG	62	NC_001661	19019	157
	Antisense	GCT TAG ACC AAC GAG AGG ATA			19155	
RB: group1 ^a	Sense	AGT GGT GGA GAT TAC GTT G	60	FJ525433	1974	628
	Antisense	TAC ACG CGA CAA ATC GAG			2584	
RB: group2 ^b	Sense	CGG AAG GGA CTA CGT GGT	60	FJ525434	1976	658
	Antisense	CGT TTG CAC GGG TTC AAT G			2615	
T36	Sense	GGT GTA AGG AAG CGT GTG TCG CAT	66	NC_001661	5641	537
	Antisense	ACC TGC ACC GTC TAA CAA CAT CAT CG			6152	
HA16-5	Sense 1	TAG GAA GGG TCA CTG CCC TGA CA	56	GQ454870	2128	610
	Antisense	GTA AGT ATC TAA AAC CAG GAG			2717	
	Sense 2	CGA CAA GTG CAT TAC GTC TCA G			2563	
Primers as per (Roy et al., 2010):						
B165 (T68)	Sense	GTT AAG AAG GAT CAC CAT CTT GAC GTT GA	59			510
	Antisense	AAA ATG CAC TGT AAC AAG ACC CGA CTC				
T3	Sense	GTT ATC ACG CCT AAA GTT TGG TAC CAC T	60			409
	Antisense	CAT GAC ATC GAA GAT AGC CGA AGC				
VT	Sense	TTT GAA AAT GGT GAT GAT TTC GCC GTC A	60			302
	Antisense	GAC ACC GGA ACT GCY TGA ACA GAA T				
T30	Sense	TGT TGC GAA ACT AGT TGA CCC TAC TG	60			206
	Antisense	TAG TGG GCA GAG TGC CAA AAG AGT				

^a RB group 1 include genotypes NZRB-TH28, NZRB-M12, NZRB-G90 and HA18-9.

^b RB group 2 include genotypes NZRB-TH30, NZRB-M17 and Taiwan- Pum/SP/T1.

Table 2. The CTV strains detected in two South African cross-protecting sources using the various strain-specific primers and the NCBI accession numbers for the sequences of the positive amplification products.

CTV source	CTV strain							
	T68	RB1	RB2	HA16-5	VT	T30	T3	T36
GFMS35	[KP721477] ^a	[KP721478]	[KP721479]	- ^b	-	-	-	-
LMS6	[KP721480]	[KP721481]	[KP721482]	[KP721483]	-	-	-	-

^a NCBI accession number of the nucleotide sequence of positive amplifications, ^b - indicates no amplification.

Table 3. The CTV strains detected in Grapefruit budwood source trees pre-inoculated with GFMS35.

Grapefruit variety	CTV strain							
	T68	RB1	RB2	HA16-5	VT	T30	T3	T36
'Star Ruby'	-	4/4 ^a	4/4	- ^b	-	-	-	-
'Marsh'	-	5/5	5/5	-	-	-	-	-
'Flame'	-	1/1	1/1	-	-	-	-	-

^a Number of trees positive/number of trees tested, ^b - indicates no amplification.

4.2.9 PROGRESS REPORT: Characterisation of *Citrus tristeza virus* variants and their influence on the symptom expression in the grapefruit host

Project 1100 (2014/15 – 2016/2017) by G. Cook (CRI), T. Jooste (SU) S.P. van Vuuren (CRI), C. Murray (CRI), J.H.J. Breytenbach (CRI), J.T. Burger (SU), H.J. Maree (SU)

Summary

The focus of this project is to investigate the CTV strains impacting stem-pitting in grapefruit and the use of cross-protection to mitigate the disease. A glasshouse trial using two commercial grapefruit varieties (Star Ruby and Marsh) is underway to evaluate the influence of specific CTV strains in single or mixed infections on stem-pitting. The CTV sources applied in the study are being fully characterised and full genome sequences for 5 strains were generated by conventional sequencing. Metagenomic, next-generation sequencing (NGS) of these isolates is in progress to ensure that they are singly-infected CTV sources that do not contain other pathogens or CTV strains. The sequences are required to refine diagnostics for strain-specific quantitative PCRs for later trial evaluation. The full-genome data additionally add to the known diversity of CTV strains worldwide and to an understanding of the South African CTV strain population. The NGS data generated is also being used to develop an automated bioinformatic pipeline for the detection and strain identification of CTV. Initial evaluations of the grapefruit glasshouse trial suggest possible interactions between the different strains as diminished symptom expression was observed in treatments containing strain mixtures. A quantitative analysis was done on all Star Ruby and Marsh trial plants, singly infected with the various strains, to evaluate potential differences in virus titre between the strains within each host. The test indicated striking titre differences within plants and between plants of the same treatment especially with the T68 strain. The average titre of this strain was higher than the titres of the other strains within Star Ruby and was also the strain that was associated with the most stem pitting thus far in the trial. An additional singly infected source of the T3 strain was identified. The single-strain status of this source is still being verified, but inoculations onto Marsh and Star Ruby demonstrated this is a severe isolate and small RNA profiles generated from these plants are being used to study virus-host interactions.

Opsomming

Hierdie projek is daarop gemik om die CTV rasse wat 'n invloed op stamgleuf in pomelo's het, te bepaal, asook om 'n oorsig te kry van die rasse wat 'n rol in kruisbeskerming speel. Verskillende kombinasies van enkelras isolate in twee pomelo gashere (Star Ruby en Marsh) word gebruik om ras-interaksies en die uitwerking op die gashere te ondersoek. Die CTV bronne wat gebruik is in die studie is ten volle gekarakteriseer en die vol-genoom basis-volgordes van 5 rasse is met behulp van konvensionele Sanger metodes vasgestel. Metagenomiese volgende-generasie volgordebepaling (NGS) is onderweg om te verseker dat die isolate inderdaad enkelras-besmette bronne is wat ook geen ander patogene bevat nie. Genoom karakterisering is ook nodig om ras-spesifieke kwantitatiewe PCR toetse te ontwikkel wat vir verdere proef-evaluering benodig word. Die volgenoom-data dra ook by tot die kennis van diversiteit van CTV rasse wêreldwyd sowel as die begrip van die Suid-Afrikaanse CTV rasse. Die NGS data wat gegenereer is, word addisioneel gebruik om 'n outomatiese CTV diagnostiese pyplyn te ontwikkel. Voorlopige resultate van die pomelo-proef dui op moontlike interaksies tussen die verskillende rasse, vanweë verlaagde simptome uitdrukking wat waargeneem is in behandelings van sekere ras-mengsels. Om verskille in virus-titer tussen die rasse te ondersoek is 'n kwantitatiewe analise gedoen op al die Star Ruby en Marsh plante wat afsonderlik besmet is met die verskillende rasse. Die toets het merkbare titer verskille binne en tussen plante van dieselfde behandeling getoon, veral met die T68-ras. Die gemiddelde titer van hierdie ras was hoër as die van die ander rasse in Star Ruby. Dit was ook die ras wat die meeste gleufstam veroorsaak het. 'n Bykomende enkelras besmette bron van die T3 ras is geïdentifiseer. Die enkelras status van hierdie bron moet nog bevestig word, maar inokulasies op Marsh en Star Ruby toon dat dit 'n aggressiewe isolaat is. Klein RNS profiele van hierdie plante is gegenereer om virus-gasheer interaksie te bestudeer.

4.2.10 PROGRESS REPORT: Evaluation of citrus material for greening resistance

Project 815 (2006 - 2015) by S.P. van Vuuren, J.H.J. Breytenbach & G. Cook (CRI)

Summary

Attempts are made to obtain greening resistance by rescuing embryos from healthy chimeras on greening infected fruit and growing them on artificial medium. Two embryo rescue clones, GTC-E2 and GTC-T2 were identified symptomless in 2006 after exposure to the citrus greening vector. PCR confirmed that they were free of the greening organism. A third clone, GTC-14, showed possible tolerance. These three clones have been multiplied on virus-free rootstocks and separately pre-immunised with two *Citrus tristeza virus* sources where after they were planted during 2007 in an orchard for field evaluations. Greening symptoms were observed on three trees of two clones after 7 years and the presence of the Liberibacter in these trees was confirmed by PCR. All the other trees were symptomless and tested negative by PCR. Trees of clone GTC-T2 were all free of greening. The fifth crop was harvested from the trees and the external fruit quality compared favourably with the Midnight Valencia control. Clone GTC-E2 had the best production and was significantly better than the Midnight Valencia. The cumulative yield for five years of clone GTC-E2 was also significantly better than that of Midnight Valencia. The three clones are currently being re-evaluated for Liberibacter resistance / tolerance at a site where the triozid population is uncontrolled.

Opsomming

Daar word gepoog om vergroening weerstandbiedendheid te verkry deur embryo's uit gesonde chimeras van vergroende vrugte te verwyder en op kunsmatige medium te kweek. Twee embryo-herwinningsklone, GTC-E2 en GTC-T2, is in 2006 geïdentifiseer as simptomeeloos na blootstelling aan besmette vektore van sitrusvergroening. PCR het getoon dat hulle vry van die organisme is. 'n Derde kloon, GTC-14, het moontlike toleransie getoon. Die drie klone is op onderstamme vermeerder en afsonderlik met twee *Citrus tristeza virus* bronne gepreïmmuniseer en gedurende 2007 in 'n boord uitgeplant vir verdere evaluasie. Na 7 jaar is vergroeningsimptome vir die eerste keer op drie bome van twee klone waargeneem. Die teenwoordigheid van die Liberibacter is deur PCR bevestig. Al die ander bome was simptomeeloos en het negatief getoets met PCR. Al die bome van kloon GTC-T2 was vry van vergroening. Die vyfde oes is van die bome verkry en eksterne vruggehalte vergelyk goed met die van Midnight Valencia kontrole. Kloon GTC-E2 het die beste produksie gelewer en was betekenisvol beter as die van Midnight Valencia. Die kumulatiewe

produksie oor vyf jaar van kloon GTC-E2 was ook betekenisvol beter as die van Midnight Valencia. Die drie klone word tans ook ge-evalueer vir Liberibacter weerstandbiedendheid / verdraagsaamheid by 'n terrain waar die trioïd insek vektor nie beheer word nie.

4.2.11 PROGRESS REPORT: Further studies on alternative hosts of “*Candidatus Liberibacter africanus*” and related Liberibacters on tree members of indigenous Rutaceae
Project 886B (2013 - 2016) by Gerhard Pietersen and Ronel Roberts (ARC-PPRI and UP)

Summary

Citrus greening has been reduced to economically acceptable levels in South Africa through stringent vector control strategies and the removal of inoculum sources, but remains a problem in cooler citrus production areas of South Africa. The perpetuation of the disease may be due to the presence of hosts other than citrus to '*Candidatus Liberibacter africanus*' (Laf). During a previous project (886) large numbers of *Calodendrum*, *Vepris*, *Zanthoxylum* and *Clausena* were collected and analyzed for the presence of Liberibacters. Laf *sensu stricto* was not detected in any indigenous members of the Rutaceae; however, Liberibacters related to Laf were found in all Rutaceae genera analysed, supporting the general hypothesis that Laf may have derived from indigenous African members of the Rutaceae. It is important to do controlled transmission tests to test the possibility that these bacteria may infect citrus. We also need to determine the potential occurrence of Liberibacters in some of the remaining indigenous Rutaceous tree host genera (*Teclea* and *Oricia*). With the introduction to the USA of "*Candidatus Liberibacter asiaticus*" (Las), associated with Huanglongbing disease, research effort, supported by millions of dollars of funding, is being used to develop fundamental understandings and control strategies for this disease. Novel control strategies are being evaluated, many based on molecular interventions. To this end it is important that we stay up to date with the latest international developments regarding Liberibacter detection, control and epidemiology and also characterize the local sources of Liberibacters by whole genome sequencing in order to exploit the sequence differences amongst the Liberibacters. Data obtained thus far suggest that the Liberibacter variants observed in South Africa appear to be restricted to specific Rutaceous hosts. If greater understanding is obtained regarding the genes that regulate host range we may be able to exploit this in the long term for improved disease control.

Opsomming

Die voorkoms van sitrus vergroening in Suid Afrika is verminder tot ekonomiese aanvaarbare vlakke deur die implementering van streng vektor beheer en die verwydering van inokulumbronne. Ten spyte hiervan, bly vergroening 'n probleem in koeler produksie areas wat daarop dui dat '*Candidatus Liberibacter africanus*' (Laf) moontlik op alternatiewe gashere voorkom. Gedurende 'n vorige projek (886) is 'n groot aantal *Calodendrum*, *Clausena*, *Vepris* en *Zanthoxylum* monsters getoets vir Liberibacters. Tipiese Laf is nie geïdentifiseer op enige van die monsters nie. Tog is ander Laf verwante Liberibacters geïdentifiseer in elk van die geanaliseerde genera. Die bestaan van alternatiewe Liberibacters ondersteun die hipotese dat Laf se oorsprong vanaf 'n inheemse Rutaceae bron kan wees. Dit is belangrik dat gekontroleerde oordragingstoetse na sitrus van die inheemse Liberibacters gedoen word om te bepaal of hierdie Liberibacters sitrus kan infekteer. Daar moet ook vasgestel word of Liberibacters in die oorblywende inheemse Rutaceae genera, *Teclea* en *Oricia*, voorkom. Met die voorkoms van '*Candidatus Liberibacter asiaticus*' (Las), die oorsaak van Huanglongbing, in Amerika, word baie geld en tyd bestee aan die navorsing van verskeie aspekte van hierdie bakterium. Alternatiewe beheer strategieë word tans ondersoek met die klem op molekulêre sisteme. Dit is belangrik om by te bly met alle internasionale verwickeling rakende Liberibakter diagnostiese toetse, beheer en epidemiologie. Addisioneel moet klem daarop geplaas word om die genome van inheemse Liberibacters se DNA volgordes te bepaal sodat verskille tussen die genome geïdentifiseer kan word. Sulke verskille kan lei tot die ontwikkeling van beter beheer strategieë.

4.2.12 PROGRESS REPORT: Comparison of shoot tip grafted citrus with old clone material
Project 1074 (2013 - 2023) by S.P. van Vuuren, J.H.J. Breytenbach & G. Cook (CRI)

Summary

Some cultivar owners and agents claim that the use of old clone material of cultivars is more profitable than material from Citrus Improvement Scheme from the Citrus Foundation Blok (CFB). CFB material has been cleaned from all graft transmissible agents by shoot tip grafting and thereafter inoculated with an approved *Citrus tristeza virus* (CTV) source for cross-protection. The objective of this study is to compare tree and fruit characteristics of shoot tip grafted material with that of old clone material. Three cultivars are involved *viz.* Benny Valencia, Cambria navel and Glen Ora Late navel. Budwood was collected from original sources of

the cultivars and budded according to normal nursery practices to Swingle citrumelo, Carrizo citrange and C35 citrange rootstocks. The same was done with material of the three cultivars that was obtained from the CFB. Strict sterilisation measures of cutting tools were maintained during budding. The CTV and Citrus viroid (CVd) status of all the bud wood sources were established by reverse transcription polymerase chain reaction (RT-PCR). All the sources originating from the CFB is supposed to have the same CTV strains than the LMS 6 cross-protecting source, viz. T68, HA16-5 and two RB variants. It was found that Benny Valencia 2 only had the HA16-5 strain, which is just a part of the complex, while the Cambria 3 navel and the Glen Ora Late navel were virus-free. Treatments containing material from the CFB were re-inoculated with the LMS 6 CTV source. The original Cambria material from Baviaanskloof, which was used to make trees of this treatment was found to be free of CEVd. Trees of the treatment containing the original Cambria source were re-inoculated with the original material from Dunbrody Estates. CVd-IIa was absent in the Baviaanskloof and Dunbrody sources and the use of these sources to evaluate the effect of CVd-IIa is not valid. Trees in the treatment containing CVd-IIa were re-inoculated with CVd-IIa from the OR 4 source. All the re-inoculations delayed the planting of the trees which will commence after winter.

Opsomming

Sommige kultivar eienaars en agente maak aanspraak daarop dat ou klone materiaal meer winsgewend is as Sitrus Verbeteringskema materiaal vanaf die Grondvesblok (GVB). GVB materiaal is gesuiwer van alle entoordraagbare siektes deur middel van groeipunt-enting en is daarna geïnkuleer met 'n goedgekeurde *Citrus tristeza virus* (CTV) bron vir kruisbeskerming. Die doel van die studie is om boom en vrug eienskappe van GVB materiaal met die van ou kloon materiaal te vergelyk. Drie kultivars is betrokke, nl. Benny Valencia, Cambria nawel en Glen Ora Late nawel. Okuleerhout is van oorspronklike bronne van die kultivars versamel en volgens normale kwekery praktyke op Swingle citrumelo, Carrizo citrange en C35 citrange onderstamme geokuleer. Dieselfde is gedoen met materiaal wat vanaf die GVB ontvang is. Streng sterilisasie voorsorgmaatreels van snygereedskap is gevolg tydens okulering. Die CTV en Citrus viroïede (CVd) status van al die enthout bronne is bepaal deur middel van polimerase kettingreaksie (PKR). Al die soetlemoen enthout bronne vanaf die GVB is veronderstel om dieselfde CTV rasse as die oorspronklike LMS6 kruisbeskerdingsbron te hê nl. T68, HA16-5 en twee RB variante. Dit is bevind dat Benny Valencia 2 slegs die HA16-5 ras bevat het, wat slegs 'n gedeelte van die kompleks is, terwyl Cambria 3 nawel en die Glen Ora Late nawel albei virusvry was. Al drie die behandelings is herinokuleer met die LMS6 CTV bron. Die oukloon Cambria materiaal van Baviaanskloof wat gebruik is om die behandeling te maak is vry bevind van CEVd. Bome wat van hierdie materiaal gemaak is, is herinokuleer met oukloon materiaal afkomstig van Dunbrody Estates. CVd-IIa was afwesig in die Baviaanskloof en Dunbrody bronne en die gebruik van die twee bronne was nie geskik nie. Bome met die CVd-IIa behandeling is herinokuleer met CVd-IIa vanaf die OR 4 bron. Al die herinokulasies het die projek vertraag en die plant van die bome sal na die winter gedoen word.

4.3 PROGRAMME: FRUIT AND FOLIAR DISEASES Programme coordinator: G.C. Schutte (CRI)

4.3.1 PROGRAMME SUMMARY

Two new systemic fungicides (RB1 and RB2) alone and alternated with mancozeb were tested on 'Nova' mandarins for the control of *Alternaria* brown spot in the Swellendam and Schagen. Results showed that both fungicides applied alone at monthly intervals performed well, but were less effective when they were alternated with mancozeb. Results would have been better if the 4th application was applied at the Schagen trial site but due to heavy rain and flooding this was not possible. Heavy fog in Swellendam in May resulted in numerous new infections and lesions just before harvest, also leading to no result (4.3.2).

The development of an improved spray deposition assessment protocol and deposition benchmarks indicative of the biological effectiveness of deposition parameters was studied to optimise spray application. High spray volumes (>10 000 l.ha⁻¹) did not result in better spray deposition and similar and even improved spray deposition quantity and uniformity at better spray efficiency can be obtained at intermediate spray volumes (5 000-8 000 l.ha⁻¹) through optimal use of equipment or through the use of more efficient sprayers. Low spray pressures and adequate, efficient spraying speeds also performed well. Leaves showed promising deposition quantity results for low volume applications at increased product concentrations. Deposition uniformity results at low volumes (2 000 to 4 000 l.ha⁻¹) indicated relatively poorer canopy penetration, and emphasises the need for canopy management to allow better penetration. Low spray volumes realised better deposition quality per leaf, but higher volumes resulted in more uniform deposition throughout the tree canopy. High spray volumes (>10 000 l.ha⁻¹) did not improve deposition due to excessive run-off, identified the need for calibration criteria for optimised application (4.3.3).

Organic matter with a low pH helps *Botrytis cinerea* Pers., the cause of blossom blight on lemons to survive and to infect blossoms. To determine which stage of blossom development is most suitable for infection and when they should be sprayed for the control of the disease, the pH, Brix and mycelium growth rates of *Botrytis* on a petal- and stamen-extract media of different citrus cultivars were compared over two seasons in White River and Addo. No pattern could be detected as results were inconsistent. *In vitro* results showed that *Botrytis* was most sensitive to benomyl, followed by iprodione. Field trials showed that benomyl and iprodione performed the best as well as two new fungicides, fenhexamid and pyrimethanil. A commercial application of Benlate in Addo showed that blossoms in the balloon stage were better protected than open blossoms. The appearance of *Botrytis*-like ridges on lemons in a field trial in Sundays' River was ascribed to excessive nitrogen. Breakthru Union, NuFilm 17 with mancozeb and mancozeb alone resulted in poor coverage of all flower parts (4.3.4).

For tree volume based sprayer calibration, necessitates that trees must be characterised in terms of dimensions (volume) and density. A LiDAR (light detection and ranging) system was procured, a mount built to facilitate PC communication and ASCII and binary translation of scanning data has commenced (4.3.5).

Previous problems experienced with poor *Alternaria* brown spot control, can be ascribed to the effect deposition quality had on disease control, as well as possible physical and/or chemical effects of adjuvant use together with copper oxychloride sprays. Poor correlations between deposition quantity, quality and disease control on leaves, show that the current method use to capture and analyse deposition on leaf surface are not accurate enough. A histopathology study showed that adjuvants did not have physical or chemical effect on the adhesion of spores to the leaf surface, germ tube length, viable conidia and conidial/germ tube stress. Copper oxychloride alone and added to adjuvants had a significant effect on these parameters. Entreé with copper oxychloride reduced conidial adhesion the most, realised the shortest germ tube length and realised the highest percent stress (4.3.6).

PROGRAMOPSOMMING

Vir die beheer van *Alternaria* bruinvlek op 'Nova' mandaryne is nuwe sistemiese swamdoders in Swellendam en Schagen (RB1 en RB2) is op hul eie asook in 'n afwisselende program met mancozeb getoets. Goeie resultate is verkry waar beide swamdoders alleen toegedien is, maar afwisseling met mancozeb het die spuitprogramme swakker gevaar. By Schagen kon die resultate beter gewees het omdat die 4de bespuiting in midsomer weens vloede nie toegedien kon word nie, terwyl swaar mis kort voor oes in Swellendam in Mei tot ontelbare nuwe infeksies en letsels aanleiding gegee het (4.3.2).

Die ontwikkeling van 'n verbeterde spuitneerslag-assesseringsprotokol asook drempelwaardes om die aanduiding van biologiese doeltreffendheid van neerslag parameters te bepaal, is gebruik om verbeterde spuittoediening te bestudeer. Hoë spuitvolumes ($> 10\ 000\ \text{l}\cdot\text{ha}^{-1}$) het nie beter spuitneerslag op blare bewerkstellig nie en soortgelyke en selfs beter spuitdeponering, eenvormigheid en beter spuitdoeltreffendheidsvlakke kan d.m.v. intermediêre volume toedienings ($5\ 000$ tot $8\ 000\ \text{l}\cdot\text{ha}^{-1}$) tesame met optimale gebruik van toerusting, of deur die gebruik van meer doeltreffende spuit-tegnologie op blare verkry word. Laer spuitdruk en effektiewe spuit-spoed het goeie resultate opgelewer. Blare het belowende deposisie kwantiteit vir lae volume toedienings by verhoogde produkonsentrasies. Deposisie uniformiteitsdata by lae volume toedienings ($2\ 000$ tot $4\ 000\ \text{l}\cdot\text{ha}^{-1}$) dui op swak lower penetrasie, wat die belangrikheid beklemtoon vir lowerbestuur om verbeterde penetrasie toe te laat. Lae volume toedienings het beter deposisie kwaliteit per blaar tot gevolg gehad, maar hoër volume toedienings het beter deposisie uniformiteit deur die boomlower tot gevolg gehad. Hoë spuitvolumes ($10\ 000\ \text{l}\cdot\text{ha}^{-1}$) het nie deposisie verbeter nie a.g.v. oormatige afloop, wat daarop dui dat kalibrasie kriteria vir toedienings optimisering van lae volume toediening nodig is (4.3.3).

Organiese materiaal met 'n lae pH help dat *Botrytis cinerea* Pers. wat bloeiselsversenging op suurlemoene veroorsaak, kan oorleef en het die vermoë om blomme te infekteer. Om te bepaal watter blomdeel en - stadium meer vir swamgroei geskik is en dus geteiken moet word vir die effektiewe chemiese beheer van die siekte, is die pH, Brix en swamgroei op 'n blomblaar- en stuifmeeldraad-ekstrak-medium van verskillende sitruskultivars oor twee seisoene in Witrivier en Addo gemeet. Geen patroon kon bepaal word nie omrede resultate wisselvallig was. *In vitro* resultate toon dat *Botrytis* mees sensief was teen benomyl, gevolg deur iprodion. Veldproewe toon dat benomyl en iprodione goeie beheer van die siekte tot gevolg gehad het, asook twee nuwe swamdoders, fenhexamid en pyrimethanil. 'n Kommersiële bespuiting van Benlate in Addo het getoon dat blomme wat toe is (ballonstadium) langer beskerm word as oop blomme. Die hoë voorkoms van *Botrytis*-agtige riuwe op suurlemoene in 'n veldproef in die Sondagsrivier is aan stikstof toegeskryf. Mancozeb met Breakthru Union, NuFilm 17 en mancozeb alleen, toon swak bedekking van alle blomdele (4.3.4).

Vir boom-ry-volume gebaseerde kalibrasie moet bomekappe gekarakteriseer word in terme van dimensies (volume) en lower-digtheid. 'n LiDAR (Light detection and ranging) sisteem is aangeskaf, 'n montering gebou om rekenaarkommunikasie te bewerkstellig en 'n ASCII binêre vertaling van geskandeerde data is begin (4.3.5).

Wisselvallige resultate wat voorheen met *Alternaria* bruinvlekbeheer verkry is, kan toegeskryf word aan die effek wat deposisie kwaliteit op siektebestuur het, asook die maontlike fisiese en/of chemiese effekte van die benatter wat gebruik is saam met koperoksichloried bespuitings. Swak korrelasies tussen deposisiekwantiteit, -kwaliteit en siektebestuur op blare, toon dat die huidige metodes wat gebruik word om deposisie op blare af te neem en te analiseer, nie akkuraat genoeg is nie. 'n Histopatologiese studie toon dat benatters nie 'n fisiese of chemiese effek op die aanhegting van spore, kiembuise lengte, spoor vatbaarheid het nie, maar wel stres verhoog in spore sowel as kiembuise. Koperoksichloried alleen en saam met benatters het 'n betekenisvolle effek op hierdie faktore gehad. Entree saam met koperoksichloried het spooraanhegting die meeste verlaag, die kortste kiembuise tot gevolg gehad en het die hoogste persentasie stres tot gevolg gehad (4.3.6).

4.3.2 **PROGRESS REPORT: Evaluation of new spray programmes for the control of *Alternaria* brown spot in the summer rainfall regions of South Africa** Project 750 (Ongoing) by G.C. Schutte & C. Kotze (CRI)

Summary

New systemic fungicides (RB1 and RB2) alone and alternating with mancozeb were tested on 'Nova' mandarins for the control of *Alternaria* brown spot in the Swellendam and Schagen areas. Results showed that both fungicides applied alone at monthly intervals performed the best, but when they were alternated with mancozeb, were less effective. Results would have been better if the 4th application were applied at Schagen but could not be performed due to heavy rain and flooding, while heavy fog in Swellendam in May resulted in numerous new infections and lesions just before harvest.

Opsomming

Nuwe sistemiese swamdoders (RB1 en RB2) is op hul eie asook in 'n afwisselende program met mancozeb getoets vir die beheer van *Alternaria* bruinvlek op 'Nova' mandaryn in Swellendam en Schagen. Resultate toon dat beide swamdoders wat alleen toegedien is die effektiëste was. In afwisseling met mancozeb het hulle swakker gevaar. Resultate by Schagen kon beter gewees het as die 4de bespuiting toegedien kon word, maar weens swaar reën en vloede kon dit nie toegedien word nie, terwyl swaar mis in Swellendam in Mei tot baie nuwe infeksies en letsels aanleiding gegee het kort voor oes.

4.3.3 **FINAL REPORT: Optimisation of fungicide spray applications in citrus orchards** Project PPL 891 (April 2007 – April 2015) by Paul Fourie (CRI) and Gideon van Zyl (CRI at SU)

Summary

In South Africa, fungicide spray application at medium to high cover (6000 to 9000 l ha⁻¹) is recommended to control fruit and foliar diseases. A large proportion of excessive spray volume is, however, lost to run-off and drift, which results in considerable environmental pollution of soils and air. High spray volumes nonetheless provides a safety buffer in cases of improper calibration, machinery and equipment use, and wrong and neglected application techniques. This study focussed on the development of an improved spray deposition assessment protocol and deposition benchmarks indicative of the biological effectiveness of deposition parameters to study optimised spray application and possible cost-saving through reduced volume foliar application. From various spray trials using various machines at spray volumes from 300 to 24000 l ha⁻¹, it was clear that deposition parameters on leaves improved with higher spray volumes, but that excessively high spray volumes (>10 000 l ha⁻¹) did not result in better spray deposition. Similar and even improved spray deposition quantity and uniformity at better spray efficiency could be obtained at intermediate spray volumes (5000-8000 l ha⁻¹) through optimal use of equipment or through the use of more efficient sprayers. Generally good results were obtained at lower spray pressures (10 to 15 bar and 1.5 bar) (depending on spray machine type) and adequate, efficient spraying speeds (1.9 to 2.9 km h⁻¹). The potential of low-energy, low cost and/or reduced volume sprayers were evaluated. Results on leaves showed promising deposition quantity results for low volume applications at increased product concentrations (2x; 4x and 8x). However, uniformity results at low volumes (2000 to 4000 l ha⁻¹) indicated relatively poorer canopy penetration, and therewith the need for canopy management to allow better penetration. Lower spray volumes generally realised better deposition quality per leaf, but higher volumes resulted in more uniform deposition throughout the tree canopy. Research to date has demonstrated that excessive spray volumes (>10000 l ha⁻¹) did not

improve deposition due to excessive run-off, identified calibration criteria for optimised application, and showed the potential of reduced volume application. It was also clear from this project that a dynamic mind shift is needed in how we apply plant protection products in South Africa, moving away from area based calibrations and conventional technology to tree volume and density based calibration using improved spray machinery.

Opsomming

In Suid-Afrika word swamdoders teen medium to hoë dek-bespuittings (6000 tot 9000 l ha^{-1}) aanbeveel om vrug- en blaarsiektes te beheer. 'n Groot deel van oormatige spuitvolumes gaan egter verlore a.g.v afloop en drif wat tot aansienlike omgewingsbesoedeling van die gronde en lug lei. Hoë spuitvolumes dien egter as n veiligheidsbuffer in die gevalle waar onvoldoende kalibrasie, masjiene en toerusting gebruik word, asook verkeerde en swak toedieningstegnieke. Hierdie studie het gefokus op die ontwikkeling van 'n verbeterde spuitneerslag-assesseringsprotokol asook drempelwaardes vir die aanduiding van biologiese doeltreffendheid van neerslag parameters te bepaal. Hierdie protokol is gebruik om verbeterde spuittoediening en moontlike kostebesparing deur verlaagde volume blaar spuit-toedienings te bestudeer. Vanuit verskeie spuitproewe met verskillende masjiene by spuitvolumes vanaf 300 tot 24000 l ha^{-1} , was dit duidelik dat die uitermate hoë spuitvolumes ($> 10000 \text{ l ha}^{-1}$), nie beter spuit neerslag op blare realiseer het nie. Soortgelyke en selfs beter spuitdeponering, eenvormigheid en beter spuitdoeltreffendheidsvlakke kan d.m.v intermediêre volume toedienings (5000 tot 8000 l ha^{-1}) tesame met optimale gebruik van toerusting, of deur die gebruik van meer doeltreffende spuit-tegnologie op blare verkry word. Laer spuit-druk (10 tot 15 bar) en effektiewe spuit-spoed (1.9 tot 2.9 km h^{-1}) (afhangende van die spuit masjienerie) het goeie resultate gelewer. Die potensiaal van lae-energie, lae-werkskoste en/of lae-volume spuitmasjiene is ook bestudeer. Resultate op blare het belowende deposisie kwantiteit vir lae volume toedienings by verhoogde produk konsentrasies ($2\times$, $4\times$ en $8\times$) gewys. Deposisie uniformiteit data by lae volume toedienings (2000 tot 4000 l ha^{-1}) het egter op swak lower penetrasie gedui, en daarvoor die nodigheid vir lower bestuur om verbeterde penetrasie toe te laat. Lae volume toedienings het gewoonlik beter deposisie kwaliteit per blaar tot gevolg gehad, maar hoër volume toedienings het beter deposisie uniformiteit deur die boomlower tot gevolg gehad. Navorsing tot op hede het gedui dat oormatige hoë spuitvolumes (10000 l ha^{-1}) nie deposisie verbeter nie a.g.v oormatige afloop, het nodige kalibrasie kriteria vir toediening optimisering en ook die potensiaal van lae volume toediening uitgewys. Dit was ook duidelik uit hierdie projek dat 'n dinamiese kopskuif benodig word oor hoe plant beskermingsprodukte in Suid-Afrika toegedien word. Dit is nodig om weg te beweeg van area gebaseerde kalibrasie metodiek en konvensionele tegnologie na 'n boom volume en digtheid gebaseerde kalibrasie metodiek en die gebruik van gevorderde spuitmasjienerie.

Introduction

South Africa is currently the 13th largest citrus producer and 3rd largest exporter of fresh market citrus in the world (almost 70% of total production), accessing more markets worldwide than any other citrus producing country (CGA Key industry statistics 2014 ref). Quality disease free fruit is needed to satisfy local and international markets. This drive local citrus producers to follow intensive disease management programs, especially for the control of important diseases such as Citrus black spot (*Phyllostica citricarpa* (McAlpine)) (EFSA, 2009, 2008; EPPO, 2009 Kotzé, 1981; 2000; Schutte, 1997) and Alternaria brown spot (*Alternaria alternata* (Fr: Fr) Keissl., tangerine pathotype) (Schutte, 1996). Acceptable disease control is primarily achieved through medium to full cover dilute fungicidal sprays ranging from 6000 to 16000 l ha^{-1} (Grout, 1997, 2003). Application is done with a variety of spray machines produced locally and internationally, varying widely in sprayer design.

Mature citrus trees are reported to hold sprays to a maximum of $2\ 300 \text{ l ha}^{-1}$ only, depending on the canopy geometry and foliage density (Cunningham and Harden, 1998, 1999). As much as 85% of the excessive spray volume is therefore lost to endo- and exodrift, which results not only in considerable environmental pollution of soils and air, but also increased run-off, reduced spray cover and therewith reduced spray efficacy (Furness et al., 2006ab; Landers and Farooq, 2004). Moreover, excessively high spray volumes are not time and cost effective. Scope for improvement of the current spray application in southern Africa certainly exist as growers for processing in Florida (USA) apply 1500 l ha^{-1} to mature trees (Pete Timmer, pers. comm.), while the use of novel spray applicators allowed a reduction in spray volumes to below 6000 l ha^{-1} in Australia (Furness et al., 2006b). Citrus trees differ in geometry and density depending on type, cultivar and growing region. Yet, in South Africa, set spray volumes exists for application types (Grout, 1997). This study therefore focussed on identifying spraying methodology and technology (spray machines) used by the citrus industry in South Africa. Identified spraying methodology was subsequently evaluated and from acquired data, novel low cost, low input and/or low volume methodologies and technologies identified and evaluated. This was ultimately done for the optimisation of fungicide application in South African citrus production.

In order to study the optimisation of spray application on grape vineyards, researchers at Stellenbosch University's Plant Pathology department (USPP) have developed a spray assessment protocol using fluorometry, photomicrography and digital image analyses (Brink *et al.*, 2004, 2006). Following the determination of benchmark levels for biologically effective spray deposits, they clearly demonstrated that the current best-practice spray applications in table and wine grape vineyards did not result in biologically effective spray deposits. One method of improving the *status quo* was to use spray applicators within specific optimal volume output ranges. USPP's research has shown that optimal use for an air shear machine (Cima™) in table or wine grape vineyards was between 250 and 500 l ha⁻¹, compared with the standard 1000-1500 l ha⁻¹. Biologically effective spray deposits on leaves and bunches were effected by increasing the fungicide concentration relative to the decrease in volume (2- or 4-fold).

Stated objectives

1. Conduct a thorough survey of current spray application materials and methods in all major citrus growing areas.
2. Determine benchmarks for biological efficacy of copper oxychloride against *Alternaria* brown spot.
3. Characterisation of spray deposition with current spray application methods.
 - a. Evaluate methods for optimisation of spray application with commonly-used applicators.
 - b. Evaluate methods for optimisation of spray application with novel applicators.
4. Development and validation of a user-friendly calibration system.
5. Develop a guideline for optimal use of commonly-used and novel spray applicators in citrus orchards.
6. Use of adjuvants for improved spray deposition on citrus leaves and fruit
7. Physical and chemical effects of adjuvants on spray deposition and control of *Alternaria* brown spot following copper oxychloride sprays on mandarin leaves
8. Modelling of the influence of quantity and quality of copper oxychloride sprays on control of *Alternaria* brown spot on mandarin leaves

Materials and methods

1. Conduct a thorough survey of current spray application materials and methods in all major citrus growing areas.

A comprehensive questionnaire comprising all aspects of spray application in citrus orchards was compiled. This questionnaire was handed out after grower study group meetings in various citrus growing areas. The questionnaire was also circulated on CRInet. The data were summarised to accurately reflect the current status of spray application in the citrus industry, which was essential for conceptualisation of following experimentation. The information will furthermore prove invaluable when future changes to the *status quo* are negotiated with growers, the agrichemical industry and the Registrar for Agricultural Remedies.

2. Determine benchmarks for biological efficacy of copper oxychloride against *Alternaria* brown spot.

The objective focussed on improving the deposition assessment protocol developed by Brink *et al.* (2004; 2006). This was done successfully by Fourie *et al.* (2009), which used the improved protocol to evaluate the effect of run-off on spray deposition and control of *Alternaria* brown spot of mandarins (Progress report 2007-8). This work was concluded and published in a scientific article: Fourie, P.H., du Preez, M., Brink, C.J., Schutte, G.C. 2009. The effect of runoff on spray deposition and control of *Alternaria* brown spot of Mandarins. *Australasian Plant Pathology* 38: 173-182.

The protocol was further improved by van Zyl *et al.* (2013) and was ultimately used for spray deposition assessment and benchmark development for the control of *Alternaria* brown spot on mandarin leaves with copper oxychloride. This work section was concluded and published in a scientific article: van Zyl, J.G., Fourie, P.H., Schutte, G.C. 2013. Spray deposition assessment and benchmarks for control of *Alternaria* brown spot on Mandarin leaves with copper oxychloride. *Crop Protection* 46: 80-87.

3. Characterisation of spray deposition with current spray application methods.

- a. **Evaluate methods for optimisation of spray application with commonly-used applicators.**

Trial layout and sampling. Trials were conducted in a uniform section of the selected orchard. For each treatment combination, a row-section with 6 to 10 trees was sprayed from both sides. Two buffer rows were left unsprayed between treatments. For logistical reasons, spray deposition on leaves was determined instead of deposition on fruit. However, in another experiment using similar methodology (CRI 918), we

observed an 80% correlation between fluorescent pigment deposition on leaves and deposition on fruit. A 76% and 90% correlation was observed between the copper residue analysed and the quantitative fluorescent pigment measurements on leaves and fruit, respectively, which supports this methodology as an effective tool for spray deposition assessment. As replications, 3 uniform trees were selected from each sprayed section from which leaves were sampled for spray deposition analysis. At least 12 intact leaves were sampled from each of various positions in the canopy: inner (>30 to 50 cm into the tree) and outer (leaves on the outside of the tree) canopy, and top, middle and bottom part of each tree. Leaves from these 6 positions were collected separately in plastic bags and transported in cool, dry conditions to the laboratories at Stellenbosch University, where it was cool-stored at 4°C until further analysis. Deposition quantity of upper and lower leaf surfaces were conducted as described previously from 12 leaves per position.

Spray deposition analysis. For deposition analysis, petioles were removed from leaves with a scissor by cutting it just in front of the start of the leaf blade. A single leaf was positioned in the middle of a back-illuminated red Perspex box (300×210×110 mm) inside a dark room to reduce any shadowing and to enhance edging of leaves in captured images during analysis. The leaf was covered with a glass pane (200×200×2 mm) and illuminated using a ultra-violet light source (UV-A; ≈ 365 nm; Labino Mid-Light; www.labino.com). Digital photos were taken in Canon RAW file format (.CR2 ≈ 10 MB) of the upper and lower leaf surfaces using a Canon EOS 40D camera equipped with a 60 mm macro lens. The camera was attached to a tripod in a fixed position directly above the leaf. RAW image files were converted to 8-bit Exif-TIFF (.TIF ≈ 30 MB) with Digital Photo Professional version 3.1.0.0 (CANON INC.; www.canon.com) files for digital image analysis to determine the deposition parameters (van Zyl et al., 2013).

Spray deposition assessment involved digital image analysis with Image Pro Plus software version 7.0 (Media Cybernetics, www.mediacy.com) to determine the deposition quantity and quality per leaf. Similar to the methodology used in van Zyl et al. (2013), deposition quantity was measured as percent total leaf area covered by pigment particles (percentage fluorescent particle coverage; FPC%) (van Zyl et al., 2013). Deposition uniformity was calculated as the CV% in pigment deposition in a 12 leaf batch (Standard Deviation × 100/mean) and deposition efficiency was expressed as deposition quantity normalised to FPC% per 1000 l ha⁻¹.

Benchmarking. Deposition data was subjected to the FPC benchmark model developed by van Zyl et al. (2013) to evaluate the effectiveness of deposition in relation to theoretical disease control that can be achieved. The FPC50 (2.07 FPC%) and FPC75 (4.14 FPC%) benchmarks indicate 50% and 75% theoretical control of Alternaria Brown Spot on mandarin leaves, respectively.

Statistical analysis. Deposition quantity (FPC%), uniformity (%) and efficiency data were subjected to appropriate analysis of variance (ANOVA). Student's T-test or Fisher's LSD was calculated to identify significant differences between treatments at a confidence interval of 95%. The skewing effect of outliers was negated by using median values for deposition analysis. Data from upper and lower leaf surfaces was analysed separately, but were combined when describing the results. Where appropriate, data were also subjected to regression analysis and Pearson's correlation to demonstrate the possible relations between deposition quantity, quality and uniformity measurements. SAS version 8.2 statistical software (SAS institute Inc., 1999) was used for analysis.

Addo, 2008. A novel multi-fan spray machine, the BSF-Multiwing was developed by Meyer Boshoff from Hoedspruit. Similar multi-fan spray technology was demonstrated to perform better and at higher tractor speeds in Australian citrus orchards (Geoff Furness, SARDI-Loxton Australia, personal communication). This machine was compared with a grower-owned Volcano oscillating boom mistblower in a mature Navel orchard at various settings.

Hoedspruit, 2008. The BSF-Multiwing was compared with a conventional oscillating boom mistblower, the BSF-Extreme, at various calibration settings in a mature Valencia orchard.

Letsitele, 2008. The BSF-Multiwing was compared with various conventional oscillating boom mistblowers, viz. the Ultima, Bateleur (1-sided ability only) and BSF-Extreme, as well as the Cima air-shear (1-sided ability only) sprayer and ESS electrostatic sprayer (1-sided ability only). Various calibration settings for the different sprayers effecting spray volumes ranging from 300 to 12000 l ha⁻¹ were evaluated.

Groblersdal, 2009. The trial was conducted in uniform sections of a Robyn navel (*Citrus sinensis* (L.) Osbeck) [3.62×3.92 m trees (H×W) with density of 3.5 on a 5-point scale; 6.5×5 m row spacing] orchard on the farm Schoemans farms (Groblersdal, Limpopo Province, South Africa) in 2009. Treatments were applied early morning as soon as trees were dry from dew. For each treatment combination, a row section of 10

trees was marked with hazard tape and sprayed from both sides with the same tractor, power take-off (PTO) calibrated with a SENTRY ST 723 contact tachometer (Sentry Optronics corp. Taipei, Taiwan) to, and set at 540 rpm. Sprays consisted of 12 separate treatments consisting of water and yellow fluorescent pigment [40% EC (SARDI, Loxton, South Australia); 1 ml l⁻¹]. Treatment one to three was sprayed with BSF extreme sprayer at different spray volumes, with volume being manipulated by varying tractor speed; treatment four to seven was sprayed with a BSF Multiwing, with spray volume being manipulated by tractor speed, nozzle selection and spray pressure; treatment eight to ten was applied with a Ultima sprayer, with spray volume being manipulated by tractor speed and PTO speed; and lastly, treatment 11 and 12 being applied again with the BSF multiwing, with volume manipulated by tractor speed and nozzle selection. Please refer to Table 4.3.3.1 for specific spray machine calibrations. The spray tank, spray nozzles, filter and pipes of the spray machine was thoroughly washed and flushed after each treatment.

Table 4.3.3.1. Trial layout of specific spray machine calibrations used on Schoemans farm, Groblersdal, 2009.

Trt	Machine	Description	Speed (km h ⁻¹)	PTO (rpm)	Pressure (bar)	Nozzles/side	Volume (l ha ⁻¹)
1	BSF-Extreme	Tower mistblower with oscillating boom	2.3	540	20	10xD4-f/ 11xD4-f	8935
2	BSF-Extreme	Tower mistblower with oscillating boom	1.5	540	20	10xD4-f/ 11xD4-f	13700
3	BSF-Extreme	Tower mistblower with oscillating boom	2.9	540	20	10xD4-f/ 11xD4-f	7086
4	BSF-Multiwing	Multifan-tower	2.3	540	10	10xD5-f/ 11xD5-f	8601
5	BSF-Multiwing	Multifan-tower	1.5	540	10	10xD5-f/ 11xD5-f	13188
6	BSF-Multiwing	Multifan-tower	2.9	540	10	10xD5-f/ 11xD5-f	6821
7	BSF-Multiwing	Multifan-tower	2.9	540	15	10xD4-f/ 11xD4-f	6129
8	Ultima	Tower mistblower with oscillating boom	1.3	500	25	12xD4-h/ 18xD4-f	23582
9	Ultima	Tower mistblower with oscillating boom	2.1	500	25	12xD4-h/ 18xD4-f	14598
10	Ultima	Tower mistblower with oscillating boom	2.7	500	25	12xD4-h/ 18xD4-f	11354
11	BSF-Multiwing	Multifan-tower	2.3	540	10	10xD4-f/ 11xD4-f	6440
12	BSF-Multiwing	Multifan-tower	1.5	540	10	10xD4-f/ 11xD4-f	9875

Citrusdal, 2010. The trial was conducted in uniform sections of a Olinda Valencia (*Citrus sinensis* (L.) Osbeck) [3.79x4.24 m trees (HxW) with density of 3.5 on a 5-point scale; 6x3 m row spacing] orchard on the farm Karringmelksvlei (Western Province, South Africa) in 2010. Treatments were applied early morning as soon as trees were dry from dew. For each treatment combination, a row section of 10 trees was marked with hazard tape and sprayed from both sides with the same tractor, power take-off (PTO) calibrated with a SENTRY ST 723 contact tachometer (Sentry Optronics corp. Taipei, Taiwan) to, and set at 540 rpm. Sprays consisted of 12 separate treatments consisting of water and yellow fluorescent pigment [40% EC (SARDI, Loxton, South Australia); 1 ml l⁻¹]. The trial consisted out of 15 different treatments. Spray volume was manipulated by varying tractor speed, nozzle selection or spray pressure. Please refer to Table 4.3.3.2 for specific spray machines and calibration per treatment used.

Table 4.3.3.2. Trial layout of specific spray machine calibrations used on Karringmelksvlei, Citrusdal, 2010.

Trt	Machine	Description	Speed (km h ⁻¹)	PTO (rpm)	Pressure (bar)	Nozzles /side	Volume (l ha ⁻¹)
1	Nieuwoudt	High-pressure hydraulic sprayer, oscillating boom	2.5	540	30	13	12501
2	Nieuwoudt	High-pressure hydraulic sprayer, oscillating boom	4	540	30	13	7813
3	Cima	Airshear sprayer with tower and under-tree turret (1-sided spraying only)	1.7	540	1.2	13-13/12-11/12-13	8000
4	Cima	Airshear sprayer with tower and under-tree turret (1-sided spraying only)	1.7	540	0.9	11-11/10-9/10-11	4000
6	Martignani	Electrostatic airshear sprayer with tower	4	540	?	None	1000
7	Martignani	Electrostatic airshear sprayer with tower	4	540	?	None	500
9	Atasa	Low profile axial fan mistblower with turret	1.7	540	15	10xD5-56	7882
10	Atasa	Low profile axial fan mistblower with turret	1.7	540	10	10x Blue Albus	4000
11	Multiwing	Multifan-tower	1.7	540	15	11xJ4-f/10xJ5-h	6501
12	Multiwing	Multifan-tower	3.3	540	15	11xJ4-f/10xJ5-h	3349
13	Jacto	Low profile axial fan mistblower	3.3	540	17	13xJ5-3	3735
14	Jacto-tower	Low profile axial fan mistblower with air duct (1-sided spraying only)	2.5	540	20	13xJ5-3/6xJ4-3	6844
15	Jacto-Valencia	Low profile axial fan mistblower with tower (1-sided spraying only)	2.5	540	17	18xJ5-3/12xJ5-2	9494

b. Evaluate methods for optimisation of spray application with novel applicators.

Sprayer selection. Based on previous spray applicator evaluation results (Citrusdal 2010), two applicators were selected for further evaluation. Selection criteria were based on cost efficiency potential, low power input and/or reduced volume applicator potential. Selected spray applicators were a Nieuwoudt sprayer (high profile, high-pressure hydraulic sprayer with oscillating boom; 13 × nozzle ports per side; also for use of high

volume control sprays; www.nieuwoudt.co.za) and a Martignani Whirlwind KWH sprayer (high profile mistblower electrostatic sprayer with two fishtails on either side at the top and bottom of the spray tower; www.martignani.com).

Spray application and Field evaluation. The first trial was conducted in uniform sections of a Washington navel (*Citrus sinensis* (L.) Osbeck) [3.6×4.2 m trees (H×W) with density of 4.5 on a 5-point scale; 4×6 m row spacing] orchard on the farm Boontjiesrivier (Citrusdal, Western Cape, South Africa) in February 2013. Treatments were applied early morning as soon as trees were dry from dew. Air movement (wind speed at $\text{m}\cdot\text{s}^{-1}$) inside the orchard row was $1.2 \text{ m}\cdot\text{s}^{-1}$ perpendicular to orchard row with air temperature and relative humidity being 20°C and 26 % (early morning) to 28°C and 17 % (mid-day; end of trial). For each treatment combination, a row section of 10 trees was marked with hazard tape and sprayed from both sides with the same 50 kW tractor, power take-off (PTO) calibrated with a SENTRY ST 723 contact tachometer (Sentry Optronics corp. Taipei, Taiwan) to, and set at 540 rpm. Sprays consisted of eight separate treatments consisting of water and yellow fluorescent pigment [40% EC (SARDI, Loxton, South Australia); 1 ml l^{-1}]. Treatment one to Four was sprayed with the Nieuwoudt sprayer at different spray volumes, with volume being manipulated by either changing nozzles (Ceramic tipped venturi nozzles with specific diameter) or spray pressure: high volume – 1.8 mm diameter @ 1500 KPa = 6229 l ha^{-1} ; high volume – 1.8 mm diameter @ 3000 KPa = 8847 l ha^{-1} ; medium volume – 1.5 mm diameter @ 1500 KPa = 4062 l ha^{-1} ; low volume – 1.2 mm diameter @ 1500 KPa = 2708 l ha^{-1} . Tractor speed was kept constant at 2.88 km h^{-1} respectively for each treatment. Treatment five to eight was sprayed with the Martignani Whirlwind sprayer (electrostatic on) at different spray volumes at a constant spray pressure and tractor speed of 150 KPa and 2.88 km h^{-1} respectively, except for treatment five, where tractor speed was reduced to 1.78 km h^{-1} : high volume @ 1.78 km h^{-1} = 4000 l ha^{-1} ; high volume @ 2.88 km h^{-1} = 4000 l ha^{-1} ; medium volume @ 2.88 km h^{-1} = 2000 l ha^{-1} ; low volume @ 2.88 km h^{-1} = 1000 l ha^{-1} . Two buffer rows were left unsprayed between treatments. The spray tank, spray nozzles, filter and pipes of the spray machine was thoroughly washed and flushed after each treatment.

The second trial was conducted in uniform sections of a Washington navel (*C. sinensis* (L.) Osbeck) [3.8×3.6 m trees (H×W) with density of 4.6 on a 5 point scale; 2×6 m row spacing] orchard on the farm HM Pieterse Boerdery (Groblersdal, Limpopo Province, South Africa) in June 2013. Treatments were applied early morning as soon as trees were dry from dew. Air movement (wind speed at $\text{m}\cdot\text{s}^{-1}$) inside the orchard row was $0.6 \text{ m}\cdot\text{s}^{-1}$ perpendicular to orchard row with air temperature and relative humidity being 17°C and 30 % (early morning) to 25°C and 28 % (mid-day; end of trial). The trial was conducted in the same manner as the previous, with the following minor differences: The tractor used had an engine power output of 63 kW. The tractor speed used of 2.39 km h^{-1} realised higher spray volumes as in the first trial for the Nieuwoudt sprayer – high volume – 1.8 mm @ 1500 KPa = 7506 l ha^{-1} ; high volume – 1.8 mm @ 3000 KPa = 10661 l ha^{-1} ; medium volume – 1.5 mm @ 1500 KPa = 4895 l ha^{-1} ; low volume – 1.2 mm @ 1500 KPa = 3263 l ha^{-1} . Treatment five was sprayed at 1.88 km h^{-1} with the Martignani sprayer. Lastly, the travel average distance for droplets before impacting foliage or fruit was further than that of in the previous trial by 0.8 m.

Sampling strategy, spray deposition analysis, benchmarking and statistical analysis was done similarly as described above. Data were also subjected to the deposition quality assessment parameter. The leaf area was divided into equally-sized squares [100 × 100 pixels (10000 pixels)] (van Zyl et al., 2013). Depending on the leaf size, this amounted to as few as 20 to more than 250 individual squares per leaf, of which the percent area covered by fluorescent pigment particle was determined for each square. The Interquartile Coefficient of Dispersion (ICD%) per leaf [$((3\text{rd quartile} - 1\text{st quartile}) / (3\text{rd quartile} + 1\text{st quartile})) * 100$] was used as a measure of deposition quality per leaf, i.e. uniformity of deposition on the leaf surface. Low interquartile coefficient of dispersion values was indicative of better deposition quality. Deposition quality was subsequently subjected to the same statistical analysis procedure as that of deposition quantity and quality.

4. Development and validation of a user-friendly calibration system.

This objective was not addressed in this project and was submitted as a new proposal and has been accepted as a new research project: Development of a tree canopy characteristic calibration formula for reduced volume fungicide application in Southern African citrus orchards (project ref no. 1089).

5. Develop a guideline for optimal use of commonly-used and novel spray applicators in citrus orchards.

This objective will be continuously addressed through extension, and by updating the relevant sections in the Production Guidelines. An extensive spray calibration checklist was developed and published: Cutting Edge No. 166. Spray application in citrus – Sprayer calibration and checklist.

6. Use of adjuvants for improved spray deposition on citrus leaves and fruit.

This objective will be fully reported in a separate final report (Gideon van Zyl's PhD thesis-1096).

7. Physical and chemical effects of adjuvants on spray deposition and control of *Alternaria* brown spot following copper oxychloride sprays on mandarin leaves.

This objective will be fully reported in a separate final report (Gideon van Zyl's PhD thesis-1096).

8. Modelling of the influence of quantity and quality of copper oxychloride sprays on control of *Alternaria* brown spot on mandarin leaves.

This objective will be fully reported in a separate final report (Gideon van Zyl's PhD thesis-1096).

Results and discussion

1. Conduct a thorough survey of current spray application materials and methods in all major citrus growing areas.

In total, 61 questionnaires were received, which was used by the investigators as indication of the industry's spraying practices and is being used continuously as a guideline for spray trial planning.

2. Determine benchmarks for biological efficacy of copper oxychloride against *Alternaria* brown spot.

This objective was concluded and results published in two scientific papers, of which the abstracts are presented below:

PH Fourie, M du Preez, JC Brink and GC Schutte, 2008. The effect of run-off on spray deposition and control of *Alternaria* brown spot of mandarins. *Australasian Plant Pathology* 38: 173-182.

Alternaria alternata pv. *citri* is the causal agent of *Alternaria* brown spot on tangerines and their hybrids and infects young leaves and fruit of all ages. In South Africa, repeated high volume (~9000 l ha⁻¹) fungicide sprays on susceptible cultivars are the only effective control measure of this disease. The effect of run-off on spray deposition and biological efficacy was largely unknown. The aims of this study were first to characterise spray deposition and runoff on fruit and leaves, and second to determine the effect of runoff on biological efficacy. Mature Nova mandarin leaves (upper and lower leaf surfaces), Valencia Late oranges and Eureka lemons were sprayed with different volumes of, or dipped in, a mixture of water and a yellow fluorescent pigment. Sprayed parts were illuminated under black light, visualised under a stereomicroscope and digitally photographed at 10 × magnification. Quantitative and qualitative deposition assessment of the spray deposition was performed by means of digital image analyses. Hoerl regression curves were fitted to quantitative and qualitative deposition values on upper and lower leaf surfaces over spray volume (R^2 -values >0.95) and trends clearly indicated that deposition on young or mature leaves and fruit improved as spray volume increased, but only until the point of runoff was reached, thereafter deposition quantity and quality decreased. Deposition values following dip treatments were in all cases significantly subordinate to those of the best spray volumes. Mature upper leaf surfaces and Eureka lemon fruit generally retained less spray deposits than lower leaf surfaces and Valencia Late orange fruit, respectively. In order to determine the effect of runoff on biological efficacy of copper hydroxide against *Alternaria* brown spot, young Nova leaves were treated in a similar fashion and subsequently drop-inoculated with a virulent strain of *A. alternata* pv. *citri* and incubated for 3.5 days in moist chambers at 25 °C. Biological efficacy of sprays followed a quadratic trend over spray volume and clearly demonstrated the detrimental effect of runoff on biological efficacy of fungicide sprays. Sigmoidal regression analyses of mean infection percentages against quantitative and qualitative deposition on upper and lower surfaces of young Nova leaves yielded very good fits indicating the correlation between biological and deposition data.

JG van Zyl, PH Fourie, GC Schutte, 2013. Spray deposition assessment and benchmarks for control of *Alternaria* brown spot leaves with copper oxychloride. *Crop Protection* 46: 80-87.

Inadequate disease control on citrus foliage and fruit is often attributed to insufficient fungicide spray deposition on target surfaces. This study describes a novel spray deposition assessment protocol and determines deposition benchmarks indicative of the biological effectiveness for better interpretation of spray deposition results. Suitability of a yellow fluorescent pigment as tracer for copper oxychloride deposition was demonstrated through its similar particle concentration and size. Spray deposition assessment of spray

targets, which were sprayed with a mixture that included the fluorescent pigment, involved photomacrography of whole leaf or fruit surfaces, followed by digital image analyses. This protocol proved to be very accurate in determining the quantity and quality of deposition. To determine deposition benchmarks, detached young 'Nova' mandarin leaves were sprayed with copper oxychloride and fluorescent pigment at different concentrations (0.1-2 times the recommended concentration) and spray deposition assessed. Subsequently, leaves were spray inoculated with a spore suspension of *Alternaria alternata* [causal agent of Alternaria brown spot (ABS) of mandarins], moist-incubated for c. 48 h and symptom expression rated. A very good linear relationship was found between fungicide concentration, leaf area covered by fluorescent pigment particles (FPC%) ($r = 0.879$) and Cu residue analysis ($r = 0.992$). A von Bertalanffy growth curve best fitted the relation between ABS control and deposition quantity (FPC%) data (91% of the percentage variance accounted for) with a good correlation between observed and predicted values ($r = 0.825$). Benchmarks for 50% and 75% disease control were calculated as 2.07 FPC% and 4.14 FPC%, respectively. These corresponded with Cu residue levels of 59.4 and 91.0 mg kg⁻¹, respectively. These FPC benchmarks can be used to evaluate spray technology research, specifically for control of ABS and similar citrus fruit and foliar diseases.

3. Characterisation of spray deposition with current spray application methods.

a. Evaluate methods for optimisation of spray application with commonly-used applicators.

Addo. The Navel trees in the Addo orchard were on average 3.7 m tall, 4.4 m wide with 0.7 m skirt. Canopy density was rated as fairly sparse, with an index-rating of 2, with 5 being a very dense canopy. Row spacing was 7x4 m. Analysis of variance indicated a significant 3-factor interaction for treatment, leaf side and canopy position or canopy height ($P < 0.05$). The treatment x leaf side x canopy position results from the Addo spray trial are summarised in Figure 4.3.3.1.

The Volcano sprayer (operating at 20 bar pressure) was used exactly according to the grower's calibration settings, which should result in a spray volume of 8436 l ha⁻¹ at the spray speed of 1.5 km h⁻¹. It should be noted, however, that the alternating nozzle setup of hollow and full cone nozzles had not been serviced in 12 months. Deposition quantity per leaf on upper and lower leaf surfaces in the outer canopy was ≈5% FP, but a relatively large variation between leaves (%RSD; indicative of spray uniformity) was observed, especially on the upper leaf surfaces (56.4% RSD). Deposition quantity per leaf values for inner canopy leaves were lower than those observed for the outer canopy (4.3 and 2.3% FP for upper and lower leaf surfaces, respectively). Interestingly, the variation between leaves was markedly lower with the %RSD <30%. For the Volcano sprayer at 8436 l ha⁻¹, the spray efficiency was poor with an average of 0.49% FP (deposition quantity per leaf) per 1000 l of spray volume.

The BSF-Multiwing sprayer (operating at 10 bar pressure) was equipped with either disc-core D4 or D2 hollow cone nozzles (45 whirler type). When using the D4 hollow cone nozzles at tractor speed of 1.3 km h⁻¹, a spray volume of 6767 l ha⁻¹ was obtained. This was 20% lower spray volume than with the Volcano, but higher deposition quantity per leaf values were retained on outer and inner canopy leaves (6.4-6.0 and 4.6-3.6% FP, respectively). Variation in deposition quantity between leaves was also lower (<≈30% RSD), except for lower leaf surfaces of inner canopy leaves (43.4% RSD). Spray efficiency at an average of 0.76% FP per 1000 l was 55% better than that observed for the Volcano. At a tractor speed of 4.0 km h⁻¹, spray volume was reduced to 2261 l ha⁻¹ with the D4 hollow cone nozzles. Deposition quantity per leaf was comparable to what was observed with the Volcano, but slightly lower than those observed with the Multiwing with the same nozzle setup at slower tractor speeds. Variation in deposition quantity between leaves following spraying at this lower spray volume was slightly higher on upper leaf surfaces (39.6% RSD) than on lower leaf surfaces (<30% RSD), but spray efficiency was markedly improved (average of 1.79% FP per 1000 l). As spraying with the D2 hollow cone nozzles resulted in several blockages of the nozzle tips, we realised that this setup will not be practicable. Hence, the results for the D2 nozzle setup will not be discussed.

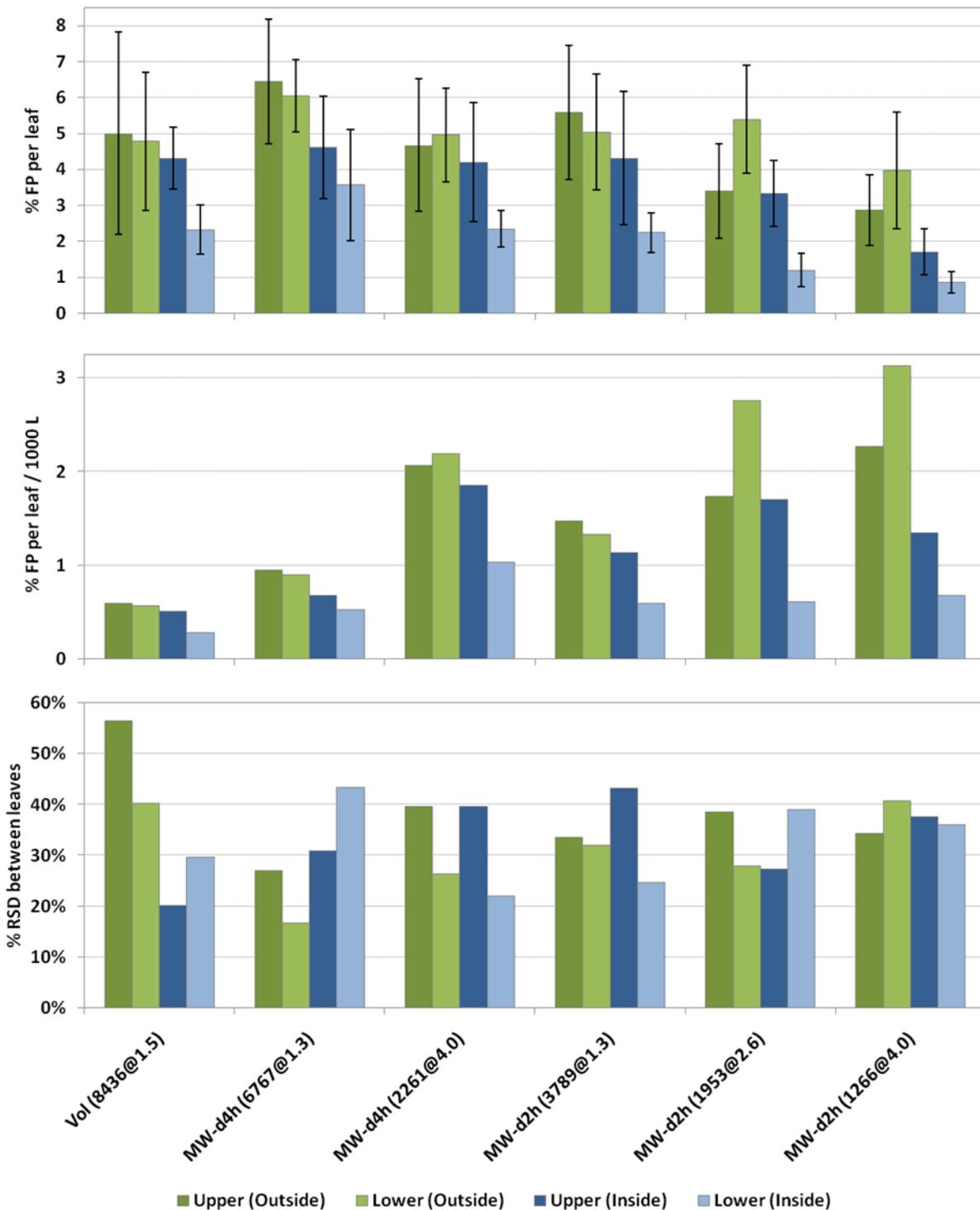


Figure 4.3.3.1. Mean deposition quantity per leaf (% FP), variation between leaves (%RSD) and spray efficiency (expressed as % FP per leaf 1000 l) on upper and lower leaf surfaces, which were sampled from top, middle and bottom sections of the inner and outer canopy, following spray application with SARDI Yellow Fluorescent Pigment (1 ml hl⁻¹) in a mature Navel orchard in Addo with a Volcano oscillating boom mistblower (Vol) and BSF-Multiwing sprayer (MW) with various nozzle selection and tractor speeds [legend = Sprayer; nozzle type (D4- or D2-hollow cone); spray volume in l ha⁻¹; tractor speed in km h⁻¹].

Hoedspruit. The Midnight Valencia trees in the Hoedspruit orchard were on average 3.9 m tall, 3.7 m wide with 0.9 m skirt. Canopy density was rated as fairly dense, with an index-rating of 4. Row spacing was 6x2 m. Analysis of variance indicated a significant 3-factor interaction for treatment, leaf side and canopy position or canopy height ($P < 0.05$). The treatment x leaf side x canopy position results from the Hoedspruit spray trial are summarised in Figure 4.3.3.2.

The BSF-Extreme (operating at 15 bar pressure) was set up with alternating Jacto J4 full cone and J5 hollow cone nozzles, which effected spray volumes of 7033, 3624, 2349 and 1554 l ha⁻¹ at tractor speeds of 1.5, 3.0, 4.6 and 6.9 km h⁻¹, respectively. At tractor speeds of 4.6 and 6.9 km h⁻¹, visual observation of fluorescent pigment deposition on trees clearly indicated an uneven deposition, which was visible as 0.75 to 1 m vertical segments of canopy with visibly variable deposition, as a result of incompatible oscillation and tractor speeds. At 1.5 km h⁻¹, the Extreme deposited 5.9 and 7.3% FP on upper and lower leaf surfaces of the outer canopy at a very low variation between leaves of 13.2 and 22.8% RSD, respectively. On the inner canopy of these dense Valencia trees, deposition quantity per leaf was lower (4.1 and 2.1% FP, respectively for upper and lower leaf surfaces) and variation between leaves was also markedly higher (46.7 and 47.2% RSD, respectively). Spray efficiency was calculated at an average of 0.69% FP per 1000 l. At 3.0 km h⁻¹, spray volume was reduced to 3525 l ha⁻¹ and deposition quantity per leaf was also lower with 4.8 and 5.3% FP deposited on upper and lower leaf surfaces of the outer canopy leaves at a variation between leaves of 23.8 and 38.2% RSD, respectively. Deposition quantity per leaf on inner canopy leaves was lower (3.3 and 2.1% FP, respectively for upper and lower leaf surfaces) and of markedly higher variation between leaves (64.3 and 96.1% RSD, respectively), which is indicative of reduced spray penetration at higher tractor speed and/or reduced spray volume. Spray efficiency was, however, markedly better at 1.06% FP per 1000 l.

The BSF-Multiwing sprayer (operating at 10 bar pressure) was again evaluated using the D2 hollow cone nozzles at 1.3 and 2.6 km h⁻¹, which resulted in spray volumes of 4421 and 2279 l ha⁻¹, respectively. The impracticability of this nozzle selection, in terms of frequent blockages, was supported by the relatively poor penetration observed in terms of deposition quantity per leaf and variation between leaves, especially at the faster tractor speed. With D4 full cone nozzles (56 whirler type), spray volume was 12632, 6512 and 4221 l ha⁻¹ at 1.3, 2.6 and 4.0 km h⁻¹, respectively, while the comparative spray volumes with D4 hollow cone nozzles were 7895, 4070 and 2638 l ha⁻¹, respectively. Deposition quantity per leaf was the highest at 1.3 km h⁻¹ tractor speed (and higher spray volumes) and fairly similar when comparing the D4 hollow (5.2-2.6% FP) and full cone (5.8-2.6% FP) nozzles. Variation between leaves was, however, slightly lower with the hollow cone nozzles (average 26.9 vs. 29.4% RSD) and spray efficiency was better (average 0.55 vs. 0.36% per 1000L). At 2.6 km h⁻¹, deposition quantity per leaf with the D4 full cone nozzles was slightly lower (5.3-1.5% FP) than that of the conventional BSF-Extreme at 1.5 km h⁻¹ (7.3-2.1% FP), but variation between leaves and spray efficiency were comparable (38.5 vs. 32.5% RSD and 0.53 vs. 0.69% per 1000 l, respectively). The D4 hollow cone nozzles showed reduced penetration at 2.6 km h⁻¹ as was clearly demonstrated by the increased variation between deposition quantity values on inner canopy leaves (70.3-95.4% RSD). Likewise, penetration at 4 km h⁻¹ with the D4 full and hollow cone nozzles was reduced, hence the high variation between upper (48.3 and 49.5% RSD, respectively) and especially lower (85.8 and 91.7% RSD, respectively) surfaces of inner canopy leaves.

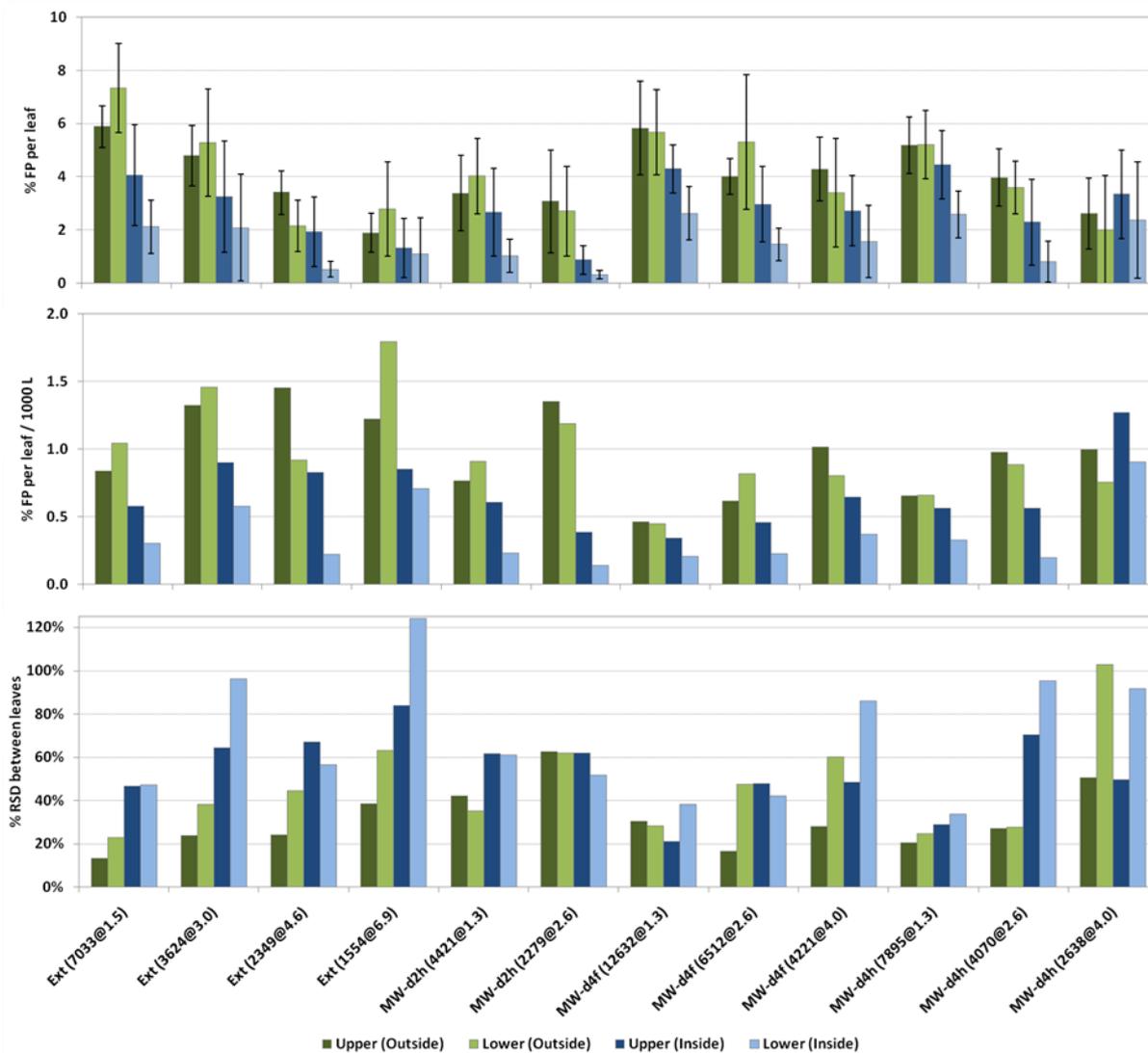


Figure 4.3.3.2. Mean spray deposition quantity per leaf (% FP), variation between leaves (%RSD) and spray efficiency (expressed as % FP per leaf 1000 l) on upper and lower leaf surfaces, which were sampled from top, middle and bottom sections of the inner and outer canopy, following spray application with SARDI Yellow Fluorescent Pigment (1 ml hl^{-1}) in a mature Valencia orchard in Hoedspruit with a BSF-Extreme oscillating boom mistblower (Ext) and BSF-Multiwing sprayer (MW) with various nozzle selection and tractor speeds [legend = Sprayer; nozzle type (D4-hollow or full cone or D2-hollow cone); spray volume in l ha^{-1} ; tractor speed in km h^{-1}].

Letsitele. The Du Roi Valencia trees in the Letsitele orchard were on average 4.3 m tall, 4.2 m wide with no skirt. Canopy density was rated as fairly dense, with an index-rating of 4. Row spacing was $7 \times 3.5 \text{ m}$. Analysis of variance indicated a significant 3-factor interaction for treatment, leaf side and canopy position or canopy height ($P < 0.05$). The treatment \times leaf side \times canopy position results from the Letsitele spray trial are summarised in Figure 4.3.3.3.

The Ultima, using an $18 \times \text{D3}$ -full and $12 \times \text{D4}$ -hollow cone nozzle setup at 20 bar pressure, was evaluated at 1.5 and 2.3 km h^{-1} , which resulted in spray volumes of 12258 and 7748 l ha^{-1} , respectively. At these tractor speeds, deposition quantity per leaf measured a mean of 12.2 and 11.1% FP on outer canopy leaves and 9.6 and 8.2% FP on inner canopy leaves, respectively, with not much difference between upper and lower leaf surfaces. Spray uniformity at 1.5 km h^{-1} was relatively good at 38.5 and 42.4% RSD for outer and inner canopy leaves, respectively, and 38.9 and 51.7% RSD at 2.3 km h^{-1} . Given the higher spray volume at 1.5 km h^{-1} , spray efficiency was relatively low at 0.89% FP per 1000 l, but was markedly better at 2.3 km h^{-1} (1.25% FP per 1000 l).

The Bateleur (operating at 20 bar pressure) was used with alternating $14 \times \text{D4}$ -hollow cone + $14 \times \text{D3}$ -full cone nozzle setup at 1.5 km h^{-1} (11507 l ha^{-1} ; Bat-i and Bat-ii), and 19 nozzles of each (10 additional nozzles on a

fixed boom without wind to spray the skirt) at 2.3 km h⁻¹ (9870 l ha⁻¹). The Bat-ii setup at 1.5 km h⁻¹ (tower angled more diagonally downward) resulted in poorer penetration of the canopy, and the results will not be discussed. At 1.5 km h⁻¹ using the Bat-i setup, the Bateleur retained significantly less fluorescent pigment (7.1 and 7.7% FP for outer and inner canopy at a variation between leaves of 51.0 and 37.0% RSD, respectively) than the Ultima at both tractor speeds. At 2.3 km h⁻¹, spray deposition quantity was slightly better at 8.5 and 8.3% FP with a variation between leaves of 37.8 and 44.2% RSD, respectively. A larger number of nozzles was used at the faster tractor speed, which did not change the volume delivery that much, but spray efficiency was improved (0.85 vs. 0.64% FP per 1000 l) by the lower spray volume and improved deposition.

The BSF-Extreme (operating at 15 bar pressure) setup differed from what was evaluated at Hoedspruit, specifically in a change from the Jacto J5-2 hollow cone nozzles to green Albus hollow cone nozzles with similar volume delivery but a wider swath angle. This setup resulted in spray volumes of 6933 and 4382 l ha⁻¹ at 1.5 and 2.3 km h⁻¹, respectively. At the slower tractor speed, spray deposition on inner and outer canopy leaves were fairly similar at an average of 6.5% FP (34.6% RSD). At 2.3 km h⁻¹, deposition on outer canopy leaves was still similar [8.4% FP (41.7% RSD)], but penetration to inner canopy leaves was slightly reduced as was evident from less deposition quantity on lower leaf surfaces and more variation between leaves [4.2% FP (91.7% RSD)] compared with upper leaf surfaces [(7.3% FP (55.1% RSD))]. Nonetheless, the BSF-Extreme proved to be relatively efficient at 0.94 and 1.61% FP per 1000 l at these tractor speeds. The BSF-Extreme was also evaluated at 3.5 km h⁻¹ (2926 l ha⁻¹), which effected fairly good deposition values on outer canopy leaves [5.0% FP (54.0% RSD)], but relatively poor penetration of the inner canopy [2.6% FP (86.1% RSD)]. Spray efficiency at an average of 1.31% FP per 1000 l was not as good as was observed at 2.3 km h⁻¹. Reduced penetration at faster tractor speeds might possibly have been accentuated by use of the wide-swath green Albus nozzles, as the latter nozzles also adversely affected the penetration ability of the BSF-Multiwing.

The BSF-Multiwing (operating at 10 bar pressure) was evaluated with either J4-full cone nozzles or green Albus hollow cone nozzles (D4-equivalent). It was also tested using the same nozzle setup as the BSF-Extreme. With the full cone nozzles at 1.5 km h⁻¹, the spray volume was 4956 l ha⁻¹, comparable to the BSF-Extreme at the same tractor speed. Deposition on the outer canopy [9.6% FP (52.7% RSD)] was significantly more than the Extreme and similar to the Ultima and Bateleur, although at somewhat higher variation between leaves. Upper leaf surface deposition of inner canopy leaves [6.7% FP (51.8% RSD)] was comparable to that following application with the Extreme at the same tractor speed, but deposition on lower leaf surfaces was significantly lower (4.4% FP) and with more variation between leaves (89.3% RSD). At 2.3 km h⁻¹ and 3958 l ha⁻¹, the Multiwing deposited statistically similar quantities of fluorescent pigment on the inner canopy leaves than at 1.5 km h⁻¹, albeit at higher variation between leaves (81.8% vs. 70.5% RSD). However, the spray efficiency at 2.3 km h⁻¹ was markedly better at 2.33% FP per 1000 l, compared with 1.53% FP at 1.5 km h⁻¹. At 3.5 km h⁻¹ and 2643 l ha⁻¹, the spray efficiency was further improved to 2.62% FP per 1000 l, markedly better than the Extreme at the same tractor speed. However, variation between leaves was relatively high at an average of 65.0 and 98.5% RSD for outer and inner canopy leaves, respectively. The use of the green Albus hollow cone nozzles alone or in combination with the J4 full cone nozzles at 2.3 km h⁻¹ and \pm 3900 l ha⁻¹ proved to be inefficient on the dense canopies and wide row spacing as penetration of the inner canopy was relatively poor in terms of deposition quantity and variation between leaves compared with the complete set of J4 full cone nozzles. These findings were confirmed when comparing the full cone and hollow cone nozzles at 3.5 km h⁻¹.

The Cima air shear sprayer was evaluated at 1.5 km h⁻¹, but at different pressure settings of 2.5 – 1 bar, which resulted in spray volumes of 3000, 2000 and 1000 l ha⁻¹. At 3000 l ha⁻¹, the Cima retained markedly less fluorescent pigment and at more variation between leaves (3.8% FP at 92.3% RSD on outer canopy and 3.0% FP at 99.3% RSD on inner canopy leaves) than the BSF-Extreme and BSF-Multiwing (using full cone nozzles) at 3.5 km h⁻¹ and \pm 2-3000 l ha⁻¹. At lower spray volumes, the deposition quantity and uniformity with the Cima declined in a linear fashion. It should be noted, however, that poor sprayer setup by the company representative might have contributed to the relatively poor results. Whilst spraying, it was noted that the tops of the canopies were not covered by the spray plume of the top fishtails. This resulted in significantly reduced deposition in the tops of canopies (results not shown) and would have contributed to the large variation between leaves. Spray efficiency with the Cima ranged from 1.14 to 1.67% FP per 1000 l.

The ESS electrostatic sprayer was used at 1.5, 2.3 and 3.5 km h⁻¹, resulting in spray volumes of 490, 310 and 207 l ha⁻¹. Given the very low spray volumes, the pigment concentration was increased to 400 ml hl⁻¹ (4x the concentration used for the other sprayers). Application of active ingredient per hectare therefore roughly equals that of the Cima sprayer at 2000 and 1000 l ha⁻¹ and despite comparable deposition quantity per leaf values, variation between leaves was very high at an average of 132.1% RSD for all treatments.

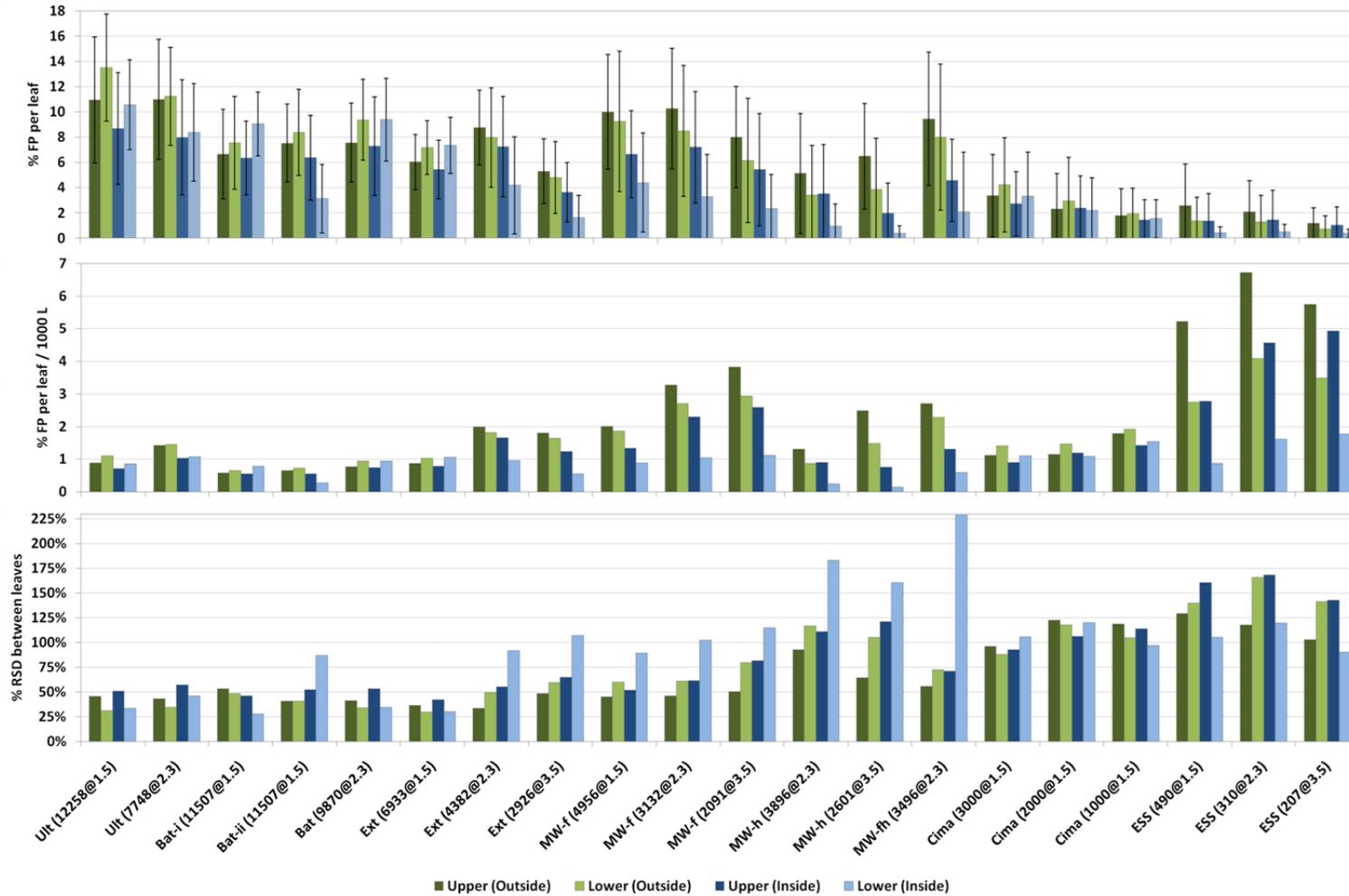


Figure 4.3.3.3. Mean spray deposition quantity per leaf (% FP), variation between leaves (%RSD) and spray efficiency (expressed as % FP per leaf 1000 l) on upper and lower leaf surfaces, which were sampled from top, middle and bottom sections of the inner and outer canopy, following spray application with SARDI Yellow Fluorescent Pigment (1 ml hl^{-1}) in a mature Valencia orchard in Letsitele with Ultima (Ult), Bateleur (Bat) and BSF-Extreme (Ext) oscillating boom mistblowers, BSF-Multiwing sprayer (MW), Cima air shear sprayer (Cima) and ESS electrostatic sprayer (ESS; used at 4x dosage of fluorescent pigment) with various nozzle selection and/or tractor speeds [legend = Sprayer; nozzle type (hollow or full cone); spray volume in l ha^{-1} ; tractor speed in km h^{-1}].

Conclusion for Addo, Hoedspruit and Letsitele

From the results obtained in these trials, it was clear that the highest deposition quantity per leaf values at the lowest variation between leaves was generally obtained with higher spray volumes. However, it was obvious that the dispersion quality of pigment deposition on individual leaves declined with increasing spray volumes due to more run-off, which might also have a detrimental effect on biological efficacy. It should furthermore be stressed that the fluorescent pigment dosage of 1x was used when comparing all the different sprayers and calibration settings (except for the ESS sprayer tested at 4x), even though spray volumes differed. Hence, the dosage per hectare differed substantially between treatments. In relative terms, spray efficiency (expressed as quantitative deposition per leaf per 1000 l of spray volume) in combination with spray uniformity (expressed by the variation in quantitative deposition between leaves) are therefore the parameters that should be used when comparing sprayers and calibration settings. Similar and even improved spray deposition can thus be obtained at lower spray volumes through optimal use of equipment or through the use of more efficient sprayers, especially if dosage per hectare is equated between treatments.

In the sparse canopies of the Navel orchard in Addo, the BSF-Multiwing performed more efficiently and spray deposition was more uniform at similar or faster tractor speeds than with the grower's Volcano sprayer. Remarkably, spray efficiency with this multi-fan tower sprayer at 4 km h⁻¹ (with D4 hollow cone nozzles) was 365% better than with the Volcano at 1.5 km h⁻¹, while uniformity was also improved (average 31.8% RSD). Sprays at lower volumes per hectare and/or faster tractor speed can result in massive savings in chemical, water, fuel and labour cost, and/or substantially improved time efficiency. Additionally, the BSF-Multiwing operated at 10 bar pump pressure compared with the 20 bar of the Volcano, and was substantially more power efficient as it used only 23 kW of tractor power. This would amount to a considerable fuel saving (as much as 50%) as a smaller tractor with less power usage can be used to spray with this machine.

As could be expected, spray uniformity was generally poorer in more dense canopies, especially on inner canopy leaves. Canopy management through pruning practices should therefore aim to reduce canopy density. This is especially pertinent should lower spray volumes and/or faster tractor speeds be used for spray application. Nonetheless, it was clear from the Letsitele results that optimised application could result in improved and more efficient application. For example, the Ultima and Bateleur at faster tractor speeds and lower spray volumes deposited similar quantities of pigment at similar uniformity levels, but at markedly improved efficiency. In these denser canopies, the BSF-Extreme and BSF-Multiwing retained on average comparable quantities of pigment per leaf, but used lower spray volumes and therewith markedly better efficiency. However, a concomitant reduction in spray uniformity between leaves was observed, and can be attributed to reduced spray penetration of the inner canopy. At higher spray volumes (7000-13000 l ha⁻¹) and slow tractor speeds (1.5 km h⁻¹) in dense canopies at Hoedspruit, these machines showed improved uniformity ($\pm 30\%$ RSD). In these dense orchards, the Multiwing proved to be as effective at faster tractor speeds.

The results with the Cima sprayer were disappointing when compared with those of the BSF-Extreme and BSF-Multiwing at similar spray volumes. Trials with this machine, however, will be repeated as poor sprayer setup undoubtedly contributed to poor performance. The ESS electrostatic sprayer, evaluated at 4x the pigment dosage used for the other sprayers, showed relatively poor results in terms of quantitative deposition per leaf and variation between leaves. From the sampled leaves, it was obvious that excellent qualitative deposition at reasonable quantities was obtained on some leaves, but with almost no deposition on others. The absence of any form of oscillation might have contributed to leaf shingling and poor penetration; a detriment which the electrostatically charged spray droplets seemed unable to overcome.

In terms of tractor speed, it seems that 3 km h⁻¹ should be the upper limit for medium cover spray application; defined as adequate cover of outer and inner canopy leaves (and fruit), without emphasis on film-wetting of the trunk and branches as would be required for full cover application. Faster tractor speed reduced spray penetration and spray uniformity, especially in denser canopies. This effect was more pronounced when using hollow cone nozzles. Nozzle selection is important and could result in reduced sprayer performance as was seen when the green Albus nozzles (wide swath angle) were used with the BSF-Extreme and -Multiwing in a dense canopy orchard with wide row spacing.

From these findings, it seems clear that medium cover sprays can be adequately delivered with 2-sided sprayers. This holds a big advantage over 1-sided sprayers as work rates are improved, costs reduced and orchard traffic and concomitant soil compaction are also halved.

Groblersdal 2009

Analysis of variance of the deposition quantity data (FPC% per leaf) indicated a significant 3-factor interaction for treatment, speed and horizontal canopy position, as well as a significant interaction between treatment, speed and vertical canopy position ($P < 0.05$). These interactions were also obvious for the normalised deposition quantity data (FPC% per 1000 l ha⁻¹). For spray uniformity (CV% between leaves) and deposition quantity (CV% per leaf) data, the treatment × speed × vertical canopy position interaction was significant, but not for horizontal canopy position.

The treatment × speed × horizontal canopy position results for deposition quantity per leaf (FPC%), uniformity (CV% between leaves), efficiency (FPC% per 1000 l ha⁻¹) and quality (CV% per leaf) from the Groblersdal spray trial are summarised in Table 1. In Table 2, the treatment × speed × vertical canopy position results are summarised for deposition efficiency (FPC% per 1000 l ha⁻¹) and uniformity (CV% between leaves) only.

Table 4.3.3.1. Mean deposition quantity per leaf (FPC%), uniformity (CV% between leaves), efficiency (FPC% per 1000 l ha⁻¹) and quality (CV% per leaf) on leaf surfaces from the inner and outer canopy of trees following spray application with SARDI Yellow Fluorescent Pigment (1 ml hl⁻¹) in a mature Robyn navel orchard in Groblersdal 2009 with three citrus sprayers at different tractor speeds and spray volumes.

Sprayer	Speed (km h ⁻¹)	Volume (l ha ⁻¹)	Deposition quantity (FPC% per leaf)			Deposition uniformity (CV% between leaves)			Deposition efficiency (FPC% per 1000 l ha ⁻¹)			Deposition quality (CV% per leaf)						
			Outer canopy		Inner canopy	Outer canopy		Inner canopy	Outer canopy		Inner canopy	Outer canopy		Inner canopy				
BSF-Extreme	1.5	13700	4.51	ghi	4.08	i-l	45.22	c-h	45.34	c-h	0.33	hi	0.30	i	10.32	b-g	8.25	hij
BSF-Extreme	2.3	8935	4.81	f-i	4.68	ghi	37.89	f-i	38.01	f-i	0.54	cd	0.52	cde	9.36	e-h	8.59	f-i
BSF-Extreme	2.9	7086	4.82	f-i	3.51	jkl	45.48	c-h	55.01	abc	0.68	b	0.50	c-g	9.55	d-h	6.13	k
BSF-Multiwing (D4)	1.5	9875	5.25	d-g	4.18	h-k	47.56	b-f	54.86	abc	0.53	cd	0.42	gf	10.34	b-f	7.43	ijk
BSF-Multiwing (D4)	2.3	6440	5.38	d-g	3.28	l	45.56	c-h	60.30	a	0.83	a	0.51	c-f	10.45	b-e	5.86	k
BSF-Multiwing (D4)	2.9	6129	5.04	e-h	3.31	kl	48.97	b-e	53.57	abc	0.82	a	0.54	cd	9.72	c-h	5.79	k
BSF-Multiwing (D5)	1.5	13188	5.61	c-f	4.23	hij	46.17	b-g	52.13	a-d	0.43	fg	0.32	hi	11.39	bc	8.05	hij
BSF-Multiwing (D5)	2.3	8601	4.36	hij	3.95	i-l	49.07	b-e	55.58	ab	0.51	c-f	0.46	d-g	8.49	ghi	6.62	jk
BSF-Multiwing (D5)	2.9	6821	4.74	f-i	3.96	i-l	48.33	b-e	53.28	a-d	0.69	b	0.58	c	9.38	e-h	7.00	ijk
Ultima	1.3	23582	7.78	a	5.88	b-e	40.12	e-i	36.36	ghi	0.33	hi	0.25	i	13.63	a	10.80	b-e
Ultima	2.1	14598	6.33	bc	6.01	bcd	43.66	d-i	35.31	i	0.43	efg	0.41	gh	11.31	bcd	10.69	b-e
Ultima	2.7	11354	6.49	b	5.76	b-e	41.34	e-i	36.31	hi	0.57	c	0.51	c-f	12.09	ab	10.50	b-e
LSD (<i>P</i> = 0.05)			0.886			9.835			0.092			1.836						

*Means of each parameter followed by the same letter do not differ significantly (*P* = 0.05)

Table 4.3.3.2. Mean deposition efficiency (FPC% per 1000 l ha⁻¹) and uniformity (CV% between leaves) on leaf surfaces from the bottom, middle and top canopy sections of trees following spray application with SARDI Yellow Fluorescent Pigment (1 ml hl⁻¹) in a mature Robyn navel orchard in Groblersdal 2009 with three citrus sprayers at different tractor speeds and spray volumes.

Sprayer	Speed (km h ⁻¹)	Volume (l ha ⁻¹)	Deposition efficiency (FPC% per 1000 l ha ⁻¹)						Deposition uniformity (CV% between leaves)					
			Bottom		Middle		Top		Bottom		Middle		Top	
BSF-Extreme	1.5	13700	0.27	stu	0.26	tu	0.40	l-r	42.20	e-k	46.84	c-i	46.80	d-i
BSF-Extreme	2.3	8935	0.49	g-n	0.47	h-o	0.63	c-f	31.06	k	43.91	d-j	38.87	h-k
BSF-Extreme	2.9	7086	0.55	e-j	0.57	d-h	0.65	cde	48.62	c-h	52.16	a-f	49.95	b-h
BSF-Multiwing (D4)	1.5	9875	0.44	g-p	0.44	g-p	0.55	e-i	51.23	b-g	48.19	c-i	54.21	a-e
BSF-Multiwing (D4)	2.3	6440	0.47	h-o	0.52	f-k	1.02	a	49.52	b-h	61.25	ab	48.01	c-i
BSF-Multiwing (D4)	2.9	6129	0.65	cde	0.72	c	0.67	dc	52.73	a-f	43.91	d-j	57.16	abc
BSF-Multiwing (D5)	1.5	13188	0.35	p-t	0.39	n-r	0.38	o-s	48.72	c-i	44.45	d-j	54.28	a-d
BSF-Multiwing (D5)	2.3	8601	0.51	g-l	0.51	g-l	0.43	k-q	44.26	d-j	48.74	c-i	63.98	a
BSF-Multiwing (D5)	2.9	6821	0.51	g-l	0.56	d-h	0.85	b	57.24	abc	50.53	b-h	44.66	d-j
Ultima	1.3	23582	0.25	tu	0.23	u	0.39	m-r	31.81	k	42.07	f-k	40.83	f-k
Ultima	2.1	14598	0.32	r-u	0.34	q-t	0.60	d-g	39.25	g-k	37.38	ijk	41.83	f-j
Ultima	2.7	11354	0.50	g-m	0.46	h-p	0.65	cde	38.94	h-k	33.62	jk	43.92	d-j
LSD (<i>P</i> = 0.05)			0.112						12.045					

*Means of each parameter followed by the same letter do not differ significantly (*P* = 0.05)

The Ultima at 11354 to 23582 I ha⁻¹ deposited the highest quantity of pigment at the best uniformity, albeit at poorest deposition quality on inner and outer canopy leaves (Table 4.3.3.1). Deposition results were generally similar on outer and inner canopy leaves. Interestingly, these deposition parameters did not differ significantly from each other irrespective of the almost doubling of spray volume when tractor speed was reduced from 2.7 to 1.3 km h⁻¹. As a consequence, spray application with the Ultima at 2.7 km h⁻¹ (11354 I ha⁻¹) was significantly more efficient than the higher volume applications at slower speeds. For the different spray volumes, deposition uniformity was generally similar (31.8 to 43.9 CV%) across vertical canopy positions, although significantly higher deposition quantities were measured in tops of tree canopies (Table 4.3.3.2).

Spray volumes with the BSF-Extreme ranged from 7068 to 13700 I ha⁻¹, but deposition quantity and uniformity levels on outer and inner canopy leaves did not differ significantly, except at the fastest spraying speed where significantly less pigment was deposited on inner leaves (Table 1). Deposition efficiency and uniformity with the BSF-Extreme at 8935 I ha⁻¹ was similar to that of the most efficient Ultima application at 11354 I ha⁻¹, but deposition quality was significantly better. At the higher spray volumes, significantly higher quantity of pigment was measured in the tops of canopies, while no statistical difference was observed between vertical canopy positions for the 7086 I ha⁻¹ application (Table 4.3.3.2). Vertical canopy position appeared not to influence deposition uniformity following sprays with the BSF-Extreme.

The BSF-Multiwing was evaluated using two nozzle combinations comprising either D4 or D5 nozzles at tractor speeds of 1.5 to 2.9 km h⁻¹, which resulted in spray volumes ranging from 9875 to 6129 I ha⁻¹ and 13188 to 6440 I ha⁻¹, respectively. Compared with outer canopy leaves, deposition quantity levels were generally lower, often significantly, on inner canopy leaves (Table 4.3.3.1). However, these values were statistically similar to those obtained with the Extreme, while the Multiwing generally deposited higher quantities of pigment on outer canopy leaves (5.04% to 5.38 FPC% and 4.36 to 5.61 FPC% for the D4 and D5 nozzles respectively) than the Extreme (4.51 to 4.82 FPC%). Equipped with the D4 nozzles, the Multiwing performed equally well on outer canopy leaves in terms of deposition quantity (5.04 to 5.38 FPC%), uniformity (45.56 to 48.97 CV%) and quality (9.72 to 10.45 CV%) as when using D5 nozzles at 1.5 km h⁻¹ (13188 I ha⁻¹), which was the best treatment using these nozzles (5.61 FPC%, 46.17 CV% and 11.39 CV%, respectively). The Multiwing was used at 10 bar pressure, but in one instance (2.9 km h⁻¹ using D4 nozzles) it was used at 15 bar. In this treatment, it was clear that the increased pump pressure improved spray penetration. The Multiwing deposited significantly higher quantities of pigment in the tops of trees at the slower tractor speeds with the D4 nozzles and at the fastest tractor speed with the D5 nozzles (Table 4.3.3.2), while no meaningful trend in deposition uniformity across vertical canopy position was obvious.

Deposition quantity results for the treatment × horizontal canopy position interaction were compared to the FPC benchmarks as determined in the previous objectives. On outer canopy leaves, all sprays deposited well above the FPC₇₅ benchmark (4.14 FPC%). On inner canopy leaves, sprays with the BSF extreme (2.3 km h⁻¹; 8935 I ha⁻¹), BSF Multiwing (D4; 1.5 km h⁻¹; 9875 I ha⁻¹ and D5; 1.5 km h⁻¹; 13188 I ha⁻¹) and all Ultima sprays realised deposition above the FPC₇₅. The rest of the treatments realised deposition well above the FPC₅₀ (2.02 %FPC) (Table 4.3.3.1).

Citusdal 2010

Analysis of variance of the deposition quantity data (FPC% per leaf) indicated a significant 2-factor interaction for treatment and horizontal canopy position, as well as a significant interaction between treatment and vertical canopy position ($P < 0.05$). These interactions were also obvious for the normalised deposition quantity data (FPC% per 1000 I ha⁻¹), although the 3-factor treatment × horizontal position × vertical position interaction was also significant ($P = 0.0024$). For spray uniformity (CV% between leaves), the treatment × vertical canopy position interaction was significant ($P = 0.0114$), and to a lesser extent also for horizontal canopy position ($P = 0.0836$). For deposition quantity (CV% per leaf) data, the treatment × vertical canopy position interaction was significant ($P = 0.0056$), as well as horizontal canopy position as main effect ($P < 0.0001$).

The treatment × horizontal canopy position results from the Citrusdal spray trial are summarised in Table 4.3.3.3, and the treatment × vertical canopy position results in Table 4.3.3.4. In general, deposition quantity was lower in inner canopy leaves, although not significantly for the low-profile Jacto, Martignani, Multiwing and Nieuwoudt. The Jacto-Valencia at 9494 I ha⁻¹ and Nieuwoudt at 12501 I ha⁻¹ deposited the highest pigment quantities on outer and inner canopy leaves. Deposition uniformity was also reasonably good (55.16 to 60.57 CV%), but quality was poor, especially with the Nieuwoudt. The Nieuwoudt at 7813 I ha⁻¹ (4 km h⁻¹) deposited significantly less pigment than its higher volume application, but uniformity was similar (c. 55 CV%), deposition efficiency better, and quality significantly better. The Nieuwoudt sprayer deposited

significantly larger quantities in the tops of trees (Table 4.3.3.4), although uniformity was better in the bottoms of canopies (49.12 to 51.26 CV% vs. 56.32 to 61.13 CV%).

Table 4.3.3.3. Mean deposition quantity per leaf (FPC%), uniformity (CV% between leaves), efficiency (FPC% per 1000 l ha⁻¹) and quality (CV% per leaf) on leaf surfaces from the inner and outer canopy of trees following spray application with SARDI Yellow Fluorescent Pigment (1 ml hl⁻¹) in a mature Olinda valencia orchard in Citrusdal 2010 with eight citrus sprayers at different tractor speeds and spray volumes.

Sprayer	Speed (km h ⁻¹)	Volume (l ha ⁻¹)	Deposition quantity (FPC% per leaf)				Deposition uniformity (CV% between leaves)				Deposition efficiency (FPC% per 1000 l ha ⁻¹)				Deposition quality (CV% per leaf)			
			Outer canopy		Inner canopy		Outer canopy		Inner canopy		Outer canopy		Inner canopy		Outer canopy		Inner canopy	
Atasa	1.7	7882	4.10	ef	2.58	i-l	52.50	hi	69.04	b-f	0.52	efg	0.33	hij	5.94	efg	4.06	hij
Atasa	1.7	4000	4.22	def	1.25	mno	56.88	e-i	70.68	b-e	1.05	a	0.31	ij	7.13	de	3.52	ijk
Cima	1.7	8000	5.04	bcd	3.08	hi	52.61	ghi	62.78	c-i	0.63	de	0.38	f-j	9.12	bc	6.52	e
Cima	1.7	4000	3.01	hi	1.94	klm	57.09	e-i	77.11	bc	0.75	cd	0.49	e-h	6.53	e	4.59	f-i
Jacto	3.3	3735	1.43	mn	1.26	mno	67.47	c-g	76.48	bc	0.38	f-j	0.34	hij	3.26	ijk	2.26	j-m
Jacto-tower	2.5	6844	3.26	ghi	1.99	j-m	65.86	c-i	66.91	c-h	0.48	e-i	0.29	j	5.79	e-h	3.49	ijk
Jacto-Valencia	2.5	9494	5.90	a	4.74	cde	56.16	e-i	60.57	d-i	0.62	de	0.50	e-h	9.54	b	7.27	cde
Martignani	4	1000	0.96	nop	0.55	op	72.50	bcd	82.95	ab	0.96	ab	0.55	ef	2.73	i-l	1.77	klm
Martignani	4	500	0.42	p	0.18	p	93.80	a	75.49	bc	0.85	bc	0.36	g-j	1.25	lm	0.60	m
Multiwing	1.7	6501	2.78	hij	2.73	ijk	51.64	i	51.72	i	0.43	f-j	0.42	f-j	6.33	ef	3.86	ij
Multiwing	3.3	3349	1.86	lm	1.68	mn	63.06	c-i	64.33	c-i	0.55	ef	0.49	e-h	4.13	ghi	4.13	ghi
Nieuwoudt	2.5	12501	5.59	ab	5.14	abc	55.56	f-i	56.11	e-i	0.45	f-j	0.41	f-j	11.63	a	10.54	ab
Nieuwoudt	4	7813	4.06	efg	3.59	fgh	54.28	f-i	56.54	e-i	0.52	efg	0.46	e-j	8.86	bcd	7.38	cde
LSD (<i>P</i> = 0.05)			0.824				14.913				0.172				1.865			

*Means of each parameter followed by the same letter do not differ significantly (*P* = 0.05)

Table 4.3.3.4. Mean deposition efficiency (FPC% per 1000 l ha⁻¹) and uniformity (CV% between leaves) on leaf surfaces from the bottom, middle and top canopy sections of trees following spray application with SARDI Yellow Fluorescent Pigment (1 ml hl⁻¹) in a mature Olinda valencia orchard in Citrusdal with eight citrus 2010 sprayers at different tractor speeds and spray volumes.

Sprayer	Speed (km h ⁻¹)	Volume (l ha ⁻¹)	Deposition efficiency (%FPC per 1000 l ha ⁻¹)						Deposition uniformity (CV% between leaves)					
			Bottom		Middle		Top		Bottom		Middle		Top	
Atasa	1.7	7882	0.38	i-m	0.49	d-l	0.41	g-m	65.25	e-k	49.44	jkl	67.62	c-j
Atasa	1.7	4000	0.67	c-f	0.89	ab	0.49	d-l	70.15	c-i	50.09	jkl	71.09	c-h
Cima	1.7	8000	0.52	d-j	0.57	d-i	0.43	g-m	41.33	l	50.65	jkl	81.09	a-e
Cima	1.7	4000	0.68	b-d	0.70	bcd	0.48	e-l	51.37	jkl	56.30	h-l	93.63	ab
Jacto	3.3	3735	0.52	d-l	0.33	j-m	0.23	m	65.90	d-k	66.01	d-j	84.02	a-d
Jacto-tower	2.5	6844	0.45	g-h	0.31	lm	0.40	h-m	56.20	h-l	67.60	c-j	75.37	c-g
Jacto-Valencia	2.5	9494	0.51	d-l	0.56	d-i	0.60	c-h	56.37	h-l	54.04	h-l	64.69	e-k
Martignani	4	1000	0.66	c-f	0.68	b-e	0.91	a	67.41	c-j	85.24	abc	80.52	a-e
Martignani	4	500	0.48	e-l	0.53	d-k	0.81	abc	76.64	b-f	82.00	a-e	95.30	a
Multiwing	1.7	6501	0.43	g-m	0.38	i-m	0.46	g-l	52.19	i-l	50.84	jkl	52.01	i-l
Multiwing	3.3	3349	0.53	d-k	0.55	d-j	0.50	d-l	64.59	e-k	49.69	jkl	76.80	b-f
Nieuwoudt	2.5	12501	0.35	j-m	0.33	klm	0.61	c-g	49.12	kl	61.13	f-k	57.25	g-l
Nieuwoudt	4	7813	0.41	g-m	0.39	h-m	0.67	c-f	51.26	jkl	56.32	h-l	58.64	f-l
LSD (<i>P</i> = 0.05)			0.211						18.264					

*Means of each parameter followed by the same letter do not differ significantly (*P* = 0.05)

The low-profile Atasa sprayer, which included a turret with nozzles, was evaluated at 7882 and 4000 l ha⁻¹. Deposition quantities and uniformity on outer canopy leaves were similar (4.10 and 4.22 FPC% and 52.50 and 56.88 CV%, respectively), although inner canopy leaves retained significantly less pigment (2.58 and 1.25 %FPC, respectively) and at poorer uniformity (69.04 and 70.68 CV%, respectively). Vertical distribution of pigment showed similar quantities on the bottom, middle and top sections of canopies, but the 4000 l ha⁻¹ application resulted in significantly higher deposition quantities in the mid-section. For both volumes, deposition uniformity was best in the mid-section (c. 50 CV%) and poorer in the bottoms and tops of trees (65.25 to 71.09 CV%).

The Cima at 8000 l ha⁻¹ deposited similar quantities on outer canopy leaves (5.04 FPC%) than the Jacto-Valencia at 9494 l ha⁻¹ and Nieuwoudt at 12501 l ha⁻¹, but inner canopy position was significantly lower (3.08 FPC%), but with similar uniformity in both canopy sections (52.61 and 62.78 CV%, respectively). At 4000 l ha⁻¹, the Cima deposited significantly less pigment on outer and inner canopy leaves (3.01 and 1.94 FPC%, respectively), at similar uniformity on outer canopies (57.09 CV%), but markedly poorer uniformity on inner canopies (77.11 CV%). For both spray volumes, vertical distribution was better in terms of quality and uniformity in the bottom and mid-sections of canopies; deposition in top sections was significantly less uniform (81.09 to 93.63 CV% vs. 41.33 to 56.30 CV%).

The low-profile Jacto operating at 3.3 km h⁻¹ and 3735 l ha⁻¹ deposited similar quantities on outer and inner canopy leaves (1.43 and 1.26 FPC%, respectively), although deposition uniformity was relatively poor (67.47 and 76.48 CV%, respectively). Vertical distribution was poor with progressively less pigment deposited at poorer uniformity toward the tops of trees. The addition of a turret and application at slower speed (2.5 km h⁻¹) and higher volume (6844 l ha⁻¹) resulted in significantly better deposition on outer canopy leaves (3.26 FPC%), but not on inner canopy leaves (1.99 FPC%). Deposition uniformity on outer canopy leaves (65.86 CV%) was similar to the low-profile application, while a marked improvement was observed on inner canopy leaves (66.91 CV%). Vertical distribution was improved with similar quantities deposited at various canopy positions, although spray uniformity showed better deposition in bottom parts of trees (56.20, 67.60 and 75.37 CV% for bottom middle and top, respectively). As mentioned previously, the Jacto-Valencia performed very well at 9494 l ha⁻¹ with an even vertical distribution of pigment, but with slightly lower uniformity in tops of canopies.

For each spray volume, deposition quantity and uniformity with the BSF-Multiwing on outer and inner canopy leaves were similar, but significantly higher/better for the 6501 l ha⁻¹ (2.78 and 2.73 FPC% at 51.64 and 51.72 CV%, respectively) than 3349 l ha⁻¹ application (1.86 and 1.68 FPC% at 63.06 and 64.33 CV%, respectively). Deposition quality was also good (3.86 to 6.33 CV%). Vertical distribution of sprayed pigment was even, although at poorer uniformity (76.80 CV%) in tops of trees at the lower spray volume and faster tractor speed.

The electrostatic Martignani operated at 4 km h⁻¹ at 1000 and 500 l ha⁻¹ and deposited markedly less pigment on inner canopy leaves (0.55 and 0.18 FPC% vs. 0.96 and 0.42 FPC% at higher volume, respectively). Deposition uniformity was relatively poor and ranged from 75.5 to 93.8 CV%. In terms of efficiency, this sprayer was as efficient as other sprayers for deposition on inner canopy leaves, and better than most on outer canopy leaves. Interestingly, it deposited significantly larger quantities of pigment in tops of trees, although deposition uniformity was better in bottoms of canopies.

Deposition quantity results for the treatment × horizontal canopy position interaction were compared to the FPC benchmarks. On outer canopy leaves, The Atasa at 4000 l ha⁻¹, the Cima at 8000 l ha⁻¹, the Jacto Valencia at 9494 l ha⁻¹ and all Nieuwoudt sprays realised deposition above the FPC₇₅ benchmark (4.22 to 5.59 %FPC). Interestingly, the Atasa at 7882 l ha⁻¹ realised deposition just below the FPC₇₅ benchmark (4.10 FPC%). The Cima at 4000 l ha⁻¹, the Jacto-tower at 6844 l ha⁻¹ and the Multiwing at 6501 l ha⁻¹ realised deposition above the FPC₅₀ benchmark (3.26 to 2.78 %FPC), with the rest of the treatments deposition below the FPC₅₀ benchmark (1.86 to 0.42 FPC%) (Table 4.3.3.3).

Conclusion for Groblersdal 2009 and Citrusdal 2010.

From the results obtained, as was also reported previously, it was clear that the highest deposition quantity per leaf values at the lowest variation between leaves was generally obtained with higher spray volumes. However, it was obvious that the dispersion quality of pigment deposition on individual leaves declined with increasing spray volumes due to more run-off, which might also have a detrimental effect on biological efficacy. It should furthermore be stressed that the fluorescent pigment dosage of 1× was used when comparing all the different sprayers and calibration settings, even though spray volumes differed. Hence, the dosage per hectare differed substantially between treatments. In relative terms, spray efficiency (expressed as deposition quantity per leaf per 1000 l of spray volume) in combination with spray uniformity (expressed

by the variation in deposition quantity between leaves) are therefore the parameters that should be used when comparing sprayers and calibration settings. Similar and even improved spray deposition can thus be obtained at lower spray volumes through optimal use of equipment or through the use of more efficient sprayers, especially if dosage per hectare is equated between treatments.

From the Groblersdal trial, where sprayers were evaluated at different tractor speeds but with constant sprayer calibration, it was clear that excessively high spray volumes ($>10\ 000\ \text{l ha}^{-1}$) do not result in better spray deposition. In fact, deposition uniformity was similar across sprayers and spray volumes ranging from 6129 to 23582 l ha^{-1} on outer canopy leaves. On inner canopy leaves, it was clear that deposition uniformity was better at higher spray volumes, although in most cases similar to outer canopy leaves.

In Citrusdal, a variety of spray machines was evaluated. The high-volume Nieuwoudt sprayer performed very well. When comparing its deposition criteria at 12501 and 7813 l ha^{-1} in this trial, it was clear that the latter spray volume, applied at 4 km h^{-1} , was the more efficient application. Several low-profile machines were evaluated and found to lack penetration and deposition in tops if canopies. This was negated to some extent by using a turret or tower. The BSF-Multiwing again proved to be a very efficient sprayer, especially when considering its low power consumption. This is also a commendable trait of the Nieuwoudt sprayer. The Cima sprayer was used at higher spray volumes than when evaluated in Letsitele (reported previously), and performed well, except for relatively poor deposition uniformity in tree tops. The Martignani performed very well when compared with another ultra-low volume electrostatic sprayer, the ESS, which was evaluated in Letsitele previously. However, spray uniformity and penetration might be problematic at the fast 4 km h^{-1} evaluated here. Slower spraying speeds (and possibly higher spray volumes) would improve these parameters and should be considered in future evaluations.

In terms of tractor speed, it seems that c. 3 km h^{-1} (as also observed previously) should be the upper limit for medium cover spray application; defined as adequate cover of outer and inner canopy leaves and fruit, without emphasis on film-wetting of the trunk and branches as would be required for full cover application. Faster tractor speed reduced spray penetration and spray uniformity, especially in denser canopies.

From the findings to date (as also reported previously), it seems clear that medium cover sprays can be adequately delivered with 2-sided sprayers. This holds a big advantage over 1-sided sprayers as work rates are improved, costs reduced and orchard traffic and concomitant soil compaction are also halved.

This research is still work in progress, and the conclusions drawn from the results obtained thus far should be viewed in this context. Most spray trials will be repeated to confirm the initial findings. Additionally, the benchmarks for biological efficacy need to be satisfactorily determined to support conclusive interpretation of the results.

b. Evaluate methods for optimisation of spray application with novel applicators.

Trial one – ‘Washington’ navel orchard (Citrusdal, Western Cape, 2013)

Deposition quantity

Analysis of variance of deposition quantity (FPC%) indicated a significant interaction ($P < 0.05$) for treatment \times horizontal canopy position \times vertical canopy position ($P < 0.0001$). The significance of the interaction can be ascribed to the high deposition quantity realised by the Nieuwoudt spray at 8847 l ha^{-1} on middle inside canopy leaves (7.37 FPC%) as well as the significantly low deposition quantity realised by the Martignani spray at 1000 l ha^{-1} on bottom inside leaves (0.273 FPC%) (results not shown). Due to the complexity of the interaction, interactions treatment \times horizontal canopy position ($P < 0.0149$) and treatment \times vertical canopy position ($P < 0.1366$) were discussed separately.

Overall, for horizontal canopy position, deposition quantity was higher on outer canopy leaves than on inner canopy leaves. On outer canopy leaves, the highest deposition was realised by Nieuwoudt sprays at 6229 l ha^{-1} (5.67 FPC%). At 8847 l ha^{-1} , Nieuwoudt sprays realised a lower deposition quantity (4.94 FPC%) than above mentioned, but still statistically similar. Nieuwoudt sprays at 4062 l ha^{-1} , 2708 l ha^{-1} and Martignani sprays at 4000 l ha^{-1} @ 1.78 km h^{-1} on outer canopy leaves deposited statistically lower deposition quantities and did not differ statistically from each other (4.01, 3.65 and 3.76 FPC% respectively). Martignani sprays at 4000 l ha^{-1} @ 2.88 km h^{-1} and 2000 l ha^{-1} did not differ statistically from each other (2.76 and 2.71 FPC%) whilst at 1000 l ha^{-1} realised the lowest deposition on the outside canopy leaves (0.99 FPC%), statistically less than all other treatments (Table 4.3.3.5).

On the inner canopy leaves, the highest deposition quantity was realised by Nieuwoudt sprays at 8847 l ha^{-1} , statistically higher than all other treatments (5.38 FPC%). At 6229 l ha^{-1} and 4062 l ha^{-1} , Nieuwoudt sprays

(4.16 and 3.50 FPC%) did not differ statistically from each other and deposited statistically less. Martignani sprays at 4000 l ha⁻¹ @ 1.78 km h⁻¹ (2.74 FPC%) realised a FPC% significantly higher than that of Nieuwoudt sprays at 2708 l ha⁻¹, Martignani sprays at 4000 l ha⁻¹ and 2000 l ha⁻¹ (1.26, 1.58 and 1.51 FPC%), which did not differ significantly from each other. At 1000 l ha⁻¹ the Martignani realised the lowest deposition quantity of all the treatments on the inner canopy leaves (0.56 FPC%) (Table 4.3.3.5).

Evaluation of the non-significant interaction treatment × vertical canopy position ($P < 0.1366$) indicated that the Nieuwoudt spray at 8847 l ha⁻¹ landed a significantly higher FPC% on middle canopy leaves (6.24 FPC%) than all other treatments at all canopy positions. This was also the case at 6229 l ha⁻¹ on leaves at the top of the tree (5.93 FPC%). All other treatments at various canopy positions did not differ in deposition quantity per treatment. However, the Martignani spray at 2000 l ha⁻¹ did realise a statistically lower FPC% on bottom canopy leaves (0.80 FPC%), in relation to middle and top canopy leaves (Table 4.3.3.6).

Deposition quality

Analysis of variance of deposition quality (ICD%) indicated a significant interaction ($P < 0.05$) for treatment × vertical canopy position × horizontal canopy position ($P = 0.0280$). The significance of the interaction can be ascribed to the lower variation in deposition quality realised by the Martignani spray on the inner middle canopy leaves at 2000 and 4000 l ha⁻¹ @ 1.78 km h⁻¹ (30.09 and 39.38 ICD% respectively) All other treatments realised significantly higher variation in deposition quality at various positions (43.68 to 76.74 ICD%), especially Martignani sprays at 1000 l ha⁻¹ and Nieuwoudt sprays at 2708 l ha⁻¹ on the inner bottom canopy leaves (75.55 and 76.74 ICD% respectively) (results not shown). Due to the complexity of the interaction, significant interactions treatment × horizontal canopy position ($P < 0.0303$) and treatment × vertical canopy position ($P < 0.0013$) were discussed separately.

For the interaction treatment × horizontal canopy position, deposition quality was generally better on outside canopy leaves. On outer canopy leaves the least variation in deposition (best deposition quality) was realised by Martignani sprays at 4000 l ha⁻¹ at the slower tractor speed (1.78 km h⁻¹) (47.66 ICD%). Martignani sprays at higher applicator speed (2.88 km h⁻¹) at 4000 and 2000 l ha⁻¹ (49.33 and 51.70 ICD% respectively) did not differ statistically from above mentioned treatment. The highest variation in deposition quality was realised by Nieuwoudt spray at 4062 and 2708 l ha⁻¹ but also the Martignani spray at 1000 l ha⁻¹ (60.25, 57.46 and 57.42 ICD% respectively). These sprays did not differ statistically nor from that of the higher volume sprays with the Nieuwoudt sprayer at 8847 and 6229 l ha⁻¹ (55.89 and 54.43 ICD%) (Table 1). As found on outer canopy leaves, significantly lower variation in deposition quality was realised by Martignani sprays at 4000 @ 1.78 km h⁻¹, 4000, 2000 l ha⁻¹ (52.97 to 53.03 ICD%) but also by the high volume Nieuwoudt applications at 8847 and 6229 l ha⁻¹ (52.43 and 55.52 ICD% respectively). Significantly higher variation in deposition quality was realised by the lower volume Nieuwoudt spray application at 4062 l ha⁻¹ (60.10 ICD%) with the 2708 l ha⁻¹ and Martignani 1000 l ha⁻¹ application realising the highest variation in deposition quality (68.93 and 63.17 ICD% respectively) (Table 4.3.3.5).

For the interaction treatment × vertical canopy position, the best deposition quality was generally found to be realised on middle canopy leaves. At the top of the canopy, Nieuwoudt sprays at 6229 l ha⁻¹ realised the highest deposition quality (49.46 ICD%). Nieuwoudt sprays at 8847 l ha⁻¹ (52.49 ICD%) and Martignani sprays at 4000 (1.78 km h⁻¹) and 2000 l ha⁻¹ did not differ statistically from this spray (53.13 and 54.4 ICD%). Significantly, the lowest deposition quality was realised by the Nieuwoudt spray at 2708 l ha⁻¹ (62.64 ICD%) and the Martignani spray at 1000 l ha⁻¹ (61.97 ICD%) (Table 4.3.3.6).

On middle canopy leaves, Martignani sprays at 4000 l ha⁻¹ at 1.78 and 2.88 km h⁻¹ applicator speeds (43.97 and 44.76 ICD% respectively) and 2000 l ha⁻¹ (43.79 ICD%) realised significantly the lowest variation in deposition quality and did not differ from each other. The remaining treatments realised significantly higher variation in deposition quality and did not differ statistically from each other (50.21 to 57.87 ICD%) (Table 4.3.3.6).

On bottom canopy leaves deposition quality was generally lower than on top and middle canopy leaves (53.85 to 69.08 ICD%). Least variation in deposition quality was realised by the Martignani spray at 4000 l ha⁻¹ at both tractor speeds (1.78 km h⁻¹ = 53.85 ICD%; 2.88 km h⁻¹ = 50.18 ICD%). The highest variation in deposition quality was realised by the Nieuwoudt spray at 2708 l ha⁻¹ and the Martignani spray at 1000 l ha⁻¹ (69.08 and 68.71 ICD% respectively), differing statistically from the other applications except for the Nieuwoudt spray at 4062 l ha⁻¹ (62.90 ICD%), which was also statistically similar to the remaining treatments (57.94 to 58.90 ICD) (Table 4.3.3.6).

Deposition uniformity

Analysis of variance of deposition uniformity between leaves (in a 12 leaf batch) indicated a significant interaction for treatment \times horizontal canopy position ($P < 0.0480$). Deposition uniformity was generally better on the outer than on the inner canopy leaves. On the outer canopy leaves, the lowest variation in deposition quantity was realised by the Nieuwoudt spray at 8847 l ha^{-1} (50.1%), statistically lower than that of the Martignani sprays at 4000 l ha^{-1} @ 2.88 km h^{-1} , 2000 and 1000 l ha^{-1} (72.31, 76.5 and 87.14% respectively). The rest of the treatments did not differ significantly in deposition uniformity (Table 4.3.3.5).

On the inside of the canopy, the Nieuwoudt spray at 2708 l ha^{-1} realised the highest variation in deposition uniformity (100.88%), significantly higher than the deposition uniformity realised by Nieuwoudt sprays at 6229 l ha^{-1} (72.65%) and 8847 l ha^{-1} (58.28%). Interestingly, Uniformity realised by the Martignani spray at 4000 l ha^{-1} @ 1.78 km h^{-1} (72.03%) did not differ statistically from that of above mentioned. (Table 4.3.3.5).

Deposition efficiency

Analysis of variance of deposition efficiency (normalised FPC% to a spray volume of 1000 l ha^{-1}) indicated significant interactions for treatment \times horizontal canopy position ($P < 0.0001$) and treatment \times vertical canopy position ($P = 0.0005$). On outer canopy leaves, the highest deposition efficiency was realised by the Martignani spray at 2000 l ha^{-1} (1.35%), statistically higher than the other treatments. Martignani sprays at 1000 (0.99%), 4000 l ha^{-1} @ 1.78 km h^{-1} (0.94%) and Nieuwoudt spray at 2708 l ha^{-1} (0.88%) realised lower but statistically similar deposition efficiency. Nieuwoudt sprays at higher spray volumes generally resulted in poorer spray efficiency (0.49 to 0.32%) (Table 4.3.3.5).

On inner canopy leaves, a similar trend was observed, with martignani sprays realising similar deposition efficiency (0.56 to 0.75%), whereas the Nieuwoudt sprays and Martignani at 4000 l ha^{-1} @ 2.88 km h^{-1} resulted in lower deposition efficiency (0.24 to 0.43%) (Table 5). For the interaction treatment vertical canopy position, the most efficient deposition was realised by the Martignani spray at 2000 l ha^{-1} on middle canopy leaves (1.64%), significantly better than all other sprays. The least efficient spray was realised by the Nieuwoudt at 6229 l ha^{-1} on the bottom canopy leaves (0.24%), statistically similar to all other sprays on bottom canopy leaves (0.34 to 0.46%) (Table 4.3.3.6).

Table 4.3.3.5. Mean deposition quantity (FPC%), quality (ICD%), uniformity (CV% between leaves) and efficiency (FPC% per 1000 l ha⁻¹) realised following sprays with water and pigment at various spray volumes with a Nieuwoudt and Martignani spray machine on inner and outer canopy leaves of a Washington naval orchard in Citrusdal 2013.

Treatment ^a	Deposition quantity (FPC%) ^b		Deposition quality (ICD%) ^b	
	Outer canopy	Inner canopy	Outer canopy	Inner canopy
	Nw-8847 l ha ⁻¹ @ 300 KPa	4.94 ab	5.38 a	55.89 cd
Nw-6229 l ha ⁻¹	5.67 a	4.16 bc	54.43 cde	55.52 cd
Nw-4062 l ha ⁻¹	4.01 bc	3.50 cde	60.25 bc	60.09 bc
Nw-2708 l ha ⁻¹	3.65 cde	1.26 fg	57.46 bcd	68.93 a
Mg-4000 l ha ⁻¹ @ 1.78 km h ⁻¹	3.76 cd	2.74 e	47.66 f	52.97 def
Mg-4000 l ha ⁻¹	2.76 e	1.58 f	49.33 ef	52.84 def
Mg-2000 l ha ⁻¹	2.71 e	1.51 fg	51.70 def	53.03 def
Mg-1000 l ha ⁻¹	0.99 fg	0.56 g	57.42 bcd	63.17 ab
	Deposition uniformity (CV% between leaves) ^b		Deposition efficiency (FPC% per 1000 l ha ⁻¹) ^b	
	Outer canopy	Inner canopy	Outer canopy	Inner canopy
	Nw-8847 l ha ⁻¹ @ 300 KPa	50.06 f	58.28 def	0.56 fgh
Nw-6229 l ha ⁻¹	53.95 ef	72.65 b-e	0.91 bcd	0.67 efg
Nw-4062 l ha ⁻¹	61.04 def	86.22 ab	0.99 b	0.86 b-e
Nw-2708 l ha ⁻¹	58.91 def	100.88 a	1.35 a	0.46 gh
Mg-4000 l ha ⁻¹ @ 1.78 km h ⁻¹	63.88 c-f	72.03 b-e	0.94 bc	0.69 c-g
Mg-4000 l ha ⁻¹	72.31 b-e	81.29 abc	0.69 c-g	0.40 h
Mg-2000 l ha ⁻¹	76.48 bcd	73.22 b-e	1.35 a	0.75 b-f
Mg-1000 l ha ⁻¹	87.14 ab	89.09 ab	0.99 b	0.56 fgh

^aTreatment layout consists of sprayer used (Nw – Nieuwoudt sprayer; Mg – Martignani sprayer) followed by specific spray volume applied

^bFor each parameter separately, values in each column followed by the same letter do not differ significantly (P > 0.05) according to Fisher's least significant difference test.

Table 4.3.3.6. Mean deposition quantity (FPC%), quality (ICD%), uniformity (CV% between leaves) and efficiency (FPC% per 1000 l ha⁻¹) realised following sprays with water and pigment at various spray volumes with a Nieuwoudt and Martignani spray machine on top, middle and bottom leaves of Washington navel orchard in Citrusdal 2013.

Treatment ^a	Deposition quantity (FPC%) ^b			Deposition quality (ICD%) ^b		
	Vertical canopy position			Vertical canopy position		
	Top	Middle	Bottom	Top	Middle	Bottom
Nw-8847 l ha ⁻¹ @ 300 KPa	4.94 bc	6.23 a	4.29 cd	52.49 def	51.69 efg	58.31 b-e
Nw-6229 l ha ⁻¹	5.93 ab	4.65 c	4.16 cd	49.46 fgh	57.53 b-e	57.94 b-e
Nw-4062 l ha ⁻¹	4.27 cd	3.77 cde	3.23 d-g	59.86 bc	57.75 b-e	62.90 ab
Nw-2708 l ha ⁻¹	2.90 c-f	2.58 e-h	1.88 hij	62.64 ab	57.87 b-e	69.08 a
Mg-4000 l ha ⁻¹ @ 1.78 km h ⁻¹	3.72c-f	4.25 cd	1.79 hij	53.13 c-f	43.97 h	53.85 c-f
Mg-4000 l ha ⁻¹	2.52 f-i	2.69 e-h	1.31 ijk	58.32 b-e	44.76 gh	50.18 fgh
Mg-2000 l ha ⁻¹	2.25 ghi	3.27 d-g	0.80 jk	54.40 c-f	43.79 h	58.90 bcd
Mg-1000 l ha ⁻¹	0.90 jk	0.95 jk	0.46 k	61.97 ab	50.21 efg	68.71 a
	Deposition uniformity (CV% between leaves) ^b			Deposition efficiency (FPC% per 1000 l ha ⁻¹) ^b		
	Vertical canopy position			Vertical canopy position		
	Top	Middle	Bottom	Top	Middle	Bottom
Nw-8847 l ha ⁻¹ @ 300 KPa	60.25 def	65.04 b-f	64.61 b-f	0.95 bcd	0.75 c-f	0.67 d-h
Nw-6229 l ha ⁻¹	54.27 ef	46.47 f	61.77 c-f	0.56 e-h	0.71 d-h	0.49 e-h
Nw-4062 l ha ⁻¹	71.50 a-e	65.71 b-f	83.70 a-d	1.05 bc	0.93 bcd	0.79 cde
Nw-2708 l ha ⁻¹	64.17 b-f	89.97 a	85.54 abc	1.07 bc	0.95 bcd	0.70 d-h
Mg-4000 l ha ⁻¹ @ 1.78 km h ⁻¹	63.22 a-e	60.33 def	80.32 a-d	0.93 bcd	1.06 bc	0.45 fgh
Mg-4000 l ha ⁻¹	73.32 a-e	77.31 a-e	79.77 a-d	0.63 d-h	0.67 d-h	0.33 h
Mg-2000 l ha ⁻¹	70.37 a-f	73.25 a-e	80.94 a-d	1.13 b	1.64 a	0.40 gh
Mg-1000 l ha ⁻¹	86.84 ab	83.50 a-d	94.01 a	0.90 bcd	0.95 bcd	0.46 fgh

^aTreatment layout consists of sprayer used (Nw – Nieuwoudt sprayer; Mg – Martignani sprayer) followed by specific spray volume applied

^bFor each parameter separately, values in each column followed by the same letter do not differ significantly (P > 0.05) according to Fisher's least significant difference test.

Deposition quantity results for the treatment × horizontal canopy position interaction were compared to the FPC benchmarks. On outer canopy leaves, all sprays realised FPC% above the FPC50 benchmark, with only the Martignani spray at 1000 l ha⁻¹ depositing quantities below the line. Only Nieuwoudt sprays at 8847 and 6229 l ha⁻¹ realised deposition above the FPC75 benchmark on outer and inner canopy leaves. On inner canopy leaves, application with the Nieuwoudt at 4062 l ha⁻¹ and Martignani at 4000 l ha⁻¹ and 1.78 km h⁻¹ showed deposition above the FPC50 line with the rest of the treatment combinations depositing below the benchmark (Figure 4.3.3.4).

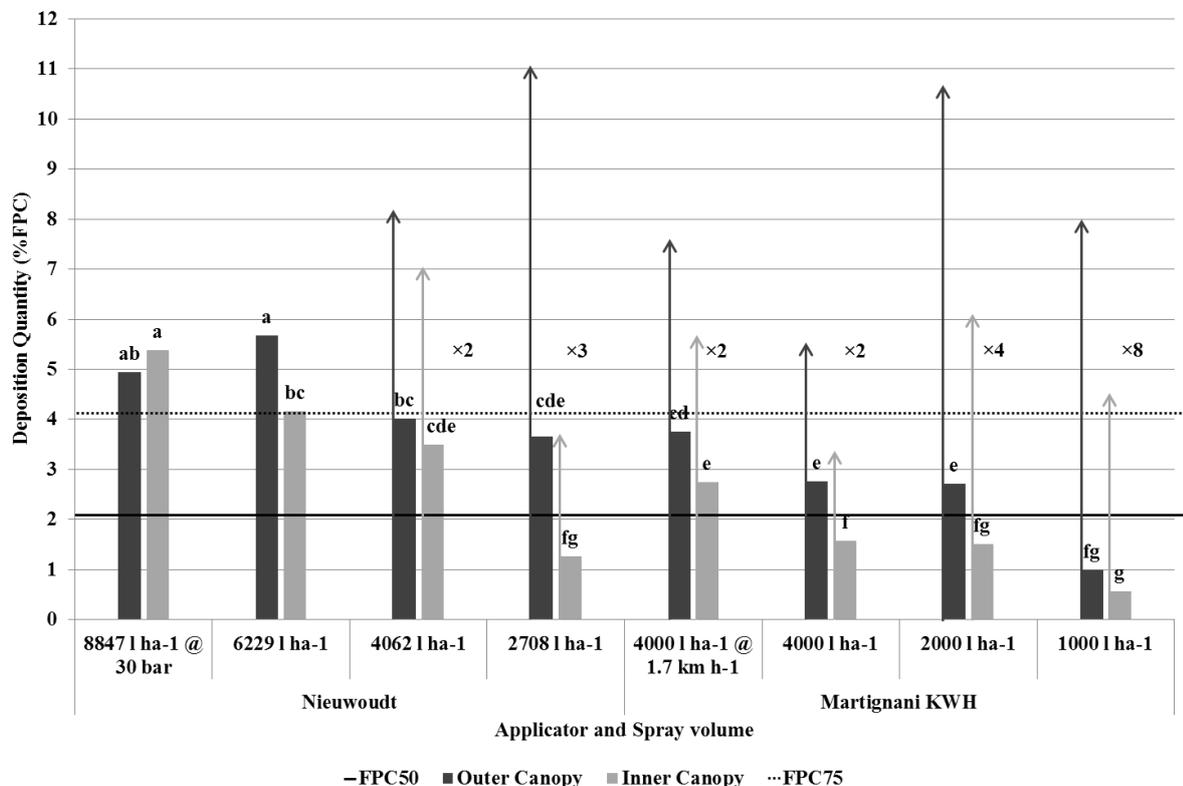


Figure 4.3.3.4. Mean deposition quantity realised by water and yellow fluorescent pigment sprays at ×1 fluorescent pigment concentration, together with lines indicating theoretical deposition quantity that can be realised when adjusting the product concentration respective to a 8000 l ha⁻¹ spray, on inner and outer canopy leaves of a Washington navel orchard following sprays with a Nieuwoudt sprayer at 8847 l ha⁻¹ @ 30 bar; 6229 l ha⁻¹; 4062 l ha⁻¹; 2708 l ha⁻¹ and a Martignani KWH sprayer at 4000 l ha⁻¹ @ 1.7 km h⁻¹; 4000 l ha⁻¹; 2000 l ha⁻¹ and 1000 l ha⁻¹, compared to FPC₅₀ and FPC₇₅ benchmarks at 2.07% and 4.14% respectively (Citrusdal 2013).

Trial two – ‘Washington’ Navel orchard (Groblersdal, Mpumalanga, 2013)

Deposition quantity

Analysis of variance of deposition quantity (FPC%) indicated significant interactions ($P < 0.05$) treatment × horizontal canopy position ($P < 0.0015$) and treatment × vertical canopy position ($P < 0.0064$).

Similarly as found in the previous trial, deposition was higher on outer canopy leaves than inner canopy leaves. The highest deposition quantity was realised with the Nieuwoudt sprayer at 7506 l ha⁻¹ (8.73 FPC%), a higher but statistically similar deposition quantity as realised by the highest spray volume of 10661 l ha⁻¹ (7.03 FPC%). Statistically similar deposition quantities to the 10661 l ha⁻¹ spray was realised by the Nieuwoudt spray at 4895 l ha⁻¹ (6.17 FPC%) and the 4000 l ha⁻¹ Martignani spray at 1.88 km h⁻¹ (5.95 FPC%). The 3263 l ha⁻¹ Nieuwoudt spray as well as the Martignani sprays at 4000 and a 2000 l ha⁻¹ realised lower, statistically similar deposition quantity on the outer canopy leaves (4.0, 3.0 and 2.5 FPC% respectively). The lowest deposition quantity on outer canopy leaves was deposited by the 1000 l ha⁻¹ Martignani spray (1.05 FPC%).

On inner canopy leaves, the best penetration (highest deposition quantity) was achieved with Nieuwoudt sprays at 4895 and 10661 l ha⁻¹ (3.93 and 3.73 FPC%). Significantly lower deposition quantities were observed on inner canopy leaves at 7506 and 3263 l ha⁻¹ (2.75 and 1.86 FPC% respectively). The

Martignani realised statistically similar deposition quantities on inner canopy leaves at 4000 (1.88 km h⁻¹), 4000, 2000 and 1000 l ha⁻¹ (1.12, 0.6, 0.4 and 0.4 FPC% respectively), however statistically similar to the 3263 l ha⁻¹ Nieuwoudt spray (Table 4.3.3.7).

Evaluation of the significant interaction treatment × vertical canopy position, deposition quantity was statistically similar for the Martignani sprays for each volume separately in the top, middle and bottom of the canopy. However this was not the case for Nieuwoudt sprays. The 4895 l ha⁻¹ Nieuwoudt spray realised significantly higher deposition in the top of the canopy (8.0 FPC%) in relation to the deposition realised in the middle (4.48 FPC%) and bottom (2.66 FPC%) of the canopy. At 3263, 15023 and 10661 l ha⁻¹, deposition quantity was higher in the middle of the canopy, with deposition quantity realised in the top and bottom of the canopy being lower (Table 4.3.3.8).

Deposition quality

Analysis of variance of deposition quality (ICD%) indicated a significant interaction treatment × horizontal canopy position ($P = 0.0351$). On outer canopy leaves the best deposition quality was realised by Martignani sprays at all sprayed volumes (52.31 to 57.79 ICD%). These sprays did not differ statistically. Nieuwoudt sprays at 10661, 7506 and 4895 l ha⁻¹ also did not differ statistically from the Martignani sprays (54.01 to 60.92 ICD%). The highest variation in deposition quality was realised by the Nieuwoudt spray at 3263 l ha⁻¹ (60.92%). On inner canopy leaves the best deposition quality was realised by the 4000 l ha⁻¹ @ 1.88 km h⁻¹ Martignani spray (51.10 ICD%), however Nieuwoudt sprays at 10661, 7506 (54.62 and 53.40 ICD% respectively) and the Martignani spray at 4000 l ha⁻¹ @ 2.88 km h⁻¹ (57.65 ICD%) did not differ statistically from this treatment. Significantly higher variation in deposition quality was realised by Nieuwoudt sprays at 4895 and 3263 l ha⁻¹ (69.17 and 70.59 ICD% respectively) and Martignani sprays at 2000 and 1000 l ha⁻¹ (66.62 and 71.89 ICD% respectively), with mentioned sprays being statistically similar (Table 4.3.3.7).

Deposition uniformity

Analysis of variance of deposition uniformity (CV%) between leaves (in a 12 leaf batch) indicated no significant interactions ($P < 0.05$) but some significant main effects [Vertical canopy position ($P = 0.0278$); Horizontal canopy position ($P < 0.0001$) and Treatment ($P = 0.0090$) were observed. Significant main effects were discussed in relation to treatment interactions: treatment × horizontal canopy position ($P = 0.0862$) and treatment × vertical canopy position ($P = 0.1000$). On outer canopy leaves, the lowest variation in uniformity was realised by the Nieuwoudt sprays at 10661 and 7506 l ha⁻¹ realising statistically similar results (44.5 and 48 CV% respectively). The Nieuwoudt spray at 4895 l ha⁻¹ (64.42 CV%) realised lower but statistically similar variation in uniformity as the 7506 l ha⁻¹ spray with the lower volume of 3263 l ha⁻¹ (68.43 CV%) realising significantly higher variation in deposition on outer canopy leaves. Deposition uniformity realised by the Martignani sprays at all volumes did not differ significantly from each other, with the lowest variation realised by the slower tractor speed spray at 4000 l ha⁻¹ (66.84 CV%) and the highest variation at 1000 l ha⁻¹ (83.62 CV%) on outer canopy leaves. Deposition uniformity on inner canopy leaves realised by the Martignani spray also did not differ statistically from each other (77.80 to 84.36 CV%) as well as not from that achieved on outer canopy leaves for volumes separately, indicating good consistency in terms of uniformity. The best deposition uniformity on inner canopy leaves was realised at 2000 l ha⁻¹ (77.8 CV%). Very high variation in distribution was realised by the Nieuwoudt sprays at all volumes, with the lowest variation being realised by the 10661 l ha⁻¹ spray (70.61 CV%) and the highest at 3263 l ha⁻¹ (97.68 CV%). Mentioned application, the 7506 and the 4895 l ha⁻¹ sprays did not differ significantly from that of the Martignani sprays, especially the 2000 l ha⁻¹ spray (77.78 to 97.68 CV%) (Table 4.3.3.7).

Deposition efficiency

Analysis of variance of deposition efficiency (normalised FPC% to a spray volume of 1000 l ha⁻¹) indicated significant interactions treatment × horizontal canopy position ($P = 0.0004$) and treatment × vertical canopy position ($P = 0.0206$).

For the significant interaction treatment × horizontal canopy position, on outer canopy leaves, the most efficient spray deposition was realised by Martignani sprays at 4000 l ha⁻¹ @ 1.88 km h⁻¹ and 2000 l ha⁻¹ (1.49 and 1.26 FPC% respectively). Martignani sprays at 4000 l ha⁻¹ @ 2.78 km h⁻¹ and 1000 l ha⁻¹ realised to be less efficient (0.75 and 1.05 FPC% respectively) with the all Nieuwoudt sprays realising significantly less efficiency than the Martignani sprays, not differing statistically between spray volumes (0.63 to 0.33 FPC%). On inner canopy leaves all sprays realised statistically similar deposition efficiency (0.17 to 0.40 FPC%) (Table 4.3.3.7).

For the significant interaction treatment x vertical canopy position, on top canopy leaves, the most efficient spray deposition was realised by the Martignani sprays at 4000 l ha⁻¹ @ 1.88 km h⁻¹ and the Nieuwoudt spray at 4895 l ha⁻¹ (1.09 and 0.82 FPC% respectively). The rest of the sprays realised significantly less deposition efficiency on top canopy leaves, with treatments being statistically similar. On middle canopy leaves, deposition efficiency was mostly similar between treatments (0.29 to 0.69 FPC%) except for Martignani sprays at 2000, 1000 and 4000 l ha⁻¹ @ 1.88 km h⁻¹ and Nieuwoudt spray at 3263 l ha⁻¹, realising the most efficient deposition (1.03 to 0.69 FPC%). On bottom canopy leaves, all sprays were statistically similar (0.58 to 0.21 FPC%) except for Martignani sprays at 4000 l ha⁻¹ @ 1.88 km h⁻¹ and 1000 l ha⁻¹, which realised the most efficient deposition (0.90 and 1.04 FPC%, respectively) (Table 4.3.3.8).

Table 4.3.3.7. Mean deposition quantity (FPC%), quality (ICD%), uniformity (CV% between leaves) and efficiency (FPC% per 1000 l ha⁻¹) realised following sprays with water and pigment at various spray volumes with a Nieuwoudt and Martignani spray machine on inner and outer canopy leaves of a Washington navel orchard in Groblersdal 2013.

Treatment ^a	Deposition quantity (FPC%) ^b		Deposition quality (ICD%) ^b	
	Outer canopy	Inner canopy	Outer canopy	Inner canopy
Nw-10661 l ha ⁻¹ @ 300 KPa	7.03 ab	3.73 c	58.08 c-f	54.62 ef
Nw-7506 l ha ⁻¹	8.73 a	2.75 de	54.01 ef	53.40 ef
Nw-4895 l ha ⁻¹	6.17 b	3.93 c	60.92 b-e	69.17 ab
Nw-3263 l ha ⁻¹	4.00 c	1.86 def	63.50 a-d	70.59 a
Mg-4000 l ha ⁻¹ @ 1.88 km h ⁻¹	5.95 b	1.12 ef	52.31 ef	51.10 f
Mg-4000 l ha ⁻¹	3.02 cde	0.59 f	52.61 ef	57.65 def
Mg-2000 l ha ⁻¹	2.51 cde	0.42 f	57.79 def	66.62 abc
Mg-1000 l ha ⁻¹	1.05 ef	0.39 f	55.29 def	71.89 a
	Deposition uniformity (CV% between leaves) ^b		Deposition efficiency (FPC% per 1000 l ha ⁻¹) ^b	
	Outer canopy	Inner canopy	Outer canopy	Inner canopy
Nw-10661 l ha ⁻¹ @ 300 KPa	44.15 g	70.61 cde	0.66 e-h	0.35 g-i
Nw-7506 l ha ⁻¹	47.95 fg	83.87 a-d	1.16 a-d	0.37 f-i
Nw-4895 l ha ⁻¹	64.42 f	90.32 ab	1.26 ab	0.80 c-f
Nw-3263 l ha ⁻¹	68.43 cde	97.68 a	1.23 abc	0.57 f-i
Mg-4000 l ha ⁻¹ @ 1.88 km h ⁻¹	66.84 def	84.37 a-d	1.49 a	0.28 hi
Mg-4000 l ha ⁻¹	76.60 b-e	86.35 abc	0.75 d-g	0.15 i
Mg-2000 l ha ⁻¹	71.12 cde	77.78 b-e	1.26 ab	0.21 i
Mg-1000 l ha ⁻¹	83.62 a-d	80.78 a-e	1.05 b-e	0.39 f-i

^aTreatment layout consists of sprayer used (Nw – Nieuwoudt sprayer; Mg – Martignani sprayer) followed by specific spray volume applied

^bFor each parameter separately, values in each column followed by the same letter do not differ significantly ($P > 0.05$) according to Fisher's least significant difference test.

Table 4.3.3.8. Mean deposition quantity (FPC%), quality (ICD%), uniformity (CV% between leaves) and efficiency (FPC% per 1000 l ha⁻¹) realised following sprays with water and pigment at various spray volumes with a Nieuwoudt and Martignani spray machine on top, middle and bottom leaves of Washington navel orchard in Groblersdal 2013.

Treatment ^a	Deposition quantity (FPC%) ^b			Deposition quality (ICD%) ^b		
	Vertical canopy position			Vertical canopy position		
	Top	Middle	Bottom	Top	Middle	Bottom
Nw-10661 l ha ⁻¹ @ 300 KPa	5.44 bcd	6.15 abc	4.55 cde	55.46 de	56.74 de	56.842 de
Nw-7506 l ha ⁻¹	5.23 bcd	7.26 ab	4.74 cde	56.04 de	53.57 de	51.50 e
Nw-4895 l ha ⁻¹	8.00 a	4.48 cde	2.66 efg	57.62 cde	62.47 bcd	75.04 a
Nw-3263 l ha ⁻¹	2.67 efg	4.48 cde	1.64 fgh	69.63 ab	62.74 bcd	68.77 ab
Mg-4000 l ha ⁻¹ @ 1.88 km h ⁻¹	4.36 cde	2.67 efg	3.59 def	53.95 de	50.48 e	50.70 e
Mg-4000 l ha ⁻¹	1.20 fgh	1.95 fgh	1.48 fgh	56.60 de	50.95 e	57.84 cde
Mg-2000 l ha ⁻¹	1.17 gh	2.06 fgh	1.16 gh	64.09 bcd	53.93 de	68.60 ab
Mg-1000 l ha ⁻¹	0.29 h	0.83 gh	1.04 gh	67.50 abc	60.08 b-e	63.19 bcd
	Deposition uniformity (CV% between leaves) ^b			Deposition efficiency (FPC% per 1000 l ha ⁻¹) ^b		
	Vertical canopy position			Vertical canopy position		
	Top	Middle	Bottom	Top	Middle	Bottom
Nw-10661 l ha ⁻¹ @ 300 KPa	65.39 d-g	46.81 g	59.95 efg	0.51 d-i	0.29 de	0.43 hi
Nw-7506 l ha ⁻¹	68.94 c-g	55.6 fg	73.19 b-f	0.70 c-i	0.97 b-f	0.63 c-i
Nw-4895 l ha ⁻¹	56.12 fg	74.2 b-f	101.79 ab	1.64 a	0.92 b-g	0.54 d-i
Nw-3263 l ha ⁻¹	84.70 a-d	71.85 b-f	92.63 ab	0.82 c-i	1.37 ab	0.50 d-i
Mg-4000 l ha ⁻¹ @ 1.88 km h ⁻¹	75.47 b-f	84.61 a-d	66.73 c-g	1.09 bc	0.67 c-i	0.90 b-h
Mg-4000 l ha ⁻¹	84.08 a-d	71.47 b-f	88.87 abc	0.50 e-i	0.49 f-i	0.37 hi
Mg-2000 l ha ⁻¹	72.37 b-f	69.28 b-g	81.70 a-e	0.58 c-i	1.03 b-e	0.58 c-i
Mg-1000 l ha ⁻¹	77.57 b-f	85.73 a-d	83.29 a-e	0.29 h-i	0.83 c-h	1.04 bcd

^aTreatment layout consists of sprayer used (Nw – Nieuwoudt sprayer; Mg – Martignani sprayer) followed by specific spray volume applied

^bFor each parameter separately, values in each column followed by the same letter do not differ significantly ($P > 0.05$) according to Fisher's least significant difference test.

Deposition quantity results for the treatment × horizontal canopy position and treatment × vertical canopy position interactions were compared to the FPC benchmarks. On outer canopy leaves, Nieuwoudt sprays at 10661, 7506 and 4895 I ha⁻¹ and Martignani sprays at 4000 I ha⁻¹ @ 1.88 km h⁻¹ realised deposition quality above the FPC75 benchmark. Nieuwoudt sprays at 3263 I ha⁻¹ and Martignani sprays at 4000 I ha @ 2.88 km h⁻¹ realised deposition quantities above the FPC50 benchmark with the 1000 I ha⁻¹ Martignani spray realising deposition quality below the FPC50 benchmark. On inner canopy leaves, none of the sprays realised deposition above the FPC75 benchmark, with Nieuwoudt applications at 10661, 7506 and 4895 I ha⁻¹ being the only sprays to realise deposition above the FPC50 benchmark.

On top canopy leaves, Nieuwoudt sprays at 10661, 7506 and 4895 I ha⁻¹ and Martignani sprays at 4000 I ha⁻¹ @ 1.88 km h⁻¹ realised deposition quality above the FPC75 benchmark. Nieuwoudt sprays at 3263 I ha⁻¹ realised deposition quantity above the FPC50 line with the rest of the sprays realising quantities below the FPC50 benchmark. On middle canopy leaves, all Nieuwoudt sprays realised deposition quantities above the FPC75 benchmark. The Martignani application at 4000 I ha⁻¹ @ 1.88 km h⁻¹ was the only spray to realise deposition above the FPC50 benchmark, with the rest of the Martignani sprays being below. On bottom canopy leaves, only Nieuwoudt sprays at 10661 and 7506 I ha⁻¹ realised sprays above the FPC75 benchmark, whilst Nieuwoudt sprays at 4895 I ha⁻¹ and Martignani sprays at 4000 I ha⁻¹ @ 1.88 km h⁻¹ realised deposition above the FPC50 benchmark. The rest of the applications all realised deposition quantities below the FPC50 benchmark (Figure 4.3.3.5).

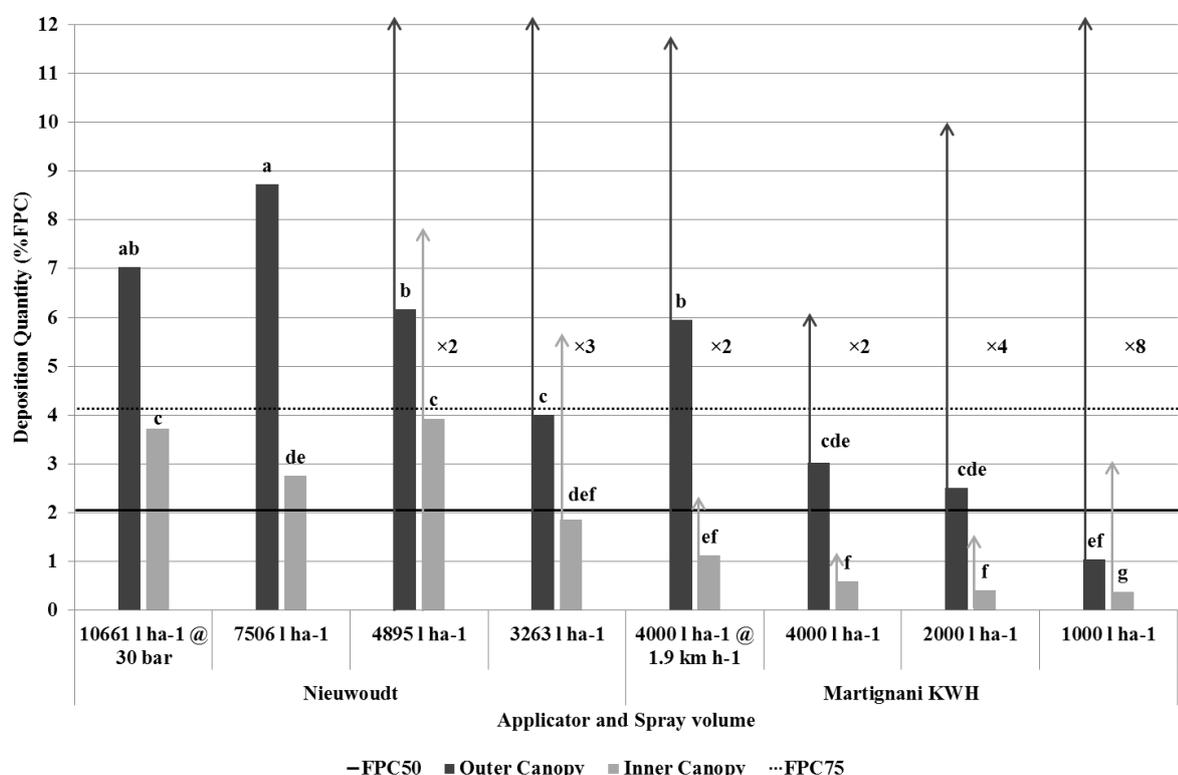


Figure 4.3.3.5. Mean deposition quantity realised by water and yellow fluorescent pigment sprays at ×1 fluorescent pigment concentration, together with lines indicating theoretical deposition quantity that can be realised when adjusting the product concentration respective to a 8000 I ha⁻¹ spray, on inner and outer canopy leaves of a Washington navel orchard following with a Nieuwoudt sprayer at 10661 I ha⁻¹ @ 30 bar; 7506 I ha⁻¹; 4895 I ha⁻¹; 3263 I ha⁻¹ and a Martignani KWH sprayer at 4000 I ha⁻¹ @ 1.7 km h⁻¹; 4000 I ha⁻¹; 2000 I ha⁻¹ and 1000 I ha⁻¹, compared to FPC₅₀ and FPC₇₅ benchmarks at 2.07% and 4.14% respectively (Groblersdal 2013).

Conclusion for Citrusdal and Groblersdal 2013.

These trials evaluated two spray applicators at different spray volumes throughout citrus tree canopies with the purpose of demonstrating to producers the redundancy of using high spray volumes and the benefits of reduced spray volumes – instigating an understanding of spray application and hopefully changing current trends and dogma in spray application. As in previous studies, the use of fluorometry, photomicrography and digital image analysis (Schutte et al., 2012; van Zyl., 2014) was effective in visualising, determining and evaluating deposition parameters of different spray treatments on citrus leaves. The implementation of spray deposition benchmarks (van Zyl et al., 2013) was insightful in evaluating deposition parameters in terms of

biological efficacy, as was also done by van Zyl et al. (2014). The fluorescent pigment used was proven by van Zyl et al. (2013) to be an accurate tracer for contact copper fungicides and have been used in other deposition studies successfully (Brink et al., 2006; Fourie et al., 2009; Schutte et al., 2012; van Zyl et al., 2010a, 2010b; van Zyl et al., 2013, 2014), therefore its use in this study. The two spray applicators evaluated, the Nieuwoudt and Martignani sprayer, both performed well in their respective fields (high- and low spray volumes respectively). Shared influencing factors such as tractor speed, PTO speed and canopy geometry and density, between spray applicators were kept constant where possible, to minimise influence from these factors on deposition parameters. Calibration was done to the best of the authors' knowledge, since improper calibration together with wrongful spraying techniques are usually the reason for poor deposition and therefore most treatment failures (Grout, 1997, 2003; Stover et al., 2002). The trial also served as an important precursor for future bio-efficacy trials, focussing on reduced volume fungicide and pesticide spray application.

In both spray trials, marked differences was found in deposition quantity, quality, efficiency and uniformity as influenced by the dynamics of different spray applicator used, spray volume and canopy density. With the Nieuwoudt sprayer high- to low volumes were sprayed (10661 to 2708 l ha⁻¹). On outer canopy leaves, deposition quantity increased with an increase in spray volume up to a point (6229 and 7506 l ha⁻¹), after which deposition quantity decreased. Loss in deposition quantity at the high volumes sprayed (8847 and 10661 l ha⁻¹) can be ascribed to aggravated run-off. Spray run-off can also be the reason for variability of deposition uniformity between different canopy positions. Spray run-off, as influenced by various factors in different cropping systems (Cross et al., 2011; Furness et al., 1998; Farooq and Salyani, 2002; Salyani and Whitney, 1990; Salyani et al., 2007; Chueca et al., 2009; Cunningham and Harden 1998; 1999; Stover et al., 2002) especially high volume sprays, is a well-documented phenomena but still ignored widely by South African citrus producers since high volume sprays act as a buffer for other shortcomings during the application process such as wrongful calibration, technique, equipment and operator error. The redundancy of this style of application (washing) needs to be addressed. Deposition quantity, quality and uniformity on inner canopy leaves increased with increase in spray volume and decreased with decreasing spray volume with the Nieuwoudt sprayer. This can be ascribed the hydraulic pressure of the spray system which increases spray volume increases and decreases with lowering spray volume. This is a substantial flaw in hydraulic spray systems which is not air assisted since it limits the Nieuwoudt spray only to be used at volumes higher than 8000 l ha⁻¹, as canopy penetration would be inefficient at higher volumes, decreasing deposition quantity, quality and uniformity. Van Zyl et al. (2014) indicated that the density of canopies complicates the penetration of it by the spray mixture. Both canopies rated 4.5/5 density scale. The very dense canopies most definitely influenced spray penetration in this trial, as indicated by low deposition quantity, quality and uniformity data achieved at lower volumes with the Nieuwoudt sprayer. Increase in spray pressure to 300 kPa also did not improve canopy penetration in both trials in relation to 150 kPa sprays. Unfortunately canopy density was not quantified. The crude 5-point scale used was effective in determining canopy density but should be improved in future studies. Furthermore, as indicated by the bio-efficacy benchmarks (van Zyl et al., 2013), dilute sprays at volumes below 8000 l ha⁻¹ would realise deposition quantity below the FPC75 benchmarks, entailing insufficient deposition for contact protectant fungicide sprays which is unacceptable when spraying for the control of zero-tolerance pathogens such as citrus black spot.

Lower spray volumes were applied with the Martignani, and subsequently realised significantly lower deposition quantity (below the FPC75 benchmark) and uniformity. Interestingly, no significant difference was found in deposition parameters between the 4000 and 2000 l ha⁻¹, whilst achieving good deposition uniformity and deposition quality in both trials. This illustrates the improved performance of the Martignani (air shear) system at reduced volumes (2000 l ha⁻¹), and is supported by the deposition efficiency achieved at this spray volume. Canopy penetration was however poor at the reduced volume sprays, realising poor deposition quantity (below the FPC50 benchmark), quality and uniformity on inner canopy leaves. Theoretically, if the deposition quantity values are increased by the concentration factor needed to achieve the same amount of active ingredient on the target surface of a "normal" 8000 l ha⁻¹ spray (1000 l ha⁻¹ × 8; 2000 l ha⁻¹ × 4; 4000 l ha⁻¹ × 2), deposition quantity would be more than sufficient for effective disease control (above the FPC₇₅) (Figure 1 and 2). This would however not be the case on inner canopy leaves, where deposition quantity would still be below the FPC₅₀ benchmark (Figure 1 and 2) due too poor canopy penetration. This highlights the fact that reduced volume applications will only be effective if canopies are managed correctly, kept well aerated, since dense canopies hinder the penetration of the spray plume (van Zyl et al., 2014; Salyani and Whitney, 1990; Farooq and Salyani, 2002). This would also be relevant for sprays with the Nieuwoudt at lower application volumes (Figures 4.3.3.1 and 4.3.3.2).

Conclusions to date

Results from these studies showed that high volume ($\geq 8000 \text{ l ha}^{-1}$) foliar applications do realise adequate deposition quantity and good deposition uniformity throughout the canopy with conventional spray application systems. High volume application also serves as a buffer for wrongful calibrations and neglected sprayer maintenance. Lower volume applications showed the potential of achieving good deposition parameters above the FPC50 and 75 benchmarks, indicating the potential to realise good disease control with improved and cheaper work rates, less product loss through run-off and drift and therefore less environmental pollution.

Excessive spray volumes ($> 10000 \text{ l ha}^{-1}$) did not improve deposition parameters and a considerable amount of water and product is wasted in terms of run-off and drift. Reduced volume sprays together with increased product concentrations resulted in better deposition parameters than that of current dilute, high volume spray applications. However, spray uniformity throughout the canopy was superior in high volume applications, indicating the necessity of adequate canopy management to allow spray penetration.

Reduced volume application will be evaluated in seasonal bio-efficacy trials especially against zero-tolerance pests such as citrus black spot and false codling moth in a new project (1132). Also, the increase in plant protection product concentrations with reduced volume sprays also needs to be evaluated in terms of safe use through evaluation of maximum residue limit (MRL) and phytotoxicity evaluations. Reduced volume spray application should be viewed and implemented as a “precision farming” tool. There would be no room for error, since there is no more “high volume” buffer to account for poorly maintained and calibrated machinery, calibration errors, spray technique, orchard management (canopy pruning) and operator error.

Spray volumes selected for use in these studies was not based on any specific measurement. For more precise calibration and application, it is of the authors' opinion that spray volumes in three dimensional crops should be based on the specific canopy volume (based on canopy geometry and density) to be sprayed and not area sprayed. This has been suggested by various other authors (Koch, 2007; Walklate et al., 2006; Walklate and Cross, 2012; Toews and Friessleben, 2012) and is proposed for implementation in other countries (Manktelow and Praat, 1997; Tumbo et al., 2001; Walklate and Cross, 2009) and other three dimensional crops (Sutton, 1988; Furness et al., 1998; Siegfried et al., 2006). However, this shift from area-to-volume based calibration is hampered by current dose rate expression in South Africa and many other countries around the world on which most product labels are registered (l ha^{-1} ; $\text{l or g Plant protection product per 100 l}$) and needs to be addressed in future.

Whilst pest and disease management is successful using the current high volume applications, it does come at the significant cost of run-off and drift losses, possible environmental pollution and expensive work rates. Optimised spray application at reduced volumes will substantially reduce these costs and has demonstrated the potential to maintain adequate spray deposition. Future research will investigate this potential in semi-commercial trials.

Future research

Two projects have been approved for funding. One from April 2014 titled: Development of a tree canopy characteristic calibration formula for reduced volume fungicide application in Southern African citrus orchards (project no. 1089) and one from April 2015 titled: Evaluation of reduced volume fungicide and pesticide sprays for control of citrus black spot and false codling moth (project no. 1132). To date spray deposition assessment has focussed on deposition on leaves (due to logistics) and leaf deposition needs to be correlated with fruit deposition. Also, cheap and efficient methodology needs to be developed to analyse deposition parameters on fruit at the same intensity that it is done on leaves.

Technology transfer

- Orchard spray demonstration (Addo, Hoedspruit) 2008
- Study group meeting and orchard spray demonstration (Constantia-Letsitele) 2008
- Orchard spray demonstration (Groblersdal, Citrusdal, Clanwilliam, Hoedspruit) 2009
- Study group meetings (Eastern and Western Cape) 2009
- Oral presentation at the 10th Workshop “Spray application techniques in fruit growing, SuproFruit 2009”, Wageningen, The Netherlands (30 Sept – 2 Oct 2009): Paul Fourie, Jan-Cor Brink, Tian Schutte, Tim Grout. 2008. Optimal use of spray machines in South African citrus orchards.
- Invited oral presentation at Cape Pomological Association, Stellenbosch (2 June 2009): Fourie PH, Brink JC, van Zyl S, Schutte T. 2009. Improving fungicide application: reality, options and impact.

- JG van Zyl and PH Fourie. "General spray application guidelines". Invited lecture at USPP Short Course day 2012.
- JG van Zyl, PH Fourie and GC Schutte. Evaluation of adjuvants to improve fungicide spray deposition and control of *Alternaria* brown spot in South African citrus orchards. Oral presentation at SASPP Western Cape PhD research day 2012.
- JG van Zyl, PH Fourie and GC Schutte. Spray deposition benchmarks for control of *Alternaria* brown spot and evaluation of adjuvants to improve fungicide spray deposition in citrus orchards. Oral presentation at 7th CRI Symposium 2012.
- JG van Zyl, D Viljoen, E Sieverding and PH Fourie. Evaluation of Break-Thru S240 and Break-Thru Union at different application volumes in South African citrus orchards. Poster presentation at 7th CRI Symposium 2012.
- JG van Zyl, PH Fourie and GC Schutte. General spray application guidelines for citrus production in South Africa. Oral presentation at CRI Road show 2012 South Africa
- JG van Zyl, PH Fourie and GC Schutte. Spray deposition benchmarks for control of *Alternaria* brown spot and evaluation of adjuvants to improve fungicide deposition in citrus orchards. Oral presentation at International Citrus Congress (ICC) 2012 Spain.
- JG van Zyl, PH Fourie and GC Schutte. Improvement of spray deposition and control *Alternaria* brown spot on mandarin leaves following sprays with copper oxychloride and selected adjuvants. Poster presentation at 48th SASPP congress 2013.
- JG van Zyl, PH Fourie and GC Schutte. General spray application guidelines for citrus production in South Africa. Oral presentation at Citrusdal producer technical study group, February 2013.
- Gideon van Zyl – Successful proposal to upgrade his MSc to PhD study
- Van Zyl J.G., Fourie P.H., Schutte G.C. 2013. Spray deposition assessment and benchmarks for control of *Alternaria* brown spot on Mandarin leaves with copper oxychloride. Research article published in *Crop Protection* 46: 80-87.
- Fourie, P.H., van Zyl, J.G., Schutte, G.C., Grout, T.G. Optimisation of spray application in South African citrus orchards: challenges and progress. 12th Workshop on spray application techniques in fruit growing (SuproFruit 2013), 26-28 June 2013; Universitat Politècnica De València, Valencia, Spain – oral presentation
- Van Zyl, J.G. P.H. Fourie, G.C. Schutte and T.G. Grout. Spray application guidelines for citrus production in South Africa. Oral presentation at CRI Pest and Disease Management Workshops throughout the country, September 2013.
- Van Zyl, J.G. , P.H. Fourie, G.C. Schutte, T.G. Grout and JG van Zyl snr. Spray application in citrus – Sprayer calibration and checklist. *CRI Cutting Edge* no 166.
- van Zyl, J.G., Grout, T.G., Schutte, G.C., Fourie, P.H. 2014. Evaluation of two novel spray applicators for fungicide spray application in South African citrus orchards. 8th CRI Citrus Research Symposium, Champagne Sports Resort, Winterton, KwaZulu-Natal, South Africa (17-20 August 2014).
- van Zyl, J.G., Grout, T.G., Schutte, G.C., Fourie, P.H. 2014. Understanding the science of spray application in citrus: getting industry back on track. 8th CRI Citrus Research Symposium, Champagne Sports Resort, Winterton, KwaZulu-Natal, South Africa (17-20 August 2014).
- van Zyl, J.G., Sieverding, E.G., Viljoen, D.J., Fourie, P.H. 2014. Evaluation of two organosilicone adjuvants at reduced foliar spray volumes in South African citrus orchards of different canopy densities. *Crop Protection* 64, 198-206.
- van Zyl, J.G., Schutte, G.C., Grout, T.G., Fourie, P.H. 2015. Understanding and optimising fungicide spray application in citrus orchards through plant pathology research. 49th Congress of the Southern African Society for Plant Pathology, Bains Lodge, Bloemfontein, Free State, South Africa (19-21 January 2015).
- van Zyl, J.G., Schutte, G.C., Grout, T.G., Fourie, P.H. 2015. Understanding and optimising fungicide spray application in citrus orchards through plant pathology research. Spray application workshop, Letaba Junction, Letsitele, Limpopo, South Africa (14 January 2015) and at Bayer Cropscience, Paarl, Western Cape, South Africa (23 January 2015).
- Claassen, J. 2014 A spraying strategy for CBS. *Farmer's weekly*, issue 15010, 13 March 2015

References cited

- JC Brink, G Holz, FJ Calitz, PH Fourie. 2004. Development of a protocol to quantify spray deposits of grape bunches. Pages 230-235 in: *Proceedings of the 7th International Symposium of Adjuvants for Agrochemicals (ISAA2004)*. Cape Town, South Africa, 8-12 November.
- Brink JC, G Holz, PH Fourie. 2006. Effect of fungicide spray cover on *Botrytis cinerea* infection in grape bunches. *South African Journal of Enology and Viticulture* 27: 51-56.
- Chang YC and Seguin-Swartz G. 2002. A rapid method for assessing the viability of fungal spores. *Can. J.*

- Plant. Pathol. 24: 230-232.
- Cunningham GP, J Harden. 1998. Reducing spray volumes applied to mature citrus trees. *Crop Protection* 17: 289 - 292.
- Cunningham GP, J Harden. 1999. Sprayers to reduce spray volumes in mature citrus trees. *Crop Protection* 18: 275 - 281.
- Fourie PH, du Preez M, Brink JC, Schutte GC. 2008. The effect of run-off on spray deposition and control of *Alternaria* brown spot of mandarins. *Australasian Plant Pathology* 38: 173-182.
- Furness GO, Magarey PA, Miller PH, Drew HJ. 1998. Fruit tree and vine sprayer calibration based on canopy size and length of row: unit canopy row method. *Crop Protection* 17: 639-644.
- Furness GO, AJ Thompson, DWL Manktelow. 2006a. Visual droplet number rating chart and fluorescent pigment sprays to estimate chemical deposition and spray coverage on plant foliage. Proceedings of the Association of Applied Biologists' conference for International advances in pesticide application. Robinson College, Cambridge, 10-12 January 2006.
- Furness GO, AJ Thompson, DWL Manktelow. 2006b. Multi-fan spray towers to improve dose efficiency and spray coverage uniformity in citrus trees. Proceedings of the Association of Applied Biologists' conference for International advances in pesticide application. Robinson College, Cambridge, 10-12 January 2006.
- Grout TG. 1997. Spray volumes and coverage requirements for citrus in southern Africa. *Citrus Journal* 6(3): 19-20.
- Landers A, M Farooq. 2004. Factors influencing air and pesticide penetration into grapevine canopies. *Aspects of Applied Biology* 71, International advances in pesticide application.
- Orbovic V, Achor D, Syvertsen JP. 2007. Adjuvants affect penetration of copper through isolated cuticles of *Citrus* leaves and fruit. *HortScience* 42: 1405-1408.
- Van Zyl JG, Fourie PH, Schutte GC. 2013. Spray deposition assessment and benchmarks for control of *Alternaria* brown spot on Mandarin leaves with copper oxychloride. *Crop Protection* 46: 80-87.

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

Project 1015 (April 2011 - March 2014) by GC Schutte, Charl Kotze and PH Fourie (CRI)

Opsomming

Botrytis cinerea Pers. is welbekend as die veroorsakende organisme wat bloeiselversenging op suurlemoene veroorsaak. Die saprofitiese aard van die patogeen lei daartoe dat dit op organiese materiaal met 'n lae pH kan oorleef en van hier af het die patogeen die vermoë om blomme te infekteer. Om te bepaal watter blomdeel en -stadium meer vir swamgroei geskik is en dus geteiken moet word vir die effektiewe beheer van die siekte, is die pH, Brix en swamgroei op 'n blomblaar- en stuifmeeldraad-ekstrak-medium van verskillende sitrus kultivars oor twee seisoene in Witrivier gemeet. 'n Verdere analiese is ook van dieselfde kultivars se blomme in Addo gemaak. Geen patroon kon bepaal word nie aangesien resultate wisselvallig was. Aangesien ons hoofdoel is om suurlemoenblomme teen Botrytis te beskerm, is spesifieke swamdoders vir *in vitro* en *in vivo* evaluasie geselekteer. *In vitro* resultate het getoon dat Botrytis mees sensief was teen benomyl, gevolg deur iprodione. 'n Metos weerstasie is op die proefperseel in Witrivier geïnstalleer om die infeksieperiodes tydens die proeftydperk te bepaal. Hieruit kon ons vasstel dat daar waarskynlik net een infeksieperiode in September 2011 was. Blomme is op 1 Oktober 2011 met Botrytis spore geïnkuleer alvorens die swamdoders toegedien is om te verseker dat daar voldoende inokulum teenwoordig is. In 2012 was die blomperiode eers in Oktober en die weerdata wat in hierdie periode gemonitor was, was ook ongunstig vir die siekte. Alhoewel daar geen blominfeksies op die onbehandelde kontrole waargeneem is nie, is blomme geoes en op 'n selektiewe medium uitgeplaat nadat hulle op 16 Oktober 2012 ook met Botrytis spore geïnkuleer is, alvorens die swamdoders toegedien is om te verseker dat daar voldoende inokulum teenwoordig is. Resultate van beide seisoene toon dat benomyl en iprodione goeie beheer van die siekte gegee het, terwyl fenhexamid en pyrimethanil in 2012 ook goeie beheer van die siekte gegee het. 'n Kommersiële bespuiting van Benlate in Addo het getoon dat blomme wat toe is (ballonstadium) langer beskerm word as oop blomme. In 'n veldproef in die Sondagsriviervallei in September 2013 is 'n uitermatige hoë voorkoms van Botrytis-agtige riuwe op suurlemoene waargeneem en geeneen van die drie swamdoderbespuitings getoets teen Botrytis kon dit verhoed nie. Uitermatige toedienings van stikstof word as oorsaak van die riuwe aangevoer. Suurlemoenblomme wat met 'n fluoriserende pigment in water gespuit is, en toe weer met die pigment met mancozeb met Breakthru Union, mancozeb met NuFilm 17 en mancozeb alleen, toon swak bedekking van alle blomdele en kon ongelukkig nie geanaliseer word nie weens die kontrasverskille tussen die wit blomdele en die agtergrond.

Summary

Botrytis cinerea Pers. has long been known as the causal pathogen of blossom blight on lemons. The saprophytic nature of the pathogen allows it to survive on a variety of organic matter with a low pH. To determine which stadium of blossom development is most suitable for infection and when they should be sprayed for the control of Botrytis, the pH, Brix and mycelium growth rates of Botrytis on a petal- and stamen-extract media of different citrus cultivars were compared over two seasons in White River. A further analysis of the same cultivars was performed on flowers from Addo. No pattern could be detected as results were inconsistent. Because it is our aim to protect lemon blossoms against Botrytis, certain fungicides were selected for *in vitro* and *in vivo* evaluation. *In vitro* results showed that Botrytis was most sensitive to benomyl, followed by iprodione. A Metos weather station was installed next to the orchard that was earmarked for the field trial to determine the infection periods during the experimental period. Only one infection period was predicted to have occurred during September 2011. The trees only started blossoming in October 2012 and the weather data showed that the conditions were unsuitable for infection. Blossoms were also pre-inoculated with Botrytis spores before the fungicides were applied to ensure sufficient inoculum. Although no blossom infections were observed on the untreated trees, blossoms were harvested after they were treated and plated out on a selective medium after they were inoculated on 16 October 2012 with Botrytis conidia. Results over two seasons showed that benomyl and iprodione performed the best in controlling the disease, while fenhexamid and pyrimethanil, also performed well in 2012. A commercial application of benomyl in Addo showed that blossoms in the balloon stage were better protected than open blossoms. Water mixed with fluorescent pigment showed that coverage of lemon petals and stamen was poor. In an orchard evaluation in the Sunday's river valley during September 2013 an excessive appearance of Botrytis-like ridges on lemon fruit were observed, which none of the three fungicide treatments could prevent. The ridges were attributed to application of excessive nitrogen. Lemon flowers sprayed with a fluorescent pigment and again with mancozeb plus Breakthru Union, mancozeb plus NuFilm 17 and mancozeb alone resulted in poor coverage of all flower parts, but deposition could not be analysed due to the poor contrast between the white flowers and the background.

Introduction

Botrytis cinerea Pers. has long been associated with flower and premature flower fall of lemons (*Citrus limon* (L.) Burn.) as well as destructive pre- and postharvest fruit rot (Klotz *et al.*, 1946; Calavan *et al.*, 1952) on lemons. Not only can infection by the pathogen lead to premature petal fall and fruit drop, but also contribute to fruit scarring and rind distortion that ultimately lead to yield loss. The saprophytic nature of the pathogen allows it to survive on a variety of organic matter such as; twigs, soil, fallen leaves and damaged or dead wood (Klotz *et al.*, 1946; Timmer *et al.*, 2000). From there the pathogen has the ability to infect weaker tissue such as petals (Palmer *et al.*, 1997). However, establishment of the pathogen relies heavily on moisture and therefore it only appears during seasons when wet, rainy conditions prevail during bloom (Klotz *et al.*, 1946; Calavan *et al.*, 1952). Fullerton *et al.* (1999) found that floral debris gets caught among the clusters and fruitlets during wet periods and thus serves as the principal source of inoculum in lemon orchards.

Management of the disease relies mainly on the application of chemicals when foggy or wet conditions are expected, the recommended chemicals for citrus being copper, dithiocarbamate and benzimidazoles (Timmer *et al.*, 2000). It has been reported that certain chemicals such as pyrimethanil, pyrrolnitrin and fenhexamid have excellent activity against *B. cinerea* on grapes (Rosslénbroich & Steubler, 2000). Furthermore, Palmer *et al.*, (1997) concluded that bicarbonates have the ability to inhibit mycelium growth of the pathogen. However, due to the saprophytic nature of the pathogen large reservoirs of inoculum are continuously present in orchards during the season, combined with the rapid development of buds and blossoms, the difficulty in managing the disease increases (Weathers, 1957). Weathers (1957) reported that the efficacy of chemical application with contact fungicides such as zineb, only last for approximately 2 weeks. With *B. cinerea* being highly capable of resistance against benzimidazole and dicarboximide fungicides (Beever *et al.*, 1989), multiple sprays during bloom could contribute to the build-up of resistance towards certain chemicals.

Searching for chemicals that are safe for humans and compatible with the environment is the impetus driving research on bicarbonates as an agent for *Botrytis* control. There is a need for new methods to control *B. cinerea*, because many chemicals will no longer be available for growers due to increasing registration costs and fungal resistance. Many salts with a basic pK_a value inhibit colony growth, indicating that pH may be a reason for the detrimental activity of bicarbonates. *Botrytis* grows better in acidic to neutral conditions than in alkaline environments and germinates in acidic conditions as well (Palmer *et al.*, 1997). Sodium bicarbonate was selected as such a chemical for field trials against *Botrytis* to search for an alternative.

The strict environmental requirements for infection, contribute to the sporadic appearance of the disease from season to season. For this reason, management of the disease is not seen as high priority by producers. Therefore, there are currently no known registered chemicals on the South African market for the control of *Botrytis* blossom blight.

Stated objectives

1. To determine which blossom parts ideally suit *Botrytis* growth; this will indicate how often blossom sprays will have to be applied.
2. To identify promising fungicides *in vitro* and the evaluation of these fungicides in field trials.
3. Disease forecasting using the grapevine model.

Materials and methods

1. Field evaluation of fungicides at 'Fountains', White River and Addo

2011 – 2012 season

Because the trees are so densely planted, five trees were marked for each block of which the middle three was used for each treatment and the other two (one on each side) as buffer trees within each row. benomyl (Benomyl, 500g/kg WG, Villa Crop Protection), iprodione (Iproflo, 500g/l SC, ICA International Chemicals), didecyl dimethyl ammonium (Sporekill, 120g/l SL, ICA International Chemicals) and sodium bicarbonate (Table 4.3.4.1.) was selected for evaluation against *Botrytis*. Five replicates per treatment were sprayed with each selected fungicide to the point of run-off with hand-guns. Sprays were only done at full bloom as no *Botrytis* infection was observed during this period. Every second row was not treated to serve as a buffer between the rows. On each of the 3 trees, 10 flower clusters were inoculated with a spore suspension (1×10^6 spores/ml) of *Botrytis* 7 days before application. After an incubation period of 7 days (8 October 2011) the trees were treated, a total of 20 flowers were removed randomly (15 October 2011) from the inoculated flower clusters for each of the treated trees and plated on a selective medium (Kerssies) for 7 to 10 days at

20°C. Individual flowers were rated for the presence or absence of Botrytis infection and means calculated as the amount of flowers infected.

2012 - 2013 season

The same orchard and the same protocol was used as described previously. benomyl, iprodione, didecyl dimethyl ammonium (DDA), fenhexamid (Teldor, 500g/l SC, Bayer) and pyrimethanil (Protector 400 SC, 400g/l SC, ICA International Chemicals) was selected for this field trial (Table 4.3.4.2.). On each of the 3 trees, 10 flower clusters were inoculated with a spore suspension (1×10^6 spores/ml) of Botrytis 7 days before application. After an incubation period of 7 days (9 October 2012) the trees were treated, a total of 20 flowers were removed randomly (16 October 2012) from the inoculated flower clusters for each of the treated trees and plated on a selective medium (Kerssies) for 7 to 10 days at 20°C.

2013 – 2014 season

A lemon orchard was selected with a history of Botrytis infection in the Sundays river valley (SRV). Three rows of trees uniform in size were grouped into 4 blocks and sprayed with an air blast sprayer on a commercial scale at 100% petal fall on 30 September 2013. Each tree received 10.4 l/tree or 8 632 l/ha. benomyl, iprodione and pyrimethanil was selected for this field trial. Unfortunately, untreated trees were not included in this semi-commercial field trial. Due to the high density of the trees, five groups of five trees each were grouped together in which fruit were randomly selected within the blocks and harvested to determine yield per tree. Fruit of each tree was bulked and weighted to determine the yield of each treatment. From the harvested fruit, 100 fruit was randomly selected and the presence of ridges on the rind associated with Botrytis recorded.

Benomyl's protective activity after a commercial application was also evaluated in this orchard. benomyl was commercially applied to a lemon orchard on the night of 2 October by the grower in Addo in the Eastern Cape. Open and closed flowers ($n = 10$) were removed the next day from the trees as well as on days 3, 7 and 14 using a pair of tweezers. Three flowers were placed onto each petri dish containing Kerssies' selective medium. A mycelium plug (2 mm in diameter) from a *Botrytis cinerea* colony was placed adjacent to the flower parts to allow mycelial overgrowth.

2. Laboratory evaluations:

- i) Determining the pH and Brix of lemon, Nadorcott, Valencia and navel orange flower parts

2011-2012 season

Flowers of lemon, Nadorcott, Valencia and navel orange cultivars were collected (± 50 g) from unsprayed orchards in the Nelspruit area. Stamens and petals were separated from the flowers, bulked and 8 g from each cultivar with 3 replicates were crushed in plastic bags using a Ryobi bench drill press with an Agdia tissue homogenizer (ACC 00930). The pH of each sample was then measured within each plastic bag using a Cyberscan 500 pH meter and the pH recorded. The Brix (%) was also measured and recorded by using the same samples with an Atago PAL 1 refractometer.

2012-2013 season

An additional set of plant material was collected from orchards in Addo and analysed using the same protocol for flowers from the same orchards were sampled as described previously.

- ii) Growth media derived from citrus flowers to determine the ideal growth medium for *B. cinerea*.

2011-2012 season

From each of the different citrus cultivars' flowers, 24 g stamen and 24 g petals were crushed using Ryobi bench drill press with tissue homogenizer. These samples were left for 24 h to soak in 1L distilled water after which the flasks were autoclaved and left for another 24 h to soak. They were then autoclaved for a second time. Thereafter, each sample was filtered through filter paper and amended with 15 g bacteriological agar per litre water and autoclaved for a third time. Onto these, a 10 mm² colony was aseptically removed from the outside circumference of a *B. cinerea* colony and placed in the centre of each plate. After 7 days the colony diameter was measured using a calliper.

2012-2013 season

An additional set of plant material was collected from orchards in Addo and analysed using the same protocol for flowers from the same orchards were sampled as described previously.

3. Accumulation of weather station data to determine the time/temperature relationships for infection

A Metos telemetric weather station was operated during the 2011-2012 period to monitor the weather conditions. This station was placed in a safe locality close to the experimental plot. Temperature, RH, rain and leaf wetness were recorded for September and October. Petals occurred during the first week of September 2011 and due to uneven blossom, 100% petal drop took place from the beginning to mid October 2011.

On grapes, slow drying conditions together with high humidity (90% or higher) favour the development of Botrytis. Suitable temperatures plus a corresponding period of slow drying conditions must exist for bunch rot infection to occur. These range from 10°C for 30 hours or more, 15.5°C for 18 hours or more, 22.5°C for 15 hours or more, 26.5°C for 22 hours or more and 39°C for 35 hours or more. These parameters were adopted and used for our prediction model on the lemons to calculate how many and when infection could have taken place.

In September 2012, a Model nr WH1081PC, Professional Touch Screen Weather Center, Tycon Systems Inc.14641 S 800 W Ste A Bluffdale, UT 84065 was installed in the orchard. Blossom was late in that season, therefore weather data for October was only recorded and analysed. Weather data for the SRV for 2013 was also obtained.

4. Spray coverage of lemon flowers

Lemon branches with flowers were collected and sprayed with a gravity feed mist spray gun which was mounted onto a compressor (ITW DEVILBISS Spray Equipment Products, 195 Internationale Blvd, Glendale Heights IL 60139 USA) and fitted with a fluid nozzle tip of 0.8 mm in diameter. Spraying was done in a laboratory with post-run-off volumes using Yellow Fluorescent pigment (400 g/L, EC, South Australian Research and Development Institute, Loxton SA 5333 Australia) at 1 ml/L water that was added to the spray mixture. After drying off, the flowers were illuminated using a Labino Mid-light (UV-A; ≈365 nm) and digital photos were taken of upper and lower surfaces of leaves and fruit using a Canon EOS 40 D camera equipped with a 50 mm macro lens. This trial was repeated and mancozeb plus Breakthru Union, mancozeb plus NuFilm 17 and mancozeb alone was applied to Valencia orange flowers and analysed as described before.

Results and discussion

1. Field evaluation of fungicides at 'Fountains', White River

2011-2012 season

No natural Botrytis infection was noticed on lemon blossoms of control trees at "Fountains" at White River. Conditions were too dry to favour infection. Because the blossoms were pre-inoculated with Botrytis, blossoms were harvested and placed on a selective medium (Kerssies) to promote Botrytis growth only. Results showed that Botrytis first occurred on the stamen and then spread to the petals (Fig. 4.3.4.2). The incidence of Botrytis recorded after 7 and 10 days was not significantly less between benomyl (25 g and 50 g/100 L water) and iprodione (100 ml/100 L water) and DDA (200 ml/100 L water) (not at 10 days) sprayed trees; only benomyl and iprodione sprayed trees were significantly better than the untreated control. Both rates of sodium bicarbonate were not significantly different from the control (Table 4.3.4.1).

2012-2013 season

Conditions during blossom were also not favourable for natural infection at 'Fountains' at White River. Blossoms had to be pre-inoculated with Botrytis and placed on a selective medium (Kerssies) to promote Botrytis growth as described previously. Results showed that all the fungicides protected the petals and stamen for 24 hours (if compared with the untreated control). Of these benomyl (both rates) and fenhexamid resulted in no growth on the stamen after 24 hours while the other fungicides showed signs of fungal growth. Between 24 and 72 hours, the fungicidal protection collapsed as no significant control was achieved (Table 4.3.4.2).

2013-2014 season

As an untreated control was not included in the semi-commercial field trial, no proper comparisons between treatments could be made. Regardless of this, none of the treatments were effective in controlling the appearance of ridges on the fruit. There were also no differences in the yield as well (Table 4.3.4.3). Discussions with other growers in the valley ascribed the high incidence of ridges on these lemons due to high levels of nitrogen application in the orchards (Fig. 4.3.4.8). Inspections and visits of lemon orchards on neighbouring farms where no ridges were detected on the same crop of the same age indicated that the ridges was most probably not caused by Botrytis infection.

2. Laboratory evaluations

i) Determining the pH and Brix of lemon, Nardorcott, Valencia and navel orange flower parts

2011-2012 season

Results showed that pH levels of lemon petals and stamen (4.95 and 5.15, respectively) were significantly lower than Clementine, navels and Valencia oranges. Although the Brix reading of lemon stamens was the highest (7.82%), it was not significant different from Valencia oranges and Clementine stamen. The Brix of lemon stamen was, however, significantly higher than lemon petals, while navel petals had the lowest Brix reading. Navel petals and stamen had the lowest Brix readings of all the cultivars (Table 4.3.4.5.).

2012-2013 season

White River

Results showed that there were no significant differences between the pH of lemon petals and stamen and the other cultivars. The pH of the petals of Clementines, lemons and navels were lower than 6 while the stamen were higher than 6. In comparison, the pH levels of Valencia oranges were significant higher (> 8.85) than the other cultivars tested. The Brix of lemon stamen was again, if compared with the 2011-2012 season, higher than the lemon petals. Navel petals had the highest Brix reading of 8.47 with a 7.57 reading for the stamen, which is between 2.5% to 3% higher than the previous season when it had the lowest reading of all the cultivars tested (Table 4.3.4.6).

Addo

Results showed that pH levels of petals and stamen of all the cultivars were closely grouped (5.28 – 6.42) of which petals of Valencia had the lowest reading of 5.28 followed by Clementines, navels and lemons. The pH of Valencia stamens was also significantly lower than the other three cultivars. The Brix of lemon petals and stamen were also significant lower in comparison with the other cultivars. The Brix readings of stamen of Clementine and navels had the highest readings of 6 and 6.4 respectively which is significant more than both lemon petals and stamen readings (Table 4.3.4.7).

ii) Determine which blossom parts ideally suit Botrytis growth

2011-2012 season

When incorporated in a bacteriological medium, lemon petals and especially lemon stamen stimulated significant more mycelium growth of Botrytis, followed by Valencia stamen. No mycelia growth was recorded on any of the petal media, except for lemons. The growth on lemon petal medium was also significant less than that for lemon stamen medium. Therefore, all the results show that lemon stamen is the ideal part for Botrytis to grow on (Table 4.3.4.5).

2012-2013 season

White River

Although lemon stamen medium resulted in the highest mycelial growth of Botrytis, it was not different from Clementine petals and stamen media. No Botrytis growth was recorded on media amended with petals and stamen of Valencia oranges (Table 4.3.4.6).

Addo

An additional set of flowers from Addo was included to see how they differ from White River in the same year. Apart from navel stamen medium, media amended with petals and stamen of Clementines resulted in significant higher mycelial growth of *Botrytis* than the media amended with flower parts of the other cultivars (Table 4.3.4.7).

Evaluation of benomyl's protective capacity after a commercial application in Addo showed that *Botrytis cinerea* can be more effectively controlled on closed or balloon stage flowers than open flowers. However, benomyl's fungicidal protection broke down after between 7 and 14 days (Figure 4.3.4.3).

3. Accumulation of weather station data to determine the time/temperature relationships for infection

White River

2011-2012 season

Temperature, relative humidity (RH), leaf wetness and rainfall were recorded during the experimental period from September to October 2011. Using the parameters for grapes to determine ideal climatic conditions for *Botrytis* infection on grapes (www.agf.gov.bc.ca), only one ideal period was recorded for September (22 to 23 September from 18h00 to 08h00). Three near ideal periods were recorded on 1 October (19h00 to 08h00: a shortfall of 2 hours), 2 October (19h00 to 08h00: a shortfall of 2 hours) and 25 October (22h00 to 11h00: a shortfall of 2 hours) (Figs. 4.3.4.3 and 4.3.4.4).

2012-2013 season

Temperature, relative humidity (RH) and rainfall were recorded during the experimental period in October 2012. There was a 3-day gap in the recordings but overall no ideal climatic conditions occurred during the blossom period for *Botrytis* infection (Fig. 4.3.4.5).

2013-2014 season

The weather data collected from SRCC unfortunately did not consist of hourly data but of daily data. Therefore, predictions and infection periods could not be determined.

4. Spray coverage of lemon flowers

Fluorescent pigment mixed with water and sprayed onto lemon flowers showed that the spray coverage was extremely poor (Fig. 4.3.4.7). Adjuvants such as Breakthru Union and NuFilm 17 were included in spray tanks with mancozeb to see if spray coverage can be increased when field trials were sprayed. Mancozeb was also sprayed alone for a comparison. Visually, spray coverage was generally extremely poor and could unfortunately not be analyzed due to difficulties in image analyses of the digital photos (Fig. 4.3.4.8 – 10).

Conclusions

Comparing the pH, Brix and determining which blossom parts ideally suit *Botrytis* growth of different citrus cultivars, did not conclusively show that lemons are more suitable for *Botrytis* infection than the other cultivars. Of the fungicides evaluated in field trials, benomyl, iprodione, DDA, pyrimethanil and fenhexamid performed very well. Of these, Benlate and Sporekill are already registered on citrus for the control of CBS and these early sprays can form part of the overall spray programme to prevent early CBS infections. Open and closed lemon flowers were protected for a short period while closed flowers (balloon stage) were better protected than open flowers. A better forecasting system will also help to determine when and how many infection periods did occur and if wind scarring and excessive nitrogen effects rather than *Botrytis* is the cause of the huge amount of ridges that was observed in the Eastern Cape the past season. Field trials are difficult to execute as conditions might not be favourable during blossom for this omnipresent fungus. The 2013-2014 season (September – October) was very dry and not favourable for infection. None of the fungicides tested were effective, resulting in equal amounts of ridges that formed and average yield per tree. The role of excessive nitrogen applications resulting in ridges on the fruit rind cannot be excluded (Fig. 4.3.4.6). Flowers are difficult to spray as spray residues do not stick to them as expected. Adjuvants also appeared not to enhance retention of the fluorescent pigment.

Technology transfer

- C.Kotze and G.C. Schutte. Control of *Botrytis cinerea* Pers. on lemons. Oral presentation at 7th CRI Symposium 2012.

References cited

- Beever, R. E., Laracy, E. P. & Pak, H. A. 1989. Strains of *Botrytis cinerea* resistant to dicarboximide and benzimidazole fungicides in New Zealand vineyards. *Plant Pathology* 38: 427-437.
- Calavan, E.C., Wampler, E. L., Sufficool, J. R. & Orsmy, H. W. 1952. Control of blossom blight of lemons. *The Californian Citrograph* 37: 180, 190.
- Fullerton, R.A., Harris, F.M. & Hallett, I.C. 1999. Rind distortion of lemon caused by *Botrytis cinerea* Pers. *New Zealand Journal of Crop and Horticultural Science* 27: 205-214
- Klotz, L. J., Calavan, E. C. & Zentmeyer, G. A. 1946. The effect of botrytis rot on lemons. *The Californian Citrograph* 31: 247, 262.
- Palmer, C. L., Horst, R. K. & Langhans, R. W. 1997. Use of bicarbonates to inhibit *in vitro* colony growth of *Botrytis cinerea*. *Plant Disease* 81: 1432-1438.
- Rosslénbroich, H-J. & Stuebler, D. 2000. *Botrytis cinerea*-history of chemical control and novel fungicides for its management. *Crop Protection* 19: 557-561.
- Timmer, L. W., Gernsey, S. M. & Graham, J. H. 2000. *Botrytis*-induced diseases. In: Compendium of citrus diseases. *American Phytopathological Society (APS Press)*: 25.
- Weathers, L. G. 1957. Controlling *Botrytis*. *The Californian Citrograph* 42: 216.

Table 4.3.4.1. Field evaluation of different fungicides applied during blossom in October 2011 at 7 and 10 day intervals after application for the control of *Botrytis cinerea* on Lemons at “Fountains” near White River.

Treatment	Rate /100L water	Botrytis incidence(%) ^x	
		7 days	10 days
Untreated control		50.00 c	51.25 c
Benlate	25 g	21.88 ab	17.19 ab
Benlate	50 g	11.25 a	7.50 a
Iprodione	200 ml	10.00 a	10.00 a
Sporekill	200 ml	27.50 abc	31.25 b
Sodium bicarbonate	1000 g	48.75 c	53.75 c
Sodium bicarbonate	2000 g	40.00 bc	52.50 c

^xMeans in a column (based on 5 replicates) followed by the same letter do not differ significantly ($P < 0.05$).

Table 4.3.4.2. Field evaluation of different fungicides applied during blossom on 16 October 2012 for the control of *Botrytis cinerea* on Lemons at “Fountains” near White River.

Treatment	Rate/ 100L Water	Botrytis incidence (%) ^x			
		24 Hours		72 Hours	
		Petal	Stamen	Petal	Stamen
Untreated control		53.33 c	40.00 bc	100.00 b	93.33 ab
Benlate	25g	13.33 a	0.00 a	73.33 ab	80.00 ab
Benlate	50g	13.33 a	0.00 a	86.67 ab	80.00 ab
Iprodione	200ml	13.33 a	6.67 a	100.00 b	86.67 ab
Sporekill	100ml	13.33 a	20.00 ab	86.67 ab	86.67 ab
Protector	120ml	20.00 ab	13.33 a	86.67 ab	86.67 ab

Teldor	75ml	6.67 a	0.00 a	86.67 ab	86.67 ab
--------	------	--------	--------	----------	----------

^xMeans in a column (based on 5 replicates) followed by the same letter do not differ significantly ($P < 0.05$).

Table 4.3.4.3. Field evaluation of different fungicides applied during blossom on 30 September 2013 for the control of *Botrytis cinerea* on lemons in the Sunday's river valley.

Treatment	Rate/ Water	100L	Percentage of fruit in each class		Yield/tree
			Clean fruit	Fruit with ridges	
Benlate	50g		50.0a	50.0a	48.59a
Iproflo	200ml		48.2a	51.8a	43.28a
Protector	120ml		50.6a	49.4a	40.64a

^xMeans in a column (based on 5 replicates) followed by the same letter do not differ significantly ($P < 0.05$).

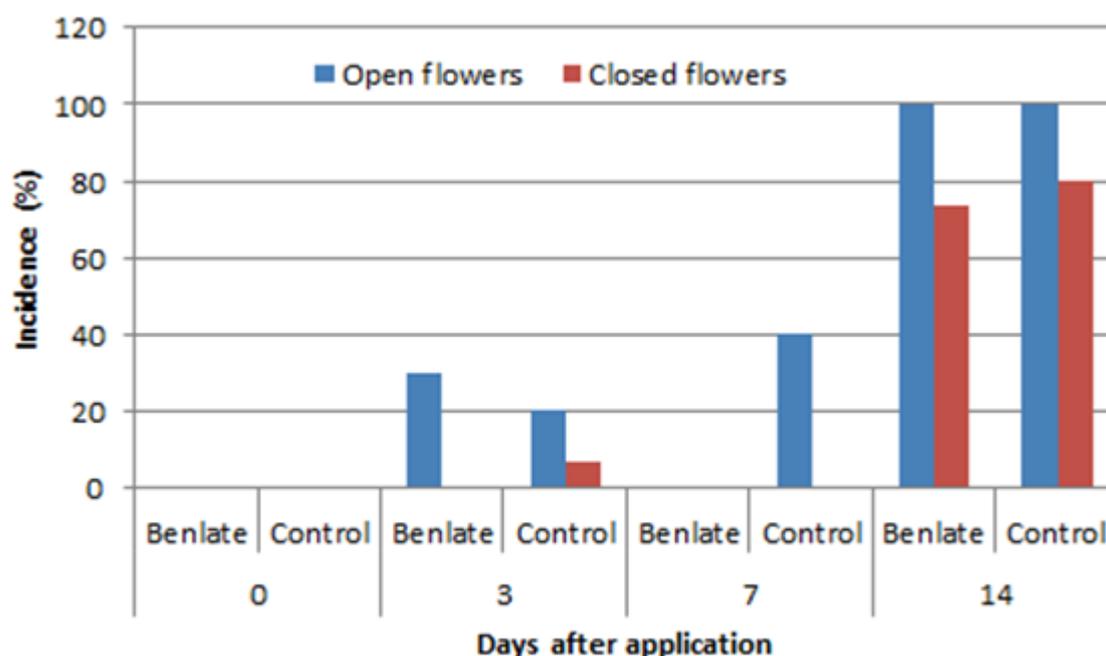


Figure 4.3.4.3. Botrytis incidence on open and closed flowers as determined in field evaluation of a commercial application of Benlate's protective capacity over time, applied during blossom in October 2012 for the control of *Botrytis cinerea* on lemons in the Sunday's river valley.

Table 4.3.4.4. Percentage mycelia inhibition of *Botrytis cinerea* treated with different fungicides at different rates compared to the untreated control.

Treatment	Mycelial inhibition (%) after 7 days ^x				
	0.1 ppm	1 ppm	10 ppm	100 ppm	1000 ppm
Benlate	100 e	100 e	100 e	100 e	100 e
Iprodione	0.14 a	100 e	100 e	100 e	100 e
Sporekill	1.30 a	15.50 b	58.14c	79.50 d	100 e

^xMeans (based on 10 replicates) followed by the same letter do not differ significantly ($P < 0.05$).

Table 4.3.4.5. Colony diameter of *Botrytis cinerea* grown on agar made from petal and stamen of different citrus cultivars, along with pH and Brix levels of petals and stamen of the same cultivars to determine flower susceptibility of lemon blossoms collected from Nelspruit, Mpumalanga province 2011 – 2012 season, to *B. cinerea* infections.

Cultivar	Botrytis colony diameter (mm ²) after 7 days ^x		pH ^x		Brix (% sucrose solution) ^x	
	Petal	Stamen	Petal	Stamen	Petal	Stamen
Clementine	0.00 a	0.00 a	5.63 b	5.70 bc	6.28 abc	6.55 bcd
Lemon	2438.03 c	5026.55 d	4.95 a	5.15 a	5.18 ab	7.82 d
Navel	0.00 a	102.10 a	5.91 cd	6.16 e	5.04 a	5.06 ab
Valencia	0.00 a	905.53 b	5.91 cd	5.99 de	6.98 cd	7.48 cd

^xMeans of each group (based on three replicates) followed by the same letter do not differ significantly ($P < 0.05$) according to Fisher's LSD test.

Table 4.3.4.6. Colony diameter of *Botrytis cinerea* on agar made from petal and stamen of different citrus cultivars, along with pH and Brix levels of petals and stamen of the same cultivars to determine flowers susceptibility of lemon blossoms for *B. cinerea* infections, collected from Nelspruit, Mpumalanga province 2012 – 2013 season.

Cultivar	Botrytis colony size (mm ²) after 7 days ^x		pH ^x		Brix (% sucrose solution) ^x	
	Petal	Stamen	Petal	Stamen	Petal	Stamen
Clementine	4736.97 cde	4781.74 de	5.68 a	6.30 c	4.90 abc	6.00 bcd
Lemon	4529.43 bcd	5178.99 e	5.72 ab	6.06 abc	6.57 cde	8.00 ef
Navel	4125.30 b	4234.59 bc	5.95 abc	6.26 bc	8.47 f	7.57 def
Valencia	0.00 a	0.00 a	8.85 d	8.87 d	4.10 a	4.27 ab

^xMeans of each group (based on three replicates) followed by the same letter do not differ significantly ($P < 0.05$) according to Fisher's LSD test.

Table 4.3.4.7. Colony diameter of *Botrytis cinerea* on agar made from petal and stamen of different citrus cultivars, pH and Brix levels of petals and stamen of the same cultivars to determine flowers susceptibility of lemon blossoms for *B. cinerea* infections, collected from Addo, Eastern Cape province 2012 -2013 season.

Cultivar	Botrytis colony size (mm ²) after 7 days ^x		pH ^x		Brix (% sucrose solution) ^x	
	Petal	Stamen	Petal	Stamen	Petal	Stamen
Clementine	4947.03c	4911.25c	5.57ab	6.18c	4.60bc	6.00d
Lemon	4165.63b	2542.06a	6.16c	6.42c	3.63ab	2.67a
Navel	2151.79a	5307.01c	5.71b	6.11c	5.70cd	6.40d
Valencia	3854.97b	4085.76b	5.28a	5.63ab	5.17cd	5.20cd

^xMeans of each group (based on three replicates) followed by the same letter do not differ significantly ($P < 0.05$) according to Fisher's LSD test.

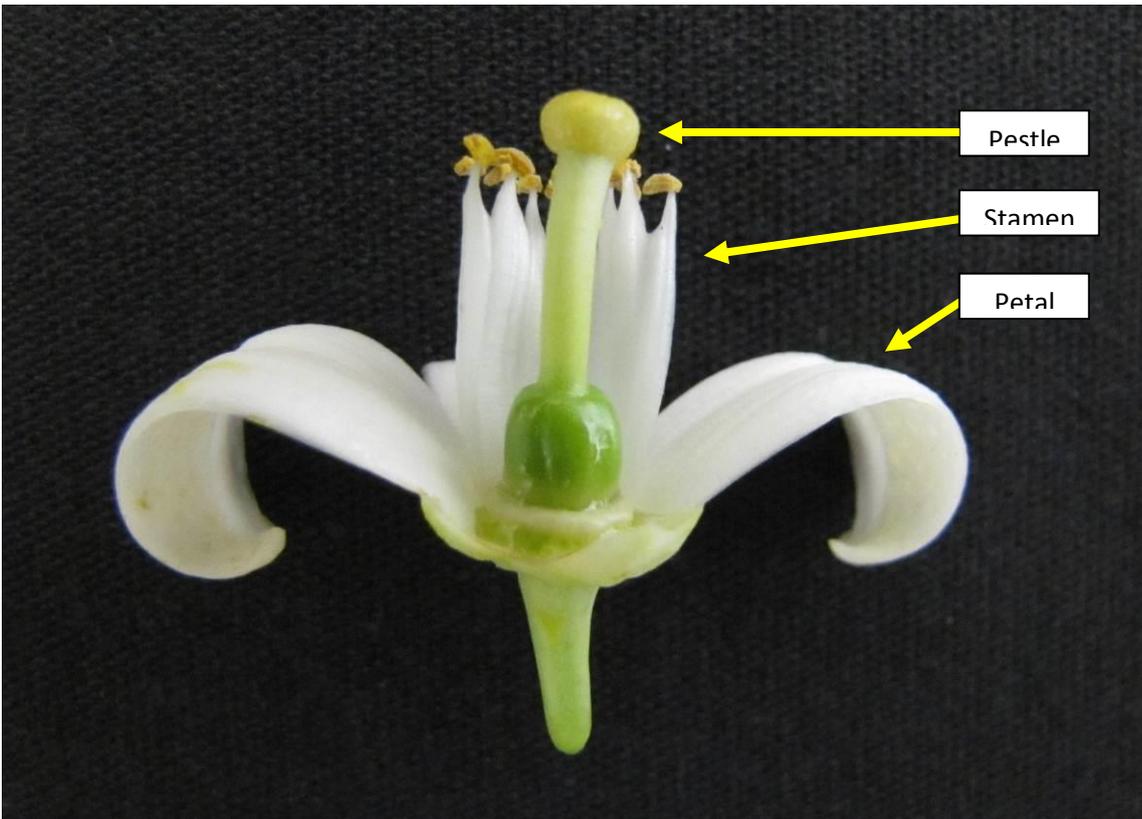


Fig. 4.3.4.1. Lemon flower showing the position of the petals, stamen and pistil.



Fig. 4.3.4.2. Lemon blossoms inoculated with *B. cinerea*, treated with fungicides before being harvested and plated on Kerssies' selective medium to determine efficacy of treatments. Note how the fungus grows on the stamen first and then establishes itself on the petals.

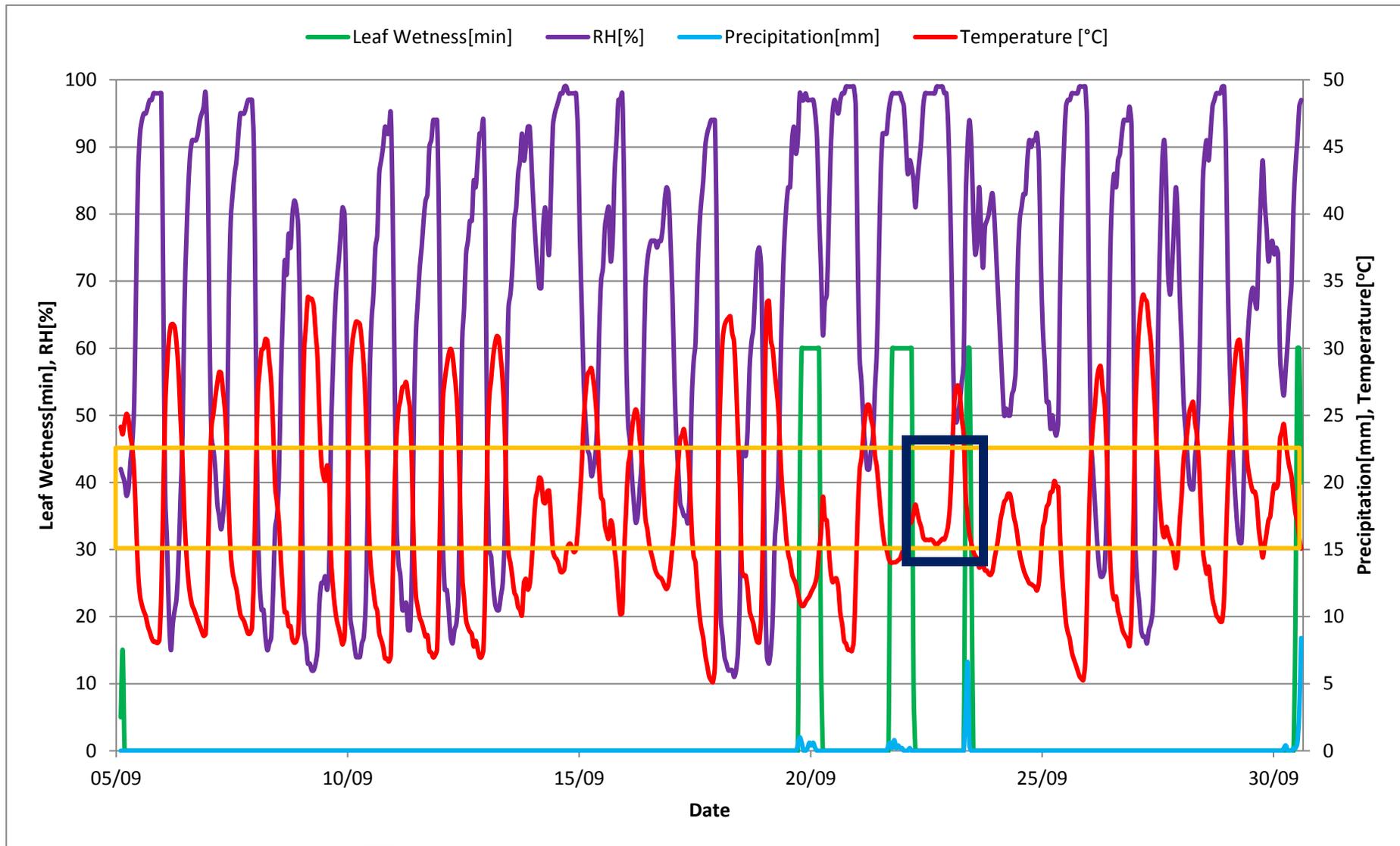


Fig. 4.3.4.3. Botrytis infection events () as determined from weather conditions for September 2011 monitored at Fountains, White River.

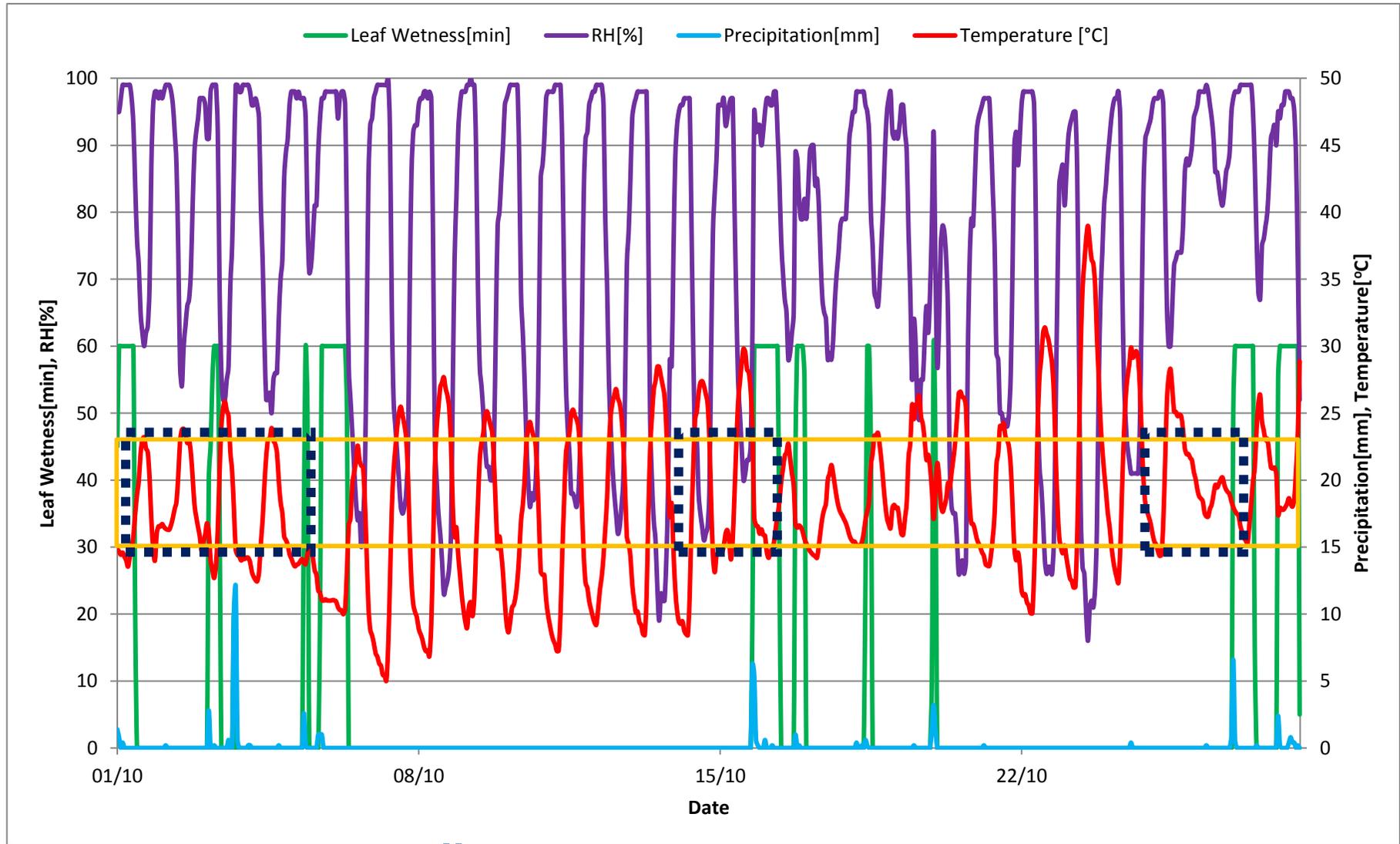


Fig. 4.3.4.4. Lack of Botrytis infection events () during October 2011 at Fountains, White River as determined by the parameters used for grapes to determine ideal climatic conditions for Botrytis infection on grapes (www.agf.gov.bc.ca).

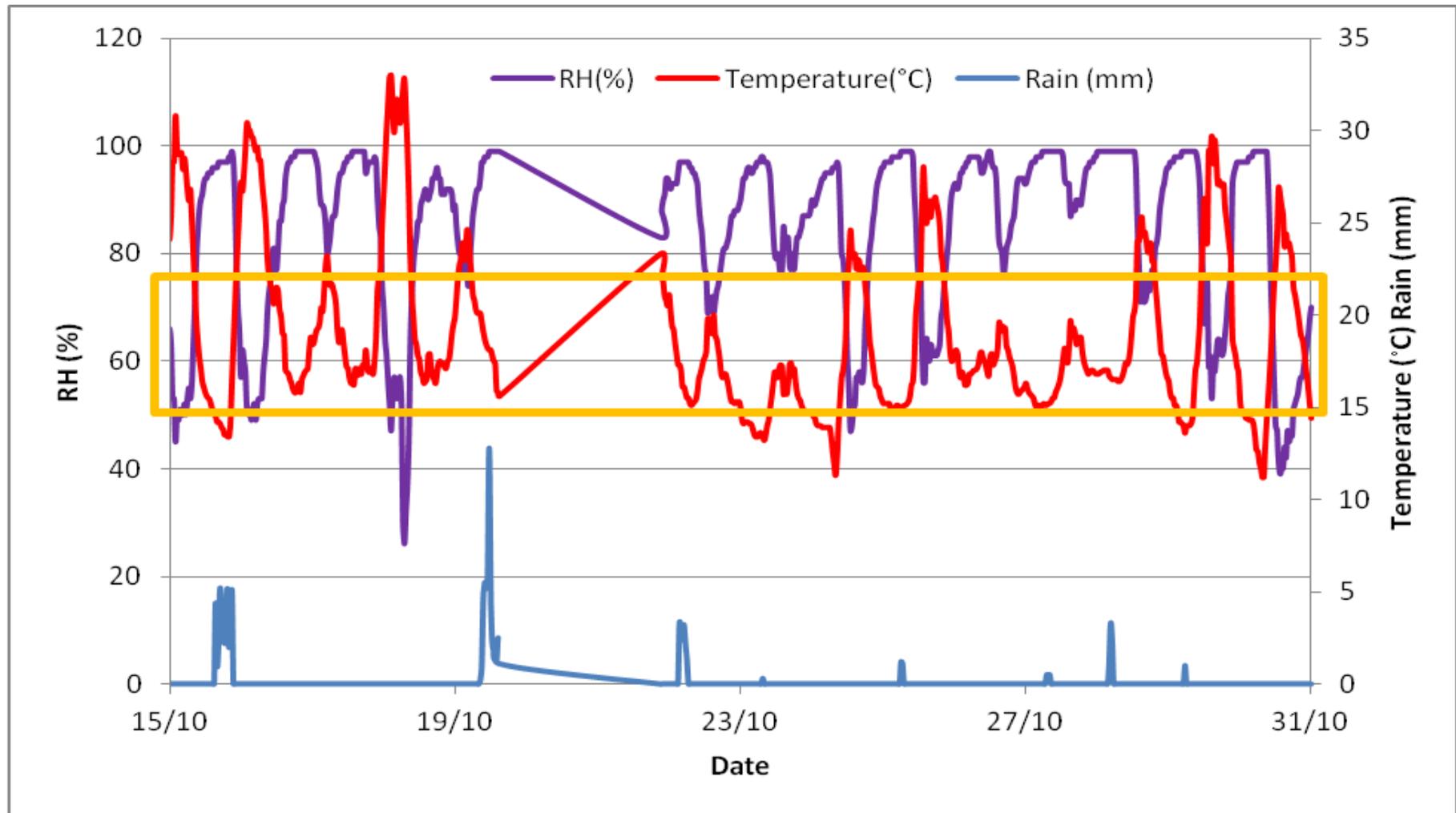


Fig. 4.3.4.5. Lack of Botrytis infection events () during October 2011 at Fountains, White River as determined by the parameters used for grapes to determine ideal climatic conditions for Botrytis infection on grapes (www.agf.gov.bc.ca).



Fig. 4.3.4.6. Ridges on lemons harvested at in Sundays river valley on 5 May 2014.



Fig. 4.3.4.7. Poor spray coverage of lemon flowers after spraying with water and fluorescent pigment.

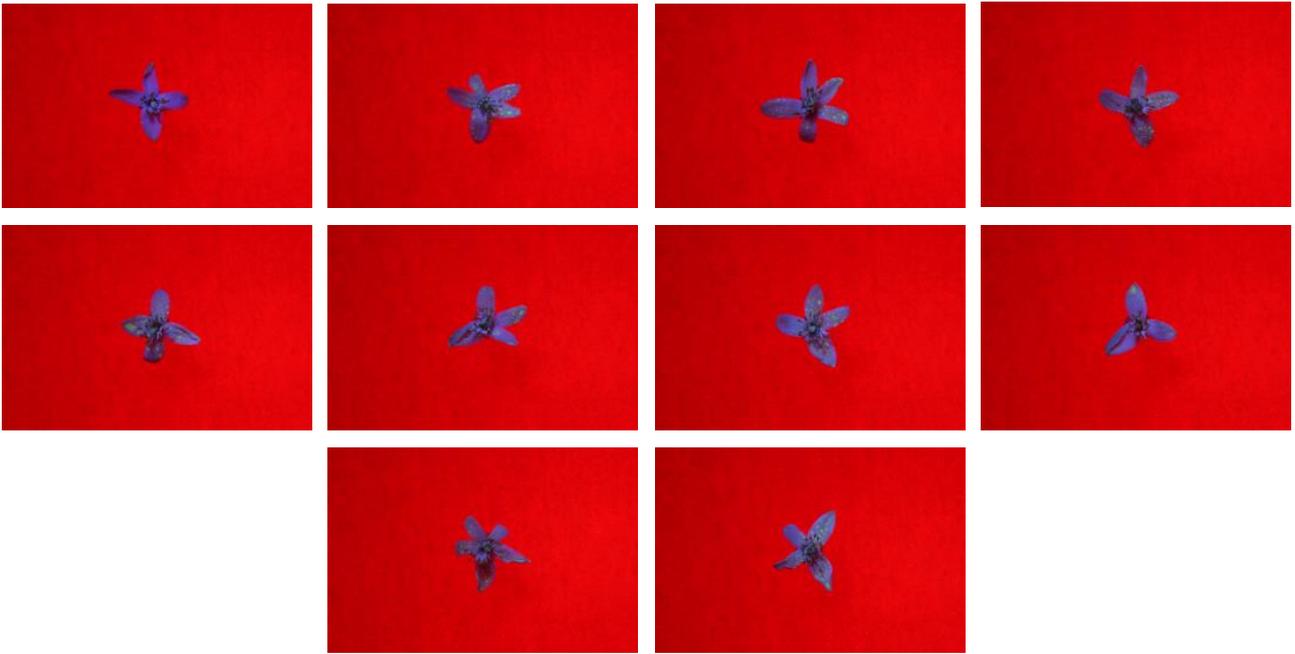


Fig. 4.3.4.8. Lack of coverage of orange blossoms sprayed with water and BreakThru Union.

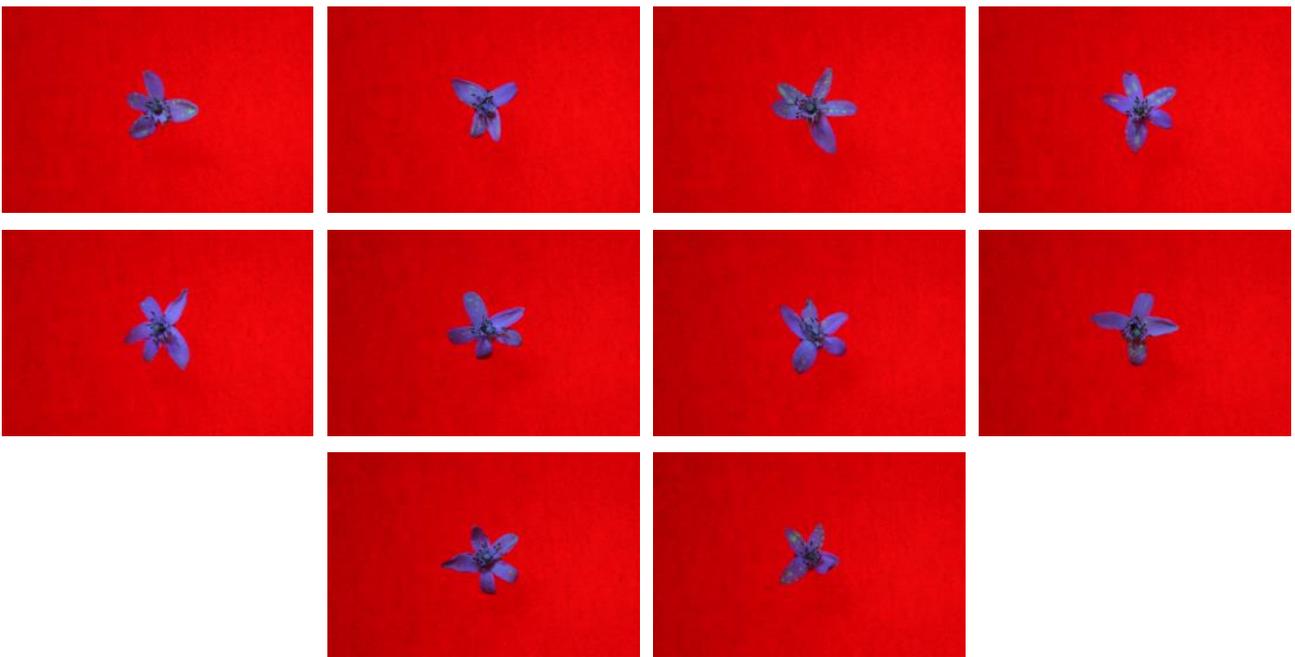


Fig.4.3.4.9. Lack of coverage of orange blossoms sprayed with mancozeb.

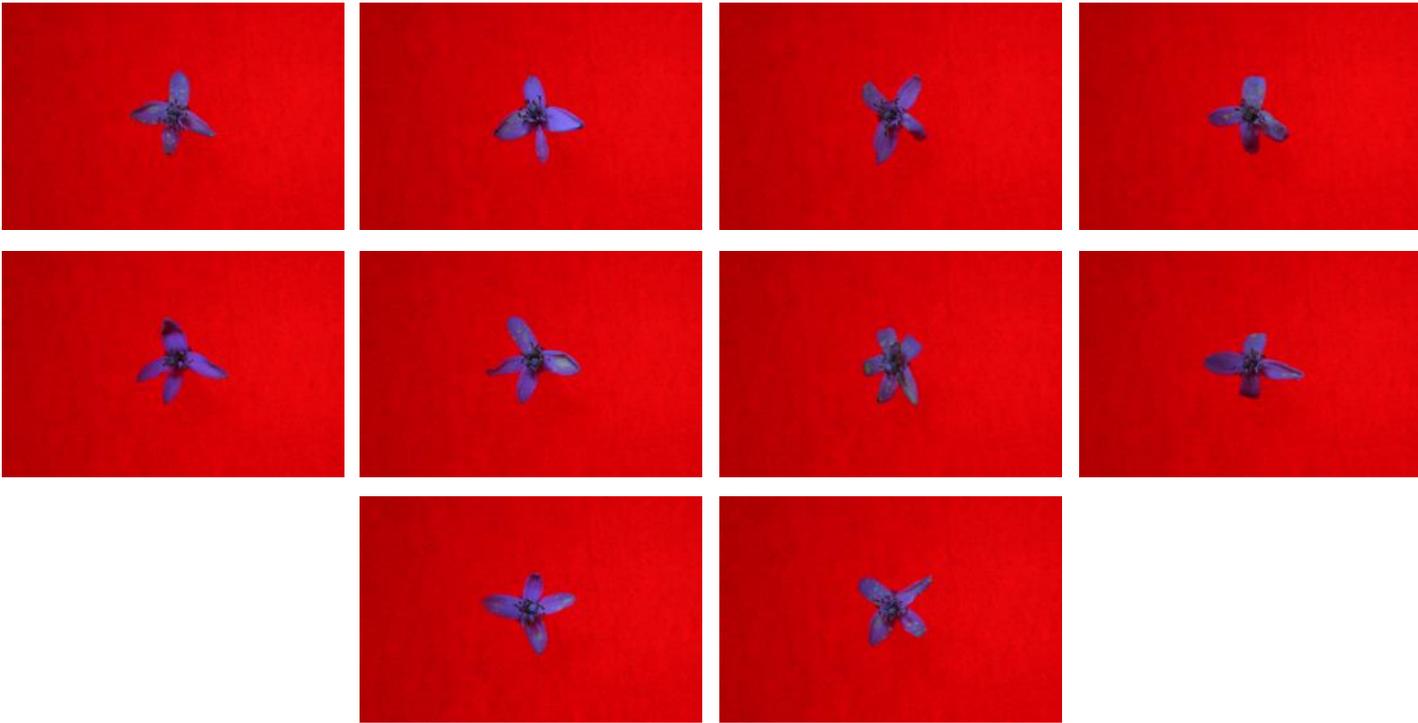


Fig. 4.3.4.10 Lack of coverage of orange blossoms sprayed with water and Nufilm 17.

4.3.5 **PROGRESS REPORT: Development of a tree canopy characteristic calibration formula for reduced volume fungicide application in citrus orchards**

Project 1089 (2014/04 – 2015/04) by JG van Zyl and PH Fourie (CRI)

Summary

Replacing ground-area based sprayer calibration with tree volume based calibration for fungicide and pesticide spray application is a necessary step to improve spray application in terms of cost and efficiency. To move to tree volume based sprayer calibration, tree canopies need to be characterised in terms of dimensions (volume) and density. A light detection and ranging (LiDAR) system was procured for this specific task. LiDAR to PC communication has been established and ASCII and binary translation of scanning data has commenced. Various mounts for the LiDAR have been designed and one has been build. First scans were initiated and a cloud image constructed.

Opsomming

Die vervanging van grond-area gebaseerde spuit kalibrasie met boom-ry-volume gebaseerde kalibrasie vir fungisied en insekdoder spuit toediening is 'n nodige stap vir die verbetering van spuit toediening in terme van koste en effektiwiteit. Om te beweeg na boom-ry-volume gebaseerde kalibrasie moet bome lowers gekarakteriseer word in terme van dimensies (volume) en lower-digtheid. 'n "Light detection and ranging" (LiDAR) sisteem is aangeskaf vir die spesifieke taak. LiDAR na rekenaar kommunikasie is bewerkstellig en ASCII en binêre vertaling van data is begin. Verskeie monterings vir die LiDAR is ontwerp en een is gebou. Eerste skanderings is gedoen en 'n 'cloud' figuur is geskep.

4.3.6 **PROGRESS REPORT: The use of adjuvants to improve fungicide foliar spray deposition and control of Alternaria brown spot on citrus**

Project 1096 (2014/04 – 2014/10) by JG van Zyl and PH Fourie (CRI)

Summary

Previous work done on adjuvants together with copper oxychloride sprays indicated improved Alternaria brown spot control. However, anomalous results were found with the improved deposition assessment protocol with deposition benchmarks over- and under-predicting control. As a hypothesis, these anomalous results were ascribed to the effect of deposition quality on disease control, as well as possible physical and/or chemical effects of adjuvant use together with copper oxychloride sprays on the pathogen and leaf surfaces. These factors were investigated through improvement of the deposition quality parameter and a histopathology study. The improved deposition quality parameter was proven to be effective and accurate.

However, as indicated by poor correlations between deposition quantity, quality and disease control on leaves, it was found that the current method use to capture and analyse deposition on leaf surface do not accurately portray deposition effects on the microscopic scale as encountered by the fungal pathogen. The histopathology study, to date, indicated that adjuvants alone did not have a significant effect on conidial adhesion, germ tube length, viable conidia or germ tube/conidial stress. The addition of copper oxychloride to adjuvants and also copper oxychloride alone did have a significant effect on these evaluated parameters. Entree together with copper oxychloride reduced conidial adhesion the most (37.81%), realised the shortest germ tube length (13.11 μm) and realised the highest percent stress (93.81%) but these values were statistically similar to that achieved by the other adjuvant treatments together with copper oxychloride and copper oxychloride alone.

Opsomming

Vorige navorsing wat gedoen is op benatters saam met koperoksichloried, het gewys op verbeterde *Alternaria* bruinvlekbeheer. Onreëlmatige resultate was gevind met die verbeterde deposisie assesserings-protokol met deposisie maatstawwe wat onder- en oor siektebeheer voorspel. Hierdie onreëlmatige resultate is hipoteties aan die effek wat deposisie kwaliteit op siektebestuur, en/of moontlike fisiese of chemiese effekte van die benatter met koperoksichloriedspuite op *Alternaria* bruinvlek en blaaroppervlaktes toegeskryf. Hierdie faktore is deur die verbetering van die deposisie kwaliteitparameter en 'n histopatologie studie ondersoek. Die verbeterde kwaliteitparameter het bewys dat dit effektief en akkuraat is. Maar, swak korrelasies tussen deposisie kwantiteit, kwaliteit en siektebestuur op blare het gewys dat die huidige metode om deposisie op blare te analiseer nie die effek van deposisie op mikroskopiese skaal, soos deur swampatogeen ervaar, akkuraat genoeg weerspieël nie. Die histopatologie studie het gewys dat benatters nie 'n fisiese of chemiese effek op die aanhegting van spore, kiembuise lengte, spoor vatbaarheid het nie, maar wel stres in spore en kiembuise verhoog. Die gebruik van koperoksichloried alleen en saam met benatters het 'n merkwaardige effek op hierdie faktore gehad. Entree saam met koperoksichloried het sporaanhegting die meeste verlaag (37.81%) en het ook die kortste kiembuise tot gevolg gehad (13.11 μm) met die hoogste persentasie stres (93.81%). Hierdie waardes het egter nie statisties verskil van die ander benatters met koperoksichloried, of koperoksichloried op sy eie nie.

4.4 PROGRAMME: SOILBORNE DISEASES

Programme coordinator: J.M. van Niekerk (CRI)

4.4.1 PROGRAMME SUMMARY

Different projects within the soilborne diseases programme address various soil related disease problems. Some projects are investigating more sustainable or softer chemicals to use in the management of *Phytophthora* and citrus nematode in the orchard; while the different factors involved in citrus decline are also being researched extensively. Apart from investigating soil related industry problems, new diseases, with yet unknown etiology are also put under the spotlight of research within project 1068.

In the evaluation of various pre-plant and post-plant treatments (project 762) results that have been gathered since 2011 indicates that it is only at this stage that treatment differences are starting to appear. This project needs to continue for another three seasons to allow clear conclusions on the best treatments. Establishment of new soil fumigation trials are being investigated – specifically in replant situations. The aim of these trials will be to evaluate a list of new soil fumigant products that have in recent times come onto the market

Project 910 concluded in 2014. This project was aimed at identifying parameters that could be used as early warning indicators for citrus decline. Work done over two years did identify some physical soil characteristics along with leaf chemical characteristics that have potential to be included in a model for the early detection of decline orchards. This management tool could have the potential to make early intervention in decline orchards possible. A new project, 1092, that follows on the work done in 910, started in April 2015. This project aims to study decline in additional orchards to determine if the factors identified in 910 are indeed associated with tree decline and if they can be included in a model for the prediction of tree decline.

In project 1030, a new trial was started in 2014. This trial was aimed at evaluating new chemical and non-chemical products for their ability to control the citrus nematode. Four potential alternative products were included in this trial. Results from one season showed some promise. However, in order to obtain conclusive results evaluation for another season is needed. Once more data are available on these products, the best products and treatment regimens will be selected to include in a longer term trial.

Two distinct decline and dieback diseases have recently been observed in orchards in the Kirkwood and Patensie areas of the Eastern Cape province and in orchards in Swaziland and Hoedspruit (project 1068). Initially *Armillaria* spp. were regarded as the causal organism for the observed symptoms and diseases. However, despite extensive sampling and isolations from diseased material, no *Armillaria* spp. isolates could be obtained from any of the areas. However, it was shown that the disease etiology in the Hoedspruit/Swaziland area is vastly different from the Eastern Cape disease etiology. Further studies will include pathogenicity tests to establish pathogen status of the isolated pathogens as well as possible interactions between these pathogens. Inoculum sources of these pathogens will also be investigated along with potential preventative and curative management strategies.

In April 2014, a project (1101) was started that focusses on the preventative and curative management of soilborne pathogens, *Phytophthora* and *Pythium* spp., in citrus nurseries. This project is aimed at characterising the pathogen species and evaluating if current management practices in nurseries are still effective and if new measures are needed. In the first year of this project a large number of *Pythium*, *Phytophthora nicotianae* and *P. citrophthora* isolates have been collected. PCR-RFLP and ITS sequence analyses identified a number of *Pythium* spp. amongst the collected isolates. Mefenoxam resistance testing for the collected isolates showed varying degrees of resistance at different fungicide concentrations. This will be put into context with further trials. Two pot trials were started and the data from the first round of these trials will be included in the 2016 annual report.

With virgin soil becoming a rare commodity in citrus production areas, growers are starting to experience citrus replant problems in situations where new orchard are being established on land where citrus had been grown for an extended period of time. A new project, aimed at the characterisation of soilborne pathogens in citrus replant soils will therefore be submitted for funding at the end of 2015.

PROGRAMOPSOMMING

Verskillende projekte binne die grondgedraagde siekte program spreek 'n verskeidenheid van grondverwante siekte probleme aan. Sommige projekte ondersoek meer volhoubare of sagter chemiese middels om te gebruik in die bestuur van *Phytophthora* en sitrus nematode in die boord. Tesame met die ondersoek van grondverwante probleme in die industrie, word nuwe siektes met huidig onbekende oorsake ook onder die soeklig geplaas.

Projek 762 is al vir 'n geruime tyd aan die gang met resultate wat sedert 2011 versamel is. Nou, na vyf jaar se monitering begin verskille tussen behandelings na vore kom wat verdere monitering vir drie jaar noodsaak. Verdere berokingsproewe word ondersoek – spesifiek in herplant situasies. Die doel van hierdie proewe sal wees om nuwe berokingsmiddels te toets wat onlangs op die mark begin verskyn het.

Projek 910 is in 2014 voltooi. Die doelwit van hierdie projek was om parameters te identifiseer wat gebruik kan word as vroeë aanwysers vir agteruitgang van bome. Werk gedoen oor twee jaar het verskeie fisiese grondeienskappe en chemiese blaareienskappe identifiseer wat potensiaal het om in 'n voorspellingsmodel opgeneem te word vir die vroeë identifisering van boorde wat begin agteruitgaan. Hierdie hulpmiddel kan dan moontlik gebruik word om op 'n vroeë stadium in te gryp in boorde wat agteruitgang toon. 'n Nuwe projek, 1092, is in April 2015 begin wat bou op die werk gedoen in 910. Hierdie projek ondersoek agteruitgang in verdere boorde om vas te stel of die faktore in 910 geïdentifiseer, wel verbind kan word met boom agteruitgang en of hulle in 'n model opgeneem kan word vir die voorspelling van boomagteruitgang.

In projek 1030 is 'n nuwe proef in September 2014 begin. Hierdie proef is gemik op die evaluasie van nuwe chemiese en nie-chemiese produkte ten opsigte van hulle vermoë om die sitrus nematode te beheer. Vier nuwe middels met potensiaal is ingesluit in hierdie proef. Na een seisoen se toetsing is die resultate nog nie duidelik nie en verdere proewe vir nog een jaar is dus nodig. Sodra verdere data beskikbaar is sal die beste produkte gekies word vir insluiting in 'n langtermyn evaluasie.

Twee duidelik verskillende terugsterwingsiektes is onlangs in boorde in die Kirkwood en Patensie areas van die Oos-Kaap en in Swaziland en Hoedspruit waargeneem (Projek 1068). Aanvanklik was die mening dat *Armillaria* spp. die oorsaak was van die waargenome simptome. Ten spyte van intensiewe monsterneming kon geen *Armillaria* spp. verkry word uit enige van bogenoemde areas. Wat egter duidelik na vore gekom het, is dat die oorsaak van die siekte drasties verskil tussen die Hoedspruit/Swaziland areas en die Oos-Kaapse areas. Patogenisiteitstoetse sal meer lig werp op die patogenisiteit van hierdie patogene op sitrus en hoe hulle interaksie werk ten einde die waargenome simptome te veroorsaak. Verder sal moontlike inokulumbronne tesame met voorkomende en kuratiewe beheerstrategieë ondersoek word.

In April 2014 is 'n nuwe projek (1101) van stapel gestuur wat fokus op die voorkomende en kuratiewe bestuur van grondgedraagde patogene, *Phytophthora* en *Pythium* spp., in sitrus kwekerye. Die projek is gemik op die karakterisering van die patogeenspesies en die evaluasie van huidige bestuurspraktyke in kwekerye ten einde vas te stel of hulle steeds effektief is en of nuwe maatreëls nodig is. In die eerste jaar van hierdie projek is 'n groot getal *Pythium* en *Phytophthora* isolate versamel en met behulp van "PCR-RFLP" en "ITS" DNA volgorde bepaling is 'n hele aantal *Pythium* spp. geïdentifiseer. Mefenoxam weerstandstoetsing op die versamelde isolate het getoon dat daar verskeie vlakke van weerstand by verskillende swamdoder konsentrasies voorkom. Hierdie weerstand sal in konteks geplaas word met verdere proewe. Twee potproewe is ook gedoen en die resultate hiervan sal in die 2016 jaarverslag opgeneem word.

Nuwe grond vir aanplantings word 'n skaars kommoditeit in sitrus produksieareas en dit lei daartoe dat produsente herplant probleme begin ondervind in situasies waar nuwe boorde gevestig word op grond waar sitrus voorheen verbou is. 'n Nuwe projek, gemik op die karakterisering van grondgedraagde patogene in sitrus herplant gronde, sal dus aan die einde van 2015 ingedien word vir befondsing.

4.4.2 **PROGRESS REPORT: The evaluation of different pre-plant products for the control of the citrus nematode, as part of an integrated nematode control approach in citrus replant situations**

Project 762 (2007 – 2017) by JM van Niekerk, MC Pretorius & C Kotze (CRI)

Opsomming

Die doel van hierdie projek is om voor-plant behandelings te vind wat grond in boorde vir so lank as moontlik na plant vry sal hou van sitrus aalwurm en *Phytophthora*. Die proef is al sedert 2010 aan die gang. Verskeie grondbehandelings is gedoen voor plant in Januarie 2010 terwyl sommige behandelings jaarliks in Januarie en November toegedien word. Parameters wat jaarliks gemonitor is sedert die begin van die proef sluit in stam deursnee, boom hoogte, nematode tellings in die grond en wortels, *Phytophthora* status in die grond en 'n visuele boom gradering. Die MeBr en twee Midas 50:50 behandelings was sedert 2011 die drie beste behandelings op grond van nematode tellings in die grond en boomwortels. Behandelde grond is ontleed met behulp van die grondlokaas metode. Die gemiddelde persentasie *Phytophthora* besmette blaarskyfies het aangedui dat die beste behandelings die MeBr, die Midas 50:50 en Telone behandelings was. Hierdie behandelings was dus die beste om die vlakke van *Phytophthora* in die grond te verminder. Die boommetings het ook aangedui dat hierdie behandelings in terme van boom groei en gemiddelde getal vrugte per boom die beste is.

Summary

The aim of this project is to find preplant treatments that are effective in keeping orchard soils free from citrus nematode and *Phytophthora* for as long as possible after planting. The trial has been ongoing since January 2010. The various treatments were applied prior to planting in January 2010 with some treatments being applied annually in January and November. Tree stem diameter, tree height, nematode soil and root analysis, *Phytophthora* status in the soil and a visual tree rating, are the parameters that were monitored yearly since the start of the trial. Based on the average percentage reduction in juvenile nematode counts in the soil and female counts in the roots, the best treatments since 2011 have been the MeBr and the two Midas 50:50 treatments. In terms of average percentage *Phytophthora* infested leaf discs resulting from soil baiting from treated soil, the best treatments were MeBr, the Midas 50:50 treatments and Telone. This indicates that these products are the best in reducing *Phytophthora* levels in the soil. From the tree measurement data and average number of fruit per tree, these treatments also stood out as the best.

4.4.3 **FINAL REPORT: Investigation into edaphic factors and their interactions on citrus tree decline**

Project 910 (2008 – 2013) by M.C. Pretorius, C. Kotze (CRI), A. McLeod (USPP), N. Labuschagne (UP), D. Fourie (NWU Potchefstroom) and T. Gottwald (USDA)

Summary

Citrus decline is a root-related disease that causes significant economic loss to the citrus industry on a global scale. The causal factors involved in this disease have not yet been fully elucidated. The aim of the study was to elucidate the biotic and abiotic factors associated with citrus decline. Two declining orchards in the Mpumalanga province of South Africa were selected for the study. Trees were classified into four decline categories based on the visual decline of the tree canopies. For each tree category, yield, soil and leaf characteristics, soilborne pathogens and root disease associated symptoms were measured. Principal component analysis indicated the two orchards were distinctly different from each other. Subsequent

statistical analyses were therefore conducted separately on the datasets of the two orchards. The same parameters were implicated in the 2 sites such as percentage soil clay content for the healthy trees and percentage silt, percentage P and Mo content in leaves for the declining trees. Most of the parameters measured in this study were associated with the decline process, but none were consistently associated with citrus decline in both orchards. Several of the indicator characteristics identified in the study will now be validated in additional studies in more orchards.

Opsomming

Sitrus agteruitgang is 'n wortelverwante siekte wat ekonomies betekenisvolle verliese in die globale sitrus industrie veroorsaak. Die faktore wat hierdie siekte veroorsaak is nog nie ten volle geïdentifiseer nie. Die doelwit van hierdie studie was om die biotiese en abiotiese faktore wat met sitrus agteruitgang geassosieer word ten volle te karakteriseer. Twee boorde met simptome van agteruitgang is in die Mpumalanga provinsie van Suid-Afrika gekies. Bome is in vier klasse van agteruitgang gegroepeer volgens die visuele agteruitgang van die blaredak. Vir elke kategorie is opbrengs, grond- en blaareienskappe en grondgedraagde patoëen analiese gedoen en data versamel. Hoofkomponent analiese het getoon dat die twee boorde duidelik van mekaar verskil. Verdere statistiese analiese het dus die data van die twee boorde apart hanteer. Dieselfde veranderlikes is uit die twee persele geïdentifiseer. Hierdie sluit in persentasie klei in die grond van gesonde bome, teenoor persentasie slik, P en Mo inhoud in blare van bome met agteruitgang simptome. Meeste van die veranderlikes wat in hierdie studie gemeet is, is met die proses van agteruitgang geassosieer, maar geen is konstant met agteruitgang in beide boorde of persele verbind nie. Verskeie van eienskappe wat in hierdie studie geïdentifiseer is het potensiaal om aangewend te word om die beginstadium van agteruitgang te identifiseer wat die tydige implimentering van voorkomende bestuurspraktyke moontlik kan maak. Verdere verifiëring van hierdie eienskappe is egter nodig en gaan dus in addisionele boorde ondersoek word.

Introduction

Citrus decline is a root-related disease that causes significant economic loss to the citrus industry on a global scale. A typical declined tree will have yellowing, discoloured and distorted leaves, loss of leaves and branch dieback (Schalau, 2010). Additional symptoms commonly associated with citrus decline are sparse foliage; dead wood and reduced growth; yield and fruit size are reduced as a result. The trees do not die from the disease, but remain in a declined state following the initial onset of symptoms. These trees wilt sooner during a dry period than the adjoining healthy trees. All trees within a decline area show the same general symptoms, with a distinct margin present at the border between the diseased and healthy trees. The disease will gradually spread from the affected trees to the adjacent apparently unaffected trees. The spread of the disease within an orchard appear to be the result of feeder root rot infections (Suit et al., 1947 a, b; 1949).

Specific growth conditions are necessary to sustain a healthy citrus tree. Many health problems occur in citrus trees as a result of improper growth conditions. Poor soils, lack of nutrient content or inappropriate watering can contribute to health problems in citrus trees. These improper growing conditions will stress the trees and increase the risk of secondary problems. Certain insects attack the citrus trees, viz. aphids and citrus psylla, which vectors for the *Citrus Tristeza virus* and the Citrus greening disease, respectively. Additional diseases such as root rot, collar rot and stem canker are caused by *Phytophthora* spp. Plant parasitic nematodes (viz. *Tylenchulus semipenetrans*) also cause slow decline in citrus.

The complexity of decline of citrus is important due to the lack of knowledge regarding the original causes. By the time the decline symptoms are clearly visible it is usually too late to take any corrective actions to reverse the situation. Numerous factors can potentially contribute to the onset and progression of citrus decline. This causes remedial actions to be costly and often ineffective because the root cause of the problem is not addressed. Currently, there are no early-warning diagnostic tools that can be utilised to identify citrus decline trees. An early-diagnosis tool would assist researchers, consultants and citrus growers to attend to the decline problem proactively. The aim of this work was to use a multivariate assessment approach to identify the biotic and abiotic factors, and their interactions that could lead to the early detection of citrus tree decline.

Objectives

To identify biotic and abiotic factors and their interactions that could lead to tree decline by means of a Multi Variate Approach (MVA) method for the possible early identification of citrus decline problems.

Materials and methods

Site and trial description

Two typical root disease-related declining orchards were identified in the Mpumalanga province of South Africa.

Site 1 was located at Valley View farm near Karino on a 20-year-old Delta Valencia orchard on Rough Lemon rootstock with visual root disease decline symptoms. Site 2 was located at Friedenheim Estate on a 19-year-old orchard planted to Midnight Valencia on rough lemon rootstock trees.

The progression of Citrus Decline was classified and documented in four categories:

- Category 1 (C1): Visually healthy trees;
- Category 2 (C2): Visually healthy trees with slight indication of decline symptoms;
- Category 3 (C3): Typical root disease declined trees;
- Category 4 (C4): Severely declined trees.

Trees exhibiting all four disease progression categories were identified at Site 1, but only three decline categories (C1-C3) were identified at Site 2 (this orchard was visually in a better condition and no C4 trees were found).

The total number of trees for the two trial sites where data was collected was 105 trees (60 trees for Trial 1 and 45 for Trial 2); 15 trees were assessed per decline category. The study was conducted over a period of two seasons (2012-2013) at both sites.

Sampling and analyses

Soil, root and leaf samples were collected in May of each year. Soil (250 g) and feeder root (500 g) samples were collected at a depth of 20 cm on the eastern side of each tree in the root zone under the tree canopy. The number of second stage nematode larvae in the soil was determined according to the method of Whitehead and Hemming (1965) and the female nematode populations in the roots according to the method of Van der Vegte (1973). *Phytophthora* in the soil was determined by means of the leaf baiting technique. These analyses were performed by the CRI Diagnostic Centre (Nelspruit, South Africa).

The chemical composition of soil and leaf samples was determined by Nvirotek Laboratories. The leaf and soil chemical and physical characteristics reported on in the results section along with their abbreviations and units of measurement are listed in Table 4.4.4.1. The chlorophyll content of 30 leaves per tree was monitored by means of a mobile SPAD meter. Thirty leaves per tree were collected and the leaf size was determined by the University of Pretoria. Penetrometer readings of the soil were furthermore collected on the northern side of each tree. Other analyses included:

1. The starch content of roots was determined on 500 g of roots taken from each tree. Samples were ground, refrigerated and sent to the University of Pretoria for analyses.
2. Blight tests were conducted by using the water test. One hole was drilled per tree with a battery operated hand drill with a 10 mm drill bit. A 100 ml syringe was filled with 10 ml of water and inserted under pressure into the drilled hole. The time of water uptake by the tree determined the disease status (< 10 sec. – healthy; > 20 sec. Blight symptoms);
3. Zink content in bark was determined by collecting 50 g of bark, grinding it to a fine powder before freezing it prior to sample analyses by Lab Serve laboratory, Nelspruit, South Africa.
4. Yield and fruit size data were collected in each season from the trees in the different decline categories.

Statistical analyses

Data analysis for both seasons were performed on the physical and chemical soil factors, plant (roots and leaves) and disease related symptoms associated with tree decline; the averages of the two years was used for the data analysis procedures. The final dataset had 44 variables. Univariate statistics were performed with XLSTAT 2015. Principal component analysis (PCA) was performed on the correlation matrix with ADE-4 (Thioulouse et al., 1997). Classification and regression tree analysis was performed in R (*ctree* package; <http://www.r-project.org>). CART analysis has been used in the field crops context for the purpose of yield gap analysis (Ferraro et al., 2009; Tiftonell et al., 2008; Zheng et al., 2009). An important advantage of CART is the ability to analyse continuous and categorical entries simultaneously; conditional inference ($\alpha = 0.05$),

based on non-parametric permutation testing. According to our knowledge, this is the first use of CART analysis for exploring yield variation in the citrus production context.

Results

Comparison of the two sites

The two trial sites were chosen as they represented typical orchards and they both had trees with different levels of decline. Both orchards had similar management practices, seemingly similar soil characteristics, similar micro irrigation systems and cultivars and rootstock. In all of the PCA graphs, the average data were represented as circles and the variability is shown by the branches radiating out from the circles. The further the branches radiate out from the circle, the more variation for that replicate. Each branch represents an individual tree analysed for all the parameters (excluding yield) measured. In Figure 4.4.3.1a, the two sites are on opposite sides of the horizontal (F1) axis. These results indicate that the two sites were clearly different in terms of factors analysed. Figure 4.4.4.1b shows that site 1 is associated with higher levels of certain leaf elements (%Na, %S), certain soil elements (%Mn, %K, %Clay) and certain root parameters (Starch & Nematodes). Site 2 is associated with other variables viz. higher levels of certain leaf elements (%P, %Ca, ppm B), certain soil elements (Mg:K, Resistance, (Ca+Mg)/K, pH, Density) and certain plant characteristics (Leaf Size). The parameters that show little/no association with either site are shown in the middle of the factorial map e.g. *Phytophthora*, %K and ppm Zn in the bark.

When analysing the factors individually, and using traditional statistics, %K and %Mn are significantly higher in site 1 whereas Leaf Size and ppm B are significantly higher in site 2 (Figure 4.4.3.1c). Similarly, %S and %N was significantly higher in site 1 than in site 2. %P was significantly higher in site 2 (Figure 4.4.3.1d). Additionally, %Ca and Blight rating were significantly lower in site 1 than site 2. Percentage Clay was significantly lower in site 2 (Figure 4.4.3.1e). These results suggest that the two sites were distinctly different to each other and the remaining results were separated and analysed separately.

Change in biotic and abiotic parameters according to tree decline on the different sites

When all 44 parameters for the two sites were analysed together for both seasons it clearly shows that in both sites, the trees can be distinguished according to disease categories. For site 1 (Figure 4.4.3.2a), there is clear separation along the vertical (F2) axis, between categories 1, 2, 3 and 4 with slight overlap in some instances. For site 2 (Figure 4.4.3.2b), there is also clear separation between categories 1, 2 and 3 along the vertical (F2) axis. The clear separation of the different disease categories shows associations between disease ratings and certain variables. For example, in site 1, healthy (category 1) trees are more associated with higher values of clay content, free living nematodes and leaf size. Whereas, diseased (category 4) trees in this site, are more associated with higher levels of %P and ppm Mo in the leaves and percentage silt in the soil. For site 2, the healthy (category 1) trees had higher values of %N, leaf size, %K and nematodes in the soil whereas the diseased category (Category 3) had higher values of % Na, % Mg, % P and starch.

The factorial values corresponding to the 4 disease categories in site 1 were then projected and shown as Gauss curves (Figure 4.4.3.3a, b). The results show that, on the horizontal (F1) axis (Figure 4.4.3.3a), there was little distinction between the category 1, 2 and 3 and only category 4 being distinguishable. Whereas, on the vertical (F2) axis (Figure 4.4.3.3b), there was clear separation between categories 1 and 2 and categories 3 and 4 being similar to each other.

The 44 parameters were then sorted according to their F2 values, ranking them from highest (%Silt, %P, ppm B, ppm Mo) to lowest (%Clay, %Mg, %Ca) and drawn on a vertical graph shown in Figure 4.4.3.3c. Comparing this curve to the factorial map of the disease categories (Figure 4.4.3.3b), we see associations between healthy (category 1) trees and percentages Clay, Mg, Ca, Na. Similarly, we see associations between diseased (category 3 and 4) trees and percentage Silt, %P, B and Mo content in ppm. This is shown in Figure 4.4.3.3d where the silt values increase as the disease categories increase from 1 to 4 and in Figure 4.4.3.3e where the clay content decreases as the disease categories increase from 1 to 4.

A similar process was performed for site 2 with the 3 disease categories (Figure 4.4.3.4.a, b, c). The results again show that there was little distinction between category 1, 2 and 3 along the horizontal (F1) axis (Figure 4.4.3.4a). There was a clearer distinction along the vertical (F2) axis (Figure 4.4.3.4b). The 44 parameters were also sorted according to their F2 values, ranking them from the highest (%Na, %C, %Mg) to the lowest (%N, leaf size, %K) and shown on a vertical graph (Figure 4.4.3.4c). Comparing this curve (Figure 4.4.3.4c) to the factorial map of the disease categories (Figure 4.4.3.4b), we see associations between healthy (category 1) trees and %N, leaf size, %K. Similarly, we see associations between diseased (category 3) trees and %Na, %C and %Mg. This is reiterated in Figure 4.4.3.4d where Mo content in the leaves increases

as the disease category increases from 1 to 3. Similarly, Figure 4.434.4e shows that silt content increases as the disease category increases from 1 to 3.

Spatial distribution of all factors/parameters in the orchard

To investigate the spatial distribution within the orchards, various soil parameters (for example, % Clay as mentioned above) were projected onto the field layout to see the variability within the orchard. For site 1, (Fig 4.4.3.5a), there was an even distribution of clay content within the orchard. However, when these values were then split by the disease categories (Fig 4.4.3.5b), it became clear that the low disease categories (1 and 2) trees were associated with higher clay content in the soil (average 7.4% for category 1 and 6.2% for category 2) compared to the more diseased trees (average 4.4% for category 3 and 5% for category 4). For site 2 (Fig 4.4.3.6a), the clay content was also evenly distributed within the orchard. When these values were also split by disease category (Fig 4.4.3.6b) there was a tendency (as for site 1) to have higher clay content in the healthier (category 1 and 2) trees compared to the more diseased (category 3) trees. This tendency was not as strong as for site 1, perhaps due to no category 4 trees occurring in this site for comparison.

CART analysis

The CART analysis results identified the variables that distinguished the four disease categories as the soil clay content, the incidence of blight and the soil Zn content (Figure 4.4.3.7). A soil clay content > 5.5% was characteristic of healthy trees (~62% incidence for disease category 1, see Node 7 in Figure 4.4.3.7). The soil clay content for all the trees exhibiting the citrus decline symptoms was < 5.5% (see internode 2 in Figure 4.4.3.7). The worst affected trees (disease category 4) were planted in soils with a clay content of < 5.5% and the incidence of blight was > 2.5% (see Node 6 in Figure 4.4.3.7). Trees showing the first signs of decline were planted in soils with a soil clay content of < 5.5%, the incidence of blight was < 2.5% and the soil Zn content was > 66.4 ppm. Trees demonstrating moderate symptoms of citrus decline had the same characteristics as the trees of disease progression class 2, except the soil Zn content was > 66.4 ppm (see Node 5 in Figure 4.4.3.7).

Discussion

The choice of the two orchards in this study proved to be in order although not visually in the same condition (site 2 had less declined trees, therefore no category 4 trees) but with more or less similar tree age, soil type and situated not far apart (same climatic conditions). The results enabled the comparison between healthy, slightly diseased and severely diseased trees. It was interesting to note from our analysis that the two sites were markedly different in terms of many of the same variables that were measured, which was not apparent at the beginning of the study. This highlights the need to study as many variables as possible when comparing results between orchards since on the surface, orchards and the decline factors seem similar. The analysis of all the parameters selected, as related to the decline process, clearly indicated that a logical process is taking place as shown by the clear distinction between the different disease categories. It also indicated that the correct parameters associated with the decline process were monitored.

Despite the fact that the two sites were obviously different, as described above, a few parameters were found in common in the healthy trees (clay content in the soil, free-living and plant parasitic nematodes in the soil and leaf size) and in the declining tree categories (silt content, %P, and Mo content) (Figures 4.4.3.3c and 4.4.3.4c). This could indicate that the decline process had the same origin. The most surprising aspect was the relationship between the level of decline and clay content in the soil. The results showed that a higher clay content was associated with the healthier tree categories (Category 1 and 2) and the opposite for the diseased (Category 3 and 4) trees (Figures 4.4.3.5b, 4.4.3.6b and 4.4.3.7). This result did not support previous associations of higher clay content and disease severity. It was not expected from a within field study in an orchard that was selected for its soil homogeneity. Everything was done to avoid a trial site that consist of one part with higher clay and the rest only sand because then it would not be possible to determine if the decline was due to the differences in soil types or the parameters analysed. In this case, we refer to a clay content difference of only 3%, which is not considered as a difference that will change the physical classification of the soil.

The free-living nematodes appear in higher numbers in the healthier category trees that might indicate a more healthy soil environment. Further studies to determine if free-living nematodes could be used as indicators for healthy soils, needs to be done. The plant parasitic nematodes (PPN) (*Tylenchulus semipenetrans*, juveniles in the soil and females in the roots) reflect the healthy root activity due to the higher numbers in the healthy categories and their declining numbers in the diseased categories. It is widely known that healthier trees can accommodate more PPN when the roots are still healthy and there are more roots available as a food source for the nematodes.

The fact that the decline in these specific orchards seems to be related to the physical character of the soil illustrates that a biological factor is potentially not involved in the decline process observed in these cases. Also, the even distribution of the declining trees within the orchards without a classical pattern of a disease spread (e.g. irrigation as vector of a pathogen) does not support the hypothesis that a biological factor is involved. However, the results reflect a diachronic study over a brief time period (trees selected with established decline symptoms) and this emphasizes that decline needs to be monitored over a longer time period than done in this study.

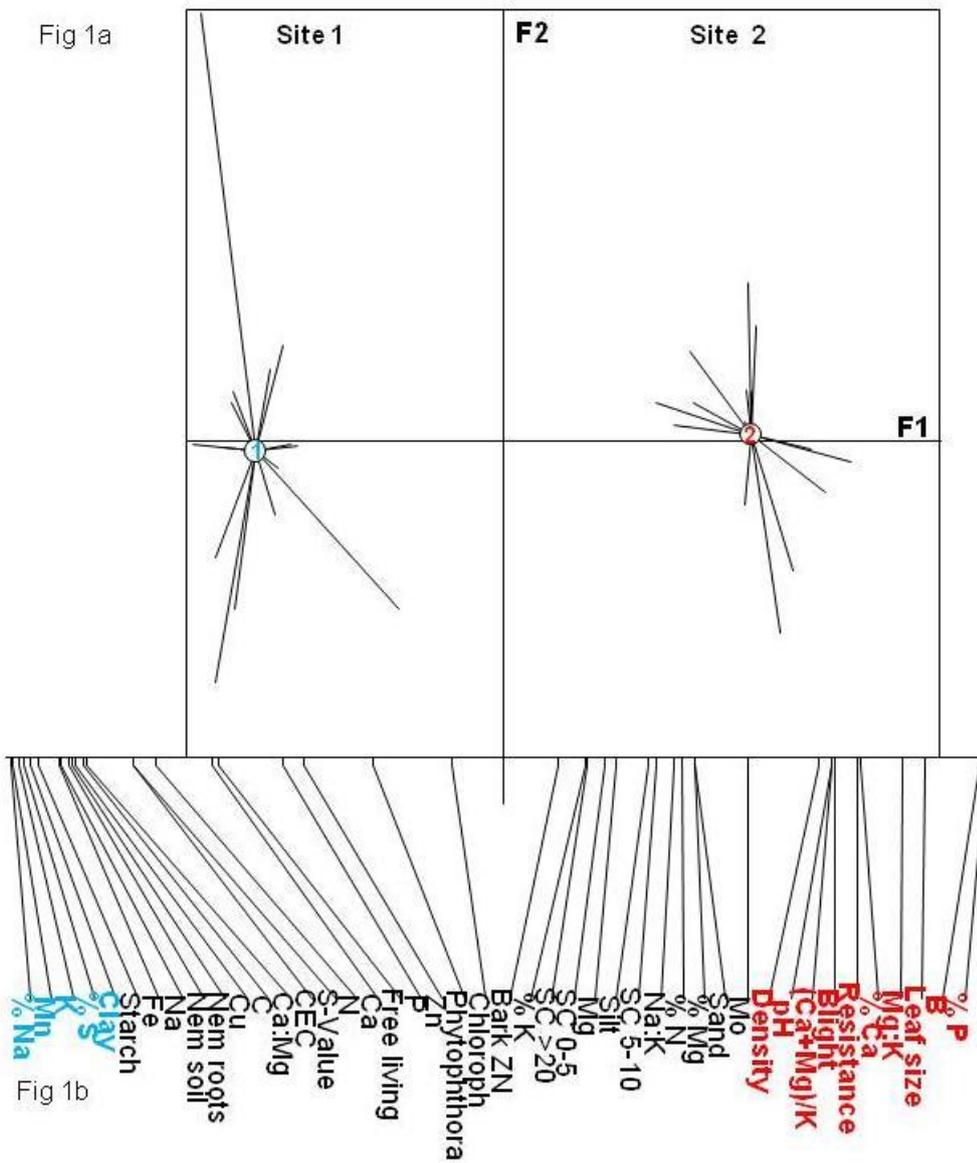
The strength of the multi-variate analysis is clearly demonstrated with the association of clay content and diseased categories, which would not be possible if the data had been analysed only with a traditional statistical model e.g. ANOVA where variables would only have been analysed one-by-one. CART analysis furthermore proved valuable for evaluating complex datasets; the CART results can be used to build a robust yet simple early-warning or predictive model for citrus decline based on three parameters only. The validity of this model will have to be verified by means of additional data collection and analyses in orchards of similar soil type.

References cited

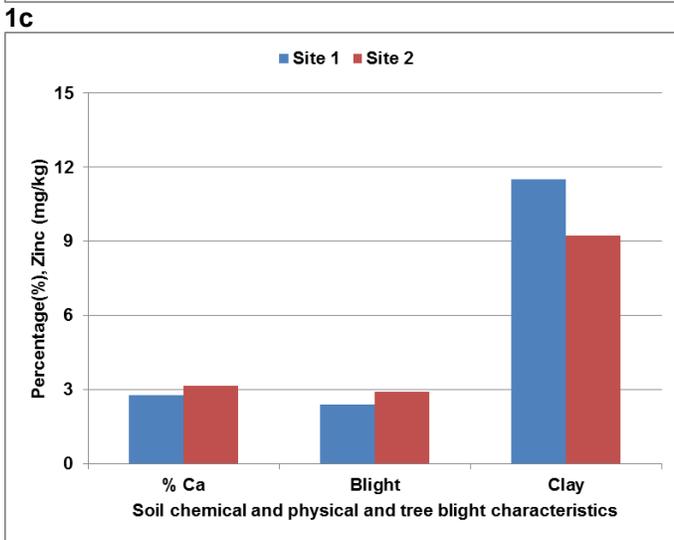
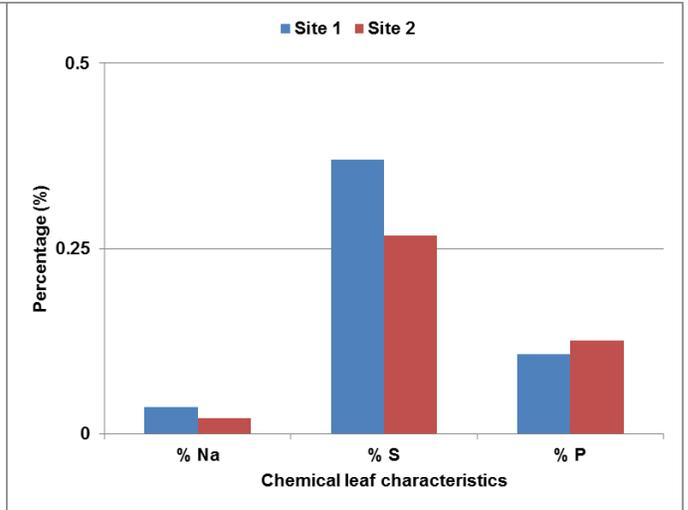
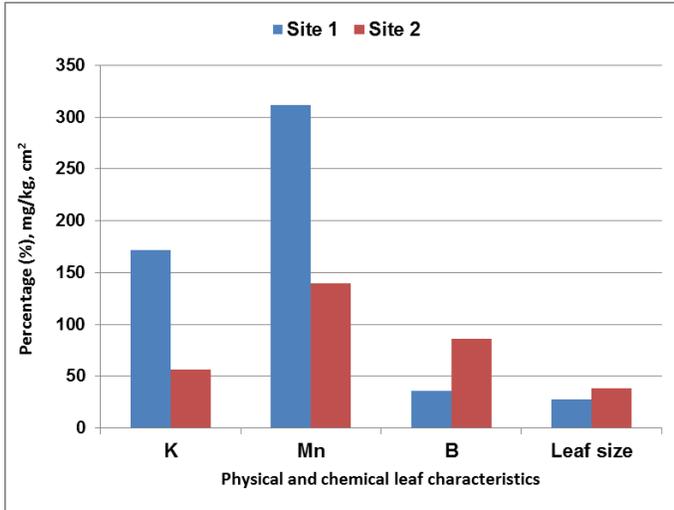
- Schalau, J. December 2010. Growing Citrus in Containers. *University of Arizona Extension Brief*.
- Suit, R.F. 1947a. Spreading decline of citrus in Florida. *Proceedings of Florida State Horticultural Society* 60: 17-23.
- Suit, R.F. and E.P. DuCharme. 1947b. Citrus decline. *Citrus Industry* 28(7): 8-13.
- Suit, R.F., and L.C. Knorr. 1949. Progress Report on citrus decline. *Proceedings of Florida State Horticultural Society* 62:45-49.
- Thioulouse, J, Chessel D, Dolédec S and Olivier J M. 1997. ADE-4: a multivariate analysis and graphical display software. *Statistics and Computing* 7: 75-83.
- Whitehead, A.G. and J.R. Hemming. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* 55: 25-38.
- Van der Vegte, F.A. 1973. A new method of estimating the numbers of citrus nematodes (*Tylenchulus semipenetrans*) in root samples. *Nematological Society of South Africa Newsletter* 4:11-12.

Table 4.4.3.1. Physical, chemical and biological measurements, their abbreviations and units of measurement, taken from soil and leaf samples collected from trees belonging to the different decline categories in the two studied orchards.

Measurement	Abbreviation	Unit of measurement	Source/area of sampling
Sodium	Na	Percentage (%)	Leaf
Sulphur	S	Percentage (%)	Leaf
Phosphorous	P	Percentage (%)	Leaf
Calcium	Ca	Percentage (%)	Leaf
Boron	B	mg/kg	Leaf
Molybdenum	Mo	mg/kg	Leaf
Zinc	Zn	mg/kg	Bark
Blight test	-	Seconds (s)	Tree trunk
Leaf size	-	cm ²	Leaf
Manganese	Mn	Percentage (%)	Soil
Potassium	K	Percentage (%)	Soil
Magnesium:Potassium	Mg:K	-	Soil
(Calcium+Magnesium)/Potassium	(Ca+Mg)/K	-	Soil
pH	pH	-	Soil
Clay content	-	%	Soil
Silt content	-	%	Soil
Sand content	-	%	Soil
Density	-	g/cm ³	Soil
Resistance	CEC	-	Soil
Citrus nematodes	-	Females/10g roots Juveniles/100g soil	Soil
<i>Phytophthora</i>	-	%	Soil

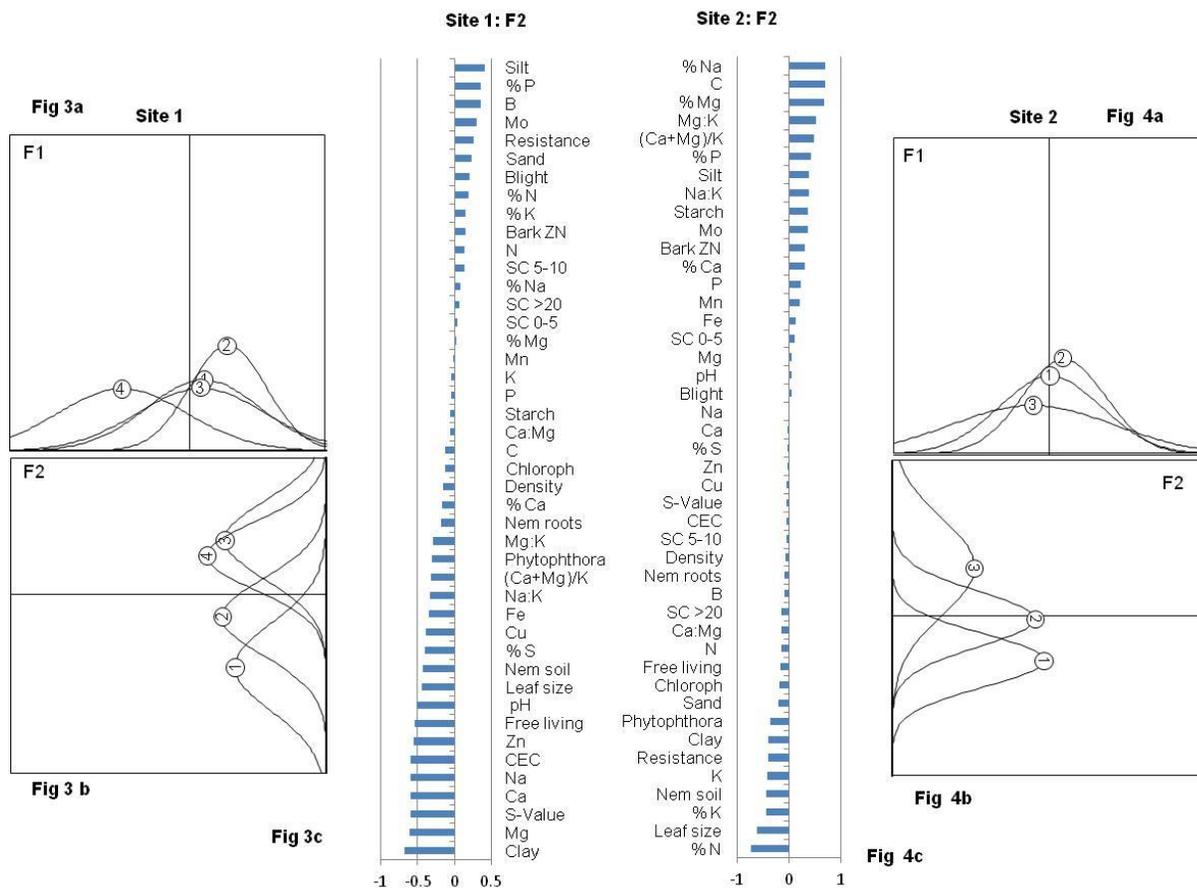


Figures 4.4.3.1a and b. (a) Site 1 and Site 2 presented on the factorial plan and (b) analysed parameters associated with Site 1 and 2.



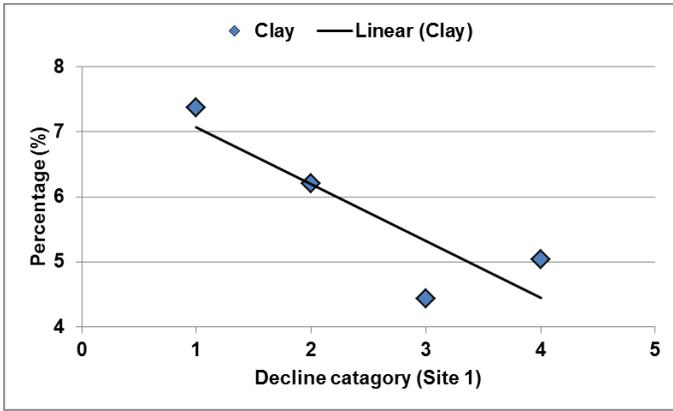
1e

Figures 4.4.3.1 c, d and e. Actual average values of certain soil, leaf and tree parameters measured at both sites over a two year period.

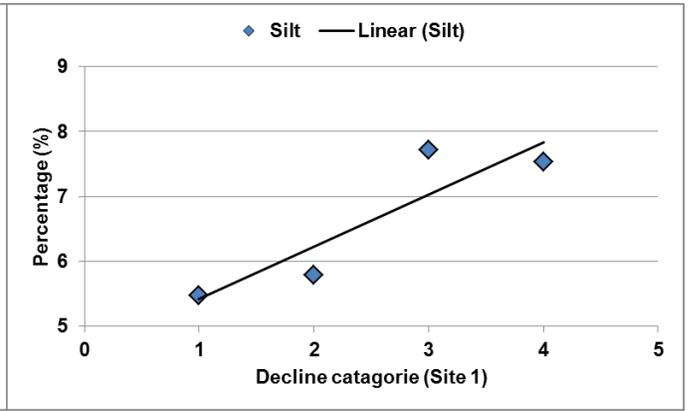


Figures 4.4.3.3a, b and 4a and b. The factorial values corresponding to the 4 disease categories in site 1 and 2 projected and shown as Gauss curves along the horizontal (F1) and vertical (F2) axis – each Gauss curve has the same area but the basis is proportional to the variability within each category.

Figures 4.4.3.3c and 4c. The 44 parameters measured on both sites sorted according to their F2 values, ranking from the highest to the lowest are presented on a vertical graph.

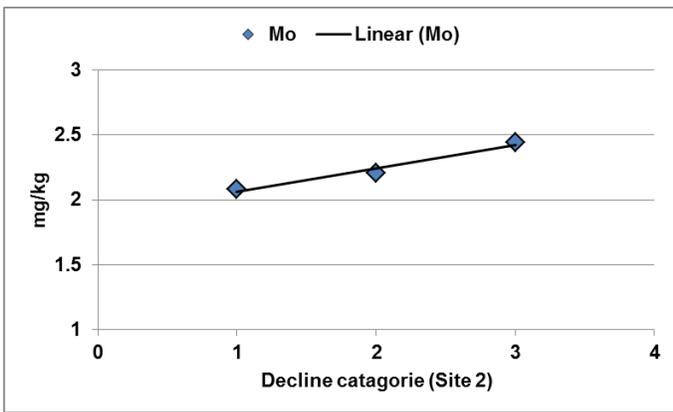


3d

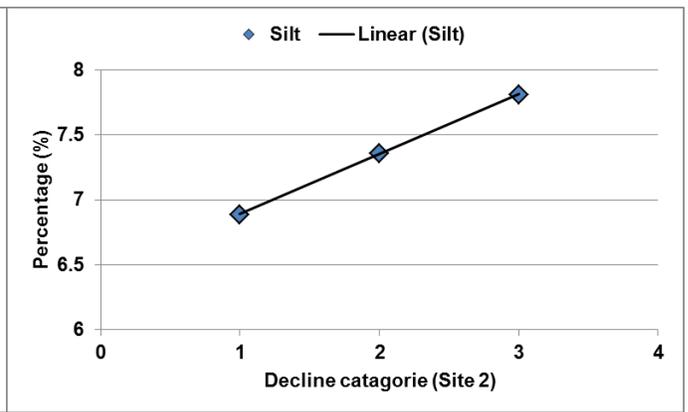


3e

Figures 4.4.4.3d and 3e. Increase and decrease of the actual percentage silt and clay values in the soil as the disease categories increase from 1 to 4



4d



4e

Figures 4.4.3.4d and 4e. Mo levels in the leaves (mg/kg) and percentage silt in the soil increases as the disease category increases from 1 to 3.

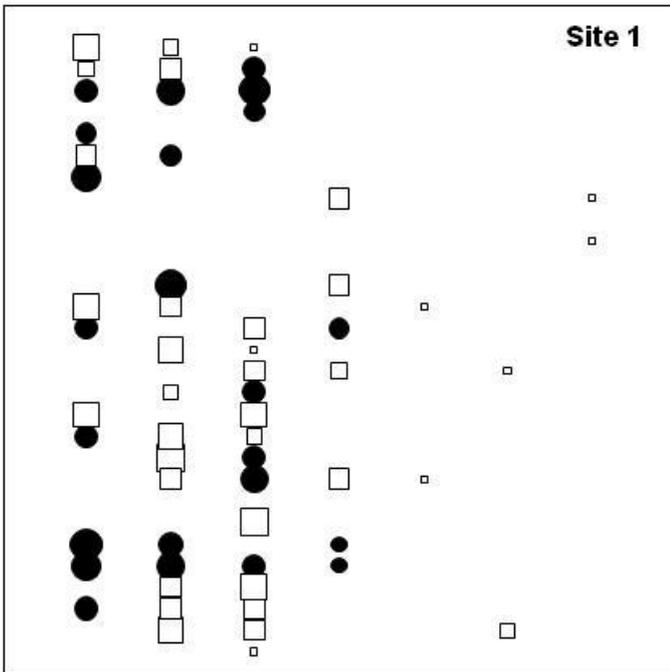


Fig 5a

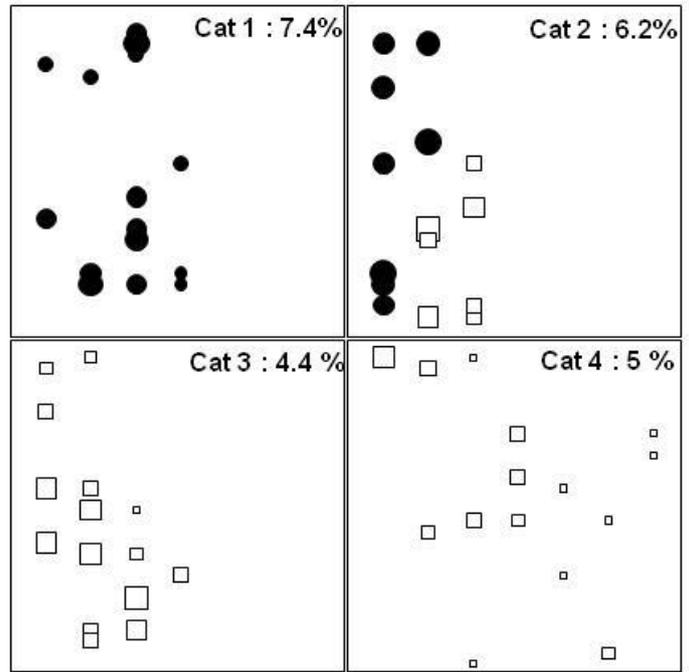


Fig 5b

Figure 4.4.3.5a. Site 1 trial plan – Each circle and square indicate the even distribution of clay content within the trial – Circles represent the clay value above the average and the squares the clay value below the average.

Figure 4.4.3.5b. Clay values split according to the four disease categories for Site 1.

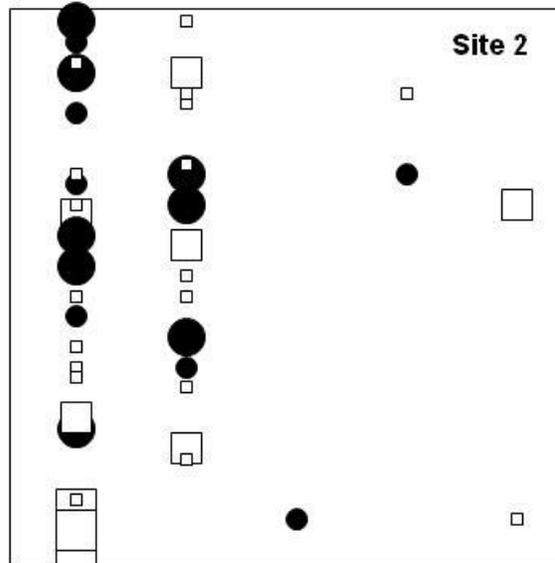


Fig 6a

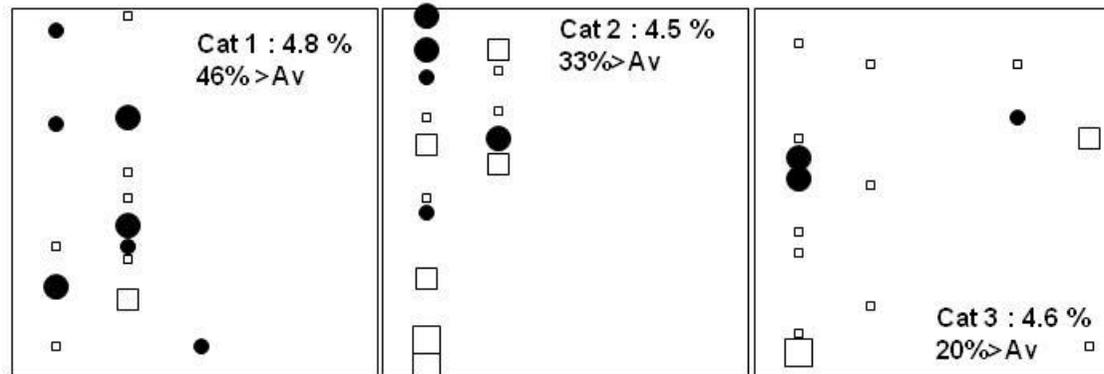


Fig 6b

Figure 4.4.3.6a. Site 2 trial plan – Each circle and square indicate the even distribution of clay content within the trial – Circles represent the clay value above the average and the squares the clay value below the average.

Figure 4.4.3.6b. Clay values split according to the three disease categories for Site 2.

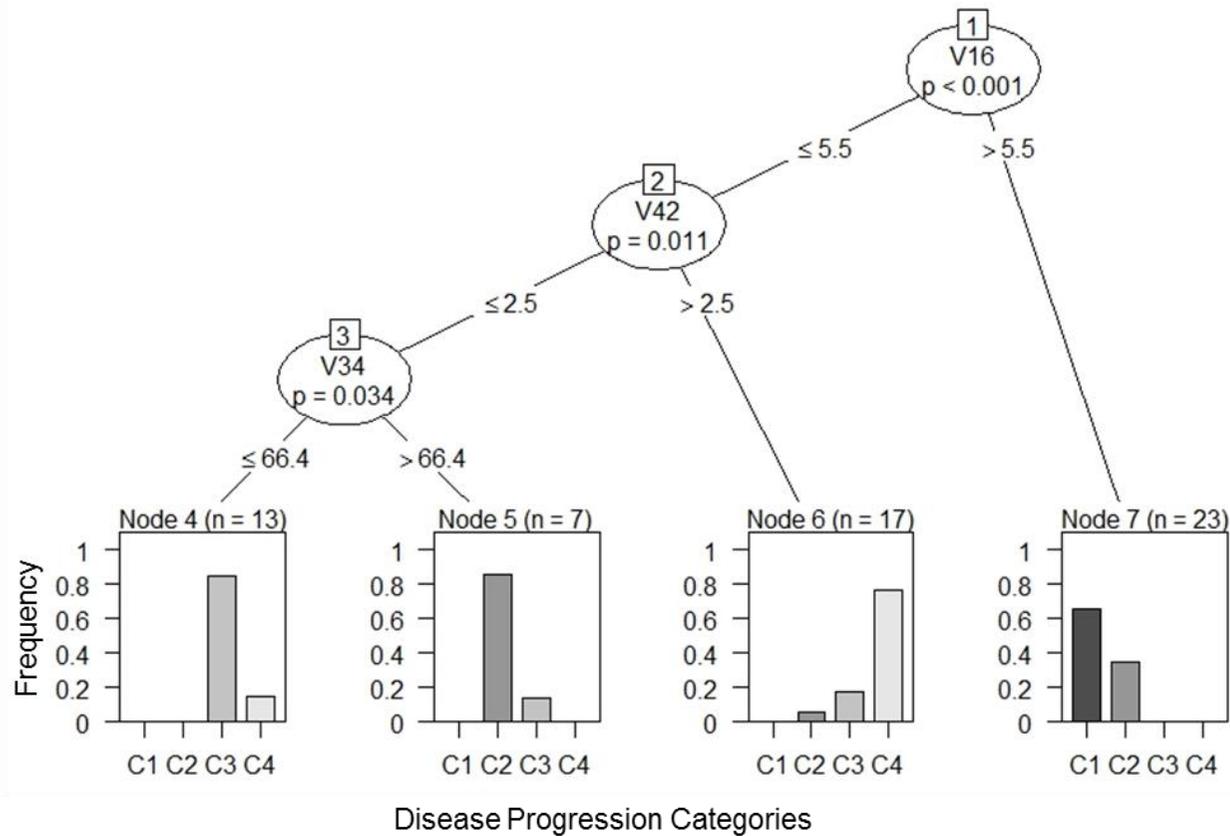


Figure 4.4.3.7. Differentiation between the citrus decline disease categories (C1 –C4) observed at Site 1 based on the soil clay content (% , V16), the incidence of blight (% , V42) and the soil zinc content (ppm, V34) as indicated by classification and regression tree analysis. The vertical axes indicate the frequency of each disease category (horizontal axes) being associated with the dependent variables (or its combinations). The disease categories indicate the progression of the decline: healthy (C1), healthy appearance with slight decline symptoms (C2), typical root disease decline symptoms (C3) and severely declined (C4) trees. Conditional inference at $\alpha = 0.05$ was used as node split criterion.

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

Project 1030 (2008 – 2016/17) by JM van Niekerk, MC Pretorius & C Kotze (CRI)

Opsomming

Die sitrus nematode, *Tylenchulus semipenetrans*, infekteer sitrus wêreldwyd en is die volopste plantparasitiese nematode in sitrusboorde. Die gebruik van toksiese nematisiedes kom toenemend plaaslik en internasionaal onder druk. Ontwikkeling van alternatiewe tot chemiese nematisiedes is dus noodsaaklik. Die volgende produkte is ingesluit in 'n nuwe proef wat gedurende September 2014 begin is. 'n Nuwe produk, OLLYS is getoets teen drie verskillende konsentrasies tesame met Cropguard en Mosblend. 'n Swambevattende nematisied, PL Gold Plus, is ook ingesluit en toegedien in sewe verskillende programme in kombinasie met standard Rugby 10G of alleen. Na een seisoen se evaluasie is duidelike gevolgtrekkings aangaande die beste behandeling nog nie moontlik nie. Op hierdie vroeë stadium wil dit voorkom asof die program waar Rugby 10G toegedien word in September gevolg deur PL Gold Plus aanwendings in Oktober en November die meeste potensiaal het. Hierdie program het konstant in beide monster periodes die nematode getalle in die wortels en grond verminder. Ten einde duidelike resultate te verkry sal die evaluasie egter vir nog 'n seisoen voorgesit moet word.

Summary

Tylenchulus semipenetrans, the citrus nematode infects citrus worldwide and is the most abundant and frequent plant-parasitic nematode in citrus orchards. The use of toxic compounds as nematicides is becoming more and more under pressure internationally and locally. Developing alternatives to chemical nematicides is therefore essential. The following products were included in a new trial that commenced in September 2014. A new product OLLYS was trailed at three different concentrations along with Cropguard and Mosblend. A fungal nematicide, PL Gold Plus was also included and applied according to seven different application regimes and in combination with the standard Rugby 10G. After only one season of testing results are still inconclusive. However, at this early stage the programme where Rugby 10G is applied in September followed by PL Gold Plus applied in October and November seems promising. This programme consistently reduced juvenile and female counts over the two sampling periods of December 2014 and February 2015. However, in order to obtain conclusive results evaluation for another season is needed.

Introduction

Nematodes are a diverse group of invertebrates, abundant as parasites or free living forms in soil, freshwater and marine environments. Soils are a particularly rich environment for nematodes, with about 26% of described genera inhabiting soil as bacterivores, fungivores, omnivores, predators or plant parasites (McSorley, 2005).

The citrus nematode, *Tylenchulus semipenetrans* Cobb, infects citrus worldwide (Van Gundy and Meagher, 1977; Heald and O'Bannon, 1987) and is the most abundant and frequent plant-parasitic nematode in citrus groves. Yield losses are estimated at about 10% worldwide. The citrus nematode is associated with poor growth of young citrus trees planted in infested groves and with poor performance of mature citrus trees. The host range of *T. semipenetrans* includes all Citrus species and most hybrids of citrus with other members of the rutaceous family such as trifoliolate orange (*Poncirus trifoliolate* L.Raf). non-rutaceous plants such as grape (*Vitis vinifera*, L), olive (*Olea europea*, L) and persimmon (*Diospyrus spp.*) are also hosts (Verdejo-Lucas, 2002).

Damage thresholds, nematode population densities that suppress tree growth and yield, are influenced by several factors including aggressiveness of the nematode population, soil type, rootstock, other diseases and grove management practices (Garabedian *et al.* 1984). Threshold values in South Africa have been set at 10 000 juveniles/250 cc soil and a 1000 females/10 g root in samples.

T. semipenetrans migrates very slowly on its own power and therefore does not readily spread from tree to tree in existing orchards. Infestation of new orchards occurs mainly through infested planting material and contaminated irrigation water (Tarjan, 1971; Baines, 1974). It is recorded that the sheath nematode, *Hemicycliophora* spp. occurs in combination with the citrus nematode in certain citrus producing countries in the world (Van Gundy, 1959) but the effect of the nematode on yields is not known. The sheath nematode was also detected in certain citrus producing regions in South Africa (L. Huisman, personal communication, CRI Diagnostic Centre, Nelspruit, 2007).

In the past, the nematicide 1,2 dibromo-3-chloropropane (DBCP) was widely used in the irrigation water against this nematode with great success. The nematode was effectively controlled while yields were also substantially increased (O'Bannon *et al.*, 1963; Philis, 1969). The activity on eggs is the most important difference between the soil fumigants used to control nematodes earlier this century, and today's non-fumigant chemicals. Following the withdrawal of DBCP, non-fumigant post-plant nematicides (carbamate or organophosphate acetylcholinesterase inhibitors) were introduced. These chemicals, however, could not eliminate, or greatly reduce, nematode populations even if applied every year. Fenamiphos is translocated systemically in the vascular system of plants, whilst the other nematicides are non-systemic and reduce nematode populations through their initial contact action only. This explains the quick recovery of nematode populations once the nematicide has been degraded in soil and emphasizes the adverse effect of enhanced degradation, as eggs hatching after the nematicide has been degraded can continue the nematode's life-cycle. The following nematicides are currently registered on citrus in South Africa: aldicarb, cadusafos, fenamiphos, terbufos, ethoprophos, fosthiazate and furfural (Nel *et al.*, 2002). When multiple nematicide applications were introduced on a commercial scale to citrus orchards in South Africa, situations occurred where growers were not successful in disrupting the nematode's life cycle despite adhering strictly to prescribed procedures. In an investigation to determine the efficacy of cadusafos in soil where aldicarb and fenamiphos failed as a result of accelerated degradation, it was found that in the absence of sufficient irrigation water none of the nematicides were distributed thoroughly through the soil profile and they consequently failed to eliminate the citrus nematode (Le Roux *et al.*, 1998).

Due to safety, environmental concerns and market pressure, only a few registered chemical nematicides remain worldwide for utilization by farmers, and the use of these products is highly restricted. Developing alternatives to chemical nematicides is essential and a great concern to researchers worldwide. Recent attempts to develop alternative methods to manage plant-parasitic nematodes include the use of entomopathogenic nematodes and various biologically derived nematicides and other organic compounds. The aim of this experiment is to: evaluate less toxic compounds for the control of the citrus nematode as well as for the control of *Phytophthora* spp. in citrus orchards.

In the field trial conducted, a range of alternative products such as non-toxic and organic compounds for the control of the citrus nematode have been evaluated. International pressure from various market organizations and governments to reduce the use of highly toxic and environmentally unfriendly products along with the final withdrawal of aldicarb in South Africa, justifies the continued testing of alternative chemicals for the control of nematodes and *Phytophthora* in South African citrus orchards.

Objective

The development and evaluation of new products for the control of soilborne pests and diseases in citrus orchards.

Materials and methods

Nematodes

A nematode infested citrus orchard with nematode female counts in excess of 5000 females per 10 g of roots was identified in 2014. This was regarded as a suitable trial site, as the standard threshold value of 1000 females per 10 g of roots was exceeded. The 17-year-old Late Valencia on Rough Lemon citrus orchard with a 10 m² drip zone is situated east of Nelspruit at Crocodile Valley Citrus Co. Single tree plots were randomly selected and replicated eight times for each treatment. The treatments included were OLLYS, an experimental product obtained from Lawrence Marais, at three different dosages. PL Gold Plus, a *Paecilomyces lilacinus* containing fungal nematicide from Becker Underwood, was applied according to seven different regimes. Also included were Cropguard from Illovo and Mosblend from Hygrotech along with Rugby 10G (cadusafos) from Philagro that was included as an industry standard treatment. The different dates of applications and dosages are presented in Table 4.4.4.1.

The products were applied by means of a 10 litre watering can to ensure an even distribution of the products under the drip zone of the trees. Cadusafos served as the standard chemical control. Protective clothing was worn to protect the researcher and assisting staff during application of these products. All the applications were executed in good weather conditions with an average day temperature of 29°C. The first applications were done in September 2014, with soil and root samples collected during December 2014, February and April 2015. These were analysed at the CRI DC using the standard techniques for *Phytophthora* and nematode analyses.

Table 4.4.4.1. Dosages and dates of application of the different products applied to determine the effect of these treatments on the citrus nematode populations in the orchard at Crocodile Valley Citrus Co. during the 2014/2015 season.

Product/Application regime	Month/Application dosage						
	Sept	Oct	Nov	Dec	Jan	Feb	Mar
Untreated	-	-	-	-	-	-	-
OLLYS Regime 1		5 ml/m ²		5 ml/m ²		5 ml/m ²	
OLLYS Regime 2		10 ml/m ²		10 ml/m ²		10 ml/m ²	
OLLYS Regime 3		20 ml/m ²		20 ml/m ²		20 ml/m ²	
Cropguard		7.5 ml/m ² + 2.5 ml/m ² -2 weeks later + 2 weeks later					7.5 ml/m ² + 2.5 ml/m ² -2 weeks later + 2 weeks later
PL Gold Plus Regime 1	1 g Gold Plus + 1 ml Gold Starter/m ²	1 g Gold Plus + 1 ml Gold Starter/m ²					
PL Gold Plus Regime 2			1 g Gold Plus + 1 ml Gold Starter/m ²	1 g Gold Plus + 1 ml Gold Starter/m ²			
PL Gold Plus Regime 3					1 g Gold Plus + 1 ml Gold Starter/m ²	1 g Gold Plus + 1 ml Gold Starter/m ²	
PL Gold Plus Regime 4	1 g Gold Plus + 1 ml Gold Starter/m ²	1 g Gold Plus + 1 ml Gold Starter/m ²	Rugby 15 ml/m ²				
PL Gold Plus Regime 5	1 g Gold Plus + 1 ml Gold Starter/m ²	1 g Gold Plus + 1 ml Gold Starter/m ²	1 g Gold Plus + 1 ml Gold Starter/m ²	1 g Gold Plus + 1 ml Gold Starter/m ²	1 g Gold Plus + 1 ml Gold Starter/m ²	1 g Gold Plus + 1 ml Gold Starter/m ²	
PL Gold Plus Regime 6	1 g Gold Plus + 1 ml Gold Starter/m ²	1 g Gold Plus + 1 ml Gold Starter/m ²		1 g Gold Plus + 1 ml Gold Starter/m ²	1 g Gold Plus + 1 ml Gold Starter/m ²		
PL Gold Plus Regime 7	Rugby 15 ml/m ²	1 g Gold Plus + 1 ml Gold Starter/m ²	1 g Gold Plus + 1 ml Gold Starter/m ²				
Rugby 10 G		Rugby 15 ml/m ²		Rugby 15 ml/m ²		Rugby 15 ml/m ²	
Mosblend	40 L/ha	40 L/ha	40 L/ha	40 L/ha	40 L/ha	40 L/ha	40 L/ha

Results and discussion

Objective / Milestone	Achievement
Apr –Jun 2014 1. Annual report	1. Annual report was written and submitted.
Jul – Sept 2014 1. Trial planning 2. First applications according to trial layout	1. Trial was planned and products obtained. 2. The first applications were done according to the trial layout in Table 1.
Oct – Dec 2014 1. Do applications according to trial layout. 2. Collect soil and root samples.	1. Applications were done according to trial layout. 2. Soil and root samples were collected.
Jan – Mar 2015 1. Do applications according to trial layout. 2. Collect soil and root samples.	1. Applications were done according to trial layout. 2. Soil and root samples were collected.

Nematodes

The juvenile count results (Table 4.4.4.2) showed that the best treatments were the PL Gold Plus Regime 7 followed by the PL Gold Plus Regime 2. The third best treatment was the Rugby 10G treatment. All of these treatments, except the Rugby 10G, reduced the juvenile counts by more than 50% on average between the three sampling dates. Although these reductions were not significantly better than the untreated control, a marked reduction was seen as mentioned above.

The results obtained for female nematode counts paint a different picture from the juvenile results. Based on the December 2014 sampling all the treatments, except PL Gold Plus Regime 3, reduced the numbers in comparison to the untreated control. However, none of these reductions were significant. The February 2015 results contradicted the December 2014 results. For this sampling date the only treatments that reduced the female counts were the PL Gold Plus Regime 7 and the Mosblend treatment. Results from the April 2015 sampling indicated that a number of treatments did reduce the female counts. However, the only one that stood out above the other treatments was the PL Gold Plus Regime 7 treatment (Table 4.4.5.3). This treatment also performed well in controlling or reducing juvenile counts and is therefore at this stage looking very promising.

This trial will also be repeated in the 2015/2016 season in order to obtain more long term data that will allow clear conclusions as to which is the best treatment or treatment regime.

Table 4.4.4.2. The evaluation of various post-plant treatments for the control of the citrus nematode and their effect on citrus nematode juvenile populations at Crocodile Valley Citrus Co. based on analysis of soil and root samples collected during December 2014 and February 2015.

Treatment	Dec-14	% decrease /increase	Feb-15	% decrease /increase	Apr-15	% decrease /increase
Untreated	2675 b		1500 abc		1769a-d	
OLLYS Regime 1	1600 ab	-40	1775 abc	18	2831ab	60
OLLYS Regime 2	1438 ab	-46	975 abc	-35	2543a-d	44
OLLYS Regime 3	1575 ab	-41	1075 abc	-28	2619a-c	48
Cropguard	1150 ab	-57	1088 abc	-28	2263a-d	28
PL Gold Plus Regime 1	2075 ab	-22	925 abc	-38	1338b-d	-24
PL Gold Plus Regime 2	775 a	-71	863 bc	-43	744d	-58
PL Gold Plus Regime 3	2438 ab	-9	1875 ab	25	1356b-d	-23
PL Gold Plus Regime 4	1250 ab	-53	1863 abc	24	793d	-55
PL Gold Plus Regime 5	788 a	-71	1300 abc	-13	2250a-d	27
PL Gold Plus Regime 6	775 a	-71	225 c	-85	2206a-d	25
PL Gold Plus Regime 7	1288 ab	-52	488 bc	-68	1113cd	-37
Rugby 10G	1038 ab	-61	1075 abc	-28	1281cd	-28
Mosblend	838 a	-69	2513 a	68	3206a	81

¹Means followed by the same letter are not significantly different at a $P \leq 0.05$ confidence level.

Table 4.4.4.3. The evaluation of various post-plant treatments for the control of the citrus nematode and their effect on citrus nematode female populations at Crocodile Valley Citrus Co. based on analysis of soil and root samples collected during December 2014 and February 2015.

Treatment	Dec-14	% decrease/inc rease	Feb-15	% decrease/inc rease	Apr-15	% decrease/inc rease
Untreated	2775 bc ¹		825 bc		1375a-d	
OLLYS Regime 1	1425 abc	-49	2775 a	236	2188a	59
OLLYS Regime 2	1000 ab	-64	1975 abc	139	2125ab	55
OLLYS Regime 3	1975 abc	-29	1175 bc	42	2038a-c	48
Cropguard	1625 abc	-41	1125 bc	36	1563a-d	14
PL Gold Plus Regime 1	2275 abc	-18	1025 bc	24	1513a-d	10
PL Gold Plus Regime 2	1025 ab	-63	2700 a	227	988b-d	-28
PL Gold Plus Regime 3	3100 c	12	1500 abc	82	1038a-d	-25
PL Gold Plus Regime 4	1975 abc	-29	2125 ab	158	963cd	-30
PL Gold Plus Regime 5	800 abc	-71	1625 abc	97	1213a-d	-12
PL Gold Plus Regime 6	1100 ab	-60	1300 abc	58	900cd	-35

PL Gold Plus Regime 7	925 ab	-67	550 c	-33	463d	-66
Rugby 10G	1300 abc	-53	1475 abc	79	638d	-54
Mosblend	1150 ab	-59	800 bc	-3	1488a-d	8

¹Means followed by the same letter are not significantly different at a $P \leq 0.05$ confidence level.

Technology transfer

Data will be presented at the biennial CRI Symposium in August 2016.

Further objectives and work plan

Continue to search for alternative products for the control of the citrus nematode and *Phytophthora* spp. Treatments for *Phytophthora* spp. control that were trailed in 2012 will again be trailed in pots with seedlings during the 2015/2016 season in order to confirm the previous results. Promising treatments from this repeat trial will be taken to a field trial at the end of 2016. The best treatments from the nematode control trial reported on above, will also be taken into a long term field trial at the end of 2016 in order to get long term nematode control data along with long term tree measurement and yield data.

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

April – June 2016

1. Conclude field trial from the 2015/2016 season.
2. Collect final soil samples.
3. Continue with *Phytophthora* control field trial with biologicals.
4. Write annual report.
5. Plan new field trials for *Phytophthora* and nematode control.

July – September 2016

1. Continue with *Phytophthora* control field trial with biologicals.
2. Plan new field trials for *Phytophthora* and nematode control.
3. Start new trials in September 2016.

October – December 2016

1. Trial applications.
2. Soil and root sample collection.

January – March 2017

1. Trial applications.
2. Soil and root sample collection.

References cited

- Baines, R.C. 1974. The effect of soil type on movement and infection rate of larvae of *Tylenchulus semipenetrans*. J. Nem. 6:60-62.
- Baines, R.C., Klotz, L.J., DeWolfe, R.H., Small, R.H., and Turner, G.O., 1966. Nematocidal and fungicidal properties of some soil fumigants. Phytopathology 56:691-698.
- Cohn, E. 1965b. The development of the citrus nematode on some of its hosts. Nematologica 11:593-600.
- Garabedian, S., Van Gundy, S.D., Mankau, R. & Radewald, J.D. 1984. Nematodes. Integrated Pest Management for Citrus. Division of Agriculture and Natural Resources Publications, University of California, Berkeley, California. Pp. 129-131.
- Heald, C.M. & O'Bannon, J.H. 1987. Citrus declines caused by nematodes. V. Slow decline. Nematology Circular No. 143. Florida Department of Agriculture and Consumer Services, Division of Plant Industry, 4 pp.
- Le Roux, Ware, A.B. & Pretorius, M.C. 1998. Comparative efficacy of preplant fumigation and postplant chemical treatment of replant citrus trees in an orchard infested with *Tylenchulus semipenetrans*. Plant Dis. 82, 1323-1327.
- McSorley, R. 2005. Adaptations of Nematodes to environmental extremes. Florida Entomologist vol. 86, 2, 138 -142.
- Nel, A., Krause, M. & Khelawanlall, N. 2002. A Guide for the control of Plant Pests. Department of Agriculture, Private Bag X144, Pretoria 0001. Thirty Ninth Edition.
- O'Bannon, J.H. & Reynolds, H.W. 1963. Response of navel orange trees to a post planting application of DBCP for control of the citrus nematode. Pl. Dis. Repr., 5:401-404.

- O'Bannon, J.H., Leather, C.R. & Reynolds, H.W. 1967. Interactions of *Tylenchulus semipenetrans* and *Fusarium* species on rough lemon (*Citrus limon*). *Phytopathology* 57, 414-417.
- Philis, J. 1969. Control of citrus nematode, *Tylenchulus semipenetrans*, with DBCP in established Cyprus citrus groves. *Pl. Dis. Reptr.*, 53:804-806.
- Tarjan, A.C. 1971. Migration of three pathogenic citrus nematodes through two Florida soils. *Soil Crop Sci. soc. Fla. Proc.* 31:253-255.
- Van der Vegte, F.A. 1973. A new method of estimating the numbers of citrus nematodes (*Tylenchulus semipenetrans*) in root samples. *Nem. Soc. S.A. Newsl.* 4:11-12
- Van Gundy, S.D. 1958. The life history of the citrus nematode *Tylenchulus semipenetrans* Cobb. *Nematologica* 3, 283-294.
- Van Gundy, S.D. 1959. The life history of *Hemicycliophora arenaria* Raski (Nematoda: Criconematidae). *Proceedings of the helminthology Society of Washington*, 26:67-72.
- Van Gundy, S.D. & Meagher, J.W. 1977. Citrus nematode (*Tylenchulus semipenetrans*) problems worldwide. 1977 Intern. Cit. Congr., Orlando, Florida. pp 7.
- Verdejo-Lucas, S. & Kaplan, D.T. 2002. The citrus nematode: *Tylenchulus semipenetrans*. In: Starr, J.L., Coo, R. and Bridge, J. (eds). *Plant Resistance to Parasitic Nematodes*. CAB International, Wallingford, UK, pp. 207-219.
- Whitehead, A.G. & J.R. Hemming. 1965. Comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Ann. Appl. Biol.* 55:25-38.

4.4.5 **PROGRESS REPORT: The status of Armillaria root rot and its management in South African citrus orchards**

Project 1068 (2012/3 – 2016/7) by J.M. van Niekerk, M.C. Pretorius and C. Kotze (CRI)

Summary

A decline and death of citrus trees have been reported from Swaziland, Hoedspruit and the Gamtoos and Sunday's River valleys for a number of years. Initial thoughts were that the symptoms are caused by *Armillaria* spp. Despite several attempts to isolate this pathogen, no isolates could be obtained from symptomatic tissue collected from declining trees. However, several other fungal genera were isolated, which led to the conclusion that *Armillaria* spp. might not be involved. Molecular identification of the isolated fungi identified *Kretzschmaria deusta* as being the only pathogen associated with the symptoms observed in Swaziland and Hoedspruit. This pathogen, previously known as *Ustulina deusta*, is known to cause *Ustulina* root and collar rot of citrus. In the two Eastern Cape areas a complex of pathogens were found to be associated with the observed symptoms. In this case the dominant ones were *Fusarium solani*, *Diaporthe neotheicola*, *Coprinellus micaceus* and *Eutypella* sp.. *Phaeoacremonium parasiticum* and *Schizophyllum commune* were also isolated but at levels much lower than the abovementioned group. *F. solani* has been associated with tree collapse and dry root rot or sudden death of citrus trees. *C. micaceus* are a coprinoid fungus that has the ability to degrade wood. *D. neotheicola* is known as a weak pathogen on several woody hosts but has been shown to cause a severe dieback of persimmons. Three different *Eutypella* spp. have been reported from California where they were associated with branch cankers and dieback of citrus trees. *Ph. parasiticum* is a known pathogen of many woody hosts such as grapevine, apple and forest trees. The last pathogen in the group is *S. commune* that is known to be associated with wood rot of citrus. Pathogenicity trials need to be conducted to confirm the pathogenicity of these pathogens on citrus and to determine how they interact. Effective management strategies will then be investigated.

Opsomming

Agteruitgang en afsterwe van sitrus bome is vir 'n aantal jare al in Swaziland, Hoedspruit en die Gamtoos en Sondagsriver valleie gerapporteer. *Armillaria* spp. is aanvanklik gereken as die veroorsakende organisme. Ten spyte van verskeie pogings om hierdie patogeen te isoleer kon geen isolate uit simptome materiaal verkry word nie. Verskeie ander swam genera is wel geïsoleer wat gelei het tot die gevolgtrekking dat *Armillaria* spp. dalk nie betrokke is nie. Molekulêre identifikasie van die geïsoleerde swamme het *Kretzschmaria deusta* geïdentifiseer as die enigste patogeen geassosieer met die simptome in Swaziland en Hoedspruit. Hierdie patogeen, vroeër bekend as *Ustulina deusta*, is bekend as die veroorsakende patogeen van *Ustulina* wortel en kraagvrot van sitrus. In die twee Oos-Kaap areas is bevind dat 'n kompleks van patogene met die waargenome simptome verbind word. Die dominante patogene was *Fusarium solani*, *Diaporthe neotheicola*, *Coprinellus micaceus* en *Eutypella* sp.. *Phaeoacremonium parasiticum* en *Schizophyllum commune* is ook geïsoleer, maar teen baie laer vlakke as bogenoemde groep. *F. solani* is bekend as die veroorsakende organisme van "dry root rot" en "sudden death" van sitrus. *C. micaceus* is welbekend as 'n houtverrotter. *D. neotheicola* is bekend as 'n swak patogeen hoewel dit ernstige

terugsterwing van persimmons veroorsaak. Drie verskillende *Eutypella* spp. is uit Kalifornië aangeteken in assosiasie met tak kankers en terugsterwing op sitrus. *Ph. parasiticum* is 'n bekende patoogeen van houtagtige gashere soos wingerd, appels en bosbou bome. Die laaste patoogeen, *S. commune*, veroorsaak weer houtverrotting van sitrus. Patogenisiteitstoetsing moet gedoen word om te bepaal of hierdie swamme almal patogene van sitrus is en moontlike interaksies te ondersoek ten einde die waargenome simptome te veroorsaak. Effektiewe beheerstrategieë sal dan ondersoek word.

4.4.6 **PROGRESS REPORT: Preventative and curative management of soilborne pathogens in citrus nurseries**

Project 1101 (2014 - 2017) by J.M. van Niekerk, M.C. Pretorius & C. Kotze (CRI)

Opsomming

In die eerste jaar van hierdie projek is 'n groot getal *Pythium*, *Phytophthora nicotianae* en *P. citrophthora* isolate versamel. "PCR-RFLP" en ITS DNA volgorde analise het verskeie *Pythium* spp. uit die versamelde isolate geïdentifiseer. Mefenoxam weerstandstoetsing vir die versamelde isolate het aangedui dat daar verskillende vlakke van weerstand by die verskillende swamdoder konsentrasies is. Hierdie resultate sal in konteks geplaas word na afloop van patogenisiteitstoetsing vir die *Pythium* spesies en ook mefenoxam residu analise van behandelde plantmedium. 'n Suksesvolle inokulasie tegniek is ontwikkel om groeimedium te inokuleer vir die toets van MeBr alternatiewe. Twee potproewe is ook suksesvol begin. Die eerste een sal in Junie 2015 en die tweede een aan die einde van 2015 voltooi word.

Summary

In the first year of this project a large number of *Pythium*, *Phytophthora nicotianae* and *P. citrophthora* isolates have been collected. PCR-RFLP and ITS sequence analyses identified a number of *Pythium* spp. amongst the collected isolates. Mefenoxam resistance testing for the collected isolates showed varying degrees of resistance at different fungicide concentrations. These results will be placed into context with mefenoxam residue analyses of treated potting medium and pathogenicity testing of the different *Pythium* species found. A successful inoculation technique was developed for inoculating potting medium in bags to be used for testing MeBr alternatives. Two pot trials were also started successfully with one concluding in June 2015 and the other at the end of 2015.

4.5 **PROGRAMME: POST-HARVEST PATHOLOGY**

Programme coordinator: Arno Erasmus (CRI)

4.5.1 **PROGRAMME SUMMARY**

Some good progress has been made during the 2014/5 period in the field of Postharvest Pathology. The team at Nelspruit has been expanded by the addition of two master's students focussing specifically on postharvest fungicide application technology. The project studying hot water, potassium silicate and biological control agents to reduce postharvest disease and chilling injury in citrus fruit at UKZN has been terminated and a final report was submitted. A new research project on effects of postharvest treatments on the reproductive potential of citrus black spot in infected fruit has been initiated at CRI in Nelspruit. The collaboration between CRI Nelspruit and the Department of Plant Pathology at the University of Stellenbosch was further strengthened by having Dr Cheryl Lennox as co-supervisor of the two master's students. A new project on essential oils by Prof Sandra Combrinck is bringing on a whole team from TUT to be part of the CRI Postharvest Pathology programme. Prof Paul Fourie still plays a pivotal roll in terms of guidance and strategising the CRI Postharvest Programme.

Following on from previous research (4.5.2), the pre-packhouse drench and flooder fungicide applications have been explored further (4.5.3). It is crucial to shorten the time from harvest to the first fungicide application (usually a drench). Flooder gives superb curative and protective green mould control as well as sporulation inhibition (4.5.4). This application has now been conclusively shown to be a real alternative to dip application. The molecular assessment of green mould resistance to imazalil has been shown to be more challenging than initially expected. Good progress was made in setting up protocols for the development of this assessment, but much more work is needed (4.5.5). The potential of hot water treatments combined with a yeast treatment for the protective control of green mould was shown. The challenge is now to test and implement these treatments on commercial level (4.5.6). Two new potential fungicidal actives (fludioxonil and propiconazole) demonstrated good action against green mould, especially on younger (≤ 18 h) infections. Propiconazole was also shown to be an acceptable alternative to guazatine, but use of this active needs to be with great caution due to its chemical similarity to imazalil (cross resistance

can be induced between these two actives). The product Fortisol was shown to inhibit guazatine burn. Some preliminary trials were conducted to indicate the “kill time” required by chlorine and hydrogen peroxide / peracetic acid to sanitise a spore suspension, as well as the effect of wetters in chlorine and fungicide solutions (4.5.7).

PROGRAMOPSOMMING

Goeie vordering is gemaak gedurende 2014/5 in die veld van Na-oespatologie. Die span by Nelspruit is uitgebrei deur die toevoeging van twee magisterstudente wat spesifiek gefokus gaan wees op na-oes swamdoder aanwendingstegnologie. Die projek op warm water, kalium silikaat en biologiese beheer van na-oes siektes en koue-skade by UKZN is afgehandel en 'n finale verslag is ingedien. 'n Nuwe projek op die effek van na-oes behandelings op die voortplantingspotensiaal van sitrus swartvlek infeksies in vrugte is by CRI in Nelspruit begin. Die samewerking tussen CRI Nelspruit en die Departement van Plantpatologie aan die Universiteit van Stellenbosch is verder versterk deur die toevoeging van Dr Cheryl Lennox as medestudieleier van die magisterstudente. 'n Nuwe projek op essensiële olies maak Prof Sandra Combrinck en 'n hele span van TUT ook deel van die CRI Naoes Patologie program. Prof Paul Fourie speel nogsteeds 'n belangrike rol in terme van leiding en strategie vir die CRI Na-oespatologie Program.

Opvolgend op vorige navorsings (4.5.2), is swamdoder aanwending deur voor-pakhuis deurdrenking (drench) en vloedtoediener (flooder) verder ondersoek (4.5.3). Dit is van kardinale belang om die tyd tussen oes en die eerste swamdoder aanwending (gewoonlik deurdrenking) so kort as moontlik te hou. Vloedtoediening gee uitstekende kuratiewe en beskermende groenskimmel beheer asook sporulasie inhibisie (4.5.4). Hierdie aanwending is nou waarlik bewys om 'n werklike alternatief vir dip aanwending te wees. Die molekulêre assessering van groenskimmel bestandheid teen imazalil het geblyk om meer uitdagend te wees as wat aanvanklik verwag is. Goeie vordering is gemaak met die opstel van protokolle vir die ontwikkeling hiervan, maar baie meer werk is nodig (4.5.5). Die potensiaal van warm water behandelings gekombineer met 'n gis behandeling vir die beskermende beheer van groenskimmel is gewys. Die uitdaging is nou om hierdie behandelings op kommersiële vlak te toets en te implementeer (4.5.6). Twee nuwe potensiese swamdoder aktiewes (fludioksonil en propiconasool) het goeie werking teen groenskimmel getoon, veral op jonger (≤ 18 h) infeksies. Propiconasool is ook bewys as 'n aanvaarbare alternatief vir guazatine, maar hierdie aktief moet met groot omsigtigheid gebruik word as gevolg van sy chemiese ooreenstemming met imasalil (kruis weerstand kan ontstaan tussen hierdie twee aktiewes). Die produk Fortisol het guazatine brand onderdruk. Voorloperproewe is gedoen om die “doodmaak tyd” van die groenskimmelswam in 'n spoorsuspensie deur chloor en waterstof peroksied / asynsuur te bepaal, asook die effek van benatters in chloor en swamdoder oplossings (4.5.7).

4.5.2 FINAL REPORT: The JBT heated flooder as an alternative application method for fungicides in citrus packhouses

Project 1050 (April 2012 - March 2013): by Arno Erasmus (CRI-Nelspruit), Paul Fourie (CRI-50USPP), Wilma du Plooy (JBT South Africa) and Charlene Jewell (JBT California)

Opsomming

Die meerderheid pakhuisse in die Suid-Afrikaanse sitrusbedryf gebruik 'n dompelbad vir die aanwending van imazalil (IMZ) om groenskimmel (veroorzaak deur *Penicillium digitatum*) te beheer. John Bean Technologies (JBT) se afdeling in Kalifornië het 'n alternatief vir die dompelbad ontwikkel wat reeds die afgelope dekade in gebruik is. Die vloedtoediener wend swamdoder in 'n water oplossing aan deur middel van 'n aantal watervalle met 'n soomlose laminêre vloei wat op die vrugte val oor roterende borsels. Hierdie tipe aanwending gee meer betroubaarheid in terme van residu-lading en siektebeheer. JBT se afdeling in Suid-Afrika het 'n eksperimentele vloedtoediener-eenheid by CRI in Nelspruit geïnstalleer. Die doel was om hierdie nuwe tegnologie te vergelyk met die huidige tegnologie in gebruik (dompelbad). Die dompelbad is met die vloedtoediener vergelyk, die effek van oplossingstemperatuur en die effek van aantal watervalle is ook ondersoek. Alle proewe is in terme van residu-lading en groenskimmel-beheer geëvalueer. Resultate toon dat die vloedtoediener 'n doeltreffende alternatiewe IMZ aanwendingsmetode vir die dompelbad is. Soortgelyke resultate in terme van residu-lading en kuratiewe beheer is met die vloedtoediener en dompelbad verkry vir vrugte behandel in oplossingstemperatuur van 25 en 35°C. Benewens dit, het die vloedtoediener ook beter beskermende beheer in vergelyking met die dompelbad getoon. Hoër temperature in die vloedtoediener het geneig om beter residu-lading en kuratiewe beheer te gee. Aanwending met drie tot vyf watervalle het meer betroubare kuratiewe beheer in vergelyking met een en twee watervalle gegee. Suurlemoen en sagte sitrus vrugte moet nie by temperature hoër as 45°C behandel word nie om skil beserings te voorkom.

Summary

The majority of South African packhouses use a dip tank to apply imazalil (IMZ) for the control of green mould (caused by *Penicillium digitatum*). John Bean Technologies' (JBT) division in California developed an alternative to the dip tank that has been in use for the past decade. The JBT heated flooder applies fungicide in an aqueous solution by means of a number of weirs that creates a seamless laminar flow that falls onto the fruit over rotating brushes. This type of application gives more consistency in terms of residue loading and disease control. JBT's division in South Africa build and installed an experimental flooder unit at CRI in Nelspruit to compare this new technology to current technology in use (dip application). The dip was compared to the flooder application by studying the effects of solution temperature on IMZ residue loading and green mould control along with the effect of number of weirs. Results show that the flooder is an effective alternative IMZ application method compared to the dip tank. Similar results were obtained for fruit treated at solution temperatures 25 and 35°C in terms of residue loading and curative control. In addition the flooder gave better protective control levels compared to the dip application. Higher temperatures tended to give better residue loading and curative control. Application using three to five weirs gave more consistent curative control compared to one and two weirs. Lemon and soft citrus fruit should not be treated at temperatures higher than 45°C to prevent rind injury.

Introduction

South Africa is the second largest exporter of fresh citrus in the world, even though not under the top ten in terms of area under citrus production. To achieve this, many inputs are intensively managed *i.e.* labour, plant nutrition and pest- and disease control. Postharvest diseases remain one of the major constraints in this fresh fruit export driven industry. Various fungicides intended as control for an array of diseases caused by both wound and latent pathogens are applied in the packhouse.

The fungicide dip tank is used by the majority (78%) of packhouses to apply mostly imazalil (IMZ). Although the dip tank was shown to be the most effective application method in terms of disease control (Erasmus et al., 2011), it has negative aspects that reflect unfavourably on its continued use. These aspects include management of fungicide concentration, sanitation and temperature. The fungicide concentration management and top-up protocols tends to be complicated and/or unreliable, and this may lead to suboptimal residue loading and the development of fungicide resistance. Due to tons of fruit going through the dip tank per day, the solution gets soiled with dirt, possibly diminishing fungicide efficacy.

The majority of dip tanks are managed at a solution temperature of 33.4°C (Erasmus et al., 2011); lower temperatures will delay the drying process before wax is applied and higher temperatures may induce injury if the exposure time is too long. However, heated (50°C) fungicide treatments improve residue loading and green mould control (Dore et al., 2009). Furthermore, treatment at higher temperatures induces fruit resistance to decay and chilling injury (Porat et al., 2004; Hasdai et al., 2004).

New fungicide application technology from John Bean Technologies (JBT) is the heated flooder (Figure 1). This technology is already implemented successfully in California (Fourie, 2011) and should be investigated for suitability for use in South African citrus packhouses. The flooder offers a more manageable and efficient method to apply IMZ as an alternative for the dip tank. The IMZ solution is applied at lower concentrations (200 – 250 ppm), yet a residue of 1 – 2 ppm is still loaded consistently. The unit is linked to a heating section, which heats the solution to 32 - 50°C, depending on the specific type of citrus being treated. During off-time the solution is heated to 60°C for 2 hours in order to pasteurise the solution.

Objectives

1. Evaluate the flooder as an alternative fungicide application method to the dip tank in South African citrus packhouses.
2. Investigate means of optimising heat therapy and fungicide application with the flooder for green mould and chilling injury control.

Materials and methods

Inoculum and spore suspensions

An IMZ sensitive strain of *P. digitatum* was used in all trials. In order to obtain inoculum for biological efficacy tests, subcultures of the isolate were grown at ambient temperature on potato dextrose agar (PDA;

Difco) medium in Petri dishes and were re-plated in 2-week cycles. Conidia were harvested from 10- to 14-day-old cultures approximately 12 hours before trials commenced and stored at 4°C. The surface of a culture was washed with sterile deionised water amended with Tween 20 (Sigma-Aldrich, St Louis, Missouri, USA) at a concentration of 0.01 mL.L⁻¹. The spore suspension was spectrophotometrically adjusted to an absorbance of 0.1 at 420 nm, which relates to a concentration of 1×10⁶ spores.mL⁻¹ (Morris and Nicholls, 1978). The conidial suspensions were placed on magnetic stirrers to maintain a uniform suspension of spores during trials.

Fruit

During the 2013 season Nova mandarin, Eureka lemon, Star Ruby grapefruit, navel oranges and Valencia oranges were collected from different areas in Mpumalanga for the various trials. At arrival, fruit were washed with an aqueous solution of 125 µg.mL⁻¹ quaternary ammonium (Sporekill, ICA) and left to dry before it was stored at 3.5 - 7°C for ±3 days. A day before a trial, fruit were transferred from cold storage to ambient in order for fruit temperature to reach ambient and to allow any condensation to evaporate.

Inoculation, incubation and evaluation

Fruit were treated protectively and curatively. Fruit destined for the curative treatments were inoculated 24 h before treatment. Fruit destined for the protective treatment were first treated, left to dry and then inoculated 24 h after treatment. The fruit were inoculated by dipping a stainless steel rod with a narrow, concave tip (2 mm long; 1 mm diameter) into the spore suspension and then wounding the fruit rind through the flavedo in to the top layer of albedo. Four inoculated wounds were induced equally apart from each other surrounding the calyx. After treatment and inoculation the fruit were incubated at 20°C.

Fruit was rated for infection and sporulation 5 and 14 days after inoculation, respectively. The number of infected wounds per fruit were evaluated using an ultra-violet light source (UV-A at 365 nm, Labino Mid-light; www.labino.com). Infected wounds can be identified as yellow fluorescence under UV light. Infection data were converted to percentage control. Sporulation was evaluated for each fruit as described by Erasmus et al. (2011). Infected fruit were given a rating from 0 to 6 related to the fruit area covered with green sporulation. Where 0 = no infection, 1 = infection with no sporulation, 2 = sporulation area less than 10 mm², 3 = sporulation area less than 50% of the fruit and more than 10 mm², 4 = sporulation area more than 50% of fruit and less than 75%, 5 = sporulation area more than 75% of the fruit and less 100%; and 6 = 100% covered with sporulating green mould. Infected fruit rated ≤ 3 was regarded as showing sporulation inhibition.

Comparison of dip application to flooder application

Imazalil was applied on fruit through either the dip tank at 500 µg.mL⁻¹ or the flooder at 250 µg.mL⁻¹ in the CRI Nelspruit postharvest laboratory. Exposure time in the dip tank solution was 60 s and 8 s in the flooder at either 20°C, 35°C or 52°C. Trials were conducted twice each on Nova mandarin, Eureka lemon, Star Ruby grapefruit and navel orange fruit. Fruit were treated curatively and protectively and sporulation was rated as described above.

The effect of IMZ concentration and solution temperature

Imazalil was applied on fruit through the flooder at a concentration of either 250 µg.mL⁻¹ or 500 µg.mL⁻¹. For each treatment concentration the temperature was increased by 5°C increments before the next batch of fruit were treated. The temperature range was 25, 30, 35, 40, 45, 50, 55 and 60°C. Exposure time was set at 8 s. This trial was conducted twice each on Eureka lemon and navel orange fruit. Fruit were treated curatively and protectively.

The effect of number of weirs and different solution temperature

Imazalil was applied on fruit through the flooder at solution temperatures of 25, 35, 45 and 55°C at a concentration of 250 µg.mL⁻¹. For each temperature treatment IMZ was applied with 1, 2, 3, 4 and 5 weirs. This trial was conducted once each on Nova mandarin and Eureka lemon fruit. Fruit were treated curatively.

Results and discussion

The commissioning of the experimental flooder was not a smooth process. After the season of 2012 it came to light that the wrong brushes have been fitted. The machine was sent back to the JBT factory in

Brackenfell, Cape Town for alterations and improvements. These alterations and fitting of the correct brushes were completed and the machine was returned to the CRI Nelspruit packline laboratory. During the season of 2013 a substantial volume of trials were conducted. Due to the changes to the applicator, the 2012 data have been omitted from this report and only the 2013 is presented.

In terms of Objective 2, extra fruit per treatment were stored with the aim to study cold injury. Although fruit were stored for extended periods of time no cold injury were induced. This could be due to storage temperatures not being cold enough. This objective was therefore not addressed in this study.

Comparison of dip application to flooder application

Residue loading

Analyses of variance for residue loading data measured on lemon and navel orange fruit showed a significant application (dip and flooder) × solution temperature (25, 35 and 52°C) interaction ($P < 0.0001$; ANOVA not shown). Residue levels loaded in the dip treatments at 25 and 35°C did not differ significantly (0.66 and $0.78 \mu\text{g.g}^{-1}$, respectively; Figure 4.5.2.1). The 52°C dip treatment residue level ($0.96 \mu\text{g.g}^{-1}$) was similar to the 35°C treatment, but significant higher than the 25°C treatment. The flooder loaded similar residue levels in the 25 and 35°C treatments (0.93 and $0.98 \mu\text{g.g}^{-1}$, respectively) and these levels were similar to the 35 and 52°C dip treatments, but significantly higher than the 25°C dip treatments. The 52°C flooder treatment loaded the highest residue level and this was significantly higher than all treatments.

Fruit batch (lemon 1 and 2, navel orange 1 and 2, nova mandarin and Star Ruby grapefruit 1 and 2) was not involved in any significant interaction, but was meaningful as main effect ($P = 0.078$). Both lemon, navel orange, and Star Ruby grapefruit fruit loaded similar residue levels ($0.97 - 1.22 \mu\text{g.g}^{-1}$; Table 4.5.2.1). The Nova mandarin fruit batch loaded the lowest residue level ($0.86 \mu\text{g.g}^{-1}$), which was significantly lower than that loaded on the two lemon and the second Star Ruby grapefruit fruit batches ($1.16 - 1.22 \mu\text{g.g}^{-1}$).

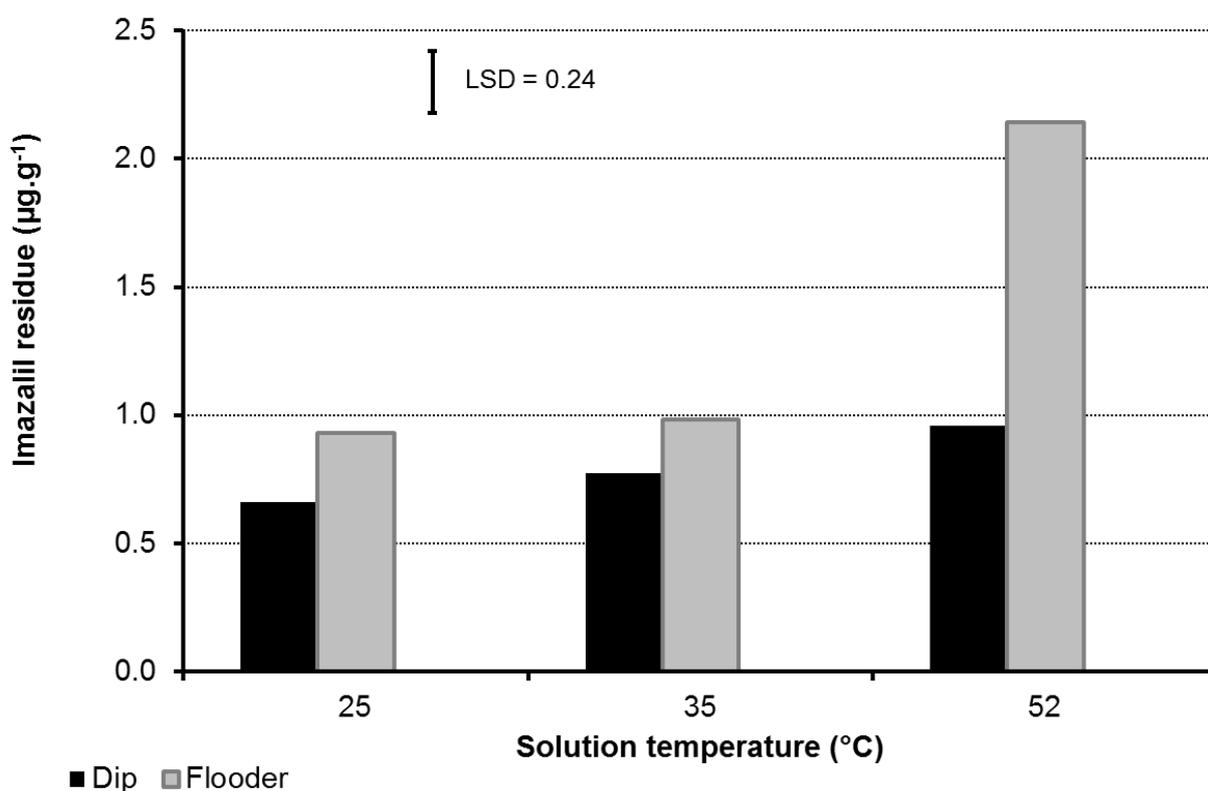


Figure 4.5.2.1. Mean imazalil (IMZ) residue loaded on lemon and navel orange, Nova mandarin and Star Ruby grapefruit fruit treated with either a dip for 60 s in $500 \mu\text{g.g}^{-1}$ IMZ or a flooder for 5 s in $250 \mu\text{g.g}^{-1}$ IMZ at solution temperatures of 25, 35 and 52°C; all fruit went through an air knife over 14 roller brushes after treatment. The LSD value (0.24) was determined by means of Fisher's test ($P \leq 0.05$).

Table 4.5.2.1. Mean imazalil (IMZ) residue loaded on various citrus types treated with either a dip for 60 s in 500 µg.g⁻¹ IMZ or a flooder for 5 s in 250 µg.g⁻¹ IMZ at solution temperatures of 25, 35 and 52°C, all fruit went through an air knife over 14 roller brushes after treatment.

Citrus type and batch	Imazalil residue ^a	
Lemon 1	1.22	a
Lemon 2	1.19	a
Navel orange 1	1.09	ab
Navel orange 2	0.97	ab
Nova mandarin	0.86	b
Star Ruby 1	1.04	ab
Star Ruby 2	1.16	a

^aEach value followed by the same letter is not significantly different according to Fisher's LSD test ($P \leq 0.05$)

Green mould control

Data for the different citrus types were analysed separately first to show the effect of the two different batches. Analyses of variance for lemon, Nova mandarin, navel orange and Star Ruby grapefruit fruit each showed a significant batch (one and two) × application (dip and flooder) × solution temperature (25, 35 and 52°C) × action (curative protective) interaction ($P = 0.018$, < 0.0001 , $= 0.048$ and $= 0.0003$, respectively; ANOVA tables not shown). In the case of lemon fruit the protective treatments of the first batch for the 25 and 35°C treatments had significantly higher control levels (61.6 and 60.8%, respectively; results not shown) than their counterparts in the second batch (26.8 and 52.0%, respectively). No other lemon batch differences were observed and control levels exceeded 73.0%. On Nova mandarin fruit, the first batch's 25°C protective dip treatment, 52°C curative flooder treatment and 35°C protective dip treatment showed significantly higher levels of control (96.9, 95.1 and 75.4%, respectively) than their second batch counterparts (55.3, 81.2 and 64.4%, respectively). Green mould in the second batch's 25°C protective flooder treatment was significantly better controlled than its first batch counterpart (94.0 vs. 62.5%, respectively). The rest of the treatments all exceeded 73.0% control. On navel orange fruit certain treatments (flooder 25°C protective, dip 25°C curative, dip 25°C protective and flooder 35°C curative) in the first batch had significantly lower levels of control than their counterparts in the second batch (94.8, 89.9, 89.3 and 86.3% vs. 100.0, 99.4, 98.9 and 98.2%, respectively). The rest of the treatments all exceeded 95.3%. On Star Ruby grapefruit fruit the first batch 25°C protective flooder treatments had a significantly higher level of control (98.8%) than the second batch counterpart (83.7%). The second batch 52°C curative flooder and 25°C protective dip treatments had significantly higher levels of control (96.7 and 89.6%, respectively) than its counterparts (57.6 and 76.9%, respectively). The rest of the treatments were all $> 91.0\%$.

Data for fruit types were combined and further analysed ignoring batch as a factor. Analyses of variance for green mould control data showed a significant citrus type × application × solution temperature × action interaction ($P < 0.0001$, ANOVA not shown). The majority of curative treatments, regardless of application, had control levels of $> 88.0\%$ with the Star Ruby 52°C flooder treatment being the only exception (76.9%; Figure 4.5.2.2). In the protective treatments results varied from citrus type to citrus type. On lemon fruit flooder treatments resulted in significantly better control than dip treatments (73.6, 97.3 and 100.0% vs. 44.2, 56.4 and 89.2 for 25, 35 and 52°C, respectively). For Nova mandarin fruit the 25°C treatments resulted in similar control (76.1 and 78.2 for dip and flooder, respectively). The flooder treatments with solution temperatures of 35 and 52°C rendered significantly higher protective control levels than its counterparts in the dip treatments (98.4 and 98.5 vs. 69.9% and 74.4%, respectively). On navel orange fruit the protective control levels were $> 94.0\%$, regardless of application or solution temperature. On Star Ruby grapefruit fruit protective control levels were all 91.0%, with the 25°C dip treatment being the only exception at 83.2%.

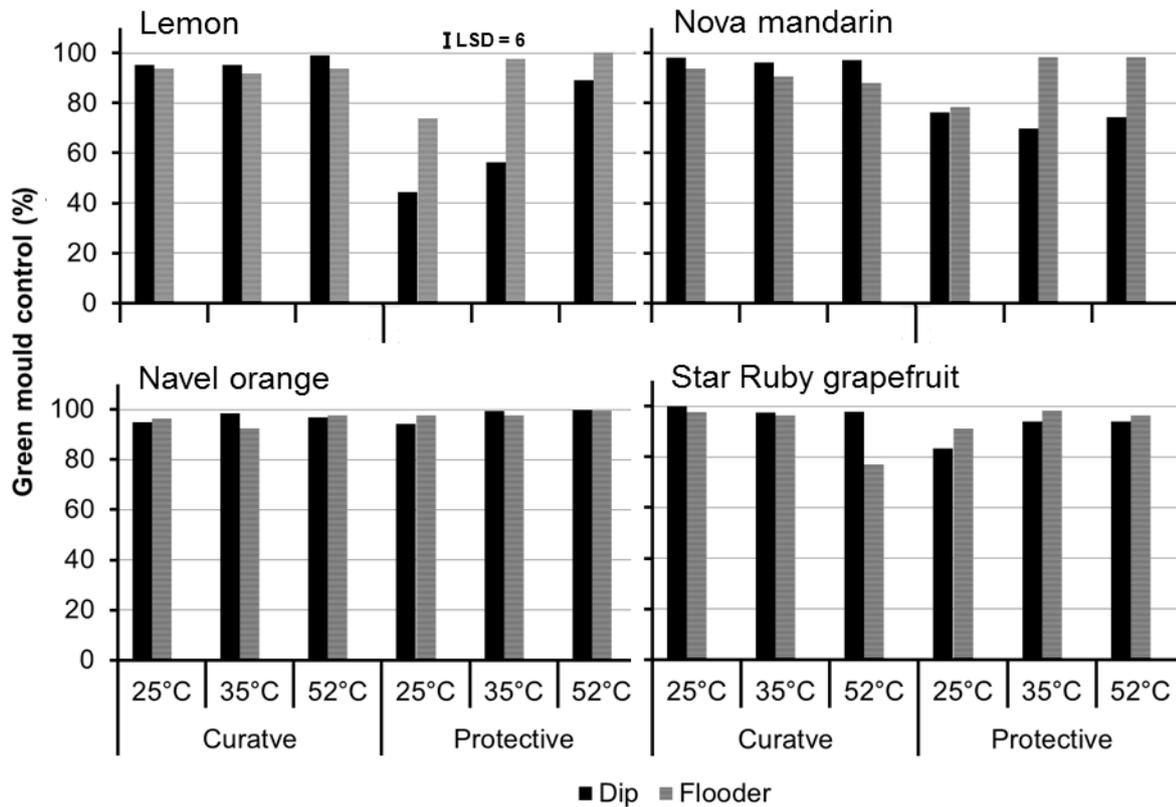


Figure 4.5.2.2. Mean percentage green mould control on various citrus types treated curatively (inoculated 24 h prior to treatment) or protectively (inoculated 24 h post treatment) with either a dip for 60 s in 500 $\mu\text{g.g}^{-1}$ imazalil or a flooder for 5 s in 250 $\mu\text{g.g}^{-1}$ imazalil at solution temperatures of 25, 35 and 52°C; all fruit went through an air knife over 14 roller brushes after treatment. The LSD value (6.00) was determined by means of Fisher's test ($P \leq 0.05$).

Sporulation inhibition

Analyses of variance for percentage green mould sporulation inhibition data showed a significant application \times action interaction ($P = 0.027$; ANOVA not shown) and a significant application \times solution temperature interaction ($P < 0.0001$). To combine these factors, results for the application \times solution temperature \times action interaction ($P = 0.464$) is presented where the previous mentioned interactions is combined. Curatively, the dip and flooder applications resulted in similar levels of sporulation inhibition (25.8 – 36.0%), with the only exception being the 52°C flooder treatment showing a significantly higher level of inhibition (67.2%). Protectively all the 25°C dip and flooder treatments showed similar results than the curative treatments (22.0 – 31.2%). The 35 and 52°C flooder treatments had significantly higher levels of inhibition compared to the other protective treatments and differed significantly from each other (47.4 and 86.5%, respectively).

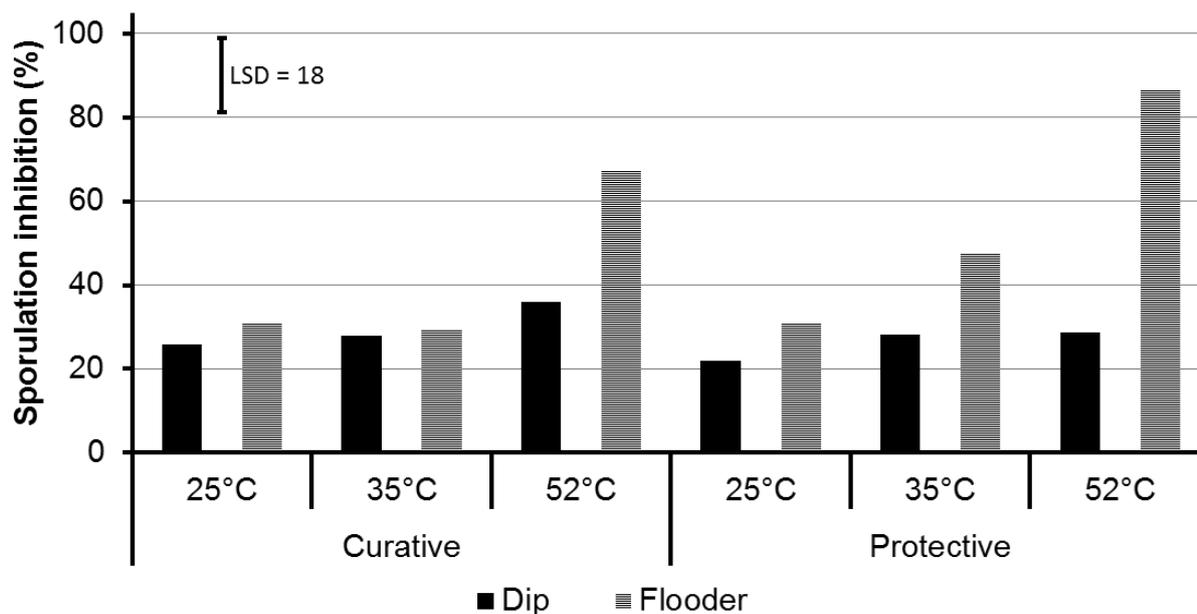


Figure 4.5.2.3. Mean percentage green mould sporulation inhibition on citrus fruit treated curatively (inoculated 24 h prior to treatment) or protectively (inoculated 24 h post treatment) with either a dip for 60 s in $500 \mu\text{g.g}^{-1}$ imazalil or a flooder for 5 s in $250 \mu\text{g.g}^{-1}$ imazalil; all fruit went through an air knife over 14 roller brushes after treatment. The LSD value (18.00) was determined by means of Fisher's test ($P \leq 0.05$).

The effect of IMZ concentration and solution temperature

Residue loading

Analyses of variance for residue loading data showed a significant concentration (250 and $500 \mu\text{g.mL}^{-1}$ IMZ) \times solution temperature ($25, 30, 35, 40, 45, 50, 55$ and 60°C) interaction ($P = 0.0003$; ANOVA not shown). For each concentration, residues levels increased with increase in solution temperature; these levels were always higher in the $250 \mu\text{g.mL}^{-1}$ treatments (Figure 4.5.2.4). The lowest residue level was loaded in the $500 \mu\text{g.mL}^{-1}$ at 25°C treatment ($0.77 \mu\text{g.g}^{-1}$) and this was similar to those loaded in the $30 - 40^\circ\text{C}$ treatments with $500 \mu\text{g.mL}^{-1}$ and the 25°C treatment with $250 \mu\text{g.mL}^{-1}$ treatments ($0.94 - 0.98 \mu\text{g.g}^{-1}$). In the $25, 30$ and 35°C in the respective 250 and $500 \mu\text{g.mL}^{-1}$ treatments the residue level did not differ significantly between the concentrations (0.97 and $0.77 \mu\text{g.g}^{-1}$ in 25°C , 1.10 and $0.94 \mu\text{g.g}^{-1}$ in 30°C and 1.26 and $0.98 \mu\text{g.g}^{-1}$ in 35°C , each respectively for 250 and $500 \mu\text{g.mL}^{-1}$). The $40, 45$ and 50°C with $250 \mu\text{g.mL}^{-1}$ treatments loaded similar residues ($1.55, 2.09$ and $2.07 \mu\text{g.g}^{-1}$, respectively), but significantly higher than the lower temperatures in the $250 \mu\text{g.mL}^{-1}$ and the similar solution temperatures in the $500 \mu\text{g.mL}^{-1}$ treatments ($0.95, 1.20$ and $1.42 \mu\text{g.g}^{-1}$, respectively). The residue level loaded in the 55°C $250 \mu\text{g.mL}^{-1}$ treatment ($2.39 \mu\text{g.g}^{-1}$) were similar to those loaded in the 45 and 50°C $250 \mu\text{g.mL}^{-1}$ treatments and significantly higher than all the lower degree $250 \mu\text{g.mL}^{-1}$ and all the $500 \mu\text{g.mL}^{-1}$ treatments. The highest residue level were loaded in the 60°C $250 \mu\text{g.mL}^{-1}$ treatment and this was significantly higher than any other treatment regardless of solution temperature or concentration. The increase in residue loading in the $250 \mu\text{g.mL}^{-1}$ treatments was more steep than the $500 \mu\text{g.mL}^{-1}$ treatments. The $25 - 40^\circ\text{C}$ treatments loaded similar residue levels ($0.77 - 0.95 \mu\text{g.g}^{-1}$). The next group that loaded similar residue levels was the $30 - 45^\circ\text{C}$ treatments ($0.94 - 1.20$). The respective 45 and 50°C , the 50 and 55°C and the 55 and 60°C treatments loaded similar residue levels ($1.20, 1.42, 1.55$ and $1.83 \mu\text{g.g}^{-1}$ for $45, 50, 55$ and 60°C , respectively). Fruit batch was significant as a main effect ($P < 0.0001$; ANOVA not shown). The first navel orange batch loaded the highest residue level (mean of $1.79 \mu\text{g.g}^{-1}$; results not shown) and this was significantly higher than the second batches of navel orange and lemon (means of 1.48 and $1.47 \mu\text{g.g}^{-1}$, respectively). The last two residue levels mentioned was also similar to each other, but significantly higher than the first batch of lemon ($1.26 \mu\text{g.g}^{-1}$).

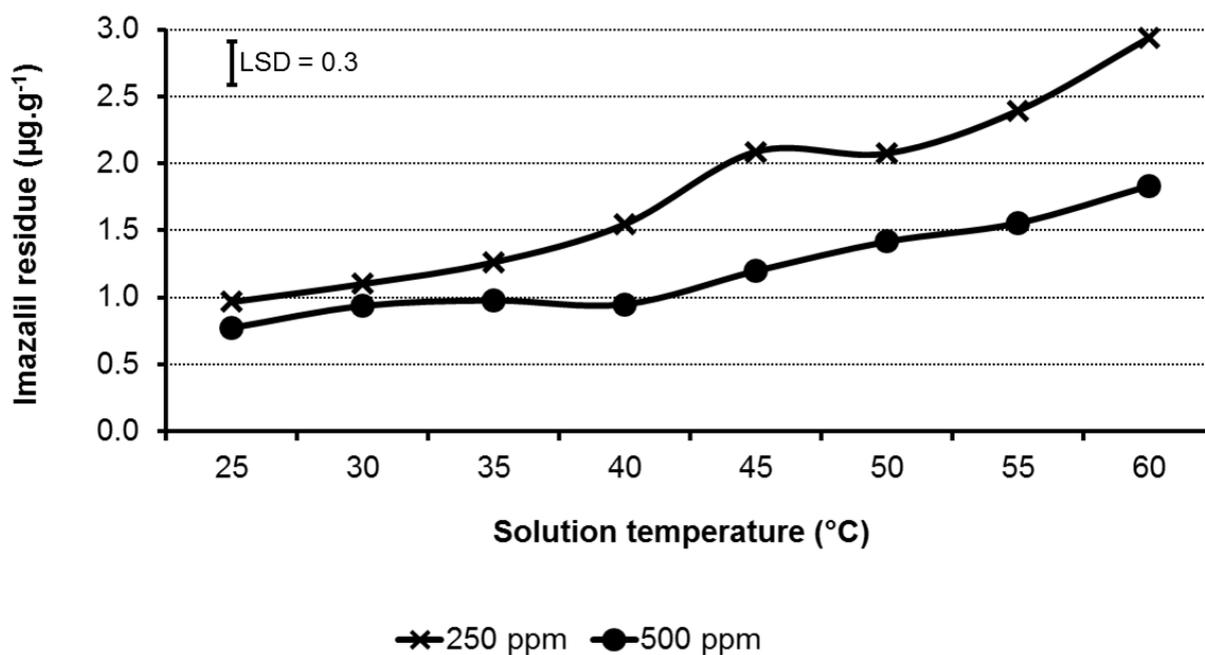


Figure 4.5.2.4. Mean imazalil (IMZ) residue loaded on citrus fruit treated with a flooder for 5 s at 250 or 500 $\mu\text{g.g}^{-1}$ IMZ, both with a solution temperature range of 25 – 60°C; all fruit went through an air knife over 14 roller brushes after treatment. The LSD value (0.3) was determined by means of Fisher's test ($P \leq 0.05$).

Green mould control

Firstly, the green mould control data were analysed separately to investigate the effect of harvest batch. Analyses of variance for the lemon data showed a significant batch \times action \times temperature interaction ($P = 0.005$; ANOVA not shown). This difference between the two batches was noted at the 25°C treatment that resulted in significantly lower curative control in the first batch than the second (89.8 and 96.3%, respectively; results not shown). In the 50°C treatment the first batch gave significantly higher curative control compared to its counterpart in the second batch (92.3 and 88.0%, respectively). In the protective control treatments the only difference between the first and second batch was in the 30°C treatment (93.6 and 98.0%, respectively). All other treatments had curative or protective control levels of $> 91.0\%$. In the navel orange fruit trials batch was only significant as a single effect ($P = 0.011$; ANOVA not shown). The first batch had significantly lower control levels compared to the second (95.6 and 96.7%, respectively; results not shown).

Data were combined and harvest batch was ignored in further analyses. Analyses of variance for green mould control data showed a significant citrus type \times action \times IMZ concentration \times solution temperature interaction ($P = 0.045$; ANOVA not shown). Curative control was always better in the 500 $\mu\text{g.mL}^{-1}$ treatments than the 250 $\mu\text{g.mL}^{-1}$ treatments, with an average improvement of 5.6% on lemon fruit and 4.0% on navel orange fruit (Figure 4.5.2.5). Curative control levels fluctuated on lemon fruit in the 250 $\mu\text{g.mL}^{-1}$ treatments with peaks at 40°C (94.4%) and 55°C (96.3%), the lowest level was at 50°C (84.0%). In the lemon 500 $\mu\text{g.mL}^{-1}$ treatments the lowest curative control level was at 25°C (93.7%) and the highest at 35°C (99.1%). The fluctuating effect was also evident in the navel orange 250 $\mu\text{g.mL}^{-1}$ treatments where peaks was noted at 35°C (93.7%), 45°C (91.0%) and 60°C (96.4%) and the lowest level was at 25°C (86.2%). In the navel orange 500 $\mu\text{g.mL}^{-1}$ treatments all levels were $> 90.0\%$ and 45, 55 and 60°C had levels of $> 95.0\%$. Protectively, control levels were higher compared to curative control regardless of citrus type, IMZ concentration or solution temperature and were at levels of ≥ 91.4 . At solution temperatures of $\geq 40^\circ\text{C}$ control levels were ≥ 96.1 .

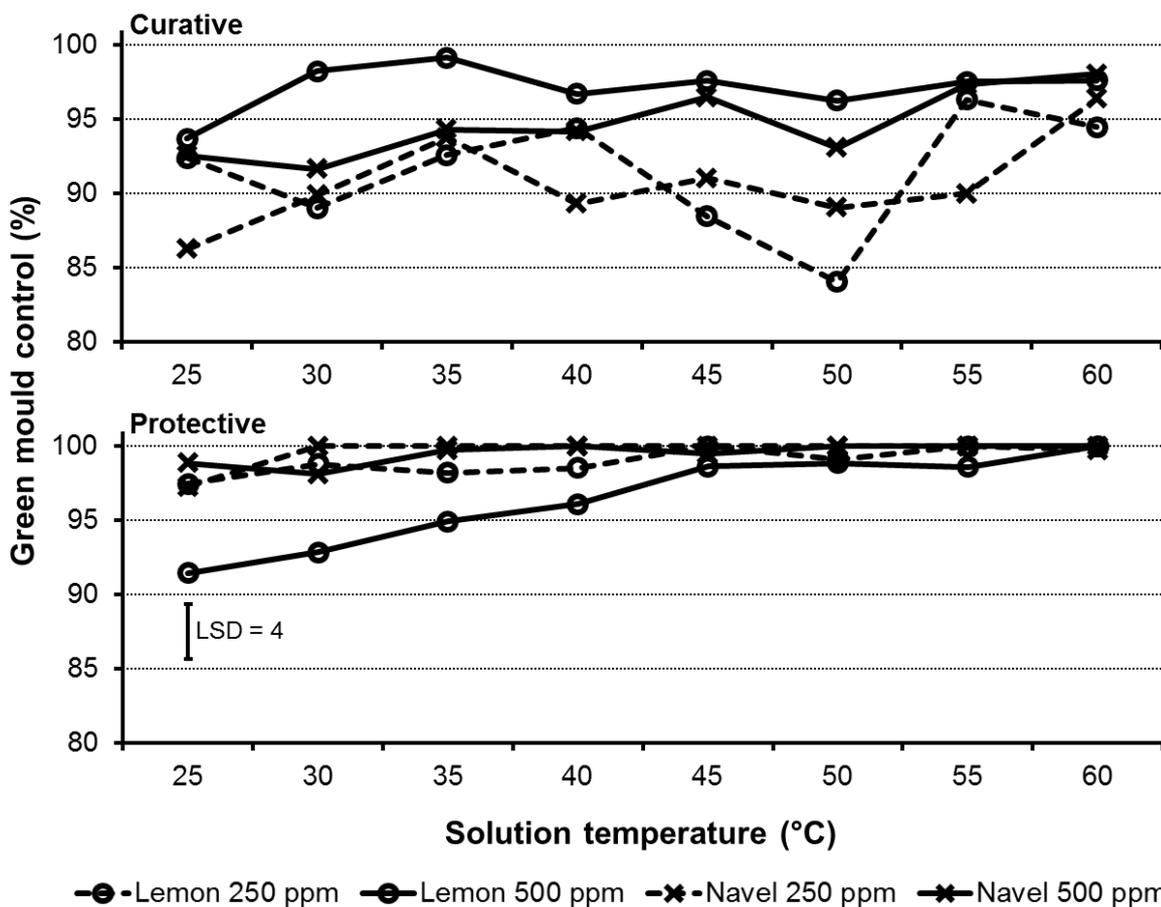


Figure 4.5.2.5. Mean percentage green mould control on Lemon and navel orange fruit treated curatively (inoculated 24 h prior to treatment) or protectively (inoculated 24 h post treatment) with a flooder for 5 s at 250 or 500 $\mu\text{g.g}^{-1}$ IMZ, both with a solution temperature range of 25 – 60°C; all fruit went through an air knife over 14 roller brushes after treatment. The LSD value (4) was determined by means of Fisher's test ($P \leq 0.05$).

The effect of number of weirs

Residue loading

Due to some missing data, data analyses were conducted in sections.

Nova mandarin fruit, 1 – 5 weirs, solution temperatures 25, 35, 45 and 55°C. Analyses of variance for residue loading data on Nova mandarin fruit showed a significant weir number \times solution temperature interaction ($P = 0.043$; ANOVA not shown). The highest residue levels were loaded with three, four and five weirs at 55°C (1.66, 1.79 and 1.99 $\mu\text{g.g}^{-1}$, respectively; Figure 4.5.2.6) and this differed significantly from all the rest. The one to two weirs at 55°C, three to five weirs at 45°C, four to five weirs at 35°C and four weirs at 25°C treatments loaded residues levels of $\approx 1.0 \mu\text{g.g}^{-1}$. All the rest were $< 0.90 \mu\text{g.g}^{-1}$ with the lowest at 0.70 $\mu\text{g.g}^{-1}$ loaded in the three weirs 25°C treatment.

Lemon and Nova mandarin fruit, two and three weirs, solution temperature 25, 35, 45 and 55°C. Analyses of variance for residue loading data from showed a significant weir number (two or three weirs) \times solution temperature interaction. Similar residue levels were loaded in the 25 – 45°C two weirs and 25 – 35°C three weirs treatments (0.73 – 0.87 $\mu\text{g.g}^{-1}$; Figure 4.5.2.7). Two weirs at 55°C and three weirs at 45°C loaded similar residue levels of 1.11 and 1.05 $\mu\text{g.g}^{-1}$, respectively. The highest level, 1.42 $\mu\text{g.g}^{-1}$ was loaded in the 55°C three weirs treatment and this was significantly higher than all the rest. The ANOVA also showed a significant citrus type \times solution temperature interaction ($P = 0.001$; ANOVA not shown). Residue levels loaded in the 25 and 35°C treatments on both lemon and Nova mandarin fruit were similar (0.73 – 0.87 $\mu\text{g.g}^{-1}$; results not shown). Residue levels loaded in the 35 and 45°C treatments were similar on lemon and Nova mandarin fruit (0.85 – 0.96 $\mu\text{g.g}^{-1}$). The highest residue level (1.44 $\mu\text{g.g}^{-1}$) was loaded in the 55°C treatments on Nova mandarin fruit and this was significantly higher than all the rest. The residue level

loaded in the 55°C treatment on lemon fruit ($1.09 \mu\text{g}\cdot\text{g}^{-1}$) were similar to the lemon 45°C treatment ($0.96 \mu\text{g}\cdot\text{g}^{-1}$), but differed significantly from the rest of the treatments.

Lemon and Nova mandarin fruit, 1 - 5 weirs, solution temperature 45 and 55°C. Analyses of variance of residue loading data showed a significant number of weirs \times solution temperature interaction ($P = 0.001$; ANOVA not shown). Treatments at 45°C and one and two weirs loaded the lowest residue levels and differed significantly from the rest of the treatments (0.80 for both respective treatments). Treatments at 45°C and one or two weirs and treatments at 55°C with one weir loaded similar residue levels ($0.80 - 0.98 \mu\text{g}\cdot\text{g}^{-1}$; Figure 4.5.2.8). Treatments at 45°C with three to five weirs and treatments at 55°C with one or two weirs loaded similar residue levels ($0.98 - 1.22 \mu\text{g}\cdot\text{g}^{-1}$). Treatments at 55°C with three, four or five weirs loaded significantly higher residue levels compared to the rest of the treatment and also differed significantly from each other ($1.42, 1.70$ and $2.00 \mu\text{g}\cdot\text{g}^{-1}$, respectively). The ANOVA also showed a significant citrus type \times solution temperature interaction ($P = 0.003$; ANOVA not shown). Residue levels loaded on Nova mandarin fruit were significantly higher than those loaded on lemon treated at 55°C (1.53 and $1.35 \mu\text{g}\cdot\text{g}^{-1}$, respectively; results not shown). Significantly lower residue levels were loaded on Nova mandarin and lemon fruit treated at 45°C (0.95 and $1.04 \mu\text{g}\cdot\text{g}^{-1}$, respectively) compared to those loaded at 55°C, these did not differ significantly from each other.

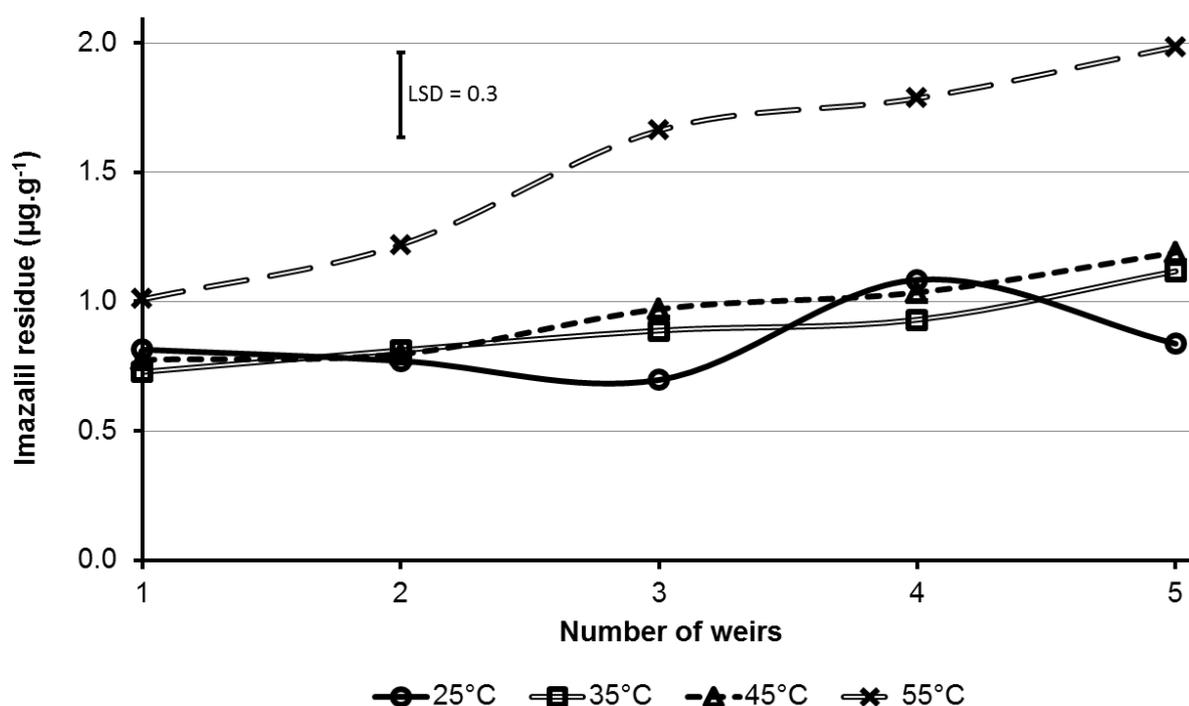


Figure 4.5.2.6. Mean imazalil (IMZ) residue loaded on Nova mandarin fruit treated with a flooder of 1 - 5 weirs at $250 \mu\text{g}\cdot\text{g}^{-1}$ IMZ, with a solution temperature range of 25, 35, 45 and 55°C; all fruit went through an air knife over 14 roller brushes after treatment. The LSD value (0.3) was determined by means of Fisher's test ($P \leq 0.05$).

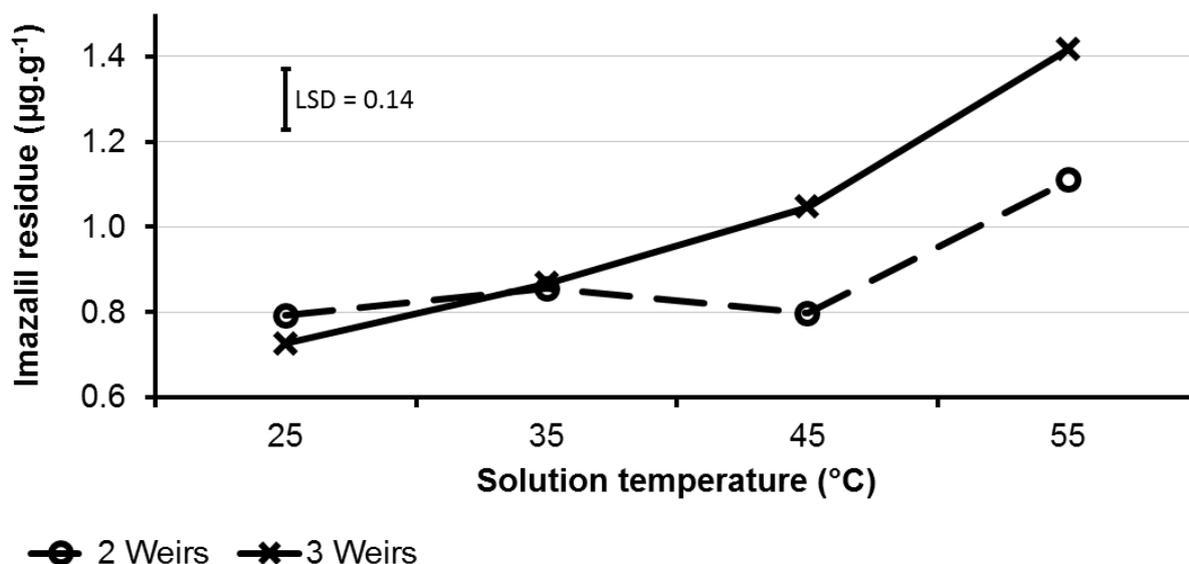


Figure 4.5.2.7. Mean imazalil (IMZ) residue loaded on Nova mandarin and lemon fruit treated with a flooder of two or three weirs at $250 \mu\text{g.g}^{-1}$ IMZ, with a solution temperature range of 25, 35, 45 and 55°C ; all fruit went through an air knife over 14 roller brushes after treatment. The LSD value (0.14) was determined by means of Fisher's test ($P \leq 0.05$).

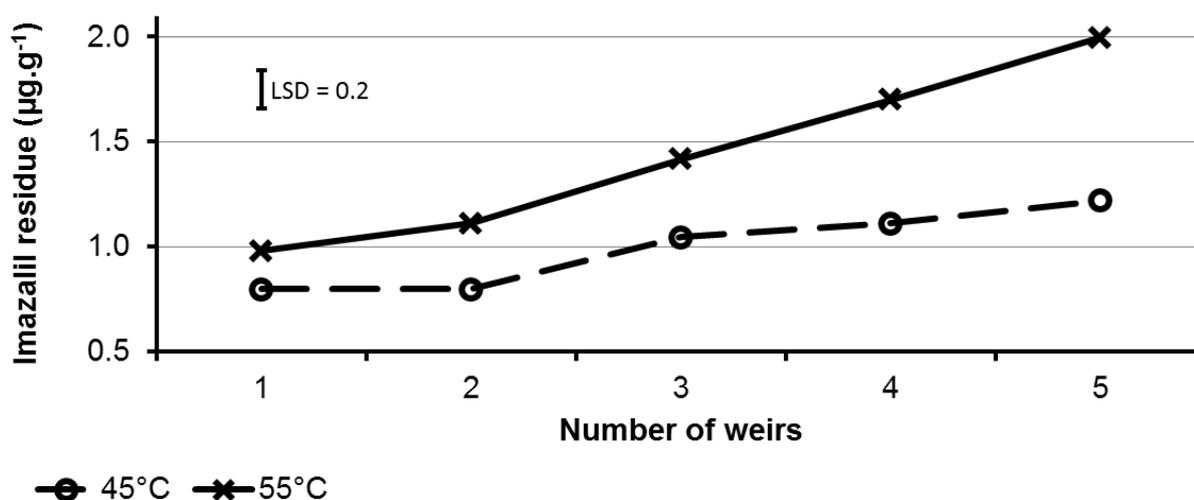


Figure 4.5.2.8. Mean imazalil (IMZ) residue loaded on Nova mandarin and lemon fruit treated with a flooder of 1 - 5 weirs at $250 \mu\text{g.g}^{-1}$ IMZ, with a solution temperature of 45 and 55°C ; all fruit went through an air knife over 14 roller brushes after treatment. The LSD value (0.2) was determined by means of Fisher's test ($P \leq 0.05$).

Green mould control

Analyses of variance of green mould control data showed a significant citrus type \times weir number \times solution temperature interaction ($P < 0.0001$; ANOVA not shown). Green mould control was generally good ($\geq 75.9\%$; Figure 4.5.2.9). The highest level within each citrus type was achieved with the two weir 35°C , one weir 25°C , three weir 55°C and five weir 25°C treatments at 99.1, 97.9, 99.2 and 98.6%, respectively (for lemon, Nova mandarin, Valencia orange 1 and Valencia orange 2, respectively). The lowest level of control within each citrus type were noted at the two weir 35°C , one weir 35°C , one weir 55°C and one weir 45°C treatments at 77.5, 75.9, 76.6 and 82.4%, respectively (for lemon, Nova mandarin, Valencia orange 1 and Valencia orange 2, respectively). The four and five weir 45°C and the five weir 35°C treatments resulted to $> 90.0\%$ control across all citrus types.

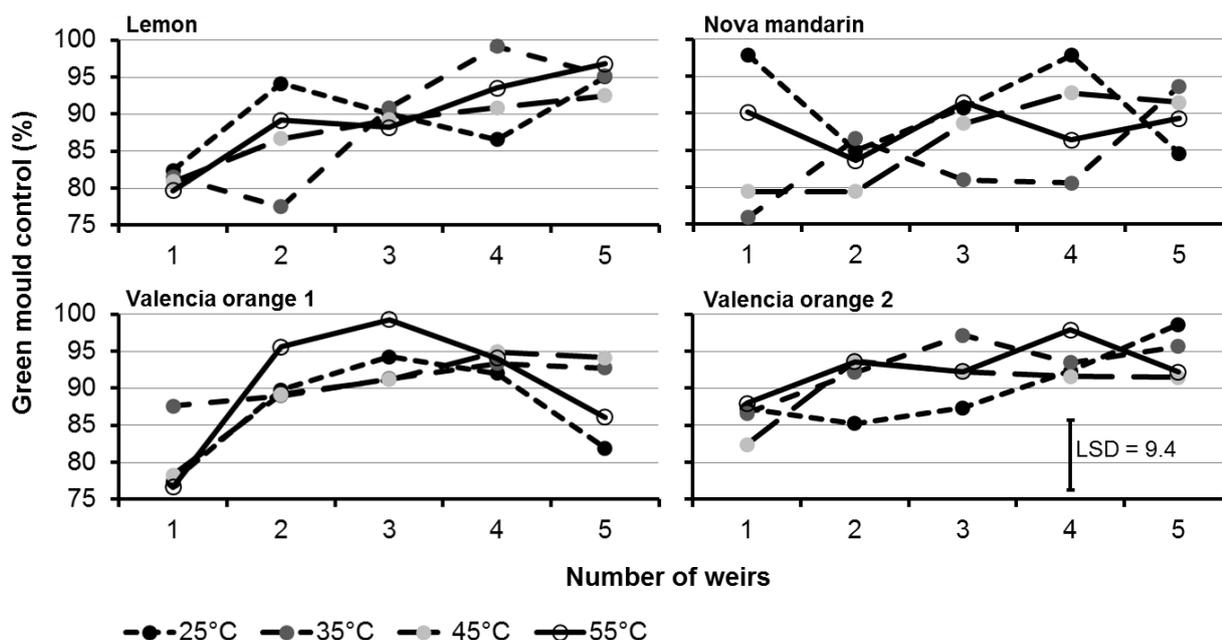


Figure 4.5.2.9. Mean percentage green mould control on Nova mandarin and lemon fruit treated curatively (inoculated 24 h prior to treatment) with a flooder of 1 - 5 weirs at 250 $\mu\text{g}\cdot\text{g}^{-1}$ IMZ, with a solution temperature of 45 and 55°C; all fruit went through an air knife over 14 roller brushes after treatment. The LSD value (0.2) was determined by means of Fisher's test ($P \leq 0.05$).

Conclusion

This work shows that the flooder is the first real alternative for dip application. It compared well with dip application in terms of curative control and in most cases gave improved protective control. At higher temperatures (52°C for curative treatments and 35 and 52°C for protective treatments) the flooder significantly improved sporulation inhibition. Residue loading was consistent at 25 and 35°C to slightly below 1.00 $\mu\text{g}\cdot\text{g}^{-1}$, but significantly increased at higher temperatures to slightly over 2.00 $\mu\text{g}\cdot\text{g}^{-1}$, which was well below the MRL. The residue level of $\approx 2.00 \mu\text{g}\cdot\text{g}^{-1}$ resulted in improved sporulation inhibition.

An increase in solution temperature resulted in an increase in residue loading. This increase was more pronounced for the 250 $\mu\text{g}\cdot\text{mL}^{-1}$ treatments than for the 500 $\mu\text{g}\cdot\text{mL}^{-1}$ treatments. This can be ascribed to the pH level being higher in the 250 $\mu\text{g}\cdot\text{mL}^{-1}$ IMZ solutions (≈ 6.0) than the 500 $\mu\text{g}\cdot\text{mL}^{-1}$ (≈ 3.5) solutions. Even though the 250 $\mu\text{g}\cdot\text{mL}^{-1}$ solutions loaded higher residues ($\approx 1.00 - 3.00 \mu\text{g}\cdot\text{g}^{-1}$) than the 500 $\mu\text{g}\cdot\text{g}^{-1}$ ($< 1.00 - < 2.00 \mu\text{g}\cdot\text{g}^{-1}$), it resulted in fluctuating and lower curative control levels compared to those gained in the 500 $\mu\text{g}\cdot\text{mL}^{-1}$ treatments. Protectively all results indicated an excellent $> 91.0\%$ control.

Increasing the number of weirs resulted in increased residue loading and this was further elevated by an increase in solution temperature. The most significant effect was seen at 55°C, although residue levels never exceeded 2 $\mu\text{g}\cdot\text{g}^{-1}$. Curative control levels did not in all cases follow the same trend as residue loading. The most consistent results ($> 90.0\%$ control) across all citrus types tested was noted in treatments with four and five weirs at 45°C and four weirs at 35°C.

In conclusion, flooder temperatures between 35 and 50°C yielded good results without rind injury. On soft citrus and lemons a lower solution temperature range should be considered to lower the risk of rind injury. Slight rind damage was observed on lemons treated at $\geq 55^\circ\text{C}$ (results not shown). Three to five weirs gave more consistent levels of residue loading and green mould control.

Future research

- The effect of solution pH (3, 4, 5 and 6) in relation to IMZ concentration (250 and 500 $\mu\text{g}\cdot\text{mL}^{-1}$) on residue loading and green mould control should be thoroughly investigated. In this study we showed that 250 $\mu\text{g}\cdot\text{mL}^{-1}$ IMZ in Nelspruit municipal water, resulted in a pH of ≈ 6 with increase residue loading, compared to 500 $\mu\text{g}\cdot\text{mL}^{-1}$ with a pH of ≈ 3.5 , but control levels fluctuated more in lower concentration compared to the higher concentration.

- The effect of flooder application of other postharvest actives (pyrimethanil, thiabendazole, guazatine, fludioxonil, and propiconazole) needs to be investigated in terms of residue loading and green mould and sour rot control.
- The contribution the flooder makes towards the double application of IMZ needs to be investigated.

Technology transfer

- W. du Plooy, A. Erasmus, C. Jewell, P. Fourie. 2012. A Heated Imazalil Flooder – New technology for South African packing houses. Oral presentation at the 7th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 19-22 August 2012.
- Arno Erasmus, Catherine Savage, Charlene Jewell, Wilma du Plooy and Paul Fourie. 2014. At last! An effective and practical alternative to the fungicide dip tank. Oral presentation at the 8th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 17-20 August 2014.
- Arno Erasmus, Catherine Savage, Charlene Jewell, Wilma du Plooy and Paul Fourie. 2015. A new and effective method for South Africa to apply imazalil for the postharvest control of citrus green mould. Poster presentation at 49th SASPP conference (Bloemfontein, Jan 2015).
- Arno Erasmus, Catherine Savage, Charlene Jewell, Wilma du Plooy and Paul Fourie. 2015. The flooder, an alternative imazalil application method for postharvest citrus green mould control. Oral presentation at 3rd International Symposium on Postharvest Pathology (Bari, Italy, June 2015).
- Arno Erasmus, Catherine Savage, Charlene Jewell, Wilma du Plooy and Paul Fourie. 2014. The heated flooder. Oral presentation at CRI Packhouse Workshops, Polokwane, Loskopdam, Durban, Port Elizabeth, Stellenbosch (January and February 2014)
- Catherine Savage, Charlene Jewell, Wilma du Plooy, Cheryl Lennox, Paul Fourie and Arno Erasmus. 2015. Update on flooder research. Oral presentation at CRI Packhouse Workshops, Tzaneen, Loskopdam, Durban, Jeffreysbaai, Stellenbosch (January and February 2015)

References cited

- Dore, A., Molinu, M.G., Venditti, T., D'hallewin, G. 2009. Immersion of lemons into imazalil mixtures heated at 50 °C alters the cuticle and promotes permeation of imazalil into rind wounds. *Journal of agricultural and food chemistry*. 57: 623 - 631
- Erasmus, A., Lennox, C.L., Jordaan, H., Smilanick, J.L., Lesar, K., Fourie, P.H. 2011. Imazalil residue loading and green mould control in citrus packhouses. *Postharvest biology and technology*
- Fourie, P.H. 2011. CRI travel report: California.
- Hasdai, M., Elmaci, C., Goldschmidt, E.E., Droby, S., Porat, R. 2004. Isolation of a thioredoxin h cDNA from grapefruit peel tissue that is induced upon infection by *Penicillium digitatum* and elicitation of pathogen resistance, *Physiological and Molecular Plant Pathology*. 65: 277-283.
- Porat R, Pavoncello D, Peretz J, Weiss B, Daus A, Cohen L, et al. 2000. Induction of resistance to *Penicillium digitatum* and chilling injury in Star Ruby grapefruit by a short hot water rinse and brushing treatment. *Journal of Horticultural Science and Biotechnology*. 75:428–32.

4.5.3 PROGRESS REPORT: Further optimisation of in-line aqueous fungicide application in citrus packhouses

Project 1104 (2014/5 – 2014/15) by Arno Erasmus & Paul Fourie (CRI), Mareli Kellerman (CRI at USPP), Cheryl Lennox (USPP) and Catherine Savage (CRI/USPP)

Opsomming

Imazalil (IMZ) word in 'n water oplossing aangewend in die meerderheid van Suid-Afrikaanse sitrus packhuise vir die beheer van groenskimmel (*Penicillium digitatum*). 'n Alternatief vir die dompelbad is onlangs na Suid-Afrika gebring. Die vloedtoediener wend die swamdoder oplossing aan deur 'n waterval meganisme terwyl die vrugte oor borsels gedraai word. Spesifikasies vir die gebruik van die vloedtoediener tydens naoes behandeling is nie deeglik geformaliseer nie. Daar is gewys dat die pH van 'n IMZ sulfaat oplossing 'n beduidende invloed het op residu lading en die daaropvolgende groenskimmel beheer. Oplossingskonsentrasie, -temperatuur en -blootstellingstyd speel ook 'n rol. Satsuma, suurlemoen en nawel lemoen vrugte is met imazalil (250 en 500 µg.mL⁻¹) behandel deur middel van die vloedtoediener by 45°C en 'n pH reeks van 3, 4, 5 en 6. Kuratiewe en beskermende beheer, sporulasie inhibisie en residu lading is in alle gevalle ondersoek. Vloedtoediener behandelings het goeie algehele beheer gegee vir alle vrugsoorte. Uitsonderings van verminderde beheer (<90%) was met die laer pH behandelings op suurlemoene en kuratiewe beheer op Satsumas. Sporulasie inhibisie het verbeter met toenemende pH en konsentrasie. Die bestuur van die pH en konsentrasie vlak van IMZ oplossings in water toepassings sal lei tot meer

konsekvente groenskimmel beheer. Weens tegniese probleme is al die doelwitte van hierdie projek nie ten volle aangespreek nie. Doelwit 1 (bestudering van die interaksie tussen 'n oplossing se pH vlak, temperatuur en blootstellingstyd) is gedeeltelik op vloedtoediener aanwending aangespreek. Doelwitte 1 sal verder aangespreek word op dip aanwending in 'n nuwe projek (1126). Die meestersgraad-student, wat op hierdie projek werk, is in die proses om resultate van hierdie projek as deel van haar eerste wetenskaplike hoofstuk te finaliseer en dit sal in 2016 as deel van die finale verslag aangebied word.

Summary

Imazalil (IMZ) is applied in aqueous solution for the control of green mould (*Penicillium digitatum*) in the majority of South African packhouses by means of the dip tank. Recently, an alternative to the dip tank was introduced to South Africa. The flooder applies the solution through a waterfall mechanism as the fruit turns over brushes. Specifications for the use of the flooder during postharvest treatment have not been thoroughly formalised. Solution pH has been shown to have a significant link to residue loading and subsequent green mould control. Solution concentration, temperature and exposure time also play a role. Satsuma, lemon and, navel and Valencia orange fruit were treated with imazalil (250 and 500 $\mu\text{g}\cdot\text{mL}^{-1}$) by means of the flooder at 45°C and a pH range (3, 4, 5, 6) and at 45, 55 and 65°C at pH 6. Curative and protective control, sporulation inhibition and residue loading were assessed in all cases. Flooder treatments showed good overall control for all fruit kinds. Exceptions of reduced control (<90%) were with lower pH treatments on lemons and curative control on Satsumas. Sporulation inhibition increased with increasing pH and concentration for all kinds. Managing the pH and concentration level of IMZ solution in aqueous application will result in more consistent green mould control. Due to technical difficulties the objectives on these trials were not fully addressed. Objective 1 (investigating the interaction between solution pH, temperature and exposure time) was partially addressed on flooder application and will be addressed further on dip application in a new project (1126). The master's degree student, working on this project, is in the process of finalising the results from this project as part of her first scientific chapter and this will be presented as part of the final report in 2016.

4.5.4 PROGRESS REPORT: Optimisation of postharvest drench application of fungicides on citrus fruit

Project 1103 (2014/4 – 2015/3) by Arno Erasmus (CRI), Paul Fourie (CRI), Mareli Kellerman (CRI at USPP), Cheryl Lennox (USPP), Catherine Savage (CRI/USPP) and Charmaine Christie (USPP)

Summary

Postharvest fungicide application for the control of green mould (*Penicillium digitatum*) is essential soon after harvest to reduce fruit infection between harvest and entering the packhouse. Drench fungicide application can be an important tool for curing orchard-borne infections or for protecting early season fruit requiring degreening. Drench application has not been standardized and information concerning drench conditions for optimal coverage and disease control is limited. Lemon and Clementine fruit were drenched with thiabendazole, pyrimethanil and 2,4-dichlorophenoxyacetic acid at a flow rate ($\text{L}\cdot\text{min}^{-1}$) of $\frac{1}{2}\text{X}$, 1X and 2X relative to the industry recommendation (250 $\text{L}\cdot\text{bin}^{-1}\cdot\text{min}^{-1}$). Assessment included curative and protective control, sporulation inhibition and residue loading. On Clementines, the higher flow rates (2X and 1X) resulted in $\geq 75\%$ curative control when treating 42 and 30 h infections, respectively. Control levels were further improved ($> 90\%$) on younger infections. At the lowest flow rate ($\frac{1}{2}\text{X}$), control was highly variable on infections > 12 h. On lemons, the lower flow rates (1X and $\frac{1}{2}\text{X}$) resulted in $> 90\%$ green mould control when treating ≤ 24 and ≤ 12 h old infections, respectively. At the highest flow rate, green mould control was $> 90\%$ and $> 80\%$ when treating ≤ 18 h and ≤ 48 h old infections, respectively. In all trials, $> 80\%$ protective control was achieved and sporulation inhibition was $< 45\%$. Residue loading and the effect of a wetter was also studied. This work shows the importance of timeous application of fungicides following harvest, and the influence of flow rate on disease control. The project has terminated and objectives that were not completed will be addressed in a new project (1126). The master's degree student working on this project is in the process of finalising the results from this project as part of her first scientific chapter and this will be presented as part of the final report in 2016.

Opsomming

Die aanwending van naoes swamdoders vir die beheer van groenskimmel (*Penicillium digitatum*) is noodsaaklik kort na die oes van vrugte om infeksie tussen oes en die pakhuis te beperk. Swamdoder stortbehandeling ('drench') kan 'n belangrike instrument vir die genesing van boord-infeksies wees, of vir die

beskerming van vroeë seisoen vrugte wat deur ontgroening moet gaan. Stortbehandeling is nie gestandariseer en aanbevelings vir optimale bedekking en siektebeheer is beperk. Suurlemoen en Clementine vrugte is stort-behandel met tiabendasool, pyrimethanil en 2,4-dichloorfenoksieasynsuur teen 'n vloeitempo ($L \cdot \text{min}^{-1}$) van $\frac{1}{2}X$, $1X$ en $2X$ relatief tot die bedryfsaanbeveling ($250 L \cdot \text{min}^{-1}$). Evaluasie het kuratiewe en beskermende beheer, sporulasie inhibisie en residu lading ingesluit. Op Clementines, het hoër vloeitempo ($2X$ en $1X$) gelei tot $\geq 75\%$ kuratiewe beheer op behandeling van 42 en 30 h oue infeksies, onderskeidelik. Beheer vlakke is verder verbeter ($> 90\%$) op jonger infeksies. Beheer was baie wisselvallig op infeksies van > 12 h en behandel teen die laagste vloeitempo ($\frac{1}{2}X$). Op suurlemoene, het die laer vloeitempo's ($1 X$ en $\frac{1}{2} X$) gelei tot $> 90\%$ groenskimmel beheer op onderskeidelik ≤ 24 en $12 \leq h$ oue infeksies. Met die hoogste vloeitempo, was groenskimmel beheer $> 90\%$ en $> 80\%$ wanneer onderskeidelik ≤ 18 h en ≤ 48 h oue infeksies behandel is. In alle proewe is $> 80\%$ beskermende beheer bereik en sporulasie inhibisie was $< 45\%$. Residulading en die effek van 'n benatter is ook ondersoek. Hierdie werk toon die belangrikheid van die tydige toediening van swamdoders na oes, en die invloed van vloeitempo op die siektebeheer. Die projek het getermineer, en onvoltooide doelwitte sal in 'n nuwe projek opgevolg word (1126). Die meestersgraad student wat op hierdie projek werk, is in die proses om resultate van hierdie projek as deel van haar eerste wetenskaplike hoofstuk te finaliseer en dit sal in 2016 as deel van die finale verslag aangebied word.

4.5.5 FINAL REPORT: Quantification of imazalil resistance in *Penicillium digitatum* populations in citrus packhouses

Project 1102 (Apr 2014 – Mar 2015) by Mareli Kellerman, Arno Erasmus and Paul Fourie (CRI)

Opsomming

Die hoeveelheid spore in 'n pakhuis beïnvloed na-oes siektebeheer en swamdoder weerstandsontwikkeling tot 'n groot mate. Dit is belangrik om swamdoder weerstand te monitor om sodoende die effektiwiteit van swamdoder toediening en pakhuis bestuurspraktyke te bepaal, asook om die verbruik periode van swamdoders te verleng. Daar is slegs enkele swamdoders beskikbaar vir die beheer van na-oes siektes, en die mees betroubare swamdoder is tans imazalil (IMZ). Die doel van die studie was om 'n metode te ontwikkel om IMZ bestande spore in pakhuis te kwantifiseer met die gebruik van kwantitatiewe PKR (qPCR), om sodoende IMZ weerstand oor tyd te monitor. Verskillende spoorvang metodes is getoets om spore in pakhuis op te vang, nl. filter papier, "double sided tape", en Petri bakkies wat met TE buffer gespuit is. Die spoorlokvalle is met twee qPCR toetse geanaliseer, een om inokulumlading te bepaal en die ander om IMZ weerstand te bepaal. Groeimedium (PDA en MEA) bakkies wat 1 dpm IMZ bevat het, is ook gebruik as addisionele weerstand indikatore. Verskillende DNA ekstraksie metodes is getoets om die qPCR protokol te optimiseer. Die beste manier om spore vir qPCR te vang, was met die gebruik van filter papier. Die qPCR toets om inokulum lading te bepaal was suksesvol, maar die IMZ weerstand qPCR toets het nie-spesifieke amplifisering gewys tydens analisering van pakhuis monsters. Die IMZ qPCR toets is weer geoptimeer met strenger parameters, en kon suksesvol DNA van miselium kwantifiseer, maar nie van pakhuis monsters nie. Dit was nodig om ten minste 3 DNA ekstraksie herhalings te doen om seker te maak die qPCR toets is akkuraat. MEA bakkies het beter resultate as PDA bakkies gegee, maar vang ook antagonisitiese giste wat resultate kan beïnvloed. Weerstandsklassifisering van IMZ-bestande isolate is herhaal, en alle isolate is geklassifiseer. Isolate van die VSA het die hoogste verskeidenheid gehad, met 13.7% van 73 isolate as R1, 19.2 % as R2 en 67.1% wat as R3 geklassifiseer is. Isolate van Chile het 1.8% R1 isolate en 98.2% R3 isolate gehad. Alle ander isolate van ander lande is as R3 geklassifiseer. Die isolate se patogenisiteit is op IMZ behandelde vrugte bevestig. DNA volgordebepaling van sekere isolate het gewys dat alle R3 isolate die verwagte 199 bp invoeging gehad het, wat afwesig was by R1 en R2 isolate.

Summary

The number of spores in a packhouse has a big influence on postharvest disease control and the development of resistance against fungicides. Monitoring fungicide resistance is important to determine the efficacy of fungicide application and packhouse management practices, and to ensure the prolonged use of the limited fungicides available for postharvest use, especially imazalil (IMZ), currently the most important postharvest fungicide. The objective of the study was the development of a method to quantify IMZ resistant spores in packhouses using quantitative real time PCR (qPCR), to monitor IMZ resistance in packhouses over time. Different kinds of spore traps were used to collect spores in packhouses. These included filter paper discs, double sided tape and Petri dishes sprayed with TE buffer. The spore traps were analysed with two qPCR assays, one to determine inoculum load and one to determine IMZ resistance. PDA and MEA plates amended with 1 ppm IMZ were also used as additional resistance indicators. Different DNA extraction methods were tested to optimize the qPCR assay. Filter paper spore traps was the best method of spore

trapping when subjected to qPCR. The qPCR assay quantifying inoculum load was successful, but the IMZ resistance qPCR assay amplified non-specific DNA. The qPCR assay was optimized again with more stringent conditions, and was able to successfully quantify mycelial DNA, but not DNA from environmental samples. It was found that DNA extractions done in 3 or more replicates was necessary to get accurate qPCR results with the qPCR assay for determining inoculum load. MEA plates gave better results than PDA plates for resistance sampling, but the presence of antagonistic yeast may influence results. Resistance characterisation of IMZ-resistant isolates was repeated, and all isolates were classified successfully. Isolates from the USA showed the highest resistance group diversity, with 13.7% of 73 isolates identified as R1, 19.2% R2 and 67.1% as R3. From Chile, 1.8% of 55 isolates were R1 and 98.2% were R3. All the isolates from the other countries were classified into the R3 group. Isolates were also tested for pathogenicity on IMZ treated fruit. All isolates were pathogenic. Sequencing of selected isolates revealed the expected 199 bp insertion in R3 isolates, which is absent in R1 and R2 isolates.

Introduction

The number of spores in a packhouse has a big influence on disease control and the development of resistance against fungicides (Wild and Eckert, 1982). Monitoring fungicide resistance is important to determine the efficacy of fungicide application and packhouse management practices, and to ensure the prolonged use of the limited fungicides available for postharvest use.

Imazalil (IMZ) is currently the most dependable fungicide used to control green mould (Ladaniya, 2008). IMZ resistance frequencies in *Penicillium digitatum* populations vary greatly over time and location, as shown by several studies (Bus *et al.*, 1991; Holmes and Eckert, 1999; Fogliata *et al.*, 2000; Zhu *et al.*, 2006; Kinay *et al.*, 2007; Fischer *et al.*, 2009 and Sánchez-Torres and Tuset, 2011). Three of the more prevalent IMZ resistance genotypes of *P. digitatum* have been characterised and termed R1, R2 and R3 (Sun *et al.*, 2011). These genotypes lead to the over-expression of genes coding for enzymes in the biochemical pathway that imazalil targets (Sánchez-Torres and Tuset, 2011).

Van Wyk (2011) argues that molecular quantification methods are more reliable than conventional methods because of higher sensitivity and specificity. Real time PCR assays can be very reliable and contamination is prevented (Chevaliez *et al.*, 2012). The potential of real time PCR for quantification of plant pathogens have been demonstrated by several studies. For example, Fraaije *et al.* (2002) quantified strobilurin resistant isolates of *Blumeria (Erysiphe) graminis* f.sp. *tritici* on wheat using SYBR green chemistry and allele-specific primers and were able to detect one resistant allele in 10 000. Michalecka *et al.* (2011) quantified strobilurin resistant *Venturia inequalis* from apple tree leaves using SYBR green chemistry. Haugland *et al.* (2004) quantified airborne fungal conidia using 65 different real time PCR assays. Schweigkofler *et al.* (2004) and Van Wyk *et al.* (2012) have quantified *Fusarium circinatum* spores from pine plantations using SYBR green chemistry. Boutigny *et al.* (2011) also quantified *Fusarium* pathogens from South African maize using the same method. Most importantly, qPCR assays for the quantification R1 and R2 genotypes of IMZ resistant *P. digitatum* have been developed (Chen *et al.*, 2008).

Previous work has shown that the most prevalent IMZ resistance genotype in South African *P. digitatum* populations is R3 (CRI project 936). Also for isolates collected from other countries, the most prevalent genotype was R3. Therefore, a molecular resistance assay could be based on the R3 genotype.

The objective of this study was the development of a method to quantify IMZ resistant spores in packhouses using quantitative real time PCR. The method will be used to monitor IMZ resistance in 10 packhouses in the Western Cape, Eastern Cape and Limpopo provinces of South Africa. Also, the isolates that could not be classified in Project 936 as R1, 2 or 3, were reclassified in this project.

Objectives

- A: Optimize spore trapping method
- B: Classify isolates, develop and validate qPCR assay
- C: Resistance sampling in packhouses

Materials and methods

Objective A: Optimize spore trapping method

Three different methods of spore trapping were tested in three packhouses in the Citrusdal area. For passive sampling, spore traps were made from filter paper dipped in TE buffer, double sided tape and Petri dishes

sprayed with TE buffer. The spore traps were left to collect spores for 7 days. The spore traps were subjected to two qPCR assays, i.e. IMZ-R3 (Project 936) and a *P. digitatum* specific assay (Haugland *et al.*, 2004). The sampling was validated by placing PDA and MEA plates alongside the spore traps, but these were only opened for 1, 3 and 5 min. Re-isolations from the PDA and MEA plates were done onto PDA with and without IMZ (1 ppm). The number of resistant colonies were counted and compared with the number of total colonies from each packhouse. The current method used by CRI diagnostic lab to test for resistance was also used: a TE buffer dipped filter paper disc placed in the packhouse for 7 days was washed in 20 mL water and serial dilutions made of the suspension, which was then plated out on PDA with and without IMZ.

For active air sampling PDA and MEA plates were placed in a handheld Burkard air sampler which was operated for 1 min in the packhouses.

Objective B: Classify isolates, develop and validate qPCR assay

In Project 936, a collection of 213 IMZ resistant *P. digitatum* isolates provided by Geert De Wever (Janssen PMP, Beerse, Belgium) was classified into resistance groups R1, R2 or R3. The isolates were all obtained from green moulded citrus fruits originating from various countries, including the USA ($n = 73$), Uruguay ($n = 4$), Spain ($n = 21$), Israel ($n = 2$), Cyprus ($n = 2$), Chile ($n = 55$), Australia ($n = 4$), Argentina ($n = 8$) and South Africa ($n = 44$). The IMZ EC₅₀ values of these isolates were previously determined by Janssen PMP and were all $>1 \mu\text{g}\cdot\text{mL}^{-1}$. An IMZ sensitive isolate (STE-U 6560), which was previously identified as *P. digitatum* (Erasmus *et al.*, 2011), was also included in the study. Not all 213 *P. digitatum* isolates could be classified into the three known different IMZ *Cyp51* resistance groups (R1, R2 or R3) in project 936 by using the multiplex PCR assay method of Sun *et al.* (2011), so they were reclassified in this project.

Template DNA for use in the multiplex PCR was obtained by first growing each isolate in 100 mL potato dextrose broth in 500-mL Erlenmeyer flasks for 10 days at 25°C in a shaking incubator (Labcon, Petaluma, CA, USA). After 10 days, the mycelia were harvested onto sterile cheese cloth and washed with sterile distilled water. The washed mycelia was placed into 2-mL Eppendorf tubes and stored at -20°C until DNA extraction was conducted. Genomic DNA was extracted from the mycelia using the Wizard® SV Genomic DNA Purification System (Promega, Madison, WI, USA) and a slight modification of the manufacturer's protocol. Glass beads (2 mm) were added to each 2-mL Eppendorf tube along with 400 μL lysis buffer. The tubes were shaken in a tissue lyser (MM 301, Retsch, Haan, Germany) for 10 min at maximum speed (30 Hz), and then incubated in a water bath at 65°C for 30 min. Tubes were centrifuged at 14 000 rpm for 8 min and the supernatant was processed according to the manufacturer's instructions. In the final step, the DNA was eluted from columns using 150 μL nuclease free water, and 1.2 μL RNase was added followed by an overnight incubation step at 25°C. The DNA concentration and A260/280 ratios were determined with a Nanodrop (ND 1000, Wilmington, DE, USA).

The multiplex PCR reaction (Sun *et al.*, 2011) contained 1x buffer (Bioline USA Inc., Taunton, MA, USA), 0.7 U BIOTAQ DNA polymerase (Bioline USA Inc.), 0.25 mM of each dNTP, 0.1 μM of each primer (B1, B2, Cyp51A1 and Cyp51A2; Chen *et al.*, 2008; Sun *et al.*, 2011; Table 1), 0.002 mg BSA (Biowest Bovine Serum Albumin Lyophilised pH ≈ 7 , Nuaille, France), 3 mM MgCl₂ and 5 μL DNA in a final volume of 20 μL . Amplification was conducted on a GeneAmp PCR System 9700 (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) using 40 cycles of 95°C for 3 min, 95°C for 40 s, 56°C for 40 s, 72°C for 1 min and a final cycle of 72°C for 7 min. Amplification products were analysed on a 1% agarose gel that were stained with GR Green DNA Stain (Excellgen, Rockville, MD, USA). A 100-bp DNA ladder (Generuler™, Fermentas Inc., Glen Burnie, MD, USA) lane was included in each gel in order to estimate the size of amplified PCR products.

Thirty-three randomly selected isolates were inoculated onto IMZ treated navel orange fruits to further confirm their resistance phenotype *in vivo*. The isolates were grown on PDA for 7 days at 22°C, and 5 x 5 mm plugs were used to inoculate fruit that were washed with 1 mL.L⁻¹ didecyl dimethyl ammonium chloride solution (Sporekill, ICA International Chemicals, Stellenbosch, South Africa) and treated with 500 $\mu\text{g}\cdot\text{mL}^{-1}$ IMZ for 60 s as a dip treatment. Each isolate was inoculated onto three control fruits and three IMZ treated fruit. After 7 days, fruit were evaluated for typical green mould symptoms, as well as the isolate's ability to grow on IMZ treated fruit, which characterised it as IMZ resistant. Previous studies have shown that IMZ sensitive isolates were not able to grow on IMZ-treated fruit (Erasmus *et al.*, 2011, 2013; Njombolwana *et al.*, 2013). Nineteen randomly selected isolates were sequenced using primers Cyp51BpromF1 and Cyp51BpromR1 (Table 1) that amplify the promoter region of *Cyp51B*, to confirm the similarity or difference in the promoter area compared to other published sequences.

Table 4.5.5.1. Sequences of primers used in this study.

Primer	Sequence (5' - 3')	Reference
B1	TATAGCGACATTAGTTTGGC	Sun <i>et al.</i> , 2011
B2	AGGAAAGTTGCAGAGAGACCCAT	
CYP51A1	TAGCTCCAAAACAAATCGTCTGCC	Chen <i>et al.</i> , 2008
CYP51A2	GGTGAAGATATTGCCGTA TAGAC	
qPdA1	AAGGGGCGGGTCTCTCGCCG	Designed for this study
qPdA2	TGTCTCGGCATGACGCCATTGAGGC	
Cyp51BpromF1	CCAACGTCTCATCGTCCCAT	Designed for this study
Cyp51BpromR1	TGCACGACTTTGGGTGAAGA	

The EC₅₀ values of the 213 isolates were analysed by K-means cluster analysis (XLSTAT version 2013.5.09, www.xlstat.com) into 5 groups and correlated with resistance groups to ascertain any possible relationship between *Cyp51* resistance group and resistance level.

A SYBR GREEN I chemistry based qPCR assay was developed to detect the R3 resistant group isolates. Four South African *P. digitatum* isolates (STE-U 2690, imb03, SR9-8 and SR-9-9) that belonged to the R3 resistance group and two IMZ-sensitive isolates (STE-U 6560 and BLC38) were sequenced using primers B1 and B2 (Sun *et al.*, 2011; Table 4.5.5.1). Sequences of two more isolates belonging to the R3 resistance group, Pdw03 (Accession number: HQ724323.1) and Pd1 (Accession number: GU124581.1) were obtained from Genbank. The sequence data were used to design the qPCR primers for the R3 resistance group using PrimerBlast (NCBI, <http://www.ncbi.nlm.nih.gov/tools/primer-blast/>). Blast analyses of the primer sequences were run in Genbank to indicate their level of species specificity.

Three primer sets were designed and evaluated for quantifying the R3 resistance group in Project 936, which targeted the PdMLE1 DNA insertion element in the promoter region of the *Cyp51B* gene and the promoter region upstream of this insertion. The primers were optimised and evaluated for species specificity using conventional PCR. The DNA that was used for determining species specificity was obtained from other closely related species and common fungi isolated from packhouses (Table 2). Primers qPdA1 and qPdA2 (Project 936) were selected for optimizing the qPCR assay with more stringent conditions than in Project 936 using template DNA extracted from mycelia from a R3 isolate (STE-U 2690). Primer annealing temperature was optimized by running a gradient qPCR with temperatures ranging from 52 - 62°C. The primer concentration was optimized by adding 50, 250 or 500 µM of each primer to the reaction. The MgCl₂ concentration was optimized by adding none, 0.75, 1 or 2 mM additional MgCl₂ to the reaction (the buffer contains 1.5 mM MgCl₂). The standard curve was drawn up by running a 4x dilution series of 20 ng.µL⁻¹ DNA in triplicate with 7 dilutions. The optimized assay was run with DNA from the sensitive isolate (STE-U 6560) as well as with that of other species (Table 4.4.5.2) to confirm the specificity of the qPCR assay. The qPCR reaction contained 1x SensimixSYBR (Bioline USA Inc., Taunton, MA, USA), 0.25 µM of each primer, 0.75 mM additional MgCl₂ and 2 µL DNA in a final volume of 20 µL. The qPCR was performed on a Bio-Rad CFX96 machine (Bio-Rad Laboratories, Hercules, CA, USA) using the following amplification conditions: 50 cycles of 95°C for 10 min, 95°C for 15 s, 62°C for 30, followed by melting curve analysis from 72°C to 95°C rising by 1°C and holding for 5 s after each step.

Three different DNA extraction methods (MO Bio PowerPlant kit, NucleoSpin Soil, and a chelex resin method) have been used to extract DNA from 10⁴, 10³ and 10² spores of the IMZ resistant isolate of *Penicillium digitatum* (STE-U 6590). Two different lysis buffers have been used for the NucleoSpin Soil kit. Four different bead sizes were used for lysing spores for the chelex resin method. The samples were subjected to qPCR using a SYBRgreen assay with primers targeting the IMZ-R3 resistance group in gene *Cyp51B* in *P. digitatum*. Efficacy of each DNA extraction method was evaluated according to the DNA starting quantity, and repeatability of the DNA extraction method between replicates. The packhouse samples obtained in objective A was subjected to the IMZ R3 qPCR assay.

Results and discussion

Objective A: Optimize spore trapping method

For filter paper spore traps, it was shown that the environmental samples could not be analysed with the IMZ-R3 qPCR assay, since non-specific amplification was observed. This assay needed to be repeated as the lower stringency of the PCR conditions might have compromised specificity. With the *P. digitatum* species specific assay, it was observed that packhouse A had the highest starting quantity of DNA and therefore the highest inoculum load (2.3 – 5.0 ng.µL⁻¹; Table 4.5.5.2). Packhouses B and C had similar amounts of inoculum (0.1 – 0.37 ng.µL⁻¹), although in packhouse C, higher starting quantity was observed with filter paper spore traps (2.1 ng.µL⁻¹) than other spore trap methods. In general, filter paper spore traps trapped the highest amount of inoculum, although in packhouse C it did not trap significantly more spores than the other 2 spore trapping methods.

Sampling with growth media showed that more colonies were observed with MEA plates than with PDA. There was also less yeast contamination on MEA plates, although it was still present. More resistant colonies could also be observed with IMZ amended MEA plates than IMZ amended PDA plates (Table 4.5.5.3).

Higher amounts of colonies were obtained with active air sampling for 1 min (Table 4.5.5.4). It gave results similar to passive sampling, specifically pertaining to which packhouse had the most resistant colonies, but not in terms of which packhouse had the highest inoculum load.

The filter paper discs placed in packhouses and analysed according to the CRI Diagnostic Centre protocol did not show any fungal colonies; only yeast colonies was observed (results not shown).

Table 4.5.5.2. Mean starting quantity of DNA obtained from different spore trapping methods in packhouses as measured by using the qPCR assay of Haugland et al. (2004).

Spore trap method	Packhouse	Starting quantity (ng.µL ⁻¹)*
Double sided tape	Packhouse A	2.3b
	Packhouse B	0.1c
	Packhouse C	0.3c
Filter paper	Packhouse A	5.0a
	Packhouse B	0.1c
	Packhouse C	2.1b
TE buffer	Packhouse A	4.2a
	Packhouse B	0.2c
	Packhouse C	0.4c

*Means with the same letter do not differ significantly

Table 4.5.5.3. Colonies counted from PDA and MEA plates opened in packhouses for 1, 3 and 5 min.

		PDA	PDA + IMZ	MEA	MEA + IMZ	MEA+ STREP	MEA+STREP+IMZ
Packhouse A	1 min	0	0	0	0	4	0
	3 min	0	0	3	1	8	2
	5 min	0	0	9	3	25	6

Packhouse B	1min	1	0	0	1	1	0
	3min	0	1	0	0	2	0
	5min	2	0	0	1	1	0
Packhouse C	1 min	0	0	12	0	9	0
	3 min	0	0	29	0	24	0
	5 min	0	0	37	0	19	1

Table 4.5.5.4. Colonies counted from active air sampling with PDA and MEA plates opened in packhouses for 1 min.

	PDA	PDA + IMZ	MEA	MEA + IMZ	MEA+ STREP	MEA+STREP+IMZ
Packhouse A	0	2	9	1	27	3
Packhouse B	42	13	34	3	36	2
Packhouse C	1	0	10	0	3	0

Objective B: Classify isolates, develop and validate qPCR assay

All isolates yielded amplification products with the multiplex PCR and these isolates could be classified into resistance groups R1 to R3 according to their banding patterns as described by Sun *et al.* (2011) (Figure 4.5.5.1). The sensitive isolate yielded a banding pattern consisting of approximately 401 and 506 bp, which was distinct from groups R1 to R3 (Figure 4.5.5.1). Isolates from the USA showed the greatest resistance group diversity with 13.7% from 73 isolates identified as R1, 19.2% as R2 and 67.1% in the R3 resistance group (Table 4.5.5.5). In Chile, 1.8% R1 and 98.2% R3 isolates were found from 55 isolates tested. All the isolates from the other countries were classified into the R3 group.

Table 4.5.5.5. Percentage of *P. digitatum* isolates obtained from green moulded citrus fruit from different countries from each genotype (R1, R2, R3 or unclassified) as determined by a conventional multiplex PCR developed by Sun *et al.* (2011), and the number of isolates used from each country.

Country	Isolates (%)			Number of isolates used
	R 1	R 2	R 3	
Argentina	0.0	0.0	100.0	8
Australia	0.0	0.0	100.0	4
Chile	1.8	0.0	98.2	55
Cyprus	0.0	0.0	100.0	2
Israel	0.0	0.0	100.0	2
South Africa	0.0	0.0	100.0	44
Spain	0.0	0.0	100.0	21

Uruguay	0.0	0.0	100.0	4
USA	13.7	19.2	67.1	73

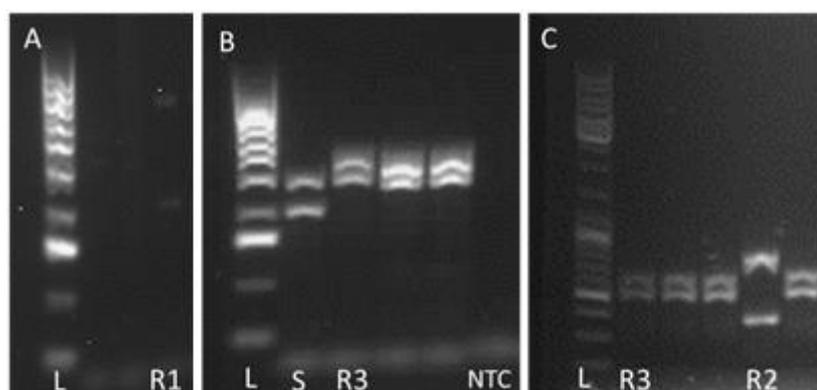


Figure 4.5.5.1. Agarose gels containing amplification products of *Penicillium digitatum* DNA amplified with a multiplex PCR (Sun *et al.*, 2011) that differentiates *Cyp51* gene associated imazalil resistance groups. The banding patterns of the R1 (401 and 1010 bp) group (A); banding patterns of R2 (401 and 705 bp) and R3 (506 and 600 bp) groups (B); and banding pattern of the sensitive isolate (401 and 506 bp; S) and R3 group (C) are shown along the 100 bp DNA ladder indicated as L.

When inoculated on fruit, all isolates showed typical green mould symptoms on untreated control fruit, as well as on the IMZ treated fruit. Some of the isolates also showed signs of sporulation inhibition on IMZ treated fruit (results not shown). There was no significant correlation between resistance grouping (R1, 2 or 3) and sporulation.

Sequencing showed highly conserved sequences among isolates belonging to separate resistance groups flanking primer binding sites of primer B1 and B2. Insertion element of R3 isolates was inserted in same location among all isolates, and absent at same location among R2 and R1 isolates.

Cluster analysis of EC_{50} values revealed five different classes among the 213 isolates analysed. The centroids for each class were 1.49, 2.29, 5.90, 9.61 and 15.19 $\mu\text{g.mL}^{-1}$ for class 1, 2, 3, 4 and 5, respectively. Isolates from the R3 resistance group occurred in all five classes, although most were in class 1 and 2 (87%; Figure 4.4.5.2). R1 and R2 isolates occurred in classes 1 and 2 only. No pattern could be observed in cluster analysis by country; isolates from most countries occurred in all classes, except for USA which only occurred in classes 1 and 2 (results not shown).

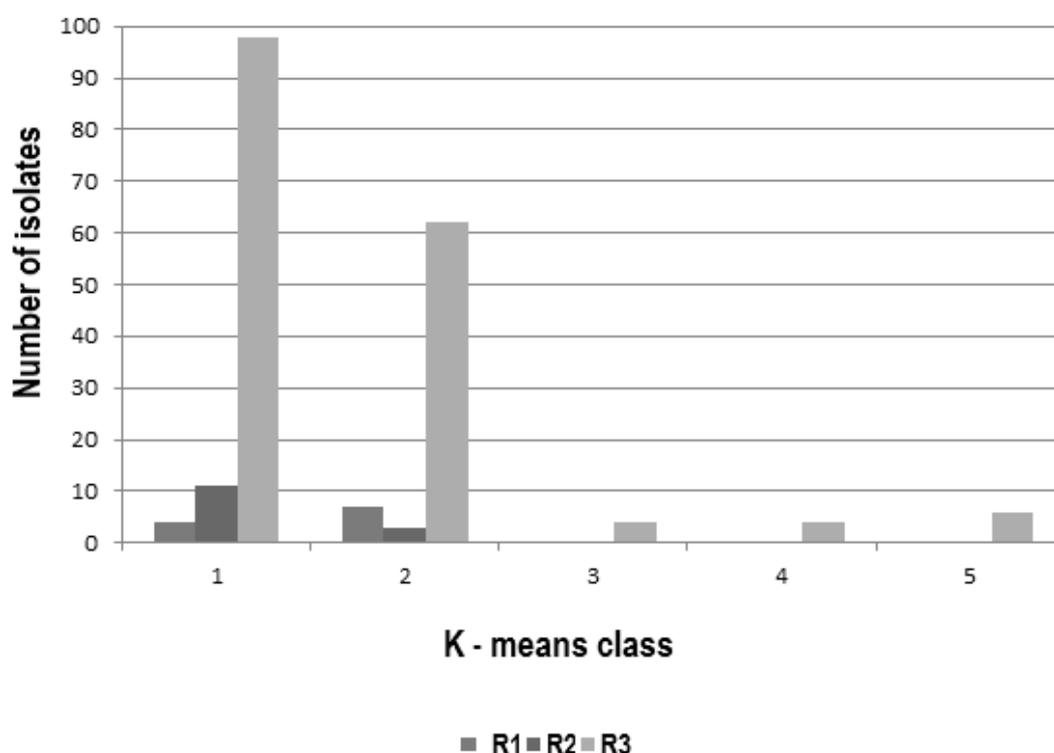


Figure 4.5.5.2. K-means cluster analysis of EC₅₀ values of 213 *Penicillium digitatum* isolates that are IMZ-resistant and classified by multiplex PCR as either R1, R2 or R3 type resistance. The centroids for each class were 1.49, 2.29, 5.90, 9.61 and 15.19 $\mu\text{g}\cdot\text{mL}^{-1}$ for class 1, 2, 3, 4 and 5, respectively.

Conventional PCR evaluation of the three primer pairs designed for quantification of the R3 resistance group showed that primer pair qPdA1 and qPdA2 did not amplify DNA from other species used in this study (Table 4.5.5.6). The qPdA1 and qPdA2 primer pair was further evaluated and optimised for use in the R3 qPCR using DNA obtained from mycelia. The primers amplified DNA from an IMZ-R3 isolate with a melting peak at 82°C, while DNA from an IMZ-sensitive isolate was amplified at a melting peak of 84°C, thus it could be clearly distinguished. IMZ sensitive DNA only amplified after 39 cycles. The quality of all qPCR amplifications was measured by the ability to draw up a standard curve, which met the following requirements: an efficiency of 90 – 100%, a R²-value of > 99%, and a slope (m-value) of -3.6 to -3.1 (Fraga *et al.*, 2008). qPCR using DNA obtained from mycelia as template yielded a standard curve with an m-value of -3.487, efficiency of 94% and R²-value of 99.9%, which was acceptable. The limit of detection was 70 $\text{fg}\cdot\mu\text{L}^{-1}$ DNA.

Table 4.5.5.6. Species and isolate numbers of isolates used to test primer specificity of primers designed for the R3 qPCR assay to quantify IMZ resistance in *P. digitatum*.

Isolate	Species
STE-U 6592	<i>Alternaria alternata</i> pv. <i>citri</i>
STE-U 6593	<i>Alternaria alternata</i> pv. <i>citri</i>
STE-U 6813	<i>Colletotrichum gloeosporioides</i>
STE-U 6814	<i>Geotrichum citri-aurantii</i>
STE-U 7649	<i>Penicillium brevicompactum</i>
STE-U 7647	<i>Penicillium brevicompactum</i>
STE-U 7651	<i>Penicillium brevicompactum</i>
STE-U 7644	<i>Penicillium chrysogenum</i>

STE-U 7646	<i>Penicillium chrysogenum</i>
STE-U 7648	<i>Penicillium chrysogenum</i>
STE-U 7639	<i>Penicillium crustosum</i>
STE-U 7645	<i>Penicillium crustosum</i>
STE-U 7650	<i>Penicillium crustosum</i>
STE-U 7638	<i>Penicillium expansum</i>
STE-U 7640	<i>Penicillium expansum</i>
STE-U 7641	<i>Penicillium expansum</i>
CV 1181	<i>Penicillium glabrum</i>
CV 36	<i>Penicillium glabrum</i>
STE-U 7643	<i>Penicillium glabrum</i>
STE-U 7406	<i>Penicillium italicum</i>
STE-U 7408	<i>Penicillium italicum</i>
STE-U 7411	<i>Penicillium italicum</i>
STE-U 7642	<i>Penicillium italicum</i>
STE-U 6378	<i>Phytophthora citricola</i>
STE-U 6558	<i>Phytophthora citricola</i>
STE-U 6815	<i>Trichoderma</i> spp.
STE-U 6816	<i>Trichoderma</i> spp.
STE-U 6818	<i>Trichoderma</i> spp.
Disease clinic isolate*	<i>Cladosporium</i> spp.
Disease clinic isolate	<i>Rhizopus</i> spp.
Disease clinic isolate	<i>Epicoccum</i> spp.

*Isolates identified to genus level that were obtained from University of Stellenbosch, Plant Pathology Disease Clinic.

For objective B, different DNA extraction methods were tested to purify DNA from filter paper spore traps. The MO Bio kit gave the most reproducible results at 10^5 spores, with a mean starting quantity of $0.004 \text{ ng.}\mu\text{L}^{-1}$, and a coefficient of variation (CV) value of 0.06 (results not shown). The NucleoSpin kit gave starting quantities of 0.02 and $0.01 \text{ ng.}\mu\text{L}^{-1}$, and CV values of 0.66 and 0.40 for buffer 1 and 2, respectively, at 10^5 spores. The Chelex resin method gave starting quantities of $0.00007 - 0.0005 \text{ ng.}\mu\text{L}^{-1}$, depending on type of beads used for lysis, and CV values of 0.34-0.73 at 10^5 spores. At lower spore counts, CV values were unacceptably high (results not shown). However, DNA yield from the packhouse samples was too variable for repeatable DNA extraction results, and thus accurate quantification using this assay was not possible.

Conclusion

It was shown that filter paper spore traps are an acceptable method of spore trapping for qPCR analysis, provided that the assay is specific enough. This was the case with the Haugland et al. (2004) assay, but not with the IMZ-R3 assay (Project 936). Further work needs to be done to investigate the latter case. The accuracy of qPCR results is influenced by the reproducibility of DNA extractions, and it was shown that the MO Bio kit gave the most reproducible results, but only at 10^5 spores and not at lower amount of spores.

The study also contributed further to our knowledge on the distribution of IMZ resistance genotypes in *P. digitatum* populations since project 936, and the molecular quantification of resistance using qPCR. The R3 resistance group was identified as the most prevalent resistance group in most citrus producing countries including South Africa. Isolates belonging to the R1 and R2 resistance groups were only detected at very low frequencies in the USA and Chile. There was no correlation between the level of resistance (measured as EC₅₀ values) of isolates and whether they were the R1, R2 and R3 genotype. Isolates from the USA showed the lowest EC₅₀ values, as well as the greatest diversity in genotypes among isolates. Isolates from Chile, South Africa and Spain had the highest EC₅₀ values. It is unknown whether differences in resistance genotype leads to different fitness penalties, virulence and levels of resistance. Dave *et al.* (1989) observed that some IMZ resistant isolates were less fit than others, and it would be interesting to see whether this can be linked to the IMZ resistance genotype. Since this study found such a small number of R1 and R2 isolates, the possibility that genotype plays a role in the level of resistance (e.g. EC₅₀) of isolates, cannot be completely ruled out.

The R3 resistance group qPCR assay was successfully optimised for DNA isolated from mycelia. However, the qPCR was not optimised for DNA extracted from environmental samples. Of some concern was the observation that IMZ-sensitive isolates were detected at a C_q (y-intercept) of 39. However, melt curve analysis showed that the product from the sensitive isolate could be differentiated from the R3 resistance group melt curve since it had a slightly higher peak melting temperature. The use of a probe assay that contains a probe that binds internal to the two currently used primers might help to overcome this problem. In order to develop highly specific and sensitive molecular assays for monitoring inoculum, primer design is very important and when more restrictions are imposed upon primers (*i.e.* it should be highly sensitive, species-specific and resistance group specific) it can be challenging to find primers that perform well in qPCR.

For exposed plate assays, MEA plates were better to use than PDA plates when using growth media as sampling technique, whether passive or active sampling was done. Interestingly, the results obtained by the various trapping techniques did not correlate. This might be due to the dynamic nature of airborne inoculum and the difference in trap times (1 week to 1 min).

Further research

Future work should include using carrier DNA in the background to improve DNA extraction repeatability, and redesigning the assay using a probe, which will add to the specificity of the assay. The current assay may still be useful in experiments using mycelia of pure cultures. The use of an exogenous internal positive control plasmid may give answers as to what causes the high variability between DNA extraction repetitions.

Technology transfer

- Kellerman, M., De Wever, G., Erasmus, A., Rose, L., Beukes, I., Viljoen, A. and Fourie, P.H. 2013. Genotyping imazalil resistance in an international collection of *Penicillium digitatum* isolates. Presented at annual American Phytopathological Society's meeting in Austin, Texas, USA. 10 – 14 Aug. 2013.
- Kellerman, M., McLeod, A., De Wever, G., Erasmus, A., Rose, L., Beukes, I., Viljoen, A. and Fourie, P.H. 2014. Fungicide resistance management. Presented at pre-season CRI packhouse workshops Jan / Feb 2014.
- Kellerman, M., McLeod, A., Erasmus, A and Fourie, P.H. 2014. Use of quantitative real-time PCR in Citrus Black Spot and *Penicillium* green mould research. Presented at 8th Citrus Research Symposium, Drakensberg, South Africa. 17 – 20 Aug. 2014.

References cited

Boutigny, A.-. 2012. Quantitative detection of *Fusarium* pathogens and their mycotoxins in South African maize. *Plant Pathology*, 61(3): 522-531.

- Bus, V., Bongers, A. & Risse, L. 1991. Occurrence of *Penicillium digitatum* and *P. italicum* resistant to benomyl, thiabendazole, and imazalil on citrus fruit from different geographic origins. *Plant Disease*, 75(11): 1098-1100.
- Chen, G., Zhang, Z., Jiang, L., Xu, F., Ma, Z. & Li, H. 2008. Real-time PCR assay for detection of the frequency of imazalil resistance of *Penicillium digitatum*. *Acta Phytopathologica Sinica*, 38(6): 561-569.
- Chevaliez, S., Rodriguez, C. & Pawlotsky, J. 2012. New virologic tools for management of chronic hepatitis B and C. *Gastroenterology*, 142(6): 1303-1313.e1.
- Dave, B., Sales, M. & Walia, M. 1989. Resistance of different strains of *Penicillium digitatum* to imazalil treatment in California citrus packinghouses. *Proceedings of the Florida State Horticultural Society*, 102:178-179.
- Erasmus, A., Lennox, C.L., Jordaan, H., Smilanick, J.L., Lesar, K., Fourie, P.H., 2011. Imazalil residue loading and green mould control in citrus packhouses. *Postharvest Biology and Technology*, 62(2): 193 - 203.
- Erasmus, A., Lennox, C.L., Smilanick, J.L., Lesar, K. & Fourie, P.H. 2013. Imazalil residue loading and green mould control on citrus fruit as affected by formulation, solution pH and exposure time in aqueous dip treatments. *Postharvest Biology and Technology*, 77(0): 43-49.
- Fischer, I.H., Lourenço, S.A., Amorim, L. & Spósito, M.B. 2009. Characterisation of the fungal population in citrus packing houses. *European Journal of Plant Pathology*, 123(4): 449-460.
- Fogliata, G., Torres Leal, G. & Ploper, L. 2000. Detection of imazalil-resistant strains of *Penicillium digitatum* Sacc. in citrus packinghouses of Tucumán province (Argentina) and their behavior against currently employed and alternative fungicides. *Revista Industrial y Agrícola De Tucumán*, 77(2): 71-75.
- Fraaije, B., Butters, J., Coelho, J., Jones, D. & Hollomon, D. 2002. Following the dynamics of strobilurin resistance in *Blumeria graminis* f. sp. *tritici* using quantitative allele-specific real-time PCR measurements with the fluorescent dye SYBR green I. *Plant Pathology*, 51(1): 45-54.
- Haugland, R.A., Varma, M., Wymer, L.J. & Vesper, S.J. 2004. Quantitative PCR analysis of selected *Aspergillus*, *Penicillium* and *Paecilomyces* species. *Systematic and Applied Microbiology*, 27(2): 198-210.
- Holmes, G.J. & Eckert, J.W. 1999. Sensitivity of *Penicillium digitatum* and *P. italicum* to postharvest citrus fungicides in California. *Phytopathology*, 89(9): 716-721.
- Kinay, P., Mansour, M.F., Mlikota Gabler, F., Margosan, D.A. & Smilanick, J.L. 2007. Characterization of fungicide-resistant isolates of *Penicillium digitatum* collected in California. *Crop Protection*, 26(4): 647-656.
- Ladaniya, M.S. 2008. 16 - Postharvest diseases and their management, *Citrus fruit*. San Diego: Academic Press. Page 417.
- Michalecka, M., Malinowski, T., Broniarek-Niemiec, A. & Bielenin, A. 2011. Real-time PCR assay with SNP-specific primers for the detection of a G143A mutation level in *Venturia inaequalis* field populations. *Journal of Phytopathology*, 159(7-8): 569-578.
- Njombolwana, N.S., Erasmus, A. & Fourie, P.H. 2013. Evaluation of curative and protective control of *Penicillium digitatum* following imazalil application in wax coating. *Postharvest Biology and Technology*, 77: 102-110.
- Sánchez-Torres, P. & Tuset, J.J. 2011. Molecular insights into fungicide resistance in sensitive and resistant *Penicillium digitatum* strains infecting citrus. *Postharvest Biology and Technology*, 59(2): 159-165.
- Schweigkofler, W., O'Donnell, K. & Garbelotto, M. 2004. Detection and quantification of airborne conidia of *Fusarium circinatum*, the causal agent of pine pitch canker, from two California sites by using a real-time PCR approach combined with a simple spore trapping method. *Applied and Environmental Microbiology*, 70(6): 3512-3520.
- Sun, X., Wang, J., Feng, D., Ma, Z. & Li, H. 2011. PdCYP51B, a new putative sterol 14 α -demethylase gene of *Penicillium digitatum* involved in resistance to imazalil and other fungicides inhibiting ergosterol synthesis. *Applied Microbiology and Biotechnology*, 1-13.
- Van Wyk, S.J.P. 2011. Epidemiology and management of *Fusarium circinatum* the Western Cape province of South Africa. *Unpublished thesis*. Stellenbosch: Stellenbosch University.
- Van Wyk, S.J.P., Boutigny, A.L., Coutinho, T.A. & Viljoen, A. 2012. Sanitation of a South African forestry nursery contaminated with *Fusarium circinatum* using hydrogen peroxide at specific oxidation reduction potentials. *Plant Disease*, 96(6): 875-880.
- Zhu, J., Xie, Q. & Li, H. 2006. Occurrence of imazalil-resistant biotype of *Penicillium digitatum* in China and the resistant molecular mechanism. *Journal of Zhejiang University-Science A*, 7362-7365.

4.5.6 FINAL REPORT: Use of hot water, potassium silicate and biological control agents to reduce postharvest disease and chilling injury in citrus fruit

Project UKZN1 (2010/11-2014/15) by Prof Mark Laing (UKZN), Dr Iona Basdew (UKZN)

Summary

The aim of this research was to investigate the integration of potassium silicate fertilisation, hot water treatment and biocontrol agents for postharvest disease and chilling injury control in citrus. Research objectives evolved from project inception to the completion of the final closing report, hence the data reported in this final closing report is a culmination of four years of research, and a detailed overview of the major findings during each year of the study. The major primary objectives of the project were to (1) optimise the preventative effects of the yeast biocontrol agent B13, combined with the best curative hot water treatment and (2) evaluate the buffering effects of pre-harvest potassium silicate applications to citrus trees on the chilling injuries suffered by lemons. In experiments testing Temperature x Time x B13 combinations for the control of *P. digitatum* on navel oranges, the most significant treatment of fruit occurred at 60°C x 20 s, 64°C x 5, 10, 15 and 20 s, followed by treatment with B13. This resulted in the lowest number of diseased fruit, 2 weeks post-treatment. Fruit showed reduction in disease development ranging from 95-98%. In similar experiments on Valencia, optimal hot water treatments were in the range 64°C for 5, 10, 15 and 20s. Fruit showed a reduction in disease development of between 95-98%, which was consistent with those results derived from the navel trials. Furthermore, all fruit also showed no physical evidence of damage due to treatment at temperatures between 58-66°C, either for 5, 10, 15 or 20 seconds. With regard to the potassium silicate studies, it was found that fruit picked from trees which received preharvest silicon treatments, either in liquid form or granular form showed superior tolerance to chilling injury. Fruit picked from trees receiving either of the control treatments, potassium sulphate or plain water, succumbed to chilling injury far quicker and far more severely, rendering them un-marketable in a simulated export situation. Furthermore, silicon uptake was assessed in the flavedo of the fruit using electron scanning microscopy. Results showed that silicon is indeed taken up by the plant and deposited to fruit, and it is postulated that this increased level in silicon in the flavedo is able to confer chilling injury protection. Preharvest silicon treatments also appeared to predispose the fruit to dehydration tolerance.

Opsomming

Die doel van hierdie navorsing was om die integrasie van kaliumsilikaat bemesting, warm water behandeling en biologiese beheer agente vir na-oes siektes en koueskade beheer in sitrus te ondersoek. Navorsings doelwitte het ontwikkel vanaf die projek se aanvang tot voltooiing, vandaar die data wat in hierdie finale verslag weergegee word as hoogtepunt van vier jaar se navorsing, en 'n gedetailleerde oorsig van die belangrikste bevindings tydens elke jaar van die studie. Die primêre doelwitte van die projek was om (1) die voorkomende effek van die biologiese beheermiddel, die gis B13, gekombineer met die beste genesende warm water behandeling te optimaliseer en (2) die bufferingseffek van vooroes kaliumsilikaat toedienings aan sitrusbome teen koue skade op suurlemoene te evalueer. In eksperimente waar die kombinasie Temperatuur x Tyd x B13 getoets is vir die beheer van *P. digitatum* op nawellemoene was die beste behandeling die van 60°C x 20 s, 64°C x 5, 10, 15 en 20 s, gevolg deur behandeling met B13. Dit het gelei tot die laagste aantal bederfde vrugte, 2 weke na behandeling. Die vermindering in siekte ontwikkeling het gewissel van 95 - 98%. In soortgelyke eksperimente op Valencia was die optimale warm water behandelings in die reeks van 64°C vir 5, 10, 15 en 20 s. 'n Vermindering in siekte ontwikkeling van tussen 95 - 98% is bereik, wat in ooreenstemming was met die resultate verkry uit die nawel proewe. Verder het alle vrugte ook geen fisiese tekens van skade getoon as gevolg van behandeling by temperature tussen 58 - 66°C, hetsy vir 5, 10, 15 of 20 s. Met betrekking tot die kaliumsilikaat studies, is daar gevind dat die vrugte gepluk van bome wat vooroes silikon behandelings ontvang het, hetsy in vloeibare vorm of korrel vorm, beter verdraagsaamheid teen koueskade getoon het. Vrugte gepluk van bome wat óf kaliumsulfaat (potte) of skoon water (kontrole behandelings) ontvang het, het koueskade baie vinniger en baie meer ernstig gekry. Dit het gelei tot die lewering van onbemarkbare vrugte in 'n gesimuleerde uitvoer situasie. Verder is silikon opname in die flavedo van die vrugte ondersoek met behulp van skandering elektron mikroskopie. Resultate het getoon dat silikon inderdaad deur die plant opgeneem word en in die vrugte gedeponeer word, en daar word gepostuleer dat hierdie verhoogde silikon vlakke die flavedo in staat stel om beskerming teen beserings as gevolg van koue te bied. Vooroes silikon behandelings het ook die vrugte vir verdraagsaamheid teen uitdroging gepredisponeer.

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

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

Opsomming

Fludioxonil en propiconazole is teen groenskimmel getoets. Beide aktiewe bestandele het potensiaal as groenskimmel swamdoders getoon, veral op ≤ 18 h infeksies. Propiconazole is ook 'n baie goeie alternatief vir guazatine teen suurvrot, maar op jonger infeksies. As gevolg van beperkte kapasiteit is die implementering van GRAS chemikalieë in die bedryf en die residu ring toets vir analitiese laboratoriums nie aangespreek nie. 'n Totaal van 68 spoor monsters is vir imazalil bestandheid in samewerking met CRI Diagnostiese Sentrum getoets. In 'n laboratoriumproef is aangetoon dat 1 min blootstellingstyd lank genoeg was vir chloor en waterstofperoksied / per-asynsuur om groenskimmel spore dood te maak. Die toevoeging van 'n benatter in 'n chlooroplossing kan die effek van chloor verbeter. Die produk Fortisol het die voorkoms van guazatine brand aansienlik verminder. Die belangrikheid van vrug droging so gou as moontlik na behandeling om die risiko van guazatine brand te verminder, is ook getoon. Loodsproewe met fluoriserende pigment het voorlopig aangedui dat 'n teenskuimmiddel eerder as 'n benatter die voorkoms van swamdoder ringe op vrugoppervlaktes, waar vrugte aan mekaar raak terwyl dit droog word, kan verminder. Sekere van hierdie projek se resultate is by die 2015 CRI Pakhuis Werkswinkels aangebied.

Summary

Fludioxonil and propiconazole were tested against green mould. Both actives showed potential as green mould fungicides, especially on ≤ 18 h infections. Propiconazole is also a very favourable guazatine alternative against sour rot, but on younger infections. Due to capacity constraints the implementation of GRAS chemicals into the industry and the residue ring test for analytical laboratories could not be addressed. A total of 68 spore samples were tested for imazalil resistance in collaboration with CRI DC. In a laboratory test it was shown that 1 min exposure time is long enough for chlorine and hydrogen peroxide / peracetic acid to kill green mould spores. Adding a wetter to a chlorine solution can enhance the effect of chlorine. The product Fortisol significantly reduced the incidence of guazatine burn. The importance of drying fruit as soon as possible after treatment to reduce the risk of guazatine burn was also shown. Pilot trials with fluorescent pigment showed the preliminary finding that antifoam agents rather than an adjuvant can reduce the effect of fungicide rings at fruit surfaces where they touch while drying. A selection of results from this project was presented at the 2015 CRI Packhouse Workshops.

Introduction

This project offers an ongoing industry service to evaluate potential new postharvest disease control products or options, as well as to conduct *ad hoc* experimentation. Products are mostly submitted from private companies, or projects/products are selected by the researchers involved. Given limited time and resources, requests are screened based on industry priorities. Below are brief reports of the activities in the project during the 2014/15 report year.

Objectives:

1. New potential products will be tested as sanitation agents and/or fungicides, the bulk of the work will be done by QMS and CRI postharvest plant pathology will have limited involvement. CRI will be involved in setting up protocols and interpreting the data and findings
2. Seek and test alternative actives for the control of sour rot and phytophthora brown rot
3. Introduce and implement the application of GRAS chemicals into the citrus postharvest industry
4. Analytical lab focus – ring test with the aim to reduce variability
5. Develop and implement a DC protocol for assessing fungicide resistance in citrus packhouses

Objective / Milestone	Achievement	
1	New potential products will be tested as sanitation agents and/or fungicides, the bulk of the work will be done by QMS and CRI postharvest plant pathology will have limited involvement. CRI will be involved in setting up protocols and interpreting the	The collaboration with QMS is slowly developing, but it was also realised that it is to the advantage of the Postharvest Pathology team to be exposed to any new potential products. Three chlorine alternatives, four imazalil alternatives and one guazatine alternative were tested. None of the chlorine alternatives can be

	data and findings.	recommended at this stage. Fludioxonil and propiconazole showed potential as new actives for the control of green mould and propiconazole for the control of sour rot.
2	Seek and test alternative actives for the control of sour rot and phytophthora brown rot.	Propiconazole has been investigated as mentioned in Objective 1. No work was done on phytophthora brown rot.
3	Introduce and implement the application of GRAS chemicals into the citrus postharvest industry.	Due to capacity constraints this objective could not be addressed.
4	Analytical lab focus – ring test with the aim to reduce variability.	Due to capacity constraints this objective could not be addressed.
5	Develop and implement a DC protocol for assessing fungicide resistance in citrus packhouses.	The Postharvest Pathology team assisted the DC with the assessment of 68 packhouse spore samples that was received for imazalil resistance testing.
	Additional objectives:	
6	Investigate the effect of exposure time of sanitation products on the germination of green mould spores.	This was successfully conducted showing that 1 min exposure time is sufficient.
7	Investigate the effect of Fortisol on reducing guazatine burn.	Fortisol significantly reduced guazatine burn.
8	Investigate the effect of adding an adjuvant to a fungicide solution to reduce the ring effect where fruit touch each other during the drying process after treatment.	Adding an adjuvant showed no effect in reducing the ring effect, but adding an antifoam agent showed promise.

Objective 1. Testing new potential sanitation and fungicide agents

Sanitation agents

Goldline

This product is a combination of hydrogen peroxide and peracetic acid. It has shown very good potential as a sanitation agent in a dip or recycle spray treatments. However, phytotoxicity was observed. This may be due to the high concentration tested, as recommended by the supplier. The full report can be seen in Addendum 1.

Xanbac-D

This product has the active ingredient dichlorophen. It showed no potential as a sanitation agent applied in a dip treatment at 200, 400 and 800 µg.mL⁻¹. The full report can be seen in Addendum 2.

eOxide

This product has the active ingredient chlorine dioxide. In laboratory trials it showed good potential as chlorine alternative (Addendum 3). In a field trial it showed weaker potential as a sanitiser in fruit wash systems (Addendum 4). Chlorine dioxide requires strict and effective management to ensure the concentration and ORP levels are maintained.

Fungicide agents

Fludioxonil (FLU) and Propiconazole (PPZ) against green mould

These products were tested for its potential as green mould fungicides. Thorough trials were conducted on Clementine, Eureka lemon and navel orange fruit and shown to be effective against 5.5-h-old infections (results not shown). Fludioxonil was less effective compared to imazalil but reduced infection to 20 – 30% compared to > 90% in untreated control treatments and can still be regarded as an option. In additional trials the effect of exposure time on green mould control was investigated (Addendum 5). It was found that fludioxonil is effective on 6 h old infections and ≈ 50% effective on 18 h old infection. Control was lost at 24 h old infections, when it was previously found that imazalil still has very good control. Propiconazole was shown to be effective on 6 and 18 h old infection and less effective on 24 h old infection (≈ 50% control;

Addendum 6). A product combining PPZ and pyrimethanil (PYR) was highly effective control 6, 18 and 24 h old infections (Addendum 7).

Propiconazole against sour rot

This product was tested for its potential as alternative sour rot fungicide. Very good control was showed on lemon (2.2% infection, results not shown), less on navel (20% infection) and moderate on Clementine (60% infection) fruit compared to untreated fruit (\approx 100% infection). In additional trials the effect of infection age on sour rot control was investigated (Addendum 6). Infection age had an effect on the level of control (0.0, 7.1 and 22.9%, respectively for 6, 18 and 24 h old infections). Similar trials were conducted with a product containing PPZ and PYR (Addendum 7). Infection levels were 0.0, 1.4 and 8.3% for 6, 18 and 24 h, respectively. These results showed a possible synergy between the two actives, but this needs to be investigated further.

Goldline

This product has been discussed as sanitation product above, but claims were made that it was also effective as a fungicide. Our work showed that it has no potential as possible green mould fungicide (Addendum 1).

Objective 4

Packhouse resistance testing

A total of 68 samples were assessed from 10 packhouses across the country. At three of these packhouses imazalil resistant *Penicillium digitatum* (green mould) isolates were detected and at six packhouses imazalil resistant isolates of other *Penicillium* species were detected. These other species were not identified, but were observed to be morphologically similar to *P. italicum* (blue mould). Only one packhouse had imazalil resistance in both species.

Objective 6

Exposure time and sanitation agents

It was shown (Addendum 8) that chlorine and Citroicide (hydrogen peroxide and paracitic acid) are very effective to kill spores after 1 min. Combining an adjuvant with chlorine improved its ability to kill spores.

Objective 7

Fortisol and its ability to reduce guazatine burn

Fortisol significantly reduced guazatine burn (Addendum 9). These trials again highlighted the importance of drying fruit quickly after treatment as fruit that stayed wet for longer had significantly more burn incidence compared to those dried immediately after treatment. Leaving guazatine treated fruit to dry too long can increase the risk of burn from 10 – 30%. Fortisol can reduce this risk by 10 – 40%.

Objective 8

Drench adjuvants and reducing fungicide rings

Adding an adjuvant to a fungicide solution did not eliminate the fungicide ring effect resulting from fruit touching each other while drying (Addendum 10). Adding antifoam to a fungicide solution did reduce the fungicide ring effect. Currently there is no antifoam product registered for citrus postharvest use.

Technology transfer

A talk dedicated to results from this project was presented at the 2015 CRI Packhouse Workshops.

Further objectives (milestones) and work plan

1. New potential products will be tested as sanitation agents and/or fungicides, this specifically include seeking actives for the control of Phytophthora brown rot and sour rot
2. Introduce and implement the application of GRAS chemicals into the citrus postharvest industry
3. Analytical lab focus – ring test with the aim to reduce variability
4. Assist CRI DC with packhouse resistance testing

Quarterly milestones for Jan-Mar, Apr-Jun, Jul-Sep, Oct-Dec 2016 and Jan-Mar 2017

Apr-June 2016

- Objective 1 – New potential products: Acquire and test products
- Objective 2 – GRAS chemicals: Find a packhouse to conduct pilot trials
- Objective 3 – Residue ring test: Prepare for ring test
- Objective 4 – Packhouse resistance testing: Assist DC when needed

Jul-Sep 2016

- Objective 1 – New potential products: Acquire and test products
- Objective 2 – GRAS chemicals: Conduct pilot trials
- Objective 3 – Residue ring test: Conduct ring test
- Objective 4 – Packhouse resistance testing: Assist DC when needed

Oct-Dec 2016

- Objective 1 – New potential products: Write reports and give feedback
- Objective 2 – GRAS chemicals: Compile and analyse data
- Objective 3 – Residue ring test: Compile data and give feedback
- Objective 4 – Packhouse resistance testing: Assist DC when needed

Jan-Mar 2017

- Objective 1 – New potential products: Present interesting results at workshops
Write progress report
- Objective 2 – GRAS chemicals: Present interesting results at workshops
Write progress report
- Objective 3 – Residue ring test: Write progress report
- Objective 4 – Packhouse resistance testing: Write progress report

Addendum 1

The evaluation of Goldline as a potential sanitation and fungicidal agent for the control of citrus green mould

Product information

Product name:	Goldline
Company:	Wessoclean
Active ingredient:	Cationic tensid (< 5%) and hydrogen peroxide (< 5%)
Trial date:	12 September 2014
Product batches:	Two different batches were used, one for the first sanitation trial and another for second sanitation trial and the fungicide trial
Fruit:	Valencia orange fruit were used in all trials

Evaluation of sanitation action

Trial 1: Sanitation 1 (Dip test)

Materials and methods

- A 5 L *Penicillium digitatum* (green mould) spore suspension containing 1×10^4 spores.mL⁻¹ was prepared
- Each treatment contained 12 fruit wounded four times beyond the flavedo (orange part) by means of a wounding tool. The wounds were inflicted around the calyx end of the fruit and each were $\approx 1 \text{ mm}^2$
- Untreated control: Wounded citrus fruit dipped for 1 min in the spore suspension
- Product treatment: In each treatment the product was added to a fresh spore suspension and after 3 min wounded citrus fruit was dipped treated for 1 min in the solution.
 - In the case of Goldline, spore suspension was added to the product that was prepared to either 100% or 50% strength
- After treatment fruit was incubated for 4 days at ambient temperature ($\approx 22^\circ\text{C}$)
- Infected wound rating data were converted to percentage infection

Results

Table 1. Percentage green mould infection on Valencia orange fruit dipped for 60 s in a 1×10^4 spore.mL⁻¹ suspension of *P. digitatum* treated for 180 s with Goldline, chlorine or left untreated.

Treatment	Concentration	Infection (%)
Goldline	50% (v/v)	16.7
Goldline	100%	2.1
Chlorine	37.5 $\mu\text{g.mL}^{-1}$	4.2
Chlorine	75.0 $\mu\text{g.mL}^{-1}$	0.0
Chlorine	150.0 $\mu\text{g.mL}^{-1}$	0.0
Untreated control	n/a	58.3

Conclusions and observations

- In comparison to chlorine this product in the recommended (100%) concentration showed potential for use as a potential sanitising agent against green mould.
- No phytotoxic damage was observed on the fruit in this trial, but phytotoxicity in the other trials with this product is a concern.
- No adverse effects were noted from working with the product.

Trial 2: Sanitation 2 (Spray applicator test)

Materials and methods

- A 5 L *Penicillium digitatum* (green mould) spore suspension containing 1×10^4 spores.mL⁻¹ was prepared
- Unwounded fruit were dipped for ≈ 10 s in the spore suspension
 - Half of the fruit were left to dry before treatment (≈ 30 minutes)
 - Half of the fruit were treated immediately (while still wet)

- Untreated control: Wounded citrus fruit dipped for 1 min in the spore suspension
- Treatment involved a high pressure spray of the product on to fruit moving over rotating brushes.
- For each treatment 3 replicates of 12 fruit each were used.
- Fruit were wounded (20 wounds per fruit) by means of a wounding tool (10 mm deep and 3.2 mm wide) after treatment and incubated for 4 days at $\approx 22^{\circ}\text{C}$
- Infected fruit rating data were converted to percentage infection
 - Statistical analyses was done using XLStats

Results

Table 1. Percentage green mould infection on Valencia orange fruit dipped for 1 min in a 1×10^4 spore.mL⁻¹ suspension of *P. digitatum* and treated for ≈ 3 s with a high pressure washer over rotating brushes with Goldline; half of the fruit were left to dry before treatment (dry fruit) and the other half were treated immediately after inoculation (wet fruit).

Treatment	Concentration	Infection (%) ^a
Goldline (Wet fruit)	100%	2.8a
Goldline (Dry fruit)	100%	12.0b
Untreated control (Wet fruit)	n/a	98.1d
Untreated control (Dry fruit)	n/a	74.8c

^aEach value followed by the same letter is not significantly different according to Fisher's LSD test ($P \leq 0.05$).

Conclusion and observations

- Fruit treated directly after inoculation (wet) had significantly lower levels of infections compared to those left to dry before treatment. This is an indication that the treatment as a whole (applicator and product combination) is less inclined to remove or kill spores from dry fruit surfaces contaminated with spores.
- Phytotoxic damage was observed on the fruit immediately after treatment (Figure 1 & 3). This was not only from the high pressure spray treatment, but also from the fungicide dip treatment reported below. This is an issue of concern and needs to be resolved before Goldline will be considered for recommendation as a chlorine alternative.
- The Goldline solution foamed excessively while circulating through the spray applicator. Resultant foam spilled out of the applicator covering the surrounding floor area, bleaching the floor. The brushes were also bleached (Figure 2).

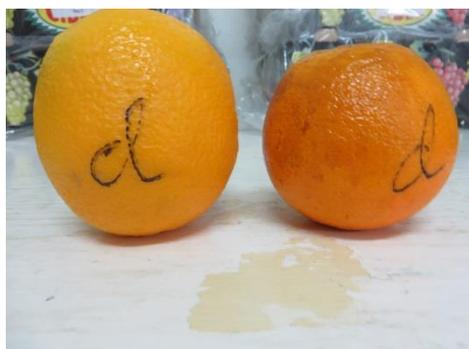


Figure 1. Fruit that was dry before treatment, with the untreated control fruit (left) showing no damage and the Goldline treatment fruit (right) with phytotoxic damage on the rind.

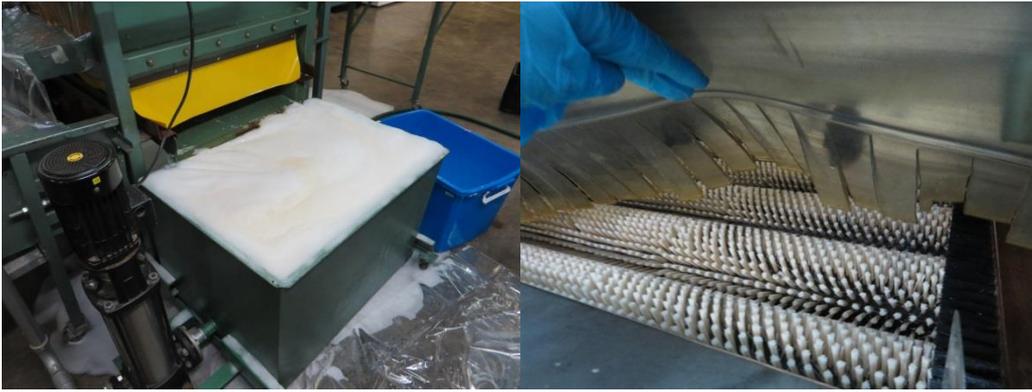


Figure 2. Excessive foaming in the spray applicator (left) due to Goldline and white bleached roller brushes (right) as a result of Goldline treatment



Figure 3. Further examples of chemical burn on fruit rind

Trial 3: Fungicide agent

Materials and methods

Test: Curative fungicidal action

- Citrus fruit were inoculated with 1×10^6 spores.mL⁻¹ spores of *Penicillium digitatum* (green mould) 6 hours prior to treatment by means of a wounding tool at four sites around the calyx end. Each wound was ≈ 2 mm deep and ≈ 1 mm in diameter.
- Inoculated fruit were dipped for 1 minute in a specific product solution.
- The fungicide imazalil was used as the fungicide standard.
- Untreated inoculated fruit served as a control treatment.
- For each treatment 3 replicates of 12 fruit were used.
- After treatment fruit was incubated for 4 days at $\approx 22^\circ\text{C}$.

Results

Table 1. Percentage green mould infection on Valencia orange fruit inoculated with *Penicillium digitatum*, incubated for 6 h and then dip treated with Goldline or imazalil

Treatment	Concentration	Infection (%) ^a
Goldline	100%	100
Imazalil	500 µg.mL ⁻¹	0
Control	0 µg.mL ⁻¹	97

Conclusion and observations

- In comparison to imazalil, Goldline at 100% concentration showed no potential for use as a fungicide against green mould.
- Some phytotoxic damage was seen on the fruit, the same as in the sanitation spray trial (Figures 1 & 3).
- No adverse effects were noted from working with the product.

Important comments

These were pilot trials. None of these results can be used in marketing or as a CRI recommendation in any citrus chemical program.

Addendum 2

The evaluation of Xanbac-D as a potential sanitising agent for the control of citrus green mould or sour rot

Product name: Xanbac-D
Company: PLAASKEM (Pty) Ltd.
Active ingredient: Dichlorophen 200g/L
Trial date: 12 September 2014
Fruit: Valencia orange fruit were used in all trials

Trial 1: Sanitation action (Dip test)

Materials and methods

- A 5 L *Penicillium digitatum* (green mould) spore suspension of 1×10^4 spores.mL⁻¹ was prepared
- Each treatment contained 12 fruit wounded four times beyond the flavedo (orange part) by means of a wounding tool. The wounds were inflicted around the calyx end of the fruit and each were $\approx 1 \text{ mm}^2$
- Untreated control: Wounded citrus fruit dipped for 1 min in the spore suspension
- Product treatment: In each treatment the product was added to a fresh spore suspension and after 3 min wounded citrus fruit was dipped treated for 1 min in the specific solution.
 - In the case of Xanbac-D spore suspension was added to the product that was prepared to either recommended, double or half the recommended concentration.
- After treatment fruit were incubated for 4 days at ambient temperature ($\approx 22^\circ\text{C}$).
- Infected wound rating data were converted to percentage infection.

Results

Table 1. Percentage green mould infection on Valencia orange fruit dipped for 1 min in a 1×10^4 spore.mL⁻¹ suspension of *P. digitatum* treated for 3 min with Xanbac-D, chlorine or untreated.

Treatment	Concentration	Infection (%)
Xanbac-D	200 $\mu\text{g.mL}^{-1}$	50.0
Xanbac-D	400 $\mu\text{g.mL}^{-1}$	39.6
Xanbac-D	800 $\mu\text{g.mL}^{-1}$	45.8
Chlorine	37.5 $\mu\text{g.mL}^{-1}$	4.2
Chlorine	75 $\mu\text{g.mL}^{-1}$	0
Chlorine	150 $\mu\text{g.mL}^{-1}$	0
Control	n/a	58.3

Conclusion and observations

- In comparison to chlorine, this product in the applied concentrations showed no potential for use as a possible sanitising agent against green mould.
- No phytotoxic damage was seen on the fruit.
- No adverse effects were noted from working with the product.

Trial 2: Fungicidal action (Dip test)

Materials and methods

Test: Fungicide against green mould and sour rot

- Citrus fruit were inoculated with 1×10^6 spores.mL⁻¹ *Penicillium digitatum* (causal agent of green mould) or 1×10^8 spores.mL⁻¹ *Geotrichum citri-aurantii* (causal agent of sour rot) 6 hours prior to treatment.
- Inoculated fruit were dipped for 1 minute in the product solution.
- The fungicide imazalil was used as a positive control for green mould.
- The fungicide guazatine was used as a positive control for sour rot.
- Untreated fruit were used as negative control.
- 1 replication was done for each treatment.
- Fruit was incubated for 4 days at 23°C for green mould.
- Fruit was incubated for 6 days at 28°C in darkness for sour rot.
- Infected wounds were rated and converted to percentage infection.
- Statistical analyses was done using XLSTAT.

Results

Table 1. The average percentage of infection on Valencia orange fruit inoculated with *P. digitatum* or *Geotrichum citri-aurantii* after treatment at 6 hours compared to the fungicide controls and untreated controls.

Treatment	Concentration	Green mould infection (%) ^a	Sour rot infection (%) ^a
Xanbac-D (6 hrs)	400 µg.mL ⁻¹	72.9b	97.9a
Imazalil (6 hrs)	500 µg.mL ⁻¹	0.0c	N/A
Guazatine (6 hrs)	500 µg.mL ⁻¹	N/A	0.0b
Control (6 hrs)	0 µg.mL ⁻¹	95.8a	93.8a

^aEach value followed by the same letter is not significantly different according to Fisher's LSD test ($P \leq 0.05$).

Conclusion and observations

- In comparison to imazalil this product in the applied concentrations showed no potential for use as a possible fungicide against green mould.
- In comparison to guazatine this product in the applied concentrations showed no potential for use as a possible fungicide against sour rot.
- No phytotoxic damage was seen on the fruit.
- No adverse effects were noted from working with the product.

Important comments

These were pilot trials. None of these results can be used in marketing or as a CRI recommendation in any citrus chemical program.

Addendum 3

The evaluation of Eoxide as a potential sanitising and fungicide agent for the control of citrus green mould

Product name: eOxide
Company: EOX Technologies
Active ingredient: ClO₂ (Chlorine dioxide)
Trial date: 12 September 2014
Fruit: Valencia orange fruit were used in all trials

Trial: Sanitation action (Dip test)

Materials and methods

- A 5 L *Penicillium digitatum* (green mould) spore suspension of 1×10^4 spores.mL⁻¹ was prepared.
- Each treatment contained 12 fruit wounded four times beyond the flavedo (orange part) by means of a wounding tool. The wounds were inflicted around the calyx end of the fruit and each were ≈ 1 mm².
- Untreated control: Wounded citrus fruit dipped for 1 min in the spore suspension.
- Product treatment: In each treatment the product was added to a fresh spore suspension and after 3 min wounded citrus fruit was dip-treated for 1 min in the specific solution.
 - In the case of eOxide spore suspension was added to the product that was prepared to either recommended, double or half strength.
- After treatment fruit were incubated for 4 days at ambient temperature ($\approx 22^\circ\text{C}$).
- Infected wound rating data were converted to percentage infection.

Results

Table 1. Percentage green mould infection on Valencia orange fruit dipped for 1 min in a 1×10^4 spore.mL⁻¹ suspension of *P. digitatum* treated for 3 min with eOxide, chlorine or untreated control.

Treatment	Concentration	Infection (%)
eOxide	50 $\mu\text{g.mL}^{-1}$	0.0
eOxide	100 $\mu\text{g.mL}^{-1}$	0.0
eOxide	200 $\mu\text{g.mL}^{-1}$	0.0
Chlorine	37.5 $\mu\text{g.mL}^{-1}$	4.2
Chlorine	75 $\mu\text{g.mL}^{-1}$	0
Chlorine	150 $\mu\text{g.mL}^{-1}$	0
Control	n/a	58.3

Conclusion and observations

- In comparison to chlorine this product in the applied concentrations showed potential for use as a possible sanitising agent against green mould.
- No phytotoxic damage was seen on the fruit.
- Despite its high sanitation potential, the product was difficult to work with. The solution had to be mixed 7 hours prior to use, which was unpractical during work hours. Furthermore the product had a powerful and unpleasant odour.

Important comments

This was a pilot trial. None of these results can be used in marketing or as a CRI recommendation in any citrus chemical program.

Addendum 4

A pilot and informal trial investigating the efficacy of eOxide as a sanitation agent prohibiting green mould infection of wounded citrus fruit

The product eOxide with active chlorine dioxide was tested at an Eastern Cape packhouse. The trial was very basic and the purpose was to get acquainted with the product and investigate its potential. Each treatment was conducted once with 13 Nova mandarin fruit. Where applicable, contaminated treatments contained 10 000 spores.mL⁻¹ of *Penicillium digitatum* (green mould). Fruit were either wounded before or after it was dipped for 60 s in the various solutions. After treatment fruit were incubated for ≈ 7 days in grape cartons on nectarine trays covered with polyethylene bags at ambient temperature. The number of infected fruit per treatment were counted and percentage infection were calculated (Table 1). In all treatments the eOxide solution was maintained by ensuring the ORP level was > 650 mV. A delay of 5 min was allowed in all treatments contaminated with spores before fruit were dipped in the specific solutions.

Table 1. Percentage infected Nova mandarin fruit that were wounded before or after treatment in clean or contaminated solutions of water or eOxide and incubated for 7 days at ambient temperature.

Trt	Detailed description	Infected fruit	
		x / 13	%
1	Wounded fruit dipped in clean water	1	7.7
2	Wounded fruit dipped in contaminated water	9	69.2
3	Wounded fruit dipped in clean eOxide solution	0	0.0
4	Wounded fruit dipped in a contaminated eOxide solution	0	0.0
5	Wounded fruit dipped operational (packhouse) eOxide solution	6	46.2
6	Unwounded fruit dipped in clean water and then wounded	1	7.7
7	Unwounded fruit dipped in contaminated water and then wounded	9	69.2
8	Unwounded fruit dipped in contaminated eOxide solution and then wounded	2	15.4

From this preliminary trial eOxide showed potential under controlled conditions (Treatments 3 and 4), but showed reduced efficacy under the condition where the organic load was higher (Treatments 5 and 8). The potential of eOxide should be further investigated and also compared to that of chlorine.

Please note: This report may not be used in any form of marketing of eOxide and if the information from this report needs to be shared with any industry role player it should be shared as a whole and in context of being a pilot trial.

Addendum 5

The evaluation of Fludioxonil as a potential fungicide agent for the control of citrus green mould

Product name: Teacher
Company: ICA International Chemicals (Pty) Ltd
Active ingredient: Fludioxonil
Trial date: 12 September 2014
Fruit: Valencia orange fruit

Trial: Fungicidal action (Dip test)

Materials and methods

Test: Fungicide against green mould

- Citrus fruit were inoculated with 1×10^6 spores.mL⁻¹ *Penicillium digitatum* (causal agent of green mould) 6, 18 and 24 h prior to treatment
- Inoculated fruit were dipped for 1 minute in the product solution
- The fungicide imazalil was used as a positive control for green mould
- Untreated fruit were used as negative control
- 3 replications were done for each treatment
- Fruit was incubated for 4 days at 23°C
- Infected wounds were rated and converted to percentage infection
- Statistical analyses was done using XLSTAT

Results

Table 1. The percentage of average green mould infection on Valencia orange fruit inoculated 6, 18 and 24 h with *P. digitatum* prior to treatment with various fungicides

Treatment	Infection age (h)	Concentration	Green mould infection (%) ^a
Teacher	6	600 µg.mL ⁻¹	0.0d
Imazalil	6	500 µg.mL ⁻¹	0.0d
Control	6	0 µg.mL ⁻¹	97.2a
Teacher	18	600 µg.mL ⁻¹	43.1c
Imazalil	18	500 µg.mL ⁻¹	0.0d
Control	18	0 µg.mL ⁻¹	75.7b
Teacher	24	600 µg.mL ⁻¹	50.7c
Imazalil	24	500 µg.mL ⁻¹	0.7d
Control	24	0 µg.mL ⁻¹	47.2c

^aEach value followed by the same letter is not significantly different according to Fisher's LSD test ($P \leq 0.05$).

Conclusion and observations

- In comparison to imazalil this product in the applied concentrations showed some potential for use as a fungicide against green mould. However, after longer incubation periods (18 and 24 h) it was significantly poorer than imazalil.
- No phytotoxic damage was seen on the fruit.
- No adverse effects were noted from working with the product.

Important comments

These were pilot trials. None of these results can be used in marketing or as a CRI recommendation in any citrus chemical program.

Addendum 6

The evaluation of Propicure as a potential fungicide agent for the control of citrus green mould or sour rot

Product name: Propicure
Company: ICA International Chemicals (Pty) Ltd
Active ingredient: Propiconazole
Trial date: 12 September 2014
Fruit: Valencia orange fruit

Trial: Fungicidal action (Dip test)

Materials and methods

Test: Fungicide against green mould

- Citrus fruit were inoculated with 1×10^6 spores.mL⁻¹ *Penicillium digitatum* (causal agent of green mould) or 1×10^6 spores.mL⁻¹ *Geotrichum citri-aurantii* (causal agent of sour rot) 6, 18, and 24 h prior to treatment
- Inoculated fruit were dipped for 1 minute in the product solution
- The fungicide imazalil was used as a positive control for green mould
- The fungicide guazatine was used as a positive control for sour rot
- Untreated fruit were used as negative controls
- 3 replications were done for each treatment
- Fruit was incubated for 4 days at 23°C
- Fruit was incubated for 6 days at 28°C in darkness for sour rot
- Infected wounds were rated and converted to percentage infection
- Statistical analyses was done using XLSTAT

Results

Table 1. The percentage of average green mould and sour rot infection on Valencia orange fruit inoculated 6, 18 and 24 h with *P. digitatum* and *G. citri-aurantii*, respectively, prior to treatment with various fungicides.

Treatment	Infection age (h)	Concentration	Green mould infection (%) ^a	Sour rot infection (%) ^a
Propicure	6	600 µg.mL ⁻¹	3.5e	0.0c
Imazalil	6	500 µg.mL ⁻¹	0.0e	N/A
Guazatine	6	500 µg.mL ⁻¹	N/A	0.0c
Control	6	0 µg.mL ⁻¹	97.2a	93.1a
Propicure	18	600 µg.mL ⁻¹	7.6e	7.1c
Imazalil	18	500 µg.mL ⁻¹	0.0e	N/A
Guazatine	18	500 µg.mL ⁻¹	N/A	0.0c
Control	18	0 µg.mL ⁻¹	75.7b	90.3a
Propicure	24	600 µg.mL ⁻¹	25.0d	22.9b
Imazalil	24	500 µg.mL ⁻¹	0.7e	N/A
Guazatine	24	500 µg.mL ⁻¹	N/A	0.0c
Control	24	0 µg.mL ⁻¹	47.2c	86.1a

^aEach value followed by the same letter is not significantly different according to Fisher's LSD test ($P \leq 0.05$).

Conclusion and observations

- In comparison to imazalil this product in the applied concentrations showed potential for use as a fungicide against green. However, after the 24-h incubation period it was significantly poorer than imazalil.
- In comparison to guazatine this product in the applied concentrations showed some potential for use as a fungicide against sour rot. However, after the 24-h incubation period it was significantly poorer than guazatine.
- No phytotoxic damage was seen on the fruit.
- No adverse effects were noted from working with the product.

Important comments - These were pilot trials. None of these results can be used in marketing or as a CRI recommendation in any citrus chemical program.

Addendum 7

The evaluation of Propirly as a potential fungicide agent for the control of citrus green mould or sour rot

Product name: Propirly
Company: ICA International Chemicals
Active ingredient: Propiconazole and Pyrimethanil
Trial date: 12 September 2014
Fruit: Valencia orange fruit

Trial: Fungicidal action (Dip test)

Materials and methods

Test: Fungicide against green mould

- Citrus fruit were inoculated with 1×10^6 spores.mL⁻¹ *Penicillium digitatum* (causal agent of green mould) or 1×10^8 spores.mL⁻¹ *Geotrichum citri-aurantii* (causal agent of sour rot) 6, 18 and 24 h prior to treatment
- Inoculated fruit were dipped for 1 minute in the product solution
- The fungicide imazalil was used as a positive control for green mould.
- The fungicide guazatine was used as a positive control for sour rot.
- Untreated fruit were used as negative controls.
- 3 replications were done for each treatment.
- Fruit were incubated for 4 days at 23°C.
- Fruit were incubated for 6 days at 28°C in darkness for sour rot.
- Infected wounds were rated and converted to percentage infection.
- Statistical analyses was done using XLSTAT.

Results

Table 1. The percentage of average green mould and sour rot infection on Valencia orange fruit inoculated 6, 18 and 24 h with *P. digitatum* and *G. citri-aurantii*, respectively, prior to treatment with various fungicides

Treatment	Infection age (h)	Concentration	Green mould infection (%) ^a	Sour rot infection (%) ^a
Propirly (PPZ and PYR)	6	600 µg.mL ⁻¹ ; 750 µg.mL ⁻¹	0.0d	0.0d
Imazalil	6	500 µg.mL ⁻¹	0.0d	N/A
Guazatine	6	500 µg.mL ⁻¹	N/A	0.0d
Control	6	0 µg.mL ⁻¹	97.2a	93.1a
Propirly (PPZ and PYR)	18	600 µg.mL ⁻¹ ; 750 µg.mL ⁻¹	2.8d	1.4d
Imazalil	18	500 µg.mL ⁻¹	0.0d	N/A
Guazatine	18	500 µg.mL ⁻¹	N/A	0.0d
Control	18	0 µg.mL ⁻¹	75.7b	90.3ab
Propirly (PPZ and PYR)	24	600 µg.mL ⁻¹ ; 750 µg.mL ⁻¹	2.8d	8.3c
Imazalil	24	500 µg.mL ⁻¹	0.7d	N/A
Guazatine	24	500 µg.mL ⁻¹	N/A	0.0d
Control	24	0 µg.mL ⁻¹	47.2c	86.1b

^aEach value followed by the same letter is not significantly different according to Fisher's LSD test ($P \leq 0.05$).

Conclusion and observations

- In comparison to imazalil this product in the applied concentrations showed potential for use as a fungicide against green mould.
- In comparison to guazatine this product in the applied concentrations showed potential for use as a fungicide against sour rot.
- No phytotoxic damage was seen on the fruit.
- No adverse effects were noted from working with the product.

Important comments - These were pilot trials. None of these results can be used in marketing or as a CRI recommendation in any citrus chemical program.

Addendum 8

The effect of sanitation product concentration and exposure time on the inhibition of *Penicillium digitatum*

Aim

To determine whether sanitation products, at various concentrations and exposure times, can inhibit the growth of *Penicillium digitatum* (PD).

Materials and Methods

- Spore suspension (1×10^6 spores.mL⁻¹ of PD) was made up an hour before the trial
- Different treatments included
 - 1) chlorine (calcium hypochloride; Control Chemicals)
 - 2) chlorine + adjuvant (metacilicate 5%)
 - 3) Citroside (hydrogen peroxide and peracetic acid; Citrosol)
- Different concentrations of each treatment product was used, with chlorine treatments applied at 0 (control), 50 and 100 ppm, Citroside at 0 (control), 6 and 12 mL/L
- The spore suspension (100 mL) was exposed to each treatment concentration for 0 (control), 1, 5, 10 or 30 min, at which time a deactivating agent (sodium thiosulphate for chlorine and sodium metabisulfite for HPPA) was added for 30 s
- Following each treatment, 500 μ L of solution was pipetted onto PDA+ plates and spread over the surface
- Plates were left to incubate for 3 to 4 days before photographs was taken of the plates

Results

Chlorine

The highest level of germination inhibition was seen at 100 ppm chlorine at 1 min exposure with only 2 – 4 colonies growing (Figure 1). Increasing the exposure time did not dramatically reduce the number of colonies (Figure 2).

Chlorine plus adjuvant

The addition of an adjuvant resulted in the 50 ppm chlorine treatments showing fewer colonies (Figure 3) compared to the same treatment without adjuvant (Figure 1). Increasing the exposure time did not have a dramatic effect (Figure 4).

Citroside

This product showed a dramatic reduction already at 1 min exposure at 0.6% with similar results at 1.2% (Figure 5). Increasing exposure time did not show any effect due to the levels being so low already after 1 min (Figure 6).

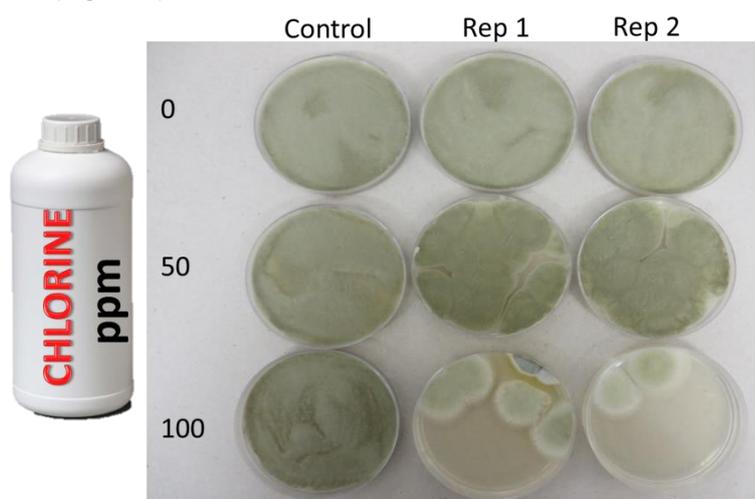


Figure 1. Green mould germination inhibition after 1 min exposure of 0, 50 and 100 ppm chlorine to a 1×10^6 spores.mL⁻¹ suspension of *Penicillium digitatum*.

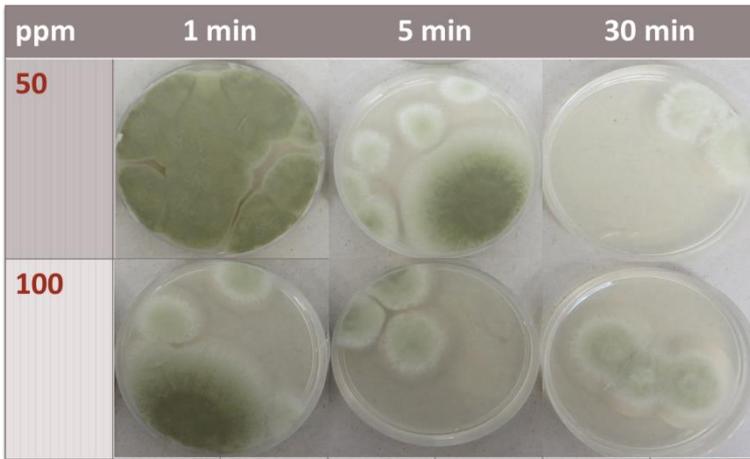


Figure 2. Green mould germination inhibition after 1, 5 and 30 min exposure of 50 and 100 ppm chlorine to a 1×10^6 spores.mL⁻¹ suspension of *Pencillium digitatum*.

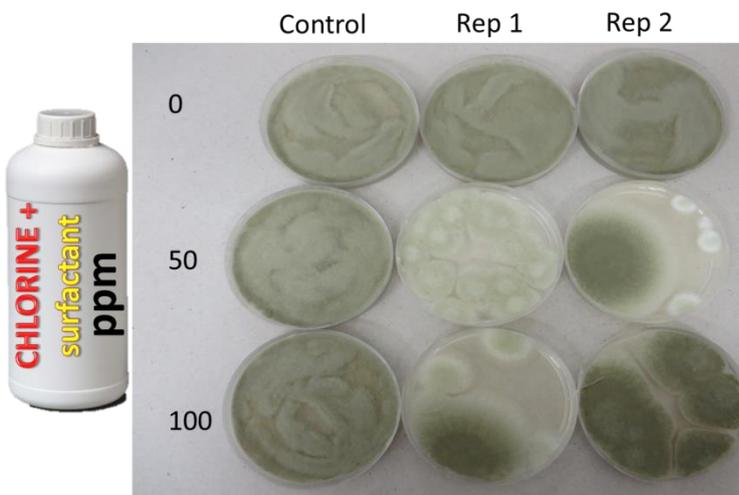


Figure 3. Green mould germination inhibition after 1 min exposure of 0, 50 and 100 ppm chlorine plus an adjuvant to a 1×10^6 spores.mL⁻¹ suspension of *Pencillium digitatum*.

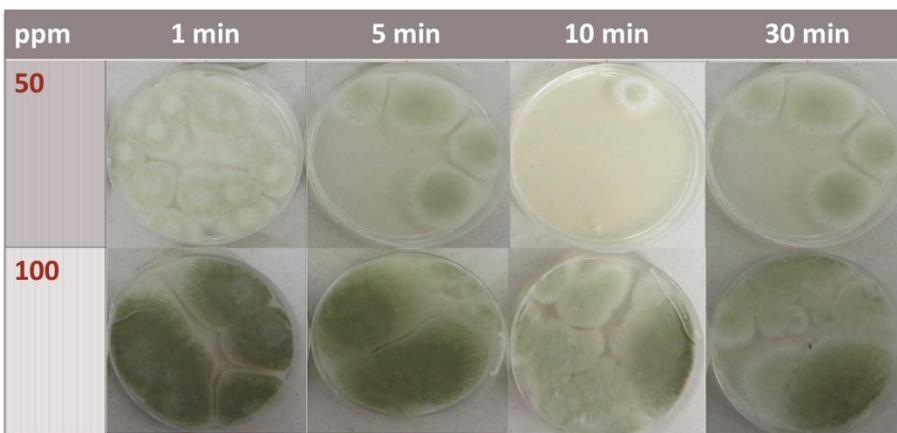


Figure 4. Green mould germination inhibition after 1, 5 and 30 min exposure of 50 and 100 ppm chlorine plus an adjuvant to a 1×10^6 spores.mL⁻¹ suspension of *Pencillium digitatum*.

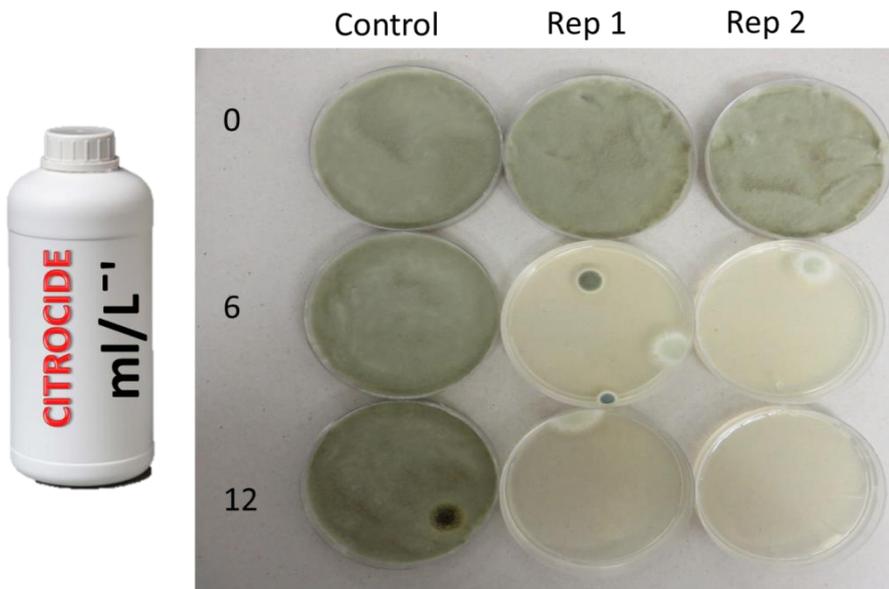


Figure 5. Green mould germination inhibition after 1 min exposure of 0, 0.6 and 1.2% Citrocide to a 1×10^6 spores.mL⁻¹ suspension of *Pencillium digitatum*.

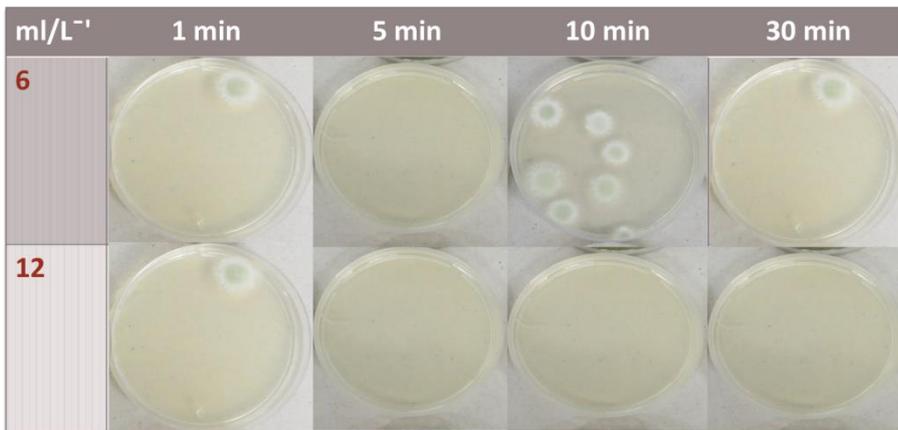


Figure 6. Green mould germination inhibition after 1, 5 and 30 min exposure of 0.6 and 1.2% Citrocide to a 1×10^6 spores.mL⁻¹ suspension of *Pencillium digitatum*.

Conclusion

The trials were from pilot laboratory trials only, and results presented should be regarded as preliminary only, and should not be used for direct comparison between these products due to the experimental design employed. Chlorine at 100 ppm was able to sufficiently inhibit green mould spore germination after 1 min exposure. The addition of an adjuvant improved the ability of chlorine to inhibit spore germination. This could be very useful as it is known that the management of a chlorine concentration in the packhouse situation is troublesome and it therefore fluctuates. However, there is very little known of the effect of adjuvants on citrus rind quality and it cannot be recommended at this stage. Citrocide is shown to be a very good alternative to chlorine.

Addendum 9

The use of Fortisol to prevent guazatine burn

Aim

To determine whether the addition of Fortisol (Wenkem and Citrosol) reduces the risk of guazatine (GZT) burn

Materials and Methods

- A 10-L solution containing either 0, 500, 1000 or 2000 ppm GZT were prepared
- Treatments were divided into two treatments: 1) GZT and 2) GZT and 0.1% Fortisol
- Chemicals were added to municipal water and mixed for 1 minute before commencing treatments
- For each treatment combination, 12 fruit were dipped for 1 min and placed in plastic bags (removed after 3 days) to simulate slow drying or placed in uncovered cartons to simulate faster drying
- All fruit were rated for burn symptoms after ± 5 days using the following scale (Fig 1 and 2): (0) no burn or new defects; (1) slight burn lesions or defects; (2) moderately severe burn lesions or defects that partly cover the fruit surface (3) intense burn lesions or defects that cover a large portion of the fruit surface.
- Two batches of Nadorcott fruit and one batch of Valencia orange fruit were used with three and four replicates each, respectively. Twelve fruit were treated per replicate.

Results

Burn symptoms significantly increased with an increase in GZT concentration (Figure 1, 2 and 3). Delaying drying of fruit after treatment significantly contributed to an increase of GZT burn. Fortisol significantly reduced the incidence of GZT burn in all treatments, except for 500 $\mu\text{g}\cdot\text{mL}^{-1}$ GZT.

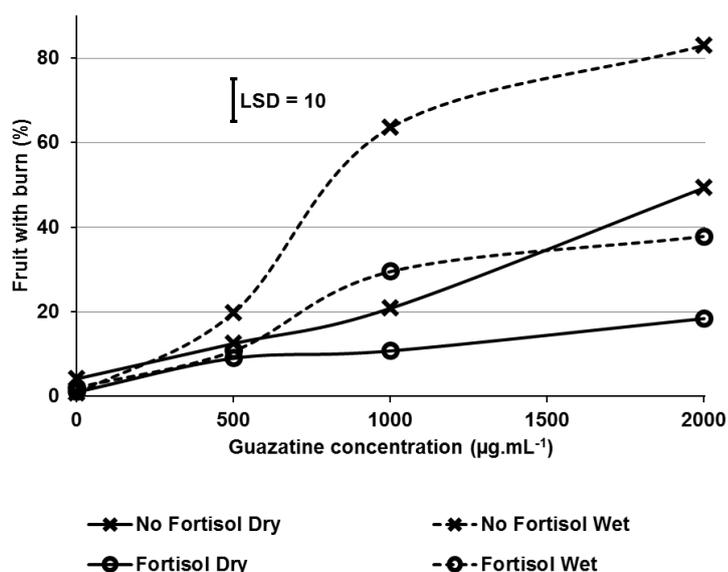


Figure 1. Percentage fruit showing guazatine burn after treatment with guazatine at either 0, 500, 1000 or 2000 $\mu\text{g}\cdot\text{mL}^{-1}$ in combination or without 0.1% Fortisol. Half of the treated fruit were placed in plastic bags for 3 d (wet) and the other half were allowed to dry immediately after treatment (dry). The LSD value (10) was determined by means of Fisher's test ($P \leq 0.05$)

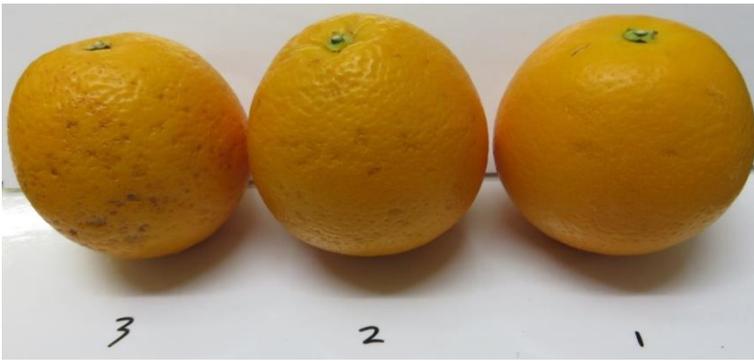


Fig 2. Burn lesions or defects on Valencia orange fruit with a rating of 1 (right), 2 (middle) and 3 (left)

Fig 3. Burn lesions or defects on Nadorcott mandarin fruit with a rating of 1 (right), 2 (middle) and 3 (left)

Conclusion

Guazatine can cause burn of fruit and the severity increases with increase GZT concentration and a delay in drying. It is therefore very important to ensure quick drying of fruit after treatment. Fortisol reduced the effect of GZT burn. Once the registration of Fortisol is finalised it can be recommended to be used in a drench solution to reduce the risk of possible GZT burn.

Addendum 10

The effect of an adjuvant added to a fungicide solution in reducing the accumulation of fungicide at places on the fruit surface where drying is prolonged

Aim

To determine whether the use of an adjuvant increases the distribution of chemicals over the fruit surface, including contact zones between fruit.

Materials and Methods

Trial 1: Testing an adjuvant

- Clementine mandarin (one batch) and Valencia orange (two batches) fruit pairs were used; no replicates
- Treatments: 1) control (water only) and 2) 0.1 ml/L⁻¹ adjuvant (a component included in the Sporekill formulation (ICA International Chemicals))

Trial 2: Testing an adjuvant and antifoaming agent

- One batch of Valencia orange fruit were used in pairs with two replicates per treatment
- Treatments: 1) control, 2) Wetcit (Oro Agri SA, Somerset West, South Africa) antifoaming agent (Foamfix, SPP, Moreleta Park, South Africa) and 4) Wetcit and antifoam

Treatment

- Pigment: all treatments contained 1 ml/L⁻¹ pigment (SARDI yellow fluorescent pigment)
- Treatment: fruit pairs were dipped for 1 min in the treatment solution and were then placed against one another (surfaces touching) for several hours until fruit were completely dry
- Once fruit were dry, photographs were then taken of the contact area using a UV light (UV-A at 365 nm, Labino Mid-light; www.labino.com) to determine the formation of rings of concentrated pigment (indicative fungicide accumulation)

Results

Trial 1

Although this is only a pilot trial it can be seen in Figure 1 that adding an adjuvant to a fungicide solution did not reduce or eliminate the ring effect where fruit were touching each other while drying.

Figure 1. Fluorescing pigment under UV light indicating the distribution of chemicals. Column (a) represents fruit treated without an adjuvant (control) and (b) with an adjuvant. The treated fruit are shown in the following order: (1) Clementine mandarin fruit, (2) Valencia orange fruit batch 1 and (3) batch 2.

Trial 2

Although this again is a pilot trial it is shown in Figure 2 that antifoam was more effective in eliminating the fungicide ring compared to an adjuvant or combining an adjuvant with antifoam

Figure 2. Fluorescing pigment under UV light indicating the distribution of chemicals. Column (a) depicts replicate 1 and column (b) replicate 2. Treatments are as follows: row (1) control, (2) surfactant, (3) antifoam and 4) surfactant and antifoam

Conclusion

Please note that this was a pilot trial only and that no conclusive findings can be made. From the initial observations, it appears that antifoam seem to be a better option to eliminate the fungicide ring effect on fruit drying after treatment. At this stage there is no registered antifoam product for citrus postharvest use.

4.5.8 **FINAL REPORT: Identification and modelling of postharvest decay risk indicators**

Project 1073 (2013/14) by Arno Erasmus (CRI), Catherine Savage (CRI), Mareli Kellerman (CRI-SU), Paul Cronje and Paul H. Fourie (CRI)

Summary

Pre-harvest risk indicators have been identified and measured during the navel orange harvest season of 2013. Fruit count, decay (in the orchard and postharvest), skirt height and wounding during harvest were measured on six different orchards throughout Mpumalanga. Despite meaningful measurements and significant differences between the orchards, these did not correlate with the postharvest decay measured due to generally low level of decay measured. Due to human resource, funding constraints, and a re-direction of research capacity this project was terminated early. The results obtained to date will be used to inform on changes to the methodology, should the project be resubmitted for funding. The outstanding

finding from this trial was that the two factors that could be related to higher levels of decay was high levels of sporulating fruit hanging in the trees during harvest time and number of wounds induced during harvest. This supports the recommendations to keep inoculum levels in the orchard as low as possible and to reduce wound inducing during harvest in order to improve disease management.

Opsomming

Voor-oes risiko-aanwysers is geïdentifiseer en gemeet tydens die nawel lemoen oes-seisoen van 2013. Dooie hout, vruglading, bederf (in die boord en naoes), inokulum lading in die boord, soom-hoogte en wonde tydens oes is gemeet in ses verskillende boorde in Mpumalanga. Ten spyte van betekenisvolle verskille tussen boorde, het geen van die aanwysers met na-oes verrotting gekorreleer weens die algemeen lae verrottingsvlakke. As gevolg van menslike hulpbron en befondsingtekorte, en nuwe navorsingsfokus is die projek vroeg getermineer. Resultate tot op hede sal gebruik word om veranderinge in metodes te maak, indien die projek weer vir befondsing voorgelê word. Die mees uitstaande bevinding van hierdie projek was dat die twee faktore wat verband met hoër vlakke van bederf gehou het, was hoë vlakke van sporulerende vrugte hangende in die bome tydens oestyd en getal woude geïnduseer gedurende oes. Dit ondersteun die aanbevelings om inokulum vlakke in die boord so laag as moontlik te hou en om wond indusering te vermy gedurende oes om siektebestuur te verbeter.

Introduction

It is well known that preharvest factors influence fruit quality, fruit's ability to resist postharvest decay organisms, as well as the infection and contamination levels of fruit by these pathogens (Michailides et al., 2010). In other fruit production systems, such as apple and mango, researchers have used various preharvest biological and environmental measurements as postharvest decay indicators (Prusky et al., 1993; Tomala, 1999; Prusky et al., 2002; Creemers and van Laer, 2006; Berrie, 2007; Sholberg, 2008). To our knowledge, this has not been attempted for Citrus fruit.

Postharvest waste as a result of decay and rind disorders remains one of the most critical research priorities of the southern African citrus industry. At present, decision-making during postharvest handling of citrus fruit is subjective or reactive in nature, which highlights the need for a risk management system based on empirical evidence.

In this cross-disciplinary project, which encompasses plant pathology and pre- and postharvest physiology, we propose to identify measurable (using mostly conventional technologies) preharvest parameters that might be used as indicators or predictors of postharvest decay and/or rind defects. These parameters will be intensively monitored / measured at 4 locations for 2 consecutive seasons, followed by assessment of rind defects, postharvest decay and other waste factors. The most influential parameters will be identified using multivariate statistics and their quantitative relation with postharvest decay and defects will be statistically modelled. This knowledge will be incorporated in a risk management system that can be used by growers and packhouses to assign risk levels to orchards / batches of fruit.

Objectives

- Identification and modelling of postharvest decay risk indicators.
- Develop a risk management system aimed at optimising postharvest citrus handling based on the risk associated with certain batches of fruit.

Materials and methods

Pre-harvest measurements and data collection

Six different orchards were identified as trial sites where various measurements were made 1 to 2 weeks before the estimated harvest date. The sites are as listed in Table 4.5.8.1. At each site 10 trees were randomly selected and marked. Measurements were taken from each tree on two sides. Due to the resignation of a key researcher situated in Stellenbosch the sites could only be in Mpumalanga and sites in Western Cape could not be included as planned originally.

Table 4.5.8.1. Site number, area, farm, block number and cultivar for each site used in this project.

Orchard no.	Area	Cultivar	Rootstock	Planting date	Planting distance (m)
A	Mpumalanga	Bahianinha	RL	1992	6 x 2.0
B	Mpumalanga	Bahianinha		2000	

C	Mpumalanga	Glenora Late	C35	2004	6 x 2.5
D	Mpumalanga	Lane Late			
E	Mpumalanga	Bahianinha	Carrizo	2001	6 x 2.5
F	Mpumalanga	Rustenburg	SC	1994	6 x 3.5

Measurements

1. Dead wood rating
Dead wood was rated and an observational rating from 1, 2, or 3 was given, where 1 = very little dead wood visible, 2 = moderately visible dead wood and 3 = abundant dead wood visible. A photo description can be seen in Figure 4.5.8.6.
2. Fruit count
Fruit were counted on each side of the tree. Fallen fruit on the orchard floor were also counted.
3. Decay
Decayed fruit were counted on the tree and on the orchard floor. Further differentiation was made between sporulating and non-sporulating decayed fruit.
4. Skirt height
The skirt height of each tree was determined. The distance from the bottom part of a skirt and the orchard floor were measured on the left and right hand side of each tree side. The lowest part of each skirt was recorded together with the lowest hanging fruit.
5. Postharvest retention samples
Twelve lug boxes of fruit from each specific site were retained, where six were stored at ambient and six stored at 5°C. The ambient fruit were rated for decay 2 weeks after harvest and the cold stored fruit 6 weeks after harvest. Six packed cartons of fruit were also retained and rated for decay 4 weeks after harvest.
6. Wounding during harvest
Six lug boxes were collected after harvest directly from the orchard. These fruit were treated with indigo carmine to determine the number of fresh wounds induced during harvest.

Results and discussion

Number of fruit per tree side

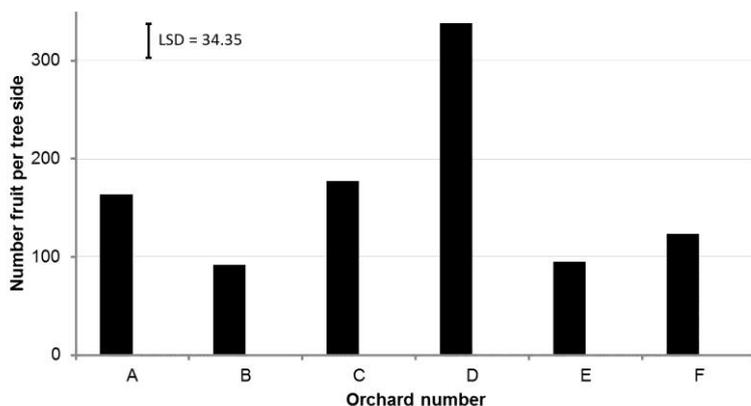


Figure 4.5.8.1. Number of navel orange fruit counted per tree side (east and west) from 10 trees in each of six different orchards in Mpumalanga. The LSD value (34.35) was determined by means of Fisher's test at $P < 0.05$.

The harvest load varied significantly between orchard where orchards B, E and F had the lowest number of fruit (≈ 100 ; Figure 1). Orchard D had the highest number (> 300). Orchard A and C had similar levels (≈ 180).

Skirt height from orchard floor

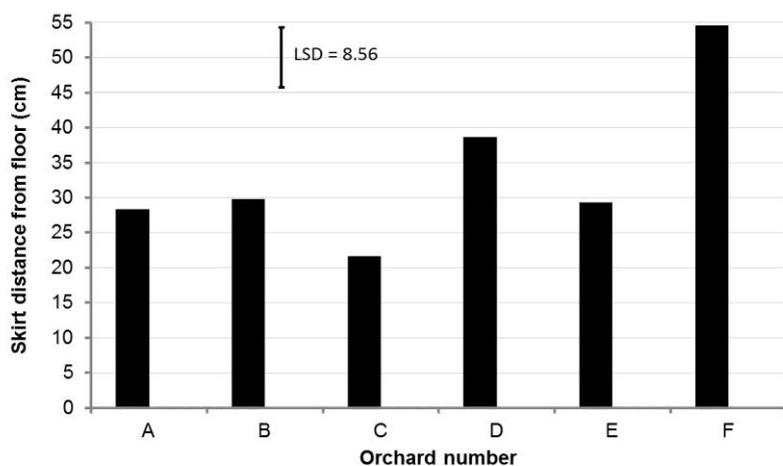


Figure 4.5.8.2. The average distance of navel orange tree skirts from the orchard floor measured on two sides of 10 trees in each of six different orchards in Mpumalanga. The LSD value (8.56) was determined by means of Fisher's test at $P < 0.05$.

Orchards A, B, C and E had the lowest and similar distance from skirt to orchard floor ($> 20 - < 30$ cm; Figure 4.5.8.2). Orchard F had the highest skirts (≈ 55 cm) and Orchard D had an average skirt height of ≈ 39 cm.

Lowest hanging fruit

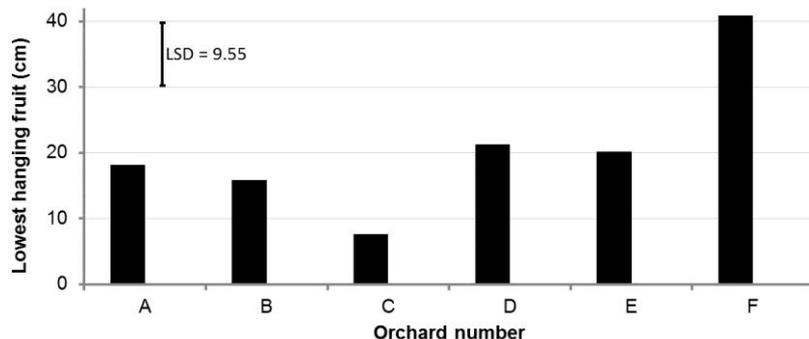


Figure 4.5.8.3. The average distance of the lowest hanging navel orange fruit from the orchard floor measured on two sides of 10 trees in each of six different orchards in Mpumalanga. The LSD value (9.55) was determined by means of Fisher's test at $P < 0.05$.

Orchard C had the lowest hanging fruit (< 10 cm; Figure 4.5.8.3). Orchards A, B, D and E had similar heights (≈ 20 cm). Orchard F had the highest hanging fruit (> 40 cm).

Number of sporulating fruit on the orchard floor

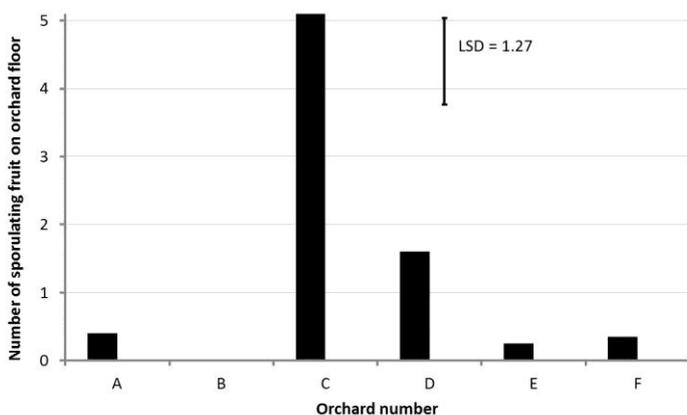


Figure 4.5.8.4. Average number of sporulating fruit counted directly under two sides of 10 navel orange trees in each of six different orchards in Mpumalanga. The LSD value (1.27) was determined by means of Fisher's test at $P < 0.05$.

No sporulating fruit were found in Orchard B, Orchard A, E and F had < 1 (Figure 4.5.8.4). Orchard D had significantly more with > 1.5. Orchard C had the highest number of sporulating fruit on the orchard floor per tree side (> 5).

Number of sporulating fruit per tree side

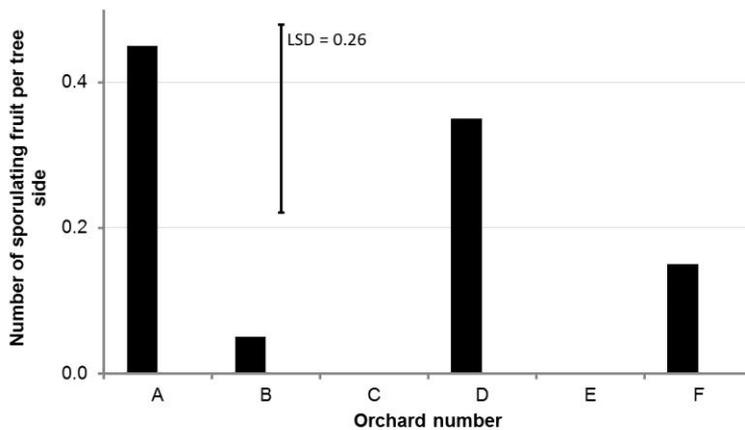


Figure 4.5.8.5. Average number of sporulating fruit hanging on two sides of 10 navel orange trees in each of six different orchards in Mpumalanga. The LSD value (0.26) was determined by means of Fisher's test at $P < 0.05$.

Orchards C and E had no sporulating fruit hanging on the specific trees (Figure 4.5.8.5). Orchards B and F had < 0.2 sporulating fruit per tree side. Orchards A and D had the highest number of sporulating fruit still hanging on the trees with ≈ 0.4 per tree side.

Dead wood rating

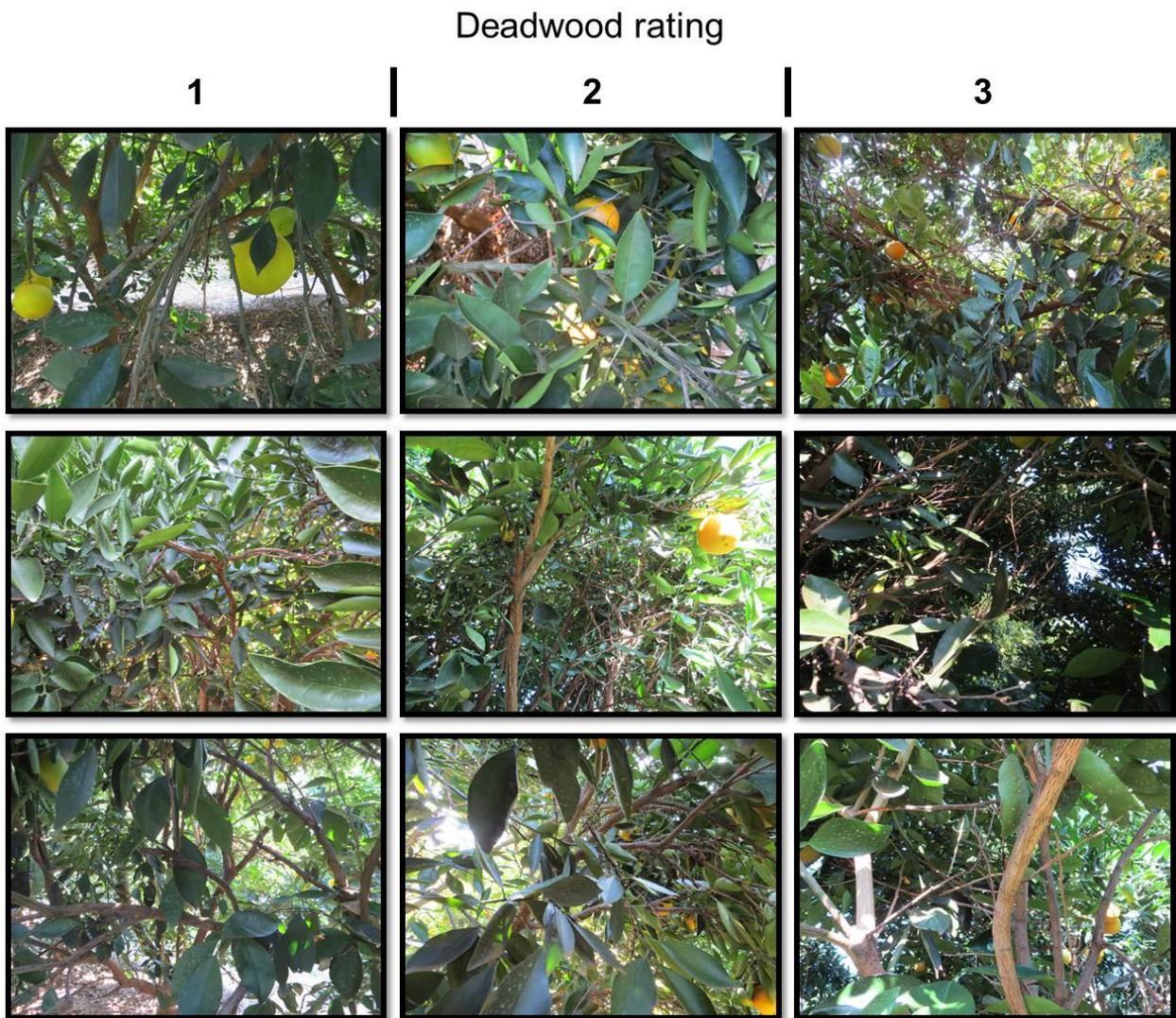


Figure 4.5.8.6. A photo description of the rating used to assess the level of deadwood per tree. Tree examples per rating are displayed, where 1 = very little dead wood visible, 2 = moderately visible dead wood and 3 = abundant dead wood visible.

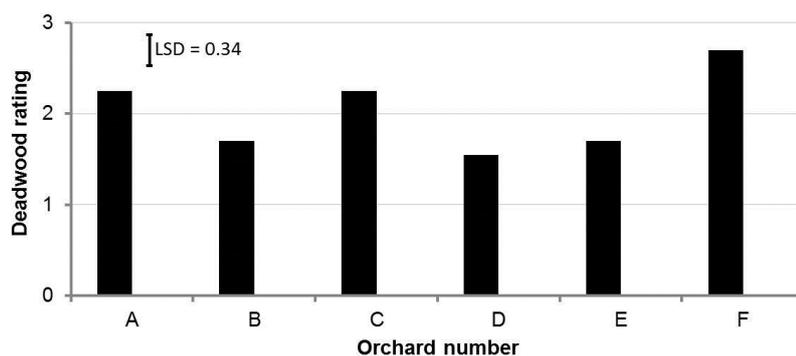


Figure 4.5.8.7. Average dead wood rating on two sides of 10 navel orange trees in each of six different orchards in Mpumalanga. The LSD value (0.34) was determined by means of Fisher's test at $P < 0.05$. Orchards B, D and E were rated with the lowest levels of dead wood (1.70, 1.55 and 1.70, respectively; Figure 4.5.8.7). Orchard A and C were rated with significantly higher dead wood levels (2.25 for both, respectively) and Orchard F rated with the highest levels (2.70).

Wounded fruit after harvest

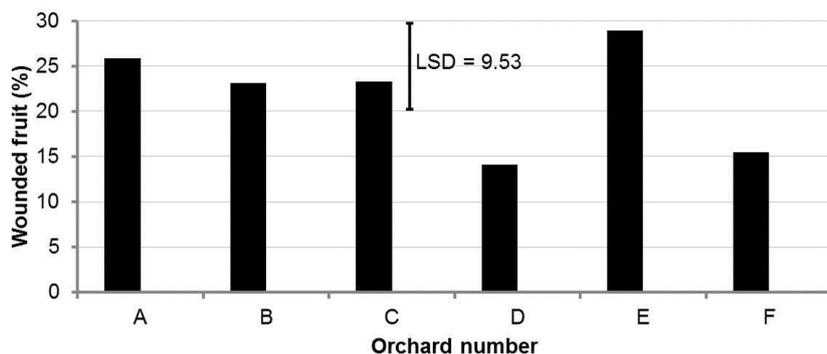


Figure 4.5.8.8. Percentage wounded navel orange fruit rated by means of indigo carmine within 4 h after harvest from six different orchards in Mpumalanga. The LSD value (9.53) was determined by means of Fisher's test at $P < 0.05$.

Orchards D and F had the lowest level of wounded fruit (14.1 and 15.5%, respectively; Figure 4.5.8.8), this was significantly lower than those from Orchard A and E (25.8 and 28.9%, respectively) and similar to Orchard B and C (23.1 and 23.3%, respectively).

Decay after harvest

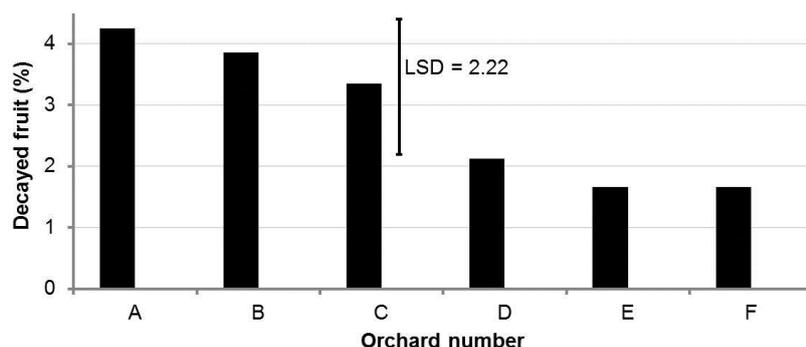


Figure 4.5.8.9. Percentage decayed navel orange fruit after harvest from six different orchards in Mpumalanga. The LSD value (2.22) was determined by means of Fisher's test at $P < 0.05$.

The only cause of decay was green mould. With a decay level of 4.3% (Figure 4.5.8.9) Orchard A had the highest level of decayed fruit, this was significantly higher than the levels of E and F (1.7% for both respective orchards), but similar to Orchard B, C and D (3.9, 3.4 and 2.1%, respectively).

When Orchard A, with the highest level of decay, is compared to Orchard E and F with the lowest levels of decay the factor number of sporulating fruit still hanging in the tree related to Orchard A having a significantly higher level of decay. Orchard A had significantly more sporulating fruit still hanging in the tree compared to Orchard E and F. Orchard E and F differed significantly in terms of skirt distance from the orchard floor, the lowest hanging fruit, dead wood rating and percentage wounded fruit, yet they still had similar and the lowest level of decay. Orchard A, E and F had sporulating fruit on the orchard floor, although it was low levels it was statistically similar. When Orchard D, which had a similar level of decay to Orchard E and F, was compared to Orchard A the factor percentage wounded fruit related to Orchard A having a higher level of decay. Orchard A had a significantly higher level of wounded fruit compared to Orchard D. Orchard D and F had similar levels of wounded fruit. Orchard E had the highest level of wounded fruit, but also the lowest level of decayed fruit.

Conclusion

No thorough conclusion can be made at this stage. Some practical realities were evident while collecting data. A harvest date is never final, it can be postponed with weeks, and this complicated certain planned measurements. The measurements stated in the materials and method section was practical and doable.

We observed that all trees have a certain levels of dead wood, this did not correlate to higher levels of decay. However, green mould was the only cause of decay, and no decay by latent pathogens originating from dead wood was observed; their absence might be attributed to unsuitable climatic conditions. The risk of dead wood as source of inoculum for the latent pathogens as well as the climatic conditions leading to infection still needs to be investigated further.

The two factors that related to higher levels of decay on fruit postharvest was sporulating fruit in the tree and wounded fruit after harvest. This support the recommendation to keep inoculum (spore) levels as low as possible in order to improve disease control. If there is no inoculum there will be no disease. Also, to improve harvest practices to have as low as possible injury on the fruit. These injuries provide opportunities for the pathogens to infect the fruit and ultimately increase disease pressure and postharvest decay.

Technology transfer

Some of this data were used in presentations at the CRI Packhouse Workshops in 2014

References cited

- Berrie AM. 2007. Using rot risk assessment to minimise losses due to rots in cold stored apples. Novel approaches for the control of postharvest diseases and disorders. Pages 443-447 in Proceedings of the International Congress, Bologna, Italy, 3-5 May, 2007.
- Creemers P, S van Laer. 2006. Key strategies for reduction of the dependence on fungicides in integrated fruit production. *Phytopathol. Pol.* 39: 19-29.
- Michailides TJ, DP Morgan, Y Luo. 2010. Epidemiological Assessments and Postharvest Disease Incidence. Pages 69-88 in *Postharvest Pathology, Plant Pathology in the 21st Century Vol. 2.* D Prusky and ML Gullino eds.
- Prusky D, Kobiler I, Zauberman G, Fuchs Y. 1993. Preharvest conditions and postharvest treatments affecting the incidence of decay in mango fruits during storage. *Acta Horticulturae* 341: 307-320.
- Prusky D, Y Shalom, I Kobiler, M Akerman, Y Fuchs. 2002. The level of quiescent infection of *Alternaria alternata* in mango fruits at harvest determines the postharvest treatment applied for the control of rots during storage. *Postharvest Biology and Technology* 25: 339-347.
- Sholberg PL. 2008. Modelling the development of postharvest diseases in fruits and vegetables. *Stewart Postharvest Review* 4: 1-6.
- Tomala K. 1999. Orchard factors affecting fruit storage quality and prediction of harvest date of apples. *Acta Horticulturae* 485: 373-382.

4.6 PROGRAMME: CITRUS BLACK SPOT Programme coordinator: G.C. Schutte (CRI)

4.6.1 PROGRAMME SUMMARY

Two spore traps were installed in lemon orchards in Hermitage and Kirkwood to monitor CBS ascospore releases. Data that were collected on a yearly basis was processed to assist the valley with informed decisions to plan effective spray programmes. Theft was a great problem, but was limited after security was improved. The project was concluded and a forecasting and monitoring system will in future be handled and operated by private companies on a commercial scale.

Microsatellite markers were used for the determination of genetic diversity of *Phyllosticta citricarpa* isolates collected from all over the world. Eight microsatellite markers were developed to genotype 388 *P. citricarpa* isolates from five SA populations and seven international populations from four countries including USA, China, Brazil and Australia. *P. citricarpa* populations in China have a greater genetic diversity than those in South Africa and in the USA. Mating types were present in the populations from South Africa at an approximately 1:1 distribution.

Various new systemic and contact fungicides as well as adjuvants in combination with registered fungicides were tested on 'Valencia' oranges for the control of citrus black spot. Kannar 202 performed well, but phytotoxicity problems were experienced. BAS70301F in a tank mixture with mineral spray oil gave excellent control of citrus black spot. New spray programmes where mancozeb were alternated with RB1 and RB2, also resulted in good control of CBS. Tank mixtures of benomyl and azoxystrobin (1x and 2x) with mancozeb and mineral spray oil sprayed as two applications with 8 week intervals also performed well in controlling CBS.

A second duplicate field trial was conducted to determine the efficacy of low, medium and high volume spray applications of Fighter (½x and 1x rates) for the control of Phytophthora brown rot. After spray applications

were completed and fruit harvested to be subjected to laboratory trials under specific environmental conditions, problems were experienced as incubation temperature could not be regulated. The trial will have to be repeated. A final report will be drafted for 2016.

Colletotrichum spp. were isolated from lesions showing similar lesions to that of CBS on grapefruit, and two predominant species were identified, namely *C. gloeosporioides* and *C. boninense*. Valencia fruit were inoculated on the trees with a spore suspension and lesion developed recorded. Photographs were taken, lesions measured and isolations made to fulfil Koch's postulates. Lesions were only observed from the wounded fruit, with 65 and 70% of fruit inoculated with *C. gloeosporioides* and *C. boninense* producing lesions, respectively.

CRI researchers collaborated in a project funded by the Florida citrus industry in USA to develop a quantitative pest risk assessment of *P. citricarpa*, with special emphasis on the fresh fruit pathway. Various critical steps in the model was identified, probabilities assigned to these steps and research gaps identified. The project was concluded and a scientific paper is being prepared.

PROGRAMOPSOMMING

Twee spoorvangers is in suurlemoenboorde te Hermitage en Kirkwood geïnstalleer vir die monitoring van askosporvrystelling. Data is versamel op 'n jaarlikse basis engeprosesseer om kwekers te help om ingeligte besluite neem t.o.v. spuitprogramme. Diefstal was 'n groot probleem, maar is later beperk toe sekuriteit verbeter is. Die projek is afgesluit en 'n voorspellings- en moniteringsstelsel sal in die toekoms deur privaat maatskappye op 'n kommersiële skaal bedryf word.

Mikrosatellietmerkers is gebruik vir die bepaling van genetiese diversiteit van *Phyllosticta citricarpa* isolate wat oor die wêreld versamel is. Agt mikrosatellietmerkers is ontwikkel om 388 *P. citricarpa* isolate van vyf SA populasies en sewe internasionale populasies afkomstig van vier lande, insluitend die VSA, China, Brasilië en Australië te genotipeer. *P. citricarpa* populasies in China het 'n groter genetiese diversiteit as die populasies in Suid-Afrika en in die VSA waar meer onlangse bekendstellings voorgekom het. Paringstipe analiese het getoon dat beide paringstipes in die Suid-Afrikaanse populasie in 'n 1:1 verhouding teenwoordig is.

Verskeie nuwe sistemiese- en kontakswamdoders asook benatters is in kombinasies met geregistreerde swamdoders op 'Valencia' lemoene vir die beheer van swartvlek beproef. Kannar 202 het goed gewerk, maar het fitotoksiese probleme getoon. BAS70301F in 'n tenkmengsel net met minerale olie het goeie beheer van swartvlek gegee. Nuwe spuitprogramme waar mancozeb met RB1 en RB2 afgewissel is, het ook goeie beheer van swartvlek tot gevolg gehad. Tenkmengsels van benomyl en azoxystrobin (1x en 2x) met mancozeb en minerale spuitolie wat met twee toedienings van 8-week intervalle, het ook goed gewerk vir die beheer van swartvlek en as dit geregistreer word, sal kwekers twee spuitronde bespaar.

'n Tweede duplikaat veldproef is uitgevoer om die effektiwiteit van lae, medium en hoë spuitvolumes van Fighter ($\frac{1}{2}x$ en $1x$ dosisse) vir die beheer van *Phytophthora* bruinvrot te bepaal. Na die spuittoedienings voltooi en vrugte geoes is om aan sekere laboratoriumtoestande onderwerp te word, is probleme met die lugversorger ondervind en het geen resultate opgelewer nie. Die proef is hierna herhaal. 'n Finale verslag sal in 2016 saamgestel word.

Colletotrichum spp. is geïsoleer van letsels met soortgelyke simptome as *Phyllosticta citricarpa* op pomelos, en as *C. gloeosporioides* en *C. boninense* geïdentifiseer. Valencia vrugte is aan die bome met spoor-suspensies van beide spesies geïnkuleer. Fotos is van die letsels geneem en letsels gemeet, waarna isolasies vanuit die letsels gemaak is om Koch se postulate te vervul. Daar het slegs letsels op die gewonde vrugte gevorm; onderskeidelik 65 en 70% vir *C. gloeosporioides* en *C. boninense*.

CRI navorsers het saamgewerk op 'n projek wat deur die sitrus-industrie in Florida, VSA, befonds is om 'n kwantitatiewe pes-risiko-bepaling van *P. citricarpa* te ontwikkel, met die klem op vars vrugte as introduksie-roete. Verskillende kritiese stappe is in 'n model bepaal, waarskynlikhede vir hierdie stappe toegevoeg en navorsingsgapings geïdentifiseer. Die projek is afgehandel en 'n wetenskaplike publikasie word voorberei.

4.6.2 **FINAL REPORT: Monitoring ascospore releases in the Eastern Cape to determine the critical period for CBS infection**

Project 919 (September 2008 – March 2014) by G.C. Schutte (CRI) and S. Serfontein (QMS)

Opsomming

In die Oos-Kaap provinsie is daar voor hierdie projek nog geen spoorvangerdata of weerdata geakkumuleer vir sitrus swartvlek (SSV) soos benodig vir die interpretasie van 'n siektevoorspellings- of siektevoorspellingsstelsel wat benodig word om die siekte meer effektief te beheer. Twee spoorvangers is in suurlemoenboorde te Hermitage en Kirkwood geïnstalleer vir die monitoring van *Phyllosticta* askospore. Tegnieke probleme en diefstal is ondervind in die kritiese tye toe askosporvystellings gemonitor moes word. In gevalle wanneer die spoorvangerdata wel beskikbaar was, het dit nietemin waardevolle data opgelewer. Soos byvoorbeeld toe askosporvystelling wel plaasgevind het, was die weerstoestande nie in alle gevalle gunstig vir infeksie soos bepaal deur die QMS infesiemodel nie. Weens die gapinge in die versamelde data, kon die kritiese infesieperiode in die Oos-Kaap nie bepaal word in die studie nie. Vir die toekoms word meer betroubare data en 'n vinniger askosporlesings verlang sodat besluitneming en spuitprogramme gedurende seisoene met voorspelde gunstige toestande vir SSV uitbrake, vroegtydig aangepas kan word. Byvoorbeeld, gedurende die 2012-13 seisoen kon sekere kwekers eers na 15 Oktober begin spuit weens baie reën in daardie periode. Insteede daarvan om dadelik oor te slaan na 'n sistemiese swamdoder met terugwerkende aksie, het meeste van hulle mancozeb kontakdoder gespuit. Vroegtydige waarskuwings sal beter besluitnemings tot gevolg hê.

Summary

No spore trap data or weather data was ever accumulated in the Eastern Cape for CBS monitoring and disease prediction as needed for the interpretation of a disease prediction or warning system which is needed for the control of the disease. Two spore traps were installed in lemon orchards in Hermitage and Kirkwood to monitor *Phyllosticta* ascospore releases. However, technical problems and theft were experienced during the critical periods of certain years when ascospore releases took place. When available, ascospore counts gave valuable data. Although ascospore releases did take place in certain cases, weather conditions were not always suitable for infection as determined by the QMS infection model. Given the gaps in the collected data, the critical infection period specifically for the Eastern Cape province could not be determined in this study. In future, more reliable data capturing and a faster turnaround in ascospore readings are required to support decision making with spray programmes during seasons with highly suitable conditions when outbreaks of CBS are predicted. For example, during 2012-13 certain growers had not completed their spray rounds before the onset of the first summer rain on 3 October 2012 and could only start spraying after 15 October 2012. Instead of spraying a systemic fungicide with kick-back action, most of them used mancozeb. Timely warning would enable better informed decisions.

Introduction

During the 2006-2007 and 2007-2008, seventeen and thirteen interceptions were recorded from lemons, oranges and Clementines infected with CBS in the Kat River and Addo areas. Rainfall patterns were studied from 1927 and it seems that the rainfall occurred a bit earlier than in the northern areas of South Africa on which current control programmes are based. Therefore, if the critical period for infection can be determined for these regions, then control programmes have to be adjusted accordingly. The average rainfall for the Fort-Beaufort area is about 650 mm per year, which is higher than what the Sundays River Valley (SRV) receives annually. There are also differences in inoculum distribution within the SRV from the Kirkwood area (with a low CBS incidence) to the Addo area (with a high CBS incidence). However, the whole SRV is subjected to a spray programme consisting of spraying lower number of applications than what is recommended when compared with the northern regions of South-Africa. For the northern areas, monitoring of the annual ascospore releases was done during the 1960s by Kotze and McOnie to determine the critical infection periods for infection. If duplicated for the Eastern Cape regions, this will improve our understanding and predictions of the disease for the Eastern Cape and to control the disease more effectively and successfully.

Stated objectives

The aim of the study was to accumulate spore trap and weather data for the interpretation of CBS epidemiology and implementation of a disease warning system needed for the control of the disease in the Eastern Cape.

Materials and methods

Two spore traps were installed during the 2009/2010 season. However, solar panels of both spore traps were stolen during harvest and had to be replaced. One was on the farm of Dave Gerber in a lemon orchard close to the technical office of SRCC (Fig. 4.6.2.1) and the other on an organic farm, Fontaine of Charles Botha, west of Kirkwood (Fig.4.6.2.2). An automatic weather station (Adcon) linked with Plant Plus in Holland, in the lemon orchard at Dave Gerber and operated by the SRCC, was also stolen. Weather data had to be obtained from another weather station at Willie Bouwer as there are no other weather stations in the vicinity of Hermitage. Spore trap discs were sent to QMS Agri Science in Letsitele for analyses on a regular basis. Ascospore releases were correlated with the weather patterns experienced during the monitoring period.

2009-2010 season

A new solar panel and battery box was ordered and installed from Interlock Systems in Pretoria. It was operational in December 2010. In certain cases, the spore trap sampled air for 1-4 days until the batteries were flat and some data could not be collected. The batteries ran flat due to the long overcast periods during summer. Apart from that, the regulator to the solar panel also broke and the battery box had to be taken down for repairs. This in itself is a huge operation as these boxes are situated 5 meters above ground on a thick steel pole. Scaffolding had to be erected or where possible, a forklift was used to hoist people to get hold of the battery box and its contents. The reaction time to get a new regulator from Pretoria was so slow that the spore trap was not operational for a long time. The filter spore trap (Fig. 4.6.2.3.) was, however, operational and was changed on a weekly basis.

2010-2011 season

A new solar panel and battery box was also ordered and installed from Interlock Systems in Pretoria for the Hermitage spore trap. Again the batteries also ran flat due to the long overcast periods during summer. After they were changed, the problems persisted and after an inspection, it was found that the new regulator to the solar panel also stopped working and the battery box also had to be taken down for repairs. This was also a huge operation as this box was also difficult to reach. Scaffolding had to be erected to get hold of the battery box and the regulator (Fig. 4.6.2.4). The filter spore trap was operational and was changed on a weekly basis.

2011-2012 season

The weather station at Hermitage was stolen. Two new Adcon weather stations were installed on 17 February 2012 at Summerville and Kirkwood. Therefore, data from another weather station had to be used. With all the cloudy days experienced, the batteries occasionally ran flat and a lot of data was lost.

2012-2013 season

Filter- and volumetric spore traps were installed and changed on a weekly basis and were fully operational throughout the season.

2013-2014 season

The spore trap batteries were all flat and had to be replaced. The spore trap's battery at Kirkwood was replaced and operational during the season and the spore trap was inspected before the onset of the 2013 season. Spore trap discs and filter paper discs were changed on a weekly basis and no operational problems were experienced.

Results and discussion

For each season, ascospore trap results as well as predicted infection periods are presented below. Predicted infection periods are based on ascospore inoculum availability and concomitant weather conditions suitable for infection (warm temperatures and leaf wetness conditions); this was calculated using a proprietary QMS Agri Science infection model. Importantly, it should be considered that the ascospore trap results do not distinguish between the CBS pathogen, *P. citricarpa*, and the harmless endophyte, *P. capitalensis*.

2009-2010 season

Hermitage/Addo. From the limited data available spore trap data from the Hermitage/Addo spore trap showed that there were two ascospore releases, viz. from 30 November to 3 December 2009, and 7 to 11 December (Fig. 4.6.2.5). Recording ceased when the solar panels were all stolen. According to the weather data, three suitable periods for ascospore release and infection potential occurred viz. from 1 to 3 and 7 to 8 December 2009. The solar panels were stolen on 23 January 2010 and after this date, data were lost. Comparing the rainfall figures between the two regions, shows that Addo also received rainfall during the same periods as Kirkwood from 22-26 February 2010 (15 mm), 2-3 March 2010 (3 mm), 10-12 March 2010 (14.2 mm) and 24-27 March 2010 (+50 mm). If these wet periods coincided with ascospore release and warm temperatures, it might have resulted in additional infection periods of fruit (until fruit becomes resistant to infection towards end-February) or leaves.

Kirkwood. Wetter conditions with ascospore releases prevailed in the Kirkwood area than the Hermitage/Addo area during 2009-2010 experimental period. In Kirkwood higher spore releases occurred during wet periods. Overall, 11 suitable periods for ascospore release and potential infection were recorded during the season with the most suitable conditions for infection that occurred from 22 – 26 February 2010 with 96 hours of continuous leaf wetness (Fig. 4.6.2.6). However, these late-season infection periods, as well as those recorded in March, should be of lesser significance for fruit infection, since fruit remain only susceptible for 4 to 5 months (October to February), after which infection no longer takes place, regardless of weather conditions and the presence of inoculums (Kotze, 2000).

2010-2011 season

Hermitage/Addo. During December and January weather data could also not be collected from Hermitage because the weather station was broken. However, three suitable periods for ascospore release and probable infection occurred from 31 December 2010 - 3 January 2011 according to weather data from an alternative source, 5 - 12 February 2011 and 4 - 9 March 2011 (Fig. 4.6.2.7). As in the previous year, Addo also received less rainfall during the same periods if compared with Kirkwood.

Kirkwood. Wetter conditions with ascospore releases prevailed in the Kirkwood area than the Hermitage/Addo area during 2010-2011 experimental period. In Kirkwood higher spore releases occurred during wet periods. Overall, 15 suitable periods for ascospore release and infection were recorded during the season with the most suitable conditions for infection that occurred from 28 November 2010, 10 – 12 March 2011, 16 – 20 March 2011 as well as 31 March - 2 April 2011. The longest wet period for the season, *i.e.* 139 hours was recorded from 4 – 11 February 2011 (Fig. 4.6.2.8A). According to Kotze (2000), fruit remain only susceptible for 4-5 months (September to January), after which infection no longer takes place, regardless of weather conditions and the presence of inoculums. Of the 15 possible infection periods, 5 extremely favourable infection periods occurred during March when the fruit is usually resistant to infection.

2011-2012 season

Spore trapping at Hermitage/Addo was a disaster and no usable ascospore trap data could be collected. Moreover, the Adcon weather station was also stolen. At Kirkwood (Fig. 4.x.x.8B), the spore trap was also not operational for the majority of the season, with ascospores trapped during 15 November to 1 December, and again at 22 December to 2 January.

2012-2013 season

Spore trapping at Hermitage was not always executed by personnel as promised. Therefore, usable ascospore trap data could not be recorded for this season. Apart from missing data during January and February 2013, data from Kirkwood showed that there was an infection period during 17 October 2012 and during 25 and 30 March 2013 (Fig. 4.6.2.9).

2013-2014 season

Few ascospores were trapped during the season, but times when it did occur, the climate was suitable. At Hermitage, the suitable periods were 15 November 2013 (no spores trapped) and 12 December 2013 with another peak during 4 to 9 January 2014 which was suitable according to the climate prediction model from QMS (Fig. 4.6.2.10).

At Kirkwood, ascospore releases coinciding with suitable weather conditions took place during 10 December 2013, 6 February 2014 and 13 February 2013 (Fig. 4.6.2.11).

Filter paper spore traps

This spore trapping technique is a new technique to distinguish and quantify between *P. citricarpa* and *P. capitalensis* ascospores on spore traps and is in the process of development at Stellenbosch University (part of project 1026) to enable quick and reliable data to be used in disease monitoring.

Conclusions to date

Spore trapping in the Eastern Cape did not start of well as technical errors and theft played a big role in the loss of data. When available, ascospore counts showed that releases coincided with rainfall. Although ascospore releases did take place in certain cases, weather conditions were not always suitable for infection.

For instance, taking weather data only into account, Hermitage/Addo would have had about 6 significant ascospore releases, which took place from 26 October 2009 to 12 March 2010. According to Dave Gerber, although it was very dry that year, the RH was higher than usual, which might have extended wetness periods and therewith contribute to infection when little rain did fall. Apart from the normal fruit susceptible period from October to January/February, ascospore release also occurred in March 2010, which should be too late for infection as fruit will have developed resistance to the disease (Kotze, 2000). The ascospore release and suitable conditions for infection at the end of the fruit susceptibility period (for example, 22 – 26 February 2010 in Kirkwood, or 4 March 2011 in Hermitage) is also at the end of the fungicide protection period and adequate protection on high-risk cultivars should be ensured in these cases.

In certain years like the 2012-2013 season for instance, uncharacteristic and highly suitable climatic conditions for CBS were experienced in the Eastern Cape. The onset of rains (October 2012 onwards) was unusually early, with heavy rain in October, resulting in favourable conditions at an early stage of fruit susceptibility and making it difficult to ensure effective fruit protection. Throughout the season, repeated rainfall periods were recorded during the fruit susceptibility period. A detailed comparison of the 2012-13 season with previous seasons is presented in Addendum A.

Given the gaps in the collected data, the critical infection period specifically for the Eastern Cape province could not be determined. In future, more reliable data capturing and a faster turnaround in ascospore readings are required to support decision making with spray programmes during seasons with highly suitable conditions when outbreaks of CBS are predicted. For example, during 2012-13 certain growers had not completed their spray rounds before the onset of the first summer rain on 3 October 2012 and could only start spraying after 15 October 2012. Instead of spraying a systemic fungicide, most of them used mancozeb instead. Timeous warning would enable better informed decisions.

Technology transfer

Talks at study groups and results were presented on the biennial CRI Symposium in 2010, 2012 and 2014.

Further research

This project was terminated March 2015. CRI will collaborate with a service providing company to continue the spore trapping service in the Eastern Cape province, if the citrus growers will subscribe to it.

References cited

- Fourie, P., Schutte, T., Serfontein, S. and Swart, S.H. 2013. Modeling the effect of temperature and wetness on *Guignardia pseudothecium* maturation and ascospore dispersal in citrus orchards. *Phytopathology* 103: 281-292.
- Kellerman, C.R. & Kotzé, J.M. 1977. The black spot disease of citrus and its control in South Africa. *Proc. Int. Soc. Citricult.* 3:992-996.
- Kiely, T.B. 1948. Preliminary studies on *Phyllosticta citricarpa* the ascigerous stage of *Phoma citricarpa* and its relation to black sot of citrus. *P. Linn. Soc. NSW* 73:249-292.
- Kotze, J.M. 2000. Black spot. *In: Compendium of of citrus diseases*, by: L.W. Timmer, S.M. Garnsey, and J.H. Graham. APS Press, ST. Paul, Minnesota.
- Kotze, J.M. 1981. Epidemiology and control of citrus black spot in South Africa. *Plant Dis.* 65(12):945-950.
- Kotze, J.M. 1996. History and epidemiology of citrus black spot in South Africa *Proc. Int. Soc. Citriculture* 1296-1299.

- McOnie, K.C. 1964. Source of inoculum of *Phyllosticta citricarpa*, the citrus black spot pathogen. *Phytopathology* 54(1):64-67.
- McOnie, K.C. 1967. Germination and infection of citrus by ascospores of *Phyllosticta citricarpa* in relation to control of black spot. *Phytopathology* 57:743-746.
- Schutte, G.C. & Beeton, K.V. 1999. Evaluation of paraquat and ethephon for monitoring latent *Phyllosticta citricarpa* infections in asymptomatic immature Valencia oranges. *Deciduous Fruit Grower* 49:S8-S9.

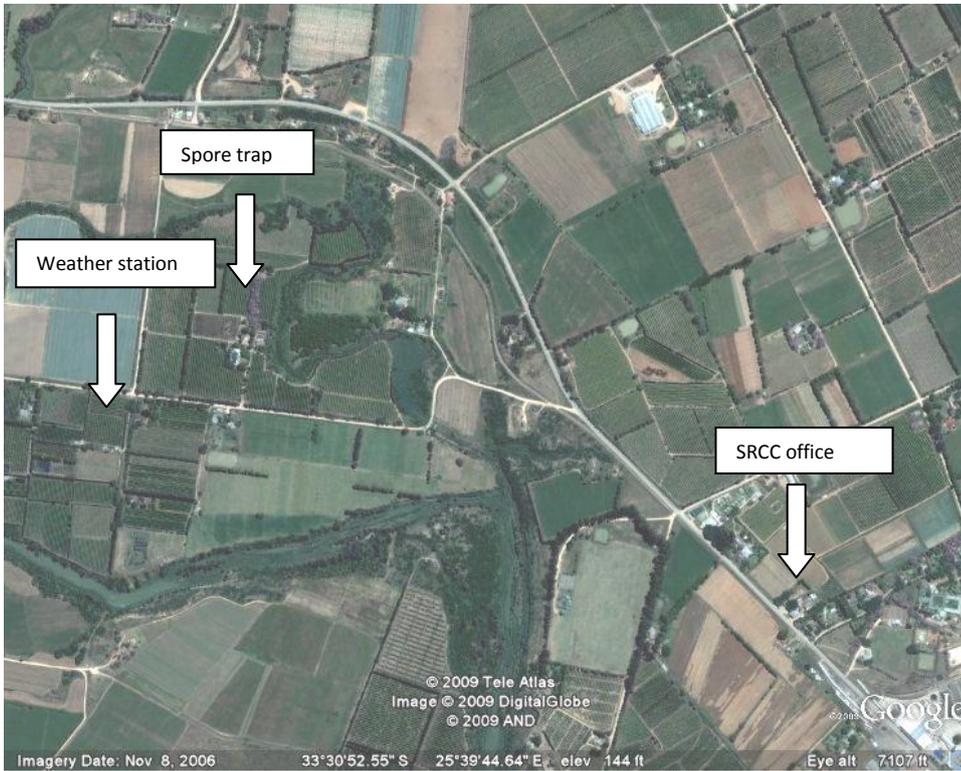


Fig. 4.6.2.1. Position of the automatic weather station and spore trap relative to the SRCC technical office in the Sunday's river valley.



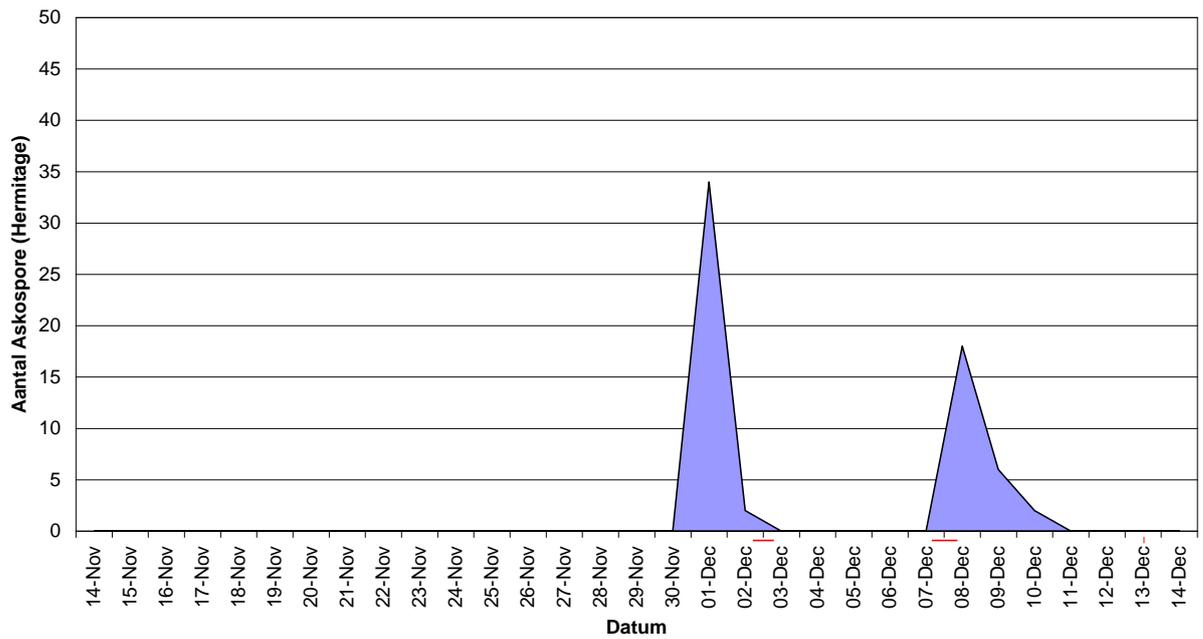
Fig. 4.6.2.2. Position of the spore trap in the lemon orchard on the farm “Fonteine” of Charles Botha north of Kirkwood



Fig. 4.6.2.3. Example of a filter paper spore trap used in the Sundays River Valley to trap ascospores.

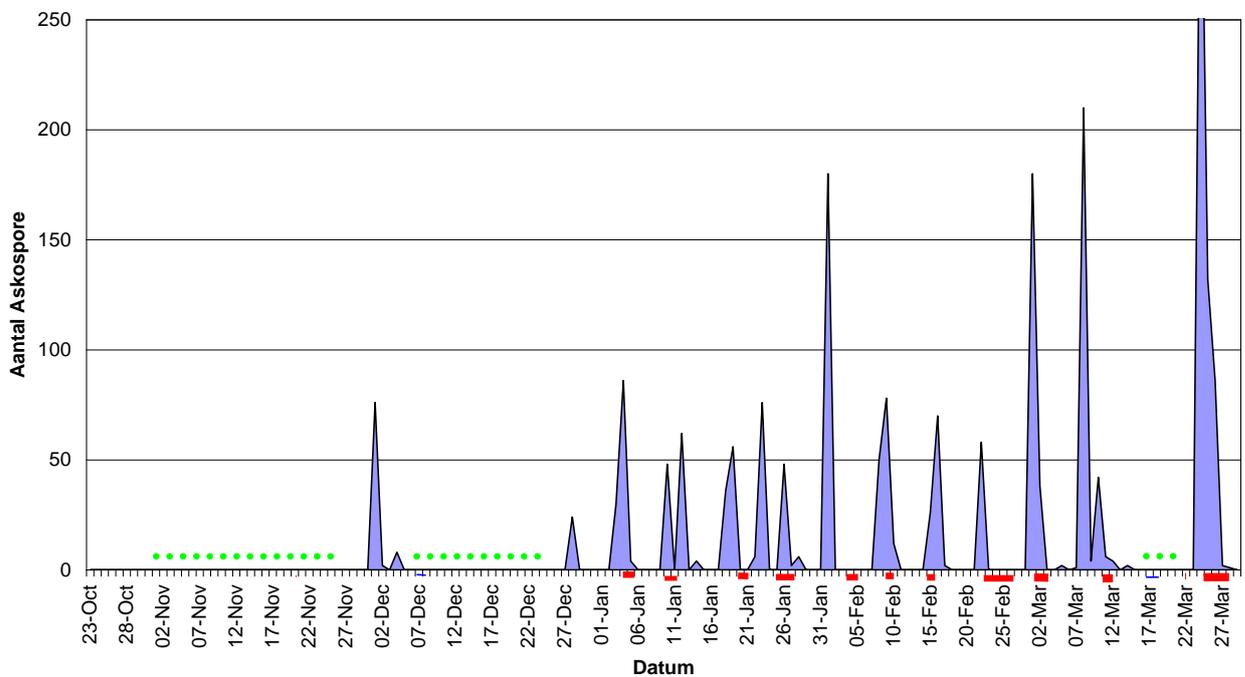


Fig. 4.6.2.4. After the theft of the solar panels and vandalism of the old battery boxes, new units were ordered and installed at both sites in the Sundays River Valley.



Ascospore  Infection period  Lost data 

Fig. 4.6.2.5. Ascospore release and possible infection period of *Phyllosticta* spp. at Hermitage in the Eastern Cape for the period 2009-2010.



Ascospore  Infection period  Lost data 
 Blue line- data from alternative weather station

Fig. 4.6.2.6. Ascospore release and possible infection period of *Phyllosticta* spp. at Kirkwood in the Eastern Cape for the period 2009-2010.

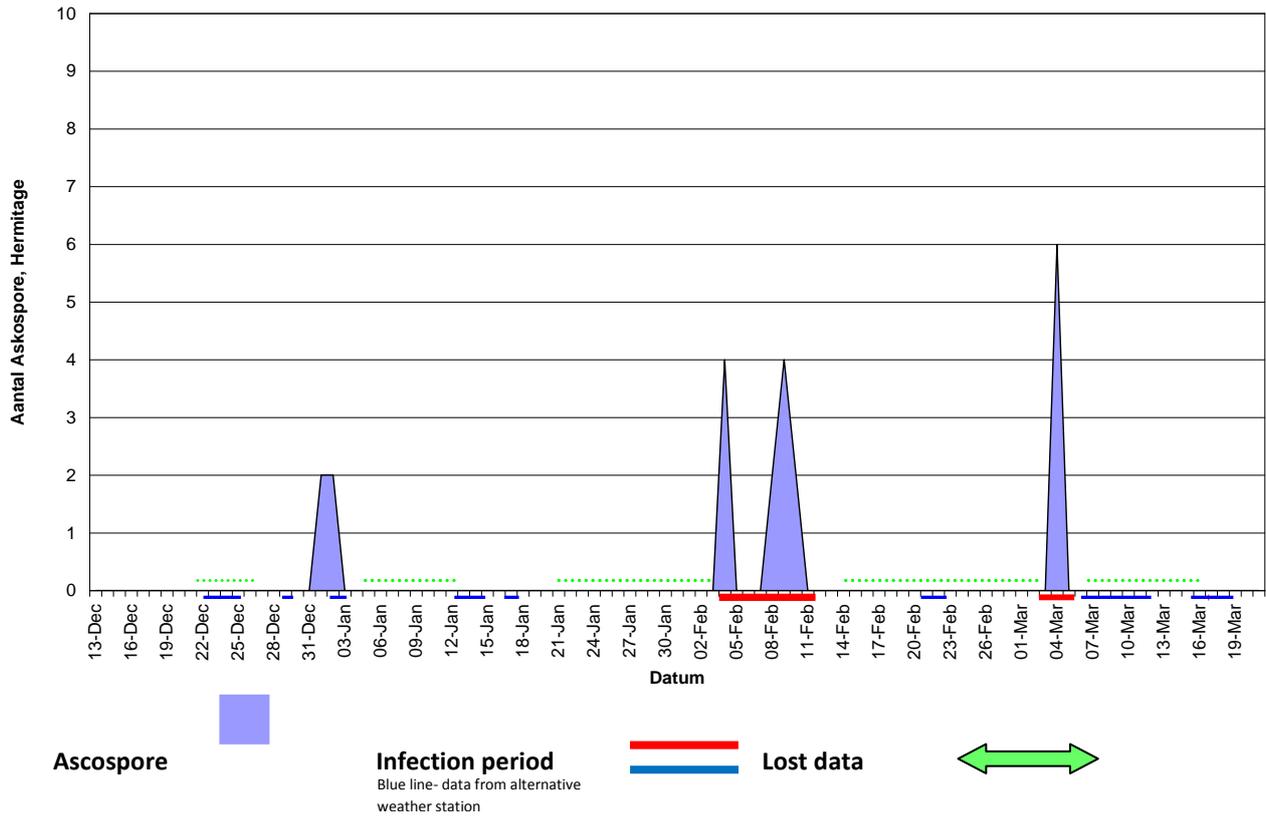


Fig. 4.6.2.7. Ascospore release and possible infection period of *Phyllosticta* spp. at Hermitage in the Eastern Cape for the period 2010-2011.

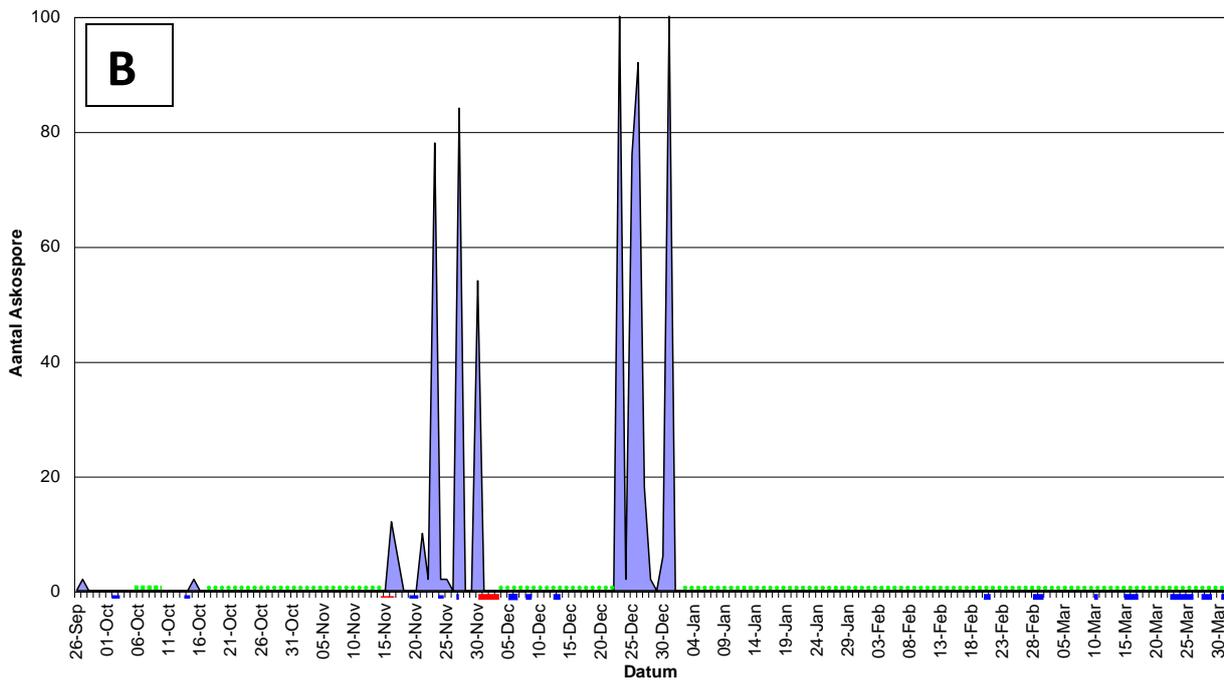
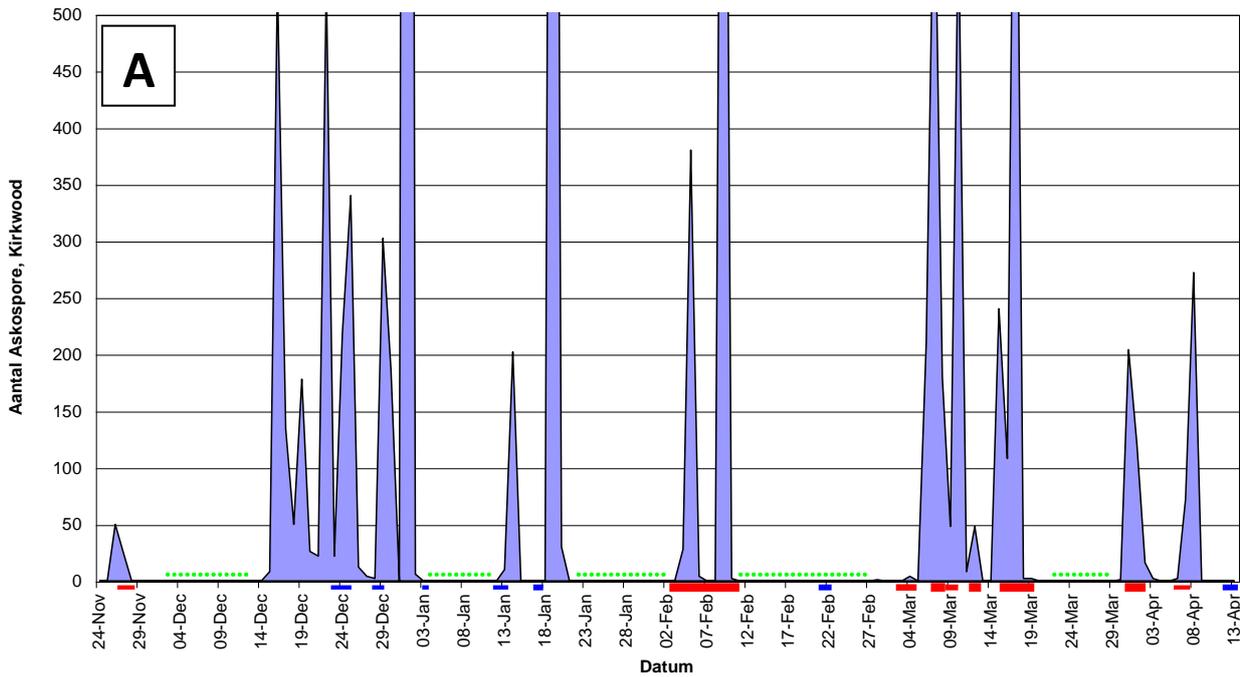


Fig. 4.6.2.8. Ascopore release and possible infection period of *Phyllosticta* spp. at Kirkwood in the Eastern Cape for the period 2010-2011 (A) and 2011-2012 (B).

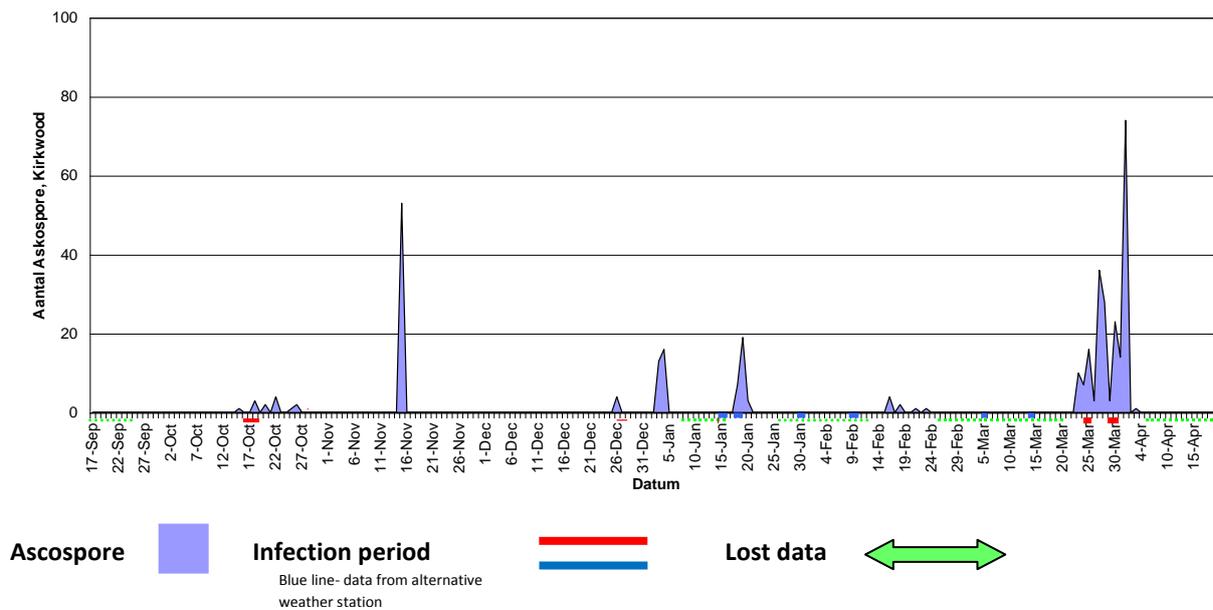


Fig. 4.6.2.9. Ascospore release and possible infection period of *Phyllosticta* spp. at Kirkwood in the Eastern Cape for the period 2012-2013.

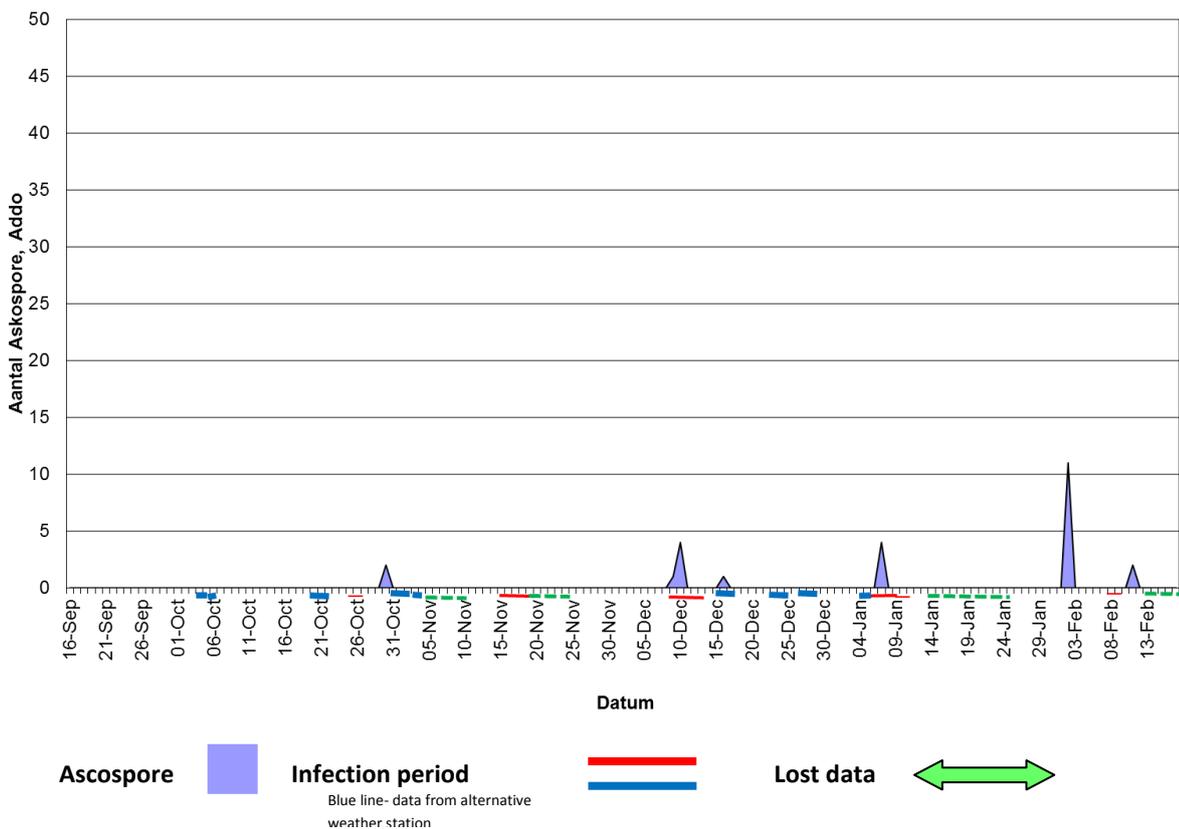


Fig. 4.6.2.10. Ascospore release and possible infection period of *Phyllosticta* spp. at Hermitage in the Eastern Cape for the period 2013-14.

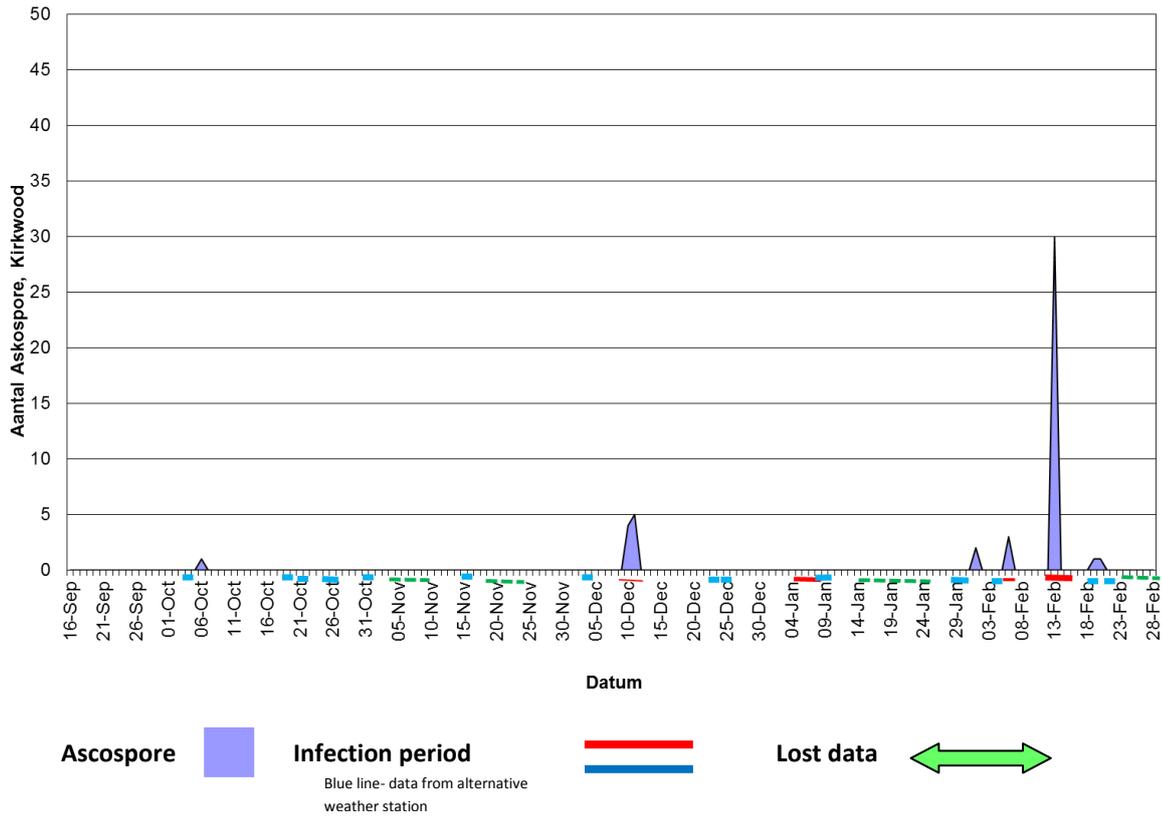


Fig. 4.6.2.11. Ascospore release and possible infection period of *Phyllosticta* spp. at Kirkwood in the Eastern Cape for the period 2013-2014.

Addendum A

Comparison of weather data and ascospore spore trap data and infection predictions for the 2012 and 2013 seasons to determine the reasons for the outbreak of CBS

To determine why there was an increase in CBS during the 2012/2013 season, a comparative climatological study was performed (see Addendum A)

a. Climate data

Weather data were obtained from the Agricultural Research Council's (www.arc.agric.za) Institute of Soil Climate and Water (ARC-ISCW) in Stellenbosch for Letsitele in the Limpopo province and Addo and Kirkwood in the Eastern Cape province. Monthly means for daily rainfall of the 2012-2013 season were compared with these rainfall figures in previous seasons.

b. Ascospore trap data and infection predictions

i) Limpopo

Weather and ascospore release data sets were obtained from growers in the Constantia, Letaba-Oranje, Mahela regions of the Letaba valley for the 2011-2012 and 2012 – 2013 seasons. The GPS coordinates of the weather and spore trap stations were: Constantia 23°40'54.96"S & 30°35'27.19"E, Letaba Oranje 23°48'42.67"S & 30°26'33.14"E, Mahela 23°52'08.07"S & 30°22'50.10"E. The total distance between the Mahela and Constantia weather stations is 30 km. A separate data set was also obtained from ARC-ISCW comprising of rainfall data for this region since 1928.

Ascospore release was monitored at Constantia, Letaba-Oranje and Mahela using Interlock Volumetric Spore Traps, which samples 20 L air per minute onto a rotating 8-day disc (Fourie *et al.*, 2013). Weather data were obtained from Adcon weather stations placed next to or in the vicinity of the spore traps. QMS Agri Science counted the trapped *Phyllosticta* ascospores per 3-hour period and compiled regular reports showing number of ascospores trapped per day to their clients. Based on ascospore counts, suitable rainfall periods and leaf wetness periods, potential CBS infection periods were also predicted. Results from 2011-2012 and 2012-2013 were compared.

ii) Eastern Cape

Weather data were obtained from ARC-ISCW for the weather stations at Addo and Kirkwood. The GPS coordinates of the weather stations were: Kirkwood T.N.K. 33.40381 S & 25.33611 E and Addo-AWS 33.56852 S & 25.69216 E. The total distance between the Kirkwood and Addo weather stations in the Sunday's River Valley is 38 km (Fig. 9).

Results

Addo (2002 - 2013)

The monthly rainfall during the critical infection period of the 2012-2013 season (October – February) for both Kirkwood and Addo was uncharacteristically higher than the long term average for October and December (Fig. 11 & 12).

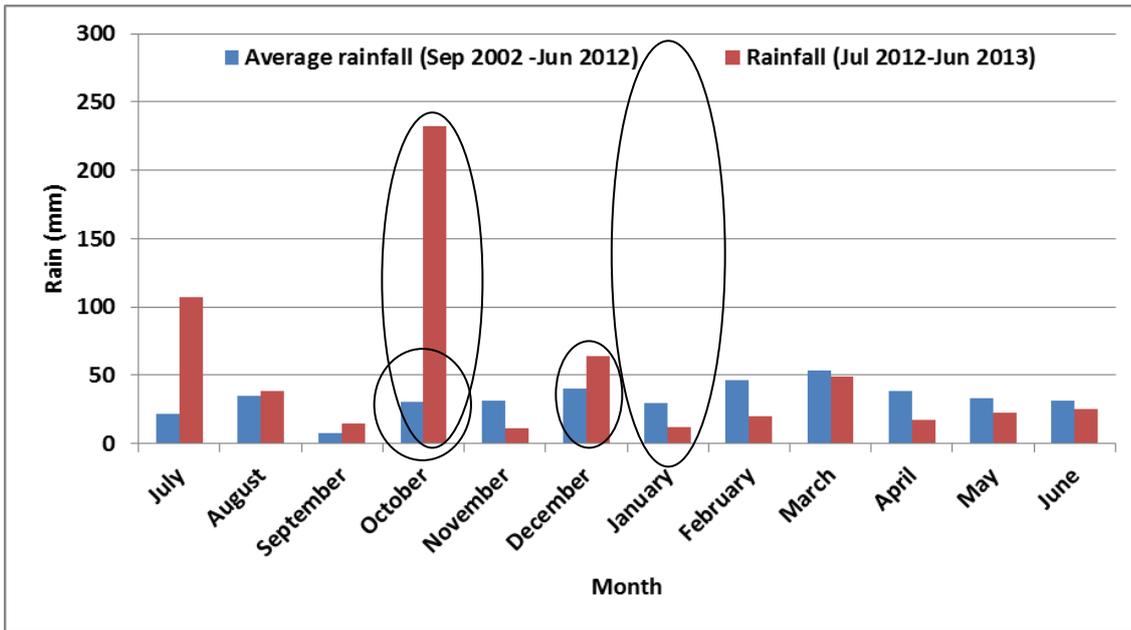


Fig. 4.6.2.11. Average monthly rainfall for Addo from 2002 to 2012 (■) and the total monthly rainfall for the 2012 – 2013 season (■), with the exceptionally high early rainfall in October highlighted, as well as higher than normal rainfall in December.

Kirkwood (1995 - 2013)

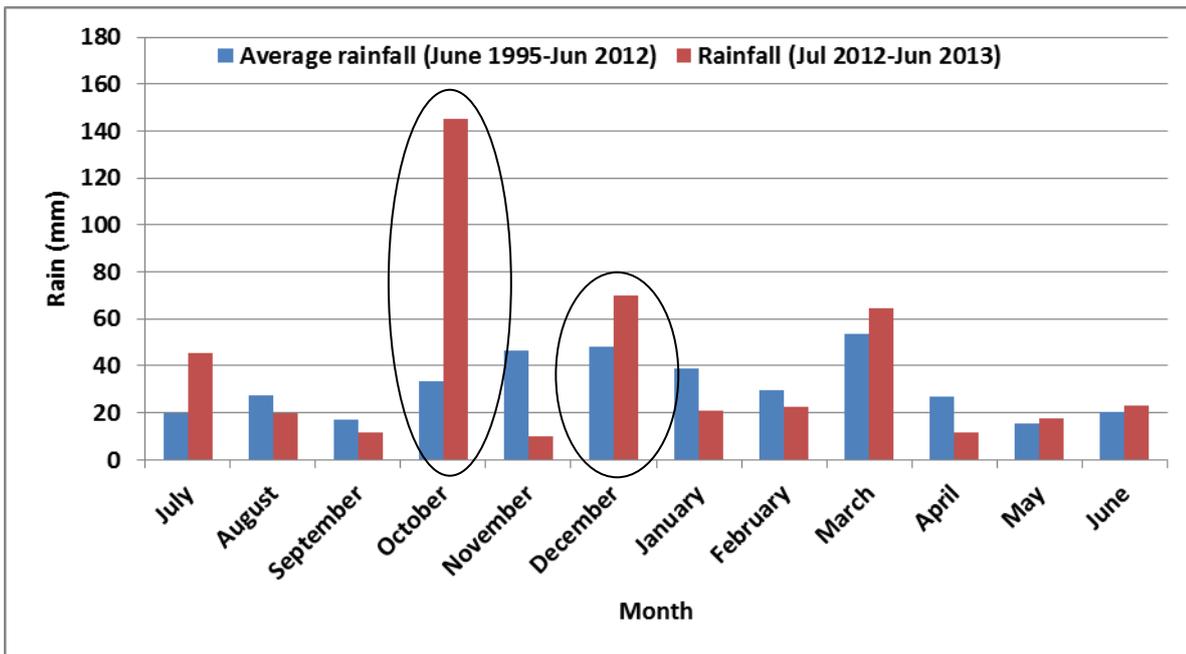


Fig. 4.6.2.12. Average monthly rainfall for Kirkwood from 1995 to 2012 (■) and the total monthly rainfall for the 2012 – 2013 season (■) with the exceptionally high rainfall in October highlighted, as well as higher than normal rainfall in December.

4.6.3 **PROGRESS REPORT: Development of new spray programmes for the control of citrus black spot**

Project 970 (Ongoing) by G.C. Schutte & C. Kotze (CRI)

Summary

Various new systemic and contact fungicides as well as adjuvants in combination with registered fungicides were tested on 'Valencia' oranges for the control of citrus black spot according to predetermined protocols from the various companies. Of the fungicides tested, Kannar 202 also performed well, but phytotoxicity problems were experienced. BAS70301F in a tank mixture with mineral spray oil gave excellent control of citrus black spot and can be recommended for registration. New spray programmes where mancozeb were altered with RB1 and RB2, also resulted in good control of CBS and can also be recommended for registration. Tank mixtures of benomyl and azoxystrobin (1x and 2x) with mancozeb and mineral spray oil sprayed as two applications with 8 week intervals, performed well in controlling CBS and if registered, will save the growers two spray rounds.

Opsomming

Verskeie nuwe sistemiese- en kontakswamdoders asook benatters in kombinasies met geregistreerde swamdoders is op 'Valencia' lemoene beproef vir die beheer van swartvlek volgens vooropgestelde protokolle van die onderskeie maatskappye. Van die swamdoders wat getoets is, het Kannar 202 goed gewerk, maar het fitotoksiese probleme gewys. BAS70301F in 'n tenkmengsel met minerale olie het goeie beheer van swartvlek gegee en kan vir registrasie aanbeveel word. Nuwe spuitprogramme waar mancozeb met RB1 en RB2 afgewissel is, het goeie beheer van swartvlek tot gevolg gehad en kan vir registrasie aanbeveel word. Tenkmengsels van benomyl en azoxystrobin (1x en 2x) met mancozeb en minerale spuitolie wat met twee toedienings van 8 week intervalle getoets is, het ook goed gewerk vir die beheer van swartvlek en as dit geregistreer word, sal dit kwekers twee spuitronde bespaar.

Introduction

Citrus black spot (CBS), caused by *Guignardia citricarpa* Kiely (anamorph *Phyllosticta citricarpa* (McAlpine) van der Aa), affects all commercial citrus cultivars only in the summer rainfall regions of the world. Control of the disease is entirely dependent on the application of fungicidal sprays during the critical period of infection from October to January in the southern hemisphere. The most important inoculum source of CBS is airborne ascospores. Pseudothecia of the fungus develop on dead infected leaves on the orchard floor within 40-180 days after leaf drop, depending on the temperature and frequency of wetting. Once mature, ascospores are discharged during rain spells. Ascospores are dependent on converging currents and favourable environmental conditions to reach a suitable host substrate, since the maximum vertical distance of ascospore ejection from a pseudothecium is 10-12 mm and the horizontal disease dispersion occurs at distances below 24.7 m. When protective fungicides such as copper and dithiocarbamates are used to control CBS, spray applications have to be carefully timed to coincide with the critical infection period. Spore trapping with an Interlock volumetric spore trap® and sampler is used to determine the first onset of ascospore release in South Africa.

A four-spray programme of copper fungicides used for CBS control can result in rind stippling and darkening of blemishes. However, alternating copper fungicides with mancozeb in a four-spray programme, solved this problem. Protective fungicides became less popular after the release of post-infection benzimidazole fungicides such as benomyl. In 1971, the introduction of a single benomyl application in a tank mixture with mancozeb and mineral spray oil came as a breakthrough as it replaced copper and dithiocarbamates that must be applied in a four-spray protective schedule (9). Since the detection of *G. citricarpa* resistance to benomyl in South Africa in 1981, emphasis has shifted back to the use of contact fungicides for disease control. Field evaluations using strobilurins for the control of CBS in 1993 also came as a breakthrough. Two applications of kresoxim-methyl and azoxystrobin at respective rates of 0.10 and 0.075 g a.i./liter in tank mixtures with mancozeb (1.2 g a.i./liter) and mineral oil (0.5% [vol/vol]/liter of water) were initially recommended. The possibility that CBS may develop resistance to the strobilurins, justifies the incorporation of two additional mancozeb applications before and after the strobilurin applications in October and January. Since the registration of strobilurins in South Africa in 1999, no new fungicides have been registered for use against CBS. Testing of novel control measures against CBS is therefore regarded as a priority even if it includes tank mixtures with current registered fungicides.

Objectives

The aim is to evaluate any new potential fungicides for the control of CBS.

Materials and methods

Valencia orange orchards with a history of CBS were selected at Croc Valley Citrus Co. Rates and dates of applications are listed in Tables 4.6.3.1 to 4. A randomised design with 5 single-tree plots per treatment was used. Fungicides were applied with a trailer-mounted, high-volume, high-pressure (2,500 - 3,000 kPa) sprayer with two hand-held spray guns. Spray volumes varied according to the size and canopy density of the tree but all trees were sprayed to the point of run-off. Certain treatments commenced in mid-October as previously recommended, depending on the climatological information required for infection during the critical infection period. Trees were selected for uniformity in canopy density and tree size. Currently, all commercial fungicide applications for CBS control in South Africa begin in mid-October, based on research findings from ascospore releases and spore trap data. At fruit maturity in July or August, CBS severity will be rated on 100 fruit per tree according to a 3-point index: 0 = clean fruit with no CBS lesions; 1 = one to three CBS lesions per fruit; and 2 = four or more CBS lesions per fruit. Proportional data will be analysed by ANOVA, using Fisher's LSD test ($P = 0.05$).

Results and discussion

Kannar 202

According to Table 4.6.3.1, the trial site at Crocodile Valley Estates showed that there were no significant differences ($P < 0.05$) between the standard registered mancozeb treatment and Kannar 202 sprayed at rates of 250 ml (1x) and 500 ml (2x) per 100 L water. All these treatments resulted in more than 87% clean exportable fruit while the untreated control had only 29.6% clean exportable fruit. Phytotoxicity was still a problem and could not be resolved by the company (Fig. 4.6.3.3).

BAS70301F

Due to the high disease pressure only 29.6% of fruit evaluated from the untreated control resulted in clean exportable fruit. This was significantly lower than the treated, which ranged between 99 and 94.4% clean exportable fruit. Although there was no significant differences between treatments, BAS70301F+ oil showed marginally more clean fruit compared to the registered commercial spray programmes. In fact, where mancozeb was sprayed in a tank mix with either one of Cabrio, Ortiva or Flint, little to no difference in performance were observed, resulting in 98.6% (Mz/Mz+Cabrio+oil/ Mz+Cabrio+oil/Mz), 97.6% (Mz/Mz+Ortiva+oil/Mz+Ortiva+oil/Mz) and 94.4% (Mz/Mz+Flint+oil/ Mz+Flint+oil/Mz) clean fruit respectively. Furthermore, when BAS70301F+ oil were sprayed without mancozeb in a tank mix, it resulted in 99% clean exportable fruit. No statistically significant differences were observed between any of the fungicide treatments in the other two criteria, but they were, however, significantly different from the untreated control. Unfortunately, trees of treatments 2 and 4 to 7 were accidentally harvested (Table 4.6.3.2).

RB1 and RB2

According to Table 4.6.3.3, the trial site at Crocodile Valley Estates showed that there were no significant differences ($P < 0.05$) between the standard registered mancozeb treatment and the spray programmes consisting of RB1 and RB2 alternated with mancozeb. Although the RB1 rate of 200ml + 2ml BT alternated with 200 g mancozeb resulted in a lower 87.4% clean exportable fruit, it was not significantly different from the standard mancozeb treatment alone that resulted in 89.4% clean exportable fruit. Both these treatments were also not significantly different from the other treatments, but were significantly different from the untreated control. There was also no significant differences between the RB1 1x (100ml/100L water) and 2x (200ml/100L water) as well as the RB2 1x (1L/100L water) and 2x (2L/100L water) treatments. Disease pressure was high as the untreated control resulted in only 29.6% clean exportable fruit. In both the other criteria used for evaluation, all the treatments were also significantly different from the control. No phytotoxicity was observed on any of the treatments.

Benomyl + Ortiva + mancozeb + oil

Where benomyl was mixed with azoxystrobin (1x and 2x rates) with mancozeb and mineral spray oil and sprayed twice (November and January), resulted in 98% and 99% clean exportable fruit respectively. These treatments were not significantly different from the four mancozeb treatments and standard azoxystrobin +

mancozeb + oil treatments and can therefore save growers two spray rounds. Results were significantly better than the untreated control (Table 4.6.3.4).

Conclusion

Kannar 202 gave control of CBS comparable with 4 mancozeb treatments, but caused phytotoxicity problems. BAS70301F mixed with mineral spray oil but without mancozeb gave excellent control of citrus black spot resulting in 99% clean exportable fruit and can be recommended for registration. New spray programmes where mancozeb was altered with RB1 and RB2 (evaluated at 1x and 2x rates), also performed well and can also be recommended for registration. Two applications consisting of tank mixtures of benomyl (Spoton-B) plus azoxystrobin (1x and 2x rates) with mancozeb and mineral spray oil, also performed well and gave more than 98% clean exportable fruit and can be recommended for registration. These treatments will save the growers two spray rounds.

Future research

There is a constant need to evaluate new and old fungicide formulations as well as fungicides that may possess activity against citrus black spot (CBS). Chemical companies frequently modify and upgrade their old products to possess new characteristics such as rain fastness and particle size and they need to be re-evaluated for efficacy. Searching for new fungicides or fungicides with new characteristics as well as some new ideas how we can alter aspects of old fungicide spray programmes to be included in effective spray programmes and to cope with fungal resistance strategies at the same time. Searching for and experimenting with cheaper and more effective fungicides sprayed alone or in tank mixtures with new or existing registered fungicides, will contribute a lot to reducing production costs and be more environmentally friendly and sustainable with regard to resistance development.

Technology transfer

Talks at study groups. Results will be presented on the bi-annual CRI Symposium in August 2016.

References cited

- Baayen, R.P., Bonants, P.J.M., Verkley, G., Carroll, G.C., Van der Aa, H.A., De Weerd, M., Van Brouwershaven, I.R., Schutte, G.C., Maccheroni, W., Glienke de Blanco, C., and Azevedo, J.L. 2002. Nonpathogenic isolates of the citrus black spot fungus, *Guignardia citricarpa*, identified as a cosmopolitan endophyte of woody plants, *G. mangiferae* (*Phyllosticta capitalensis*). *Phytopathology* 92:464-477.
- Brodrick, H.T. 1970. Investigations into blemishes on citrus fruit. IV. Accentuation of blemish marks by copper fungicide sprays. *S. Afr. Citrus J.* 441:13,15,17,25.
- Kellerman, C.R. & Kotzé, J.M. 1977. The black spot disease of citrus and its control in South Africa. *Proc. Int. Soc. Citricult.* 3:992-996.
- Kiely, T.B. 1948. Preliminary studies of *Guignardia citricarpa* n. sp. the ascigerous state of *Phoma citricarpa* McAlp. and its relation to black spot of citrus. *Proc. Linn. Soc. N.S.W.* 73:249-292.
- Kotzé, J.M. 1963. Studies on the black spot disease of citrus caused by *Guignardia citricarpa* Kiely, with particular reference to its epiphytology and control at Letaba Estates. D.Sc. (Agric) thesis. University of Pretoria, Pretoria, South Africa.
- Kotzé, J.M. 1981. Epidemiology and control of citrus black spot in South Africa. *Plant Dis.* 65:945-950.
- Kotzé, J.M. 2000. Black spot. Pages 23-25 in: *Compendium of citrus diseases*, 2nd ed. L.W. Timmer, S.M. Garnsey, and J.H. Graham, eds. The American Phytopathological Society, St. Paul, MN.
- McOnie, K.C. 1964a. Source of inoculum of *Guignardia citricarpa*, the citrus black spot pathogen. *Phytopathology* 54:64-67.
- McOnie, K.C. 1964b. Orchard development and discharge of *Guignardia citricarpa* and the onset of infection in relation to the control of citrus black spot. *Phytopathology* 54:1448-1453.
- McOnie, K.C., and Smith, J.H. 1964. Dithiocarbamates versus copper fungicides for the control of black spot disease. *S. Afr. Citrus J.* 367:13-19.
- Nel, A., Krause, M., and Khelawanlall, N. 2003. *A Guide for the control of Plant Diseases*. 2nd ed. National Department of Agriculture. Directorate: Agricultural Production Inputs. Pretoria, South Africa.
- Schutte, G.C., Beeton, K.V., and Kotzé, J.M. 1997. Rind stippling on Valencia oranges by copper fungicides used for control of citrus black spot in South Africa. *Plant Dis.* 81:851-854.
- Schutte, G.C., Tollig, B., Mansfield, R.I., and Kotzé 1996. Effect of kresoxim-methyl and azoxystrobin for the control of a benzimidazole resistant strain of citrus black spot. *Proc. Int. Soc. Citriculture* 8:345-350.
- Schutte, G.C., Mansfield, R.I., Smith, H., and Beeton, K.V. 2003. Application of azoxystrobin for control of benomyl-resistant *Guignardia citricarpa* on 'Valencia' oranges in South Africa. *Plant Dis.* 87:784-788.

- Spósito, M.B., Amorim, L., Ribeiro, P.J., Jr., Bassanezi, R.B., and Krainski, E.T. 2007. Spatial pattern of trees affected by black spot in citrus groves in Brazil. *Plant Dis.* 91:36-40.
- Timmer, L.W., Zitko, S.E., and Albrigo, L.G. 1998. Split applications of copper fungicides improve control of melanose on grapefruit in Florida. *Plant Dis.* 82:983-986.

Table 4.6.3.1. Evaluation of Kannar 202 applied from October 2013 to January 2014 for evaluation of Kannar 202 applied during the susceptible period from October to January for the control of citrus black spot on Valencia oranges at Nelspruit, South Africa.

Treatment	Rate / 100L water	Percentage of fruit in each class ^x		
		Lesions/fruit		
		0	1-3	≥4
Untreated control		29.6a	14.0b	56.4b
Mancozeb	200g	89.4b	5.0a	5.6a
Kannar 202	250ml	87.4b	5.8a	6.8a
Kannar 202	500ml	88.8b	5.8a	5.4a

^yMeans 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.

^zSpray dates were 15 October 2013, 12 November 2013, 10 December 2013 and 7 January 2014.

Table 4.6.3.2. Evaluation of BAS70301F applied from October 2013 to January 2014 for the control of citrus black spot on Valencia oranges at Nelspruit, South Africa.

Treatment	Rate per 100L water	Percentage of fruit in each class ^w		
		Lesions/fruit		
		0	1-3	≥4
Control		29.6b	14.0a	56.4a
Mz/BAS70301F+oil/BA S70301F+oil/Mz ^z	200g/10ml + 250ml/10ml + 250ml/200g	99.0a	0.8b	0.2b
Mz/Mz+Cabrio+oil/Mz+Cabrio+oil/Mz	200g/150g + 10ml + 250ml/150g + 10ml + 250ml/200g	98.6a	1.4b	0.0b
Mz/Mz+Ortiva+oil/Mz+Ortiva+oil/Mz	200g/150g + 20ml + 250ml/150g + 10ml + 250ml/200g	97.6a	1.6b	0.8b
Mz/Mz+Flint+oil/Mz+Flint+oil/Mz	200g/150g + 10g + 250ml/150g + 10ml + 250ml/200g	94.4a	2.8b	2.8b

^wMeans 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.

^zSpray dates were 15 October 2013, 5 November 2013, 27 December 2013 and 27 January 2014

Table 4.6.3.3. Evaluation of spray programmes consisting of RB1 and RB2 alternated with mancozeb and Breakthru (BT) in tank mixtures applied during the susceptible period from October to January for citrus black spot (CBS) control on Valencia oranges at Crocodile Valley Estates during 2013 and 2014.

Treatment	Rate / 100L water	Percentage of fruit in each class		
		Lesions/fruit ^y		
		0	0-3	≥4
Untreated control		29.6b	14.0a	56.4b
Mancozeb ^z	200g	89.4a	5.0b	5.6a
Mancozeb/RB1+BT/Mancozeb/RB1+BT	200g/100ml+2ml/200g/100ml+2ml	94.0a	3.6b	2.4a
Mancozeb/RB1+BT/Mancozeb/RB1+BT	200g/200ml+2ml/200g/200ml+2ml	87.4a	4.6b	8.0a
Mancozeb/RB2+BT/Mancozeb/RB2+BT	200g/1000ml+2ml/200g/1000ml+2ml	91.0a	6.0b	3.0a
Mancozeb/RB2+BT/Mancozeb/RB2+BT	200g/2000ml+2ml/200g/2000ml+2ml	96.8a	1.0b	2.2a

^yMeans 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.

^zSpray dates were 15 October 2013, 12 November 2013, 10 December 2013 and 7 January 2014.

BT = BreakThru

Table 4.6.3.4. Evaluation of alternating spray programmes consisting of tank mixtures of benomyl and azoxystrobin with mancozeb and Breakthru (BT) applied during the susceptible period from October to January for citrus black spot (CBS) control on Valencia oranges at Crocodile Valley Estates during 2013 and 2014.

Treatment	Rate / 100L water	Percentage of fruit in each class ^x		
		Lesions/fruit		
		0	1-3	≥4
Untreated control		29.6c	14.0a	56.4a
Mancozeb ^y	200g	89.4b	5.0b	5.6b
Benlate + Ortiva + MZ+ oil (x 2) ^z	25g + 10ml +150g + 250 ml	98.0ab	0.8c	1.2b
Benlate + Ortiva + MZ+ oil (x 2) ^z	50g + 20ml + 150g + 250 ml	99.4a	0.4c	0.2b
Mancozeb/Ortiva + MZ+oil/ Ortiva + MZ +oil/Mancozeb ^z	200g/20ml + 150g + 250ml/ 200g/20ml + 150g + 250ml	97.6ab	1.6c	0.8b

^xMeans 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 15 October 2013, 12 November 2013, 10 December 2013 and 7 January 2014.

^z Spray dates were 12 November 2013 and 7 January 2014.

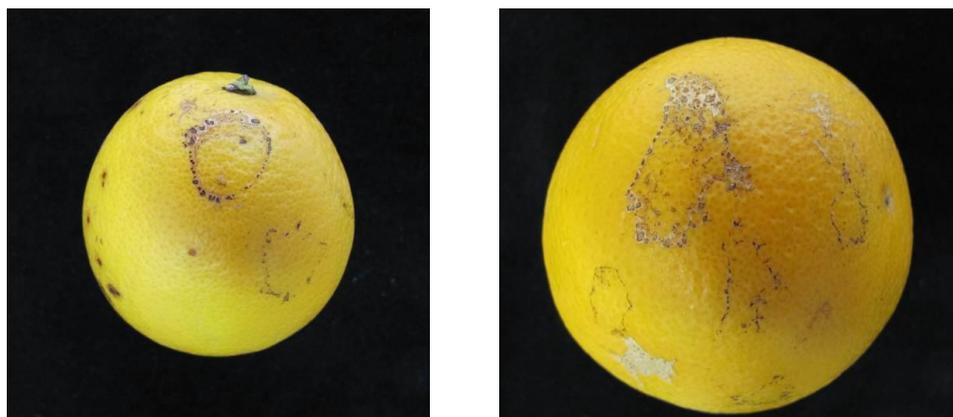


Fig. 4.6.3.1. Concentric rings formed on Valencia oranges after 4 foliar applications of Kannar 202 at rates of 250 and 500 ml/100L water.

4.6.4 FINAL REPORT: The global population structure and reproductive biology of the fungal pathogen, *Phyllosticta citricarpa* Kiely

Project 977 (2010/11 – 2016/17) by E. Carstens (CRI)

Summary

Genetic markers such as microsatellite markers are very useful and widely used in population genetic studies, since they show high levels of allelic variation. This enables the quantification of genetic diversity within and amongst pathogen populations. Genomic sequence data of *Phyllosticta citricarpa* was generated with the Ion Torrent Personal Genome Machine, which was used to mine for microsatellites and for primer design. Of the 57 primers designed, eight were shown to be polymorphic. These eight markers were used to genotype 388 *P. citricarpa* isolates from five SA populations and seven international populations from four countries including USA, China, Brazil and Australia. The study confirms that *P. citricarpa* populations in China and Australia have a greater genetic diversity than those in South Africa and in the USA where more recent introductions have occurred. Mating type analysis revealed that both mating types were present in the populations from South Africa at an approximately 1:1 distribution. Funding for this project has terminated in March 2014; however, final analyses and writing of a PhD dissertation is underway. A final report will be submitted in April 2017.

Opsomming

Genetiese merkers soos mikrosatelite is handig en word baie in genetiese studies gebruik omdat hulle hoë vlakke van alleliese variasie toon. Dit maak die kwantifisering van genetiese diversiteit binne en tussen patogeen populasies moontlik. Data van genomvolgordes van *Phyllosticta citricarpa* is met die "Ion Torrent Personal Genome" Masjien gegenereer, wat gebruik is om vir mikrosatelite en vir inleier-ontwikkeling te myn. Van die 57 inleiers wat ontwikkel is, het agt geblyk om polimorfies te wees. 'n Totaal van 388 *P. citricarpa* isolate van vyf SA populasies en sewe internasionale populasies afkomstig van vier lande, insluitend die VSA, China, Brasilië en Australië is met hierdie agt SSR merkers gegenotipeer. Die studie het bevestig dat *P. citricarpa* populasies in China en Australië 'n groter genetiese diversiteit het as die populasies in Suid-Afrika en in die VSA waar meer onlangse introduksies voorgekom het. Paringstipe analise het getoon dat beide paringstipes in Suid-Afrikaanse populasies in 'n 1:1 verhouding teenwoordig is. Befondsing vir hierdie projek is in Maart 2014 getermineer. Die finale analises en die opskryf as 'n PhD tesis is in die proses. 'n Finale verslag sal in April 2017 ingehandig word.

Introduction

The fungal pathogen, *Phyllosticta citricarpa* Kiely (anamorph *Phyllosticta citricarpa* (McAlpine) Aa), is the causative organism of the economically important disease known as Citrus Black Spot (CBS). CBS has an almost worldwide distribution, but is currently not known to occur in Europe, Chile, Japan and New Zealand. In the countries where CBS does occur, it often does not occur in all the production areas (Truter, 2010; Carstens *et al.*, 2011; Carstens *et al.*, 2012; Miles *et al.*, 2013). Some of South Africa's most important trading partners such as the European Union, Japan, United States of America, India, Iran and Reunion have identified CBS as being of quarantine importance. Although *P. citricarpa* has been known since 1895 (Benson, 1895) and was first reported in South Africa in 1929 (Doidge, 1929), almost no information is available on the global population structure, and no studies have been conducted on the genetic variability of South African populations. Currently, it is believed that this pathogen has originated with its host from South East Asia (Smith *et al.*, 1997) from where several migrations across the globe occurred. This hypothesis, however, requires investigation using a population genetics approach.

Although the epidemiology of the CBS disease in SA and other countries has been studied extensively (Kotzé, 1963, 1981, 2000; Truter, 2010), little is known about the reproductive biology of the pathogen in the world and in South Africa. The fungus can infect fruit, leaves and twigs. Two types of spores can be produced namely waterborne conidia and windborne ascospores. Ascospores are considered to be the main source of infection, but it is not known whether ascospore production is through a homothallic or heterothallic mechanism.

Objectives

1. To determine how many genetically differentiated populations of *P. citricarpa* exist in the world and to determine if migration or introductions (trade) have influenced the population structure of this pathogen.
2. To determine the mating system of the pathogen in South Africa.
3. To determine the relative contribution of sexual and asexual spores to disease development in different climatic regions in South Africa.

Materials en methods

To determine how many different genetically differentiated populations of *P. citricarpa* exist in the world and to determine if migration or introductions (trade) have influenced the population structure of this pathogen

Samples will be collected from different geographical areas in six different continents, three orchards per continent, including South Africa. Symptom descriptions will also be included in the study. International collaborators will assist with sampling. A total number of samples in the order of 300- 400 are envisioned.

Since there are currently no polymorphic markers for use in population genetic studies in *P. citricarpa*, new markers will be developed. Firstly, sequence data from known gene regions will be investigated for polymorphisms. If this approach does not yield polymorphic markers, SSR markers will be developed using next generation sequencing. The data will be analysed using several different population genetics and phylogenetic software programs such as GenALEX and R package Poppr.

To determine the mating system of the pathogen in South Africa

In order to determine the reproductive biology of the pathogen in South Africa, *P. citricarpa* isolates of seven populations will be tested following a PCR based strategy to determine the mating system. The *P. citricarpa* mating types (MAT 1-1-1 and MAT 1-2-1) was recently identified (Wang, *et al.*, 2013).

To determine the relative contribution of sexual and asexual spores to disease development in different climate regions in South Africa

Three orchards will be selected where isolates will be collected in two consecutive years. The populations will be characterized by SSR genotyping, to determine whether clonal isolates can be detected between seasons and whether populations are randomly mating. The mating types of isolates will also be determined to investigate whether frequency dependent selection for mating types is occurring.

The contribution of sexual and asexual reproduction may lead to future investigations into improving the predictions in the onset of the disease as well as developing improved control strategies.

Results and discussion

Objective/milestone	Achievement
To determine how many genetically differentiated populations of <i>P. citricarpa</i> exist in the world and to determine if migration or introductions (trade) have influenced the population structure of this pathogen.	Fifty seven SSR primer pairs were designed of which eight were shown to amplify polymorphic loci when tested on a subset of ten international isolates. Eight of the markers were used to genotype 388 <i>P. citricarpa</i> isolates from five SA populations and seven international populations from four countries including USA, China, Australia and Brazil. The genotype data were analysed using the programs, Genemapper (Applied Biosystems), GeneAlex 6.5 and R package Poppr. A draft article on these results is in preparation.
To determine the mating system of the pathogen in South Africa	Five of the seven South African <i>P. citricarpa</i> populations have been tested following a PCR based strategy to determine the mating type system.
To determine the relative contribution of sexual and asexual spores to disease development in different climate regions in South Africa	Sixty of the 400 <i>P. citricarpa</i> isolates that were collected over two seasons from two lemons orchards in two different production areas namely Mpumalanga and North West provinces were genotyped with the eight markers that were developed.

Very low levels of sequence polymorphisms were obtained when four known gene regions (Chitin synthase I, Calmodulin, second largest subunit of RNA polymerase II and β -tubulin) were sequenced in a subset of *P. citricarpa* isolates. As these markers could not be used for population genetic analyses, SSR markers were developed using next generation sequencing techniques. A South African *P. citricarpa* isolate was sequenced using the Ion Torrent Personal Genome Machine. These short sequences were assembled using the Torrent Suite 2.2 and a total of 123 contigs were sorted according to size using Galaxy (Giardine *et al.*, 2005) and were mined for microsatellites. Fifty seven primers pairs were designed using the online tool, BatchPrimer3 and tested for amplification success and yielding polymorphic loci. To determine the level of polymorphisms, a subset of ten isolates, representing three countries, was used. Eight loci were shown to be polymorphic. The markers amplified loci containing dinucleotide repeats to pentanucleotide repeats, with half of the primers amplifying trinucleotide repeats. The markers were all selectively neutral according to the Ewens-Watterson test (Yeh *et al.*, 2000). A total of 388 *Phyllosticta* isolates (12 populations from five countries) were genotyped with the eight SSR markers. Data was analysed using Genemapper (Applied

Biosystems). GeneAlix v6.5 (Peakall and Smouse, 2012) and R package Poppr (Kamvar *et al.* 2014; R Core Team, 2013) to determine the genetic and genotype diversity amongst and within populations. To determine the relationships among populations, minimum spanning networks were constructed based on Bruvo's distance using the R package Poppr. The allele and allele richness for each population was determined using HP-RARE to account for populations with different sample sizes (Kalinowski, 2005). The populations from China contained the highest gene diversity. Analysis of molecular variance (AMOVA) showed that there was significant sub-structuring among countries and within populations. Principal component analyses (PCA) differentiated the Chinese populations from all other populations. Estimates of pairwise Φ_{ST} values supported the PCA results and revealed a significant and high genetic differentiation between the Chinese populations and those from other continents. The low genetic diversity among the five South African *P. citricarpa* populations and in the USA population is an indication of founder populations.

In order to determine the mating system of the pathogen in South Africa, isolates from five of the seven South African populations were tested. Both mating types were found to be present in the five populations in a ratio that did not deviate significantly from 1:1.

In order to determine the relative contribution of sexual and asexual spores to disease development in different climate regions in South Africa, fruit samples were collected in two lemon orchards in Mpumalanga and North West Provinces. DNA was extracted from 400 isolates. To date, sixty of the *P. citricarpa* isolates from one orchard was genotyped with the eight markers that were developed.

Conclusion to date

Eight polymorphic markers were developed using next generation sequencing techniques. Twelve populations were genotyped with the eight markers. Analysis of molecular variance (AMOVA) showed that there was significant sub-structuring among countries and within populations. The populations from China contained the highest gene diversity. The low gene diversity of the South Africa populations and in the USA population is an indication of recent introductions (founder populations), which is consistent with the first report of the pathogen in South Africa in 1929 and in the USA in 2010.

Technology transfer

Oral presentations:

8th Citrus Research International Symposium - Use of simple sequence repeat (SSR) markers to evaluate genetic diversity among South African isolates of *Phyllosticta citricarpa*. Carstens E, Linde CC, Slabbert R, Langenhoven S, Schutte T, Fourie P, Adele McLeod A. (2014). 8th Citrus Research International Symposium, Champagne Sports Resort, Central Drakensberg, South Africa

Friday Forum – Department Plant Pathology, University of Stellenbosch: The global population structure and reproductive biology of the fungal pathogen, *Phyllosticta citricarpa* Kiely

Australian National University, Canberra, Australia – R package Poppr Course: The global population structure and reproductive biology of the fungal pathogen, *Phyllosticta citricarpa* Kiely

Further objectives and work plan

The eight SSR markers developed could not effectively distinguish among the MLGs, which was evident from the fact the same MLGs occurred in multiple countries and isolates from the same MLG had different mating types (*MAT1-1* and *MAT1-2*). More loci are thus needed to reach saturation of the markers that will allow efficient differentiation of MLGs. Therefore, we decided to genotype all of the populations with seven additional SRR markers recently developed by another research group.

Future work will also include the writing up of research results as a thesis and scientific articles.

Quarterly milestones for Apr-Jun, Jul-Sep, Oct-Dec 2016 and Jan-Mar 2017

April – June: Writing up these research results as a thesis and scientific articles

July – Sept: Writing up these research results as a thesis and scientific articles.

Oct - Dec: Writing up these research results as a thesis and scientific articles.

Jan – Mar: Writing up these research results as a thesis and scientific articles.

References cited

- Carstens E, Le Roux HF, Van Rooyen L, Coetzee J, Wentzel R, Schutte GC, Laubscher W, Dawood Z, Holtzhausen MA, Fourie PH, Hattingh V (2011) 47th SASPP Congress, Kruger National Park, South Africa.
- Carstens E, HF le Roux, MA. Holtzhausen, L van Rooyen, J Coetzee, R Wentzel, W Laubscher, Z Dawood, E Venter, GC Schutte, PH Fourie, V Hattingh (2012) Citrus black spot is absent in the Western Cape, Northern Cape and Free State Provinces. *South African Journal of Science* 108(7/8): 56-61.
- Doidge EM (1929) Some diseases of citrus prevalent in South Africa. *S. Afr. J. Sci.* 26: 320–325.
- Benson A. (1895) Black spot of the orange. *Agricultural Gazette of New South Wales* 6: 249.
- Giardine B, Riemer C, Hardison RC, Burhans R, Elnitski L, Shah P, Zhang Y, Blankenberg D, Albert I, Taylor J, Miller W, Kent WJ, Nekrutenko A (2005) Galaxy: a platform for interactive large-scale genome analysis. *Genome Research* 15: 1451 – 1455.
- Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Molecular Ecology Notes* 5: 187–189.
- Kamvar ZN, Tabima JF, Grünwald NJ (2014) Poppr: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2:e281.
- Kotzé JM (1963) Studies on the black spot disease of citrus caused by *Guignardia citricarpa* Kiely, with particular reference to its epiphytology and control at Letaba. D.Sc. (Agric.) thesis. University of Pretoria.
- Kotzé JM (1981) Epidemiology and Control of citrus black spot in South Africa. *Plant Disease* 65: 945–950.
- Kotzé JM (2000) Black spot. In: *Compendium of Citrus Diseases*, Whiteside JO, Garsney SM and Timmer LW, eds., The American Phytopathological Society Press, St. Paul.MN: 23-25.
- Miles AK, Tan YP, Tan MK, Donovan NJ, Ghalayini A, Drenth A (2013) *Phyllosticta* spp. on cultivated Citrus in Australia. *Australasian Plant Pathology* 42: 461-467.
- Nei M (1973) Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences, USA* 70: 3321-3323.
- Peakall R and Smouse PE (2012) 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28, 2537-2539.
- R Core Team (2013) R: A language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. <http://www.R-project.org/>
- Truter M (2010) Epidemiology of citrus black spot disease in South Africa and its impact on phytosanitary trade restrictions. PhD. Thesis. University of Pretoria.
- Wang N-Y, Rollins JA, Dewdney MM (2013) Characterization of the mating-type locus (MAT) of *Guignardia citricarpa*, the fungal causal agent of citrus black spot. *Phytopathology* 103: S2. 156.
- Yeh FC, Yang R, Boyle TJ, Ye ZM, Xiyang J (2000) POPGENE 32, Microsoft Windows-Based Freeware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre, University of Alberta; Edmonton, Canada.
- 4.6.5 **FINAL REPORT: Improving the retention of suspension liquid phosphonate fungicides on citrus fruit and leaves**
Project 1012 (April 2011- March 2014) by G.C. Schutte, C. Kotze, M.C. Pretorius (CRI)

Opsomming

'n Duplikaat veld- en laboratoriumproef is in 2014 uitgevoer vir publikasie doeleindes. Ongelukkig het dit geen resultate opgelewer nie omrede daar probleme met die lugversorger was in die vertrek waarin die laboratorium gedeelte uitgevoer was. Die proef is herhaal gedurende Julie en Augustus 2015 en 'n finale verslag sal in 2016 gelewer word.

Summary

A duplicate field and laboratory trial was executed in 2014 for publication purposes. Unfortunately no results could be obtained because we had problems with a faulty air conditioner in the room where the trial was conducted. The trial has been repeated during July and August 2015 and a final report will be drafted for 2016.

Introduction

Suspension liquid (SL) formulations like phosphonates are registered as foliar applications on citrus for the control of Phytophthora induced diseases for instance, but they do have a variety of phytotoxicity problems. Apart from field trials using different rates with different spray intervals to establish effective spray programmes, no one has ever looked scientifically at the target areas (leaves and fruit) and how SL fungicides adhere to the surfaces of citrus leaves and fruit and if adjuvants can increase/decrease their retention. Experiment 918 showed that fluorometry analyses can serve as an indicator of the deposition quantity and quality of residues on citrus fruit and leaves when SC formulations of copper and dithiocarbamates are compared with WP formulations. Residue analysis was done simultaneously with good effect and the variation of residues on citrus fruit and leaves was less if compared with fluorometry.

From associated studies (Fourie *et al.* 2008), we know that run-off curves on leaves and fruit of different ages and cultivars differ significantly. Knowledge of this aspect is vital in customised development of adjuvants, as over- or under-dosing for a given spray volume might respectively lead to excessive run-off or no additional benefit. Likewise, for a given adjuvant concentration, a pronounced difference in the adjuvant effect is experienced with varying spray volumes, especially on hydrophobic surfaces. Through the use of the deposition assessment protocol using fluorometry, digital photomacrography and image analyses the effect of adjuvants in tank mixtures with phosphonates on run-off and spray deposition can be studied. By using both fluorescent pigment deposition analyses and residue analyses of the same samples, one can determine the deposition quantity and quality as well as degradation of phosphonates over time under natural conditions.

Turrell (1961) studied the growth of the photosynthetic area of Valencia orange trees over a 29-year period. The number, size and distribution of leaves on Valencia orange (*Citrus sinensis*) trees of various ages were determined. The trees used were 3, 6, 12 and 29 years old and 3 to 5 meter in height and 7 to 15 meter in circumference. He determined that the increase in the total leaf number with tree age is given by an equation where N is the estimated number of leaves, c_1 and n_1 are constants, and α is the age of the tree in years:

$$\text{Log } N = 3.613 + 1.249 \log \alpha$$

Therefore, if this equation is proved to be useful to determine the amount of leaves of Valencia orange trees of a certain age and the amount of spray mix that each leaf should receive and correlate that with the actual %Fluo that it did receive, then one can see if adjuvants can contribute in the adhesiveness of phosphonates (and other fungicides) onto citrus leaves and fruit.

Objectives

The objective of this study is to determine if deposition quantity and quality and retention of phosphonates can be increased/improved using different phosphonate + adjuvant combinations. Additionally, the effect of these combinations on potential phytotoxicity will be evaluated.

Materials and methods

Laboratory trial

a) Quantitative spray deposition

Fresh Valencia orange leaves were picked from trees at Crocodile Valley Co. and brought to CRI. Here the leaves (three replicates; $n = 5$) were mounted onto a steel frame with a steel grid (4m x 40 cm) using stainless steel clamps spaced at equal distances from each other with leaves slanted at a 60° angle. Phosphonates X and Y were mixed in water taken from taps at Crocodile Valley Citrus Co. and made up registered rates in 1 L glass bottles. A gravity feed mist spray gun was mounted onto a compressor and a spray gun (ITW DEVILBISS Spray Equipment Products, 195 Internationale Blvd, Glendale Heights IL 60139 USA) with a fluid nozzle tip of 0.8 mm in diameter in a laboratory with post-run-off volumes (*circa* 3 ml per

leaf/fruit) with selected phosphonate formulations and varying concentrations of selected adjuvants (2x and 1x). Yellow Fluorescent pigment (400 g/L, EC, South Australian Research and Development Institute, Loxton SA 5333 Australia) at 1 ml/L was added. The spray gun was mounted onto a metal frame at a distance of 60 cm from the target with a spray angle of 90° relative to the target. The phosphonates were sprayed at 1, 2, 3 and 5 ml per leaf, first upper and then lower side and left to dry vertically after each application before they were removed for deposition assessment.

The leaves were then illuminated using a Labino Mid-light (UV-A; ≈365 nm) and digital photos were taken of upper and lower surfaces of leaves and fruit using a Canon EOS 40 D camera equipped with a 50 mm macro lens. Spray deposition assessment involved digital image analyses with Image-Pro Plus version 6.2 software to determine deposition quantity of the fluorescent pigment particles. Deposition quantity analysis involved the measurement of the area covered by pigment particles, expressed as a percentage of total fruit and leaf area. Data were subjected to analysis of variance and Student's T-test for least significant difference ($P = 0.05$).

b) Residue analyses

After the deposition assessment, the same leaves were put plastic bags and frozen and submitted for PO_3 analysis (Absolute Science, Silverton, Pretoria, South Africa). Up to date no results were obtained from the laboratory and will only be available at a later date.

Methods to determine loss of spray drift

a) Black paper

Black paper (24 x 24 cm) were cut to fit the light box and then sprayed with yellow fluorescent pigment. After the pigment dried off, the paper was illuminated in the dark room to determine how spray coverage would look under UV light and if it can be used to determine loss of spray material.

b) White paper

A3 white paper was mounted and sprayed with Fuschin with and without leaves that were mounted in front of the paper. Interesting spray patterns were observed, with some Fuschin spray drift that extended over the 24 x 24 cm borders for the light box. This is still not a technique to determine the amount of spray drift that get lost after 0.5 and 1 ml spray application.

Field trial

2014 field trial

A Navel orange (*Citrus sinensis* (L.) Osbeck) orchard on 'Cleopatra' mandarin rootstock (*C. reshni*) at Crocodile Valley Citrus Co., Nelspruit, was selected. The trees were 17 years old and c. 4 m high. The rows ran directly north to south. Trees were selected for uniformity in canopy density and tree size. Fungicides were applied on 2 July 2013 with a Jacto Arbus 2000 airblast sprayer. One treatment consisted of a standard rate as generally operated by the Estate was applied for high volume application. For low volume application the same spray machine was used and calibrated to deliver 2 500 l/ha operated at 10 bar at 3 km/h using one set of Teejet D3 with dc25 hollowcone whirlers alternated with another set of Teejet D2 with dc35 hollowcone whirlers. For full cover sprays (about 8 000 l/ha), a trailer-mounted, high-volume, high-pressure (2 500 to 3 000 kPa) sprayer with two hand-held spray guns were used to the point of run-off. The fungicide tested was phosphorous acid (Fighter, 200 SL 555 g/l Ag Chem Africa, Silverton, South Africa). Hundred milliliters SARDI Yellow Fluorescent Pigment (40% EC; South Australian Research and Development Institute, Loxton SA 5333 Australia) per 100 liter water was added to the spray mixture of each fungicide formulation.



Fig. 4.6.5.1. Jacto Arbus 2000 airblast sprayer applying Fighter at low volume applications.

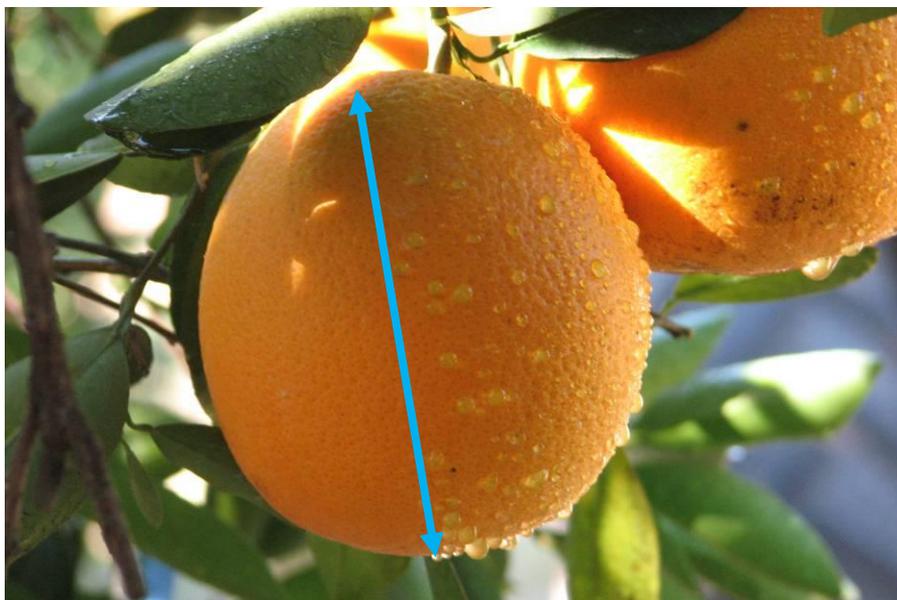


Fig.4.6.5. 2. Low volume application of Fighter with SARDI fluorescent pigment showing coverage of one half of Navel fruit only.

Fruit for bio-assay

Twenty mature Navel oranges were randomly harvested from all azimuths 1 and 2 m above ground height from the outside circumference of each tree on a weekly basis from the day of application for two weeks. They were taken to CRI where they were subjected to a ridged test where fruit were marked to distinguish between the protected and unprotected areas using a UV light to illuminate the SARDI yellow fluorescent pigment using a water resistant permanent marking pen (Fig. 4.6.5.3). The fruit were subjected to an inhouse method to determine the protection capacity of algaecides against *Phytophthora* as previously described (Fig. 4.6.5.4).

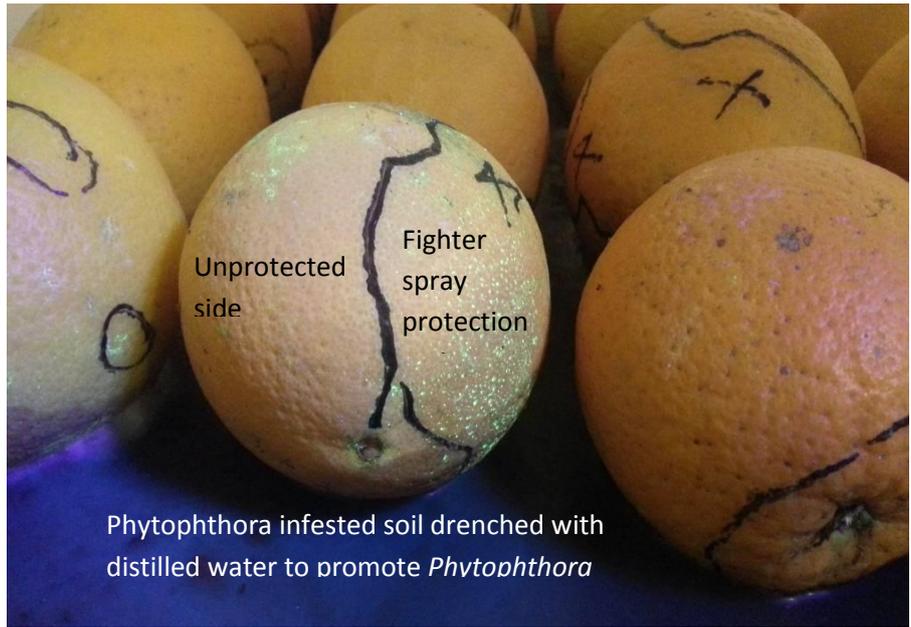


Fig. 4.6.5.3. Navel oranges sprayed with Fighter and SARDI yellow fluorescent pigment to distinguish between protected and unprotected sides of the fruit.

The fruit was placed in the water in such a manner that at least one half of the fruit with and without protection was exposed to the water containing *Phytophthora nicotianae* var *parasitica* (Pnp). The soil was tested before hand to contain Pnp using the leaf disc analysis technique and PARP selective media. The fruit was inspected on a daily basis for Pnp infection and was also sub-divided into two criteria viz. infection on and outside the protected zones (Fig. 4.6.5.4). The trial was terminated after two weeks due to problems with the aircons.



Fig. 4.6.5.4. Evaluation of Navel fruit after 7 days of exposure to infested soil with Pnp showing protective activity after spray application with algaecides.

Results and discussion

Objective / Milestone	Achievement
1. Characterization of spray deposition with full cover spray application in a field trial	One orchard spray trial was conducted.

2. Characterization of spray deposition on detached leaves in a lab. trial using different spray volumes per leaf.	Retention and quantitative deposition of these phosphonates were good if they were sprayed on their own.
3. New objectives	Determine what portion of spray mix is lost Compare light cover applications with medium cover field applications Test fruit thereafter for their susceptibility for <i>P. nicotianae</i> Determine the protection period for phosphonates Determine spray volumes of less than 1ml/leaf Are green and small fruit susceptible for brown rot?

Conclusion to date

Quantitative spray deposition analysis of phosphonate X and Y showed that adjuvants A and B did not enhance their spray deposition. Residue-analysis will confirm this. More than 0.5 ml spray application per side per leaf or 1 ml per whole leaf is required for sufficient spray coverage of Valencia leaves with phosphonates. Of the two adjuvants tested, adjuvant B is the only one that shows promise. Retention of phosphonate X on its own also performed better than phosphonate Y as the spray deposition of phosphonate X showed an increase even at 5 ml/leaf/upper and lower side. After one year's trial, it was found that Fighter applied as low volume applications at a rate of 285ml/100 L water performed well for the control of Phytophthora brown rot of citrus. However, it must be noted that the application must be done at least 3 weeks before harvest to be effective.

2014 field trial

No results could be obtained because we experienced problems with the air conditioner in the room that was used for the trial. The trial was terminated after two weeks and was repeated during the 2015 season.

Technology transfer

Lecture at 2014 citrus symposium and study groups

Future research

a) Phytophthora

Protocols will be used to determine the persistence of SL phosphonate formulations under natural conditions and to determine if growers should use either high volume or low volume applications to achieve effective control of Phytophthora root rot and brown rot. This project was expanded to include two field trials for biological testing (June 2013 and 2014) with which we can screen new products or combinations thereof with adjuvants. With the formula to calculate the amount of leaves per tree per tree age, one can confirm if this can be used by lowering the spray volume to light cover sprays. Their efficacy on fruit will however be determined using biological dip test using *P. nicotianae* as test organism.

References cited

- Brink, J.C., Holz, G., Calitz, F.J., & Fourie, P.H. 2004. Development of a protocol to quantify spray deposits of grape bunches. Pages 230-235 in: *Proceedings of the 7th International Symposium of Adjuvants for Agrochemicals (ISAA2004)*. Cape Town, South Africa, 8-12 November.
- Brink J.C., Holz, G., & Fourie, P.H. 2006. Effect of fungicide spray cover on *Botrytis cinerea* infection in grape bunches. *South African Journal of Enology and Viticulture* 27: 51-56.
- Fourie, P.H., Du Preez M., Brink, J.C., Schutte G.C. 2009. The effect of run-off on spray deposition and control of *Alternaria* brown spot of mandarins. *Australasian Plant Pathology* 38: 173-182.
- Furness, G.O., Thompson, A.J. & Manktelow, D.W.L. 2006a. Visual droplet number rating chart and fluorescent pigment sprays to estimate chemical deposition and spray coverage on plant foliage. Proceedings of the Association of Applied Biologists' conference for International advances in pesticide application. Robinson College, Cambridge, 10-12 January 2006.
- Furness, G.O., Thompson, A.J., & Manktelow, D.W.L. 2006b. Multi-fan spray towers to improve dose efficiency and spray coverage uniformity in citrus trees. Proceedings of the Association of Applied Biologists' conference for International advances in pesticide application. Robinson College, Cambridge, 10-12 January 2006.
- Grout, T.G. 1997. Spray volumes and coverage requirements for citrus in southern Africa. *Citrus Journal*

6(3): 19-20.

Landers, A., & Farooq, M. 2004. Factors influencing air and pesticide penetration into grapevine canopies. *Aspects of Applied Biology* 71, International advances in pesticide application.

Orbovic, V., Achor, D., & Syvertsen, J.P. 2007. Adjuvants affect penetration of copper through isolated cuticles of *Citrus* leaves and fruit. *HortScience* 42: 1405-1408.

Salyani, M., & McCoy, C.W. 1989. Deposition of different spray volumes on citrus trees. *Proc. Fla. State Hort. Soc.* 102: 32-36.

Turrell, F.M. 1961. Growth of the photosynthetic area of citrus. *Botanical Gazette* 284-298.

4.6.6 **PROGRESS REPORT: Epidemiology and pest risk assessment of *Phyllosticta citricarpa***
Project 1026 (April 2011 - March 2015) by Paul Fourie, Vaughan Hattingh and Tian Schutte (CRI)

Opsomming

Sitrus swartvlek is die belangrikste sitrus-siekte in Suid-Afrika, veral gegewe sy impak op marktoegang. Baie aandag en *ad hoc* navorsing is onlangs hieraan gespandeer. Hierdie projek formaliseer die *ad hoc* navorsing en sal fokus op die ontwikkeling en verbetering van 'n model vir *Phyllosticta pseudotesium* rypwording en spoorvrystelling gebaseer op meso- en mikroklimate data. Op hierdie onderwerp is aanvanklike modelering afgehandel en 'n artikel in 'n toonaangewende wetenskaplike joernaal gepubliseer. Verder is sekere CRI navorsers betrokke in 'n samewerkingsprojek wat deur die Florida sitrusbedryf in VSA befonds word. Hierdie doelwit beoog om 'n kwantitatiewe pes risiko analiese vir *Phyllosticta citricarpa*, met spesifieke fokus op vars vrugte as verspreidingsweg, te ontwikkel. Twee verdere werksinkels is gehou, en die model is gefinaliseer. 'n Wetenskaplike publikasie word tans geskryf. 'n Kwantitatiewe PCR metode om tussen die swartvlek patogeen en 'n endofitiese *Phyllosticta* sp. te onderskei, is ge-optimeer, maar was nie voldoende akkuraat sodat voortgegaan kan word om die versamelde filtreerpapier spoorvangers te analiseer nie. Verdere verbeterings word aan die PCR tegniek gedoen en vorm deel van 'n nuwe RCE befondsde projek.

Summary

Citrus Black Spot is the most important citrus disease in South Africa, specifically given its impact on market access. A considerable amount of effort and *ad hoc* research is conducted on an ongoing basis to service market access to these markets. This project formalises the *ad hoc* research and will focus on developing and improving a model for *Phyllosticta pseudotesium* maturation and ascospore dispersal based on meso-climatic weather data. On this topic, initial modelling research was completed and an article published in a leading scientific journal. Additionally, three CRI-researchers are collaborating on a project funded by the Florida citrus industry in USA to develop a quantitative pest risk assessment of *Phyllosticta citricarpa*, with special emphasis on the fresh fruit pathway. Two further workshops were held in Florida and the model was finalised. A scientific paper is being prepared. Quantitative PCR methods to distinguish between the CBS pathogen and endophytic *Phyllosticta* sp. have been optimised, but further improvements are required before collected filter paper spore trap discs can be analysed. This aspect of the study will continue as part of a new RCE funded project.

Introduction

Citrus Black Spot (CBS) is the most important citrus disease in South Africa as it is regarded as a quarantine pathogen in some of our most important markets, such as the USA and European Union. A considerable amount of effort and *ad hoc* research is conducted on an ongoing basis to service market access to these markets. This has increased in intensity following the recent EFSA critical commentary on SA's proposed pest risk assessment (PRA) and a subsequent imposition of more stringent CBS management / inspection criteria (EFSA, 2008).

Recently, CBS has also been detected in Florida, USA, leading the US pathologists to invite CRI researchers to collaborate on a project aiming at developing a quantitative PRA for the CBS pathogen, *Phyllosticta citricarpa*. This quantitative PRA will be an improvement on SA's qualitative PRA. Moreover, it should also be less disputable since this PRA will be a collaborative effort between renowned scientists from the USA, as well as other countries where CBS occurs, and not only SA scientists as was the case previously.

Part of the above-mentioned proposal will be to identify epidemiological parameters for CBS, assign proportionate risk to fruit being a pathway for introduction of the pathogen, and subsequently to conduct gap-fill research to obtain or validate parameters / estimates. CRI has recently obtained longterm spore trap and meso-climatic weather data from which initial modelling has been attempted with mixed success. Similar spore trap data but with micro-climatic weather data have been collected by local collaborators in the Marble Hall district, and are also being collected in CRI-CBS Exe 919. These data will be used to validate the developed models as well as to determine the epidemiological parameters required for the quantitative PRA.

New objectives proposed: Following the initial modelling research, a scientific paper has been submitted. However, the major weakness of that study was our inability to distinguish between the ascospores of the CBS pathogen, *Phyllosticta citricarpa*, and the endophyte *Phyllosticta capitalensis* (syn. *G. mangiferae*). It has further transpired that we might not be successful in our collaboration attempt to obtain microclimatic and ascospore trap data from the Marble Hall district, which we anticipated to use in refining the models. The latter dataset also does not distinguish between the *Phyllosticta* pathogen and endophyte.

The objectives of this study are therefore:

1. Model *Phyllosticta* pseudothecium maturation and ascospore dispersal based on meso-climatic weather data
2. Quantitative PRA of *Phyllosticta citricarpa*, with special emphasis on the fresh fruit pathway (collaborative project with Florida researchers)
3. Quantitative detection of *Phyllosticta citricarpa* and *P. capitalensis* ascospores on aerial spore traps in three localities.
4. Validate and refine *Phyllosticta* pseudothecium maturation and ascospore dispersal models using new data and new modelling approaches.

Materials and methods

1. Model *Phyllosticta* pseudothecium maturation and ascospore dispersal based on meso-climatic weather data

The proposed study is largely a desktop study using large sets of previously collected data. Methodology will include verification of data, consolidation into required format, descriptive statistics, identification and computation of suitable parameters, multivariate analyses to identify likely parameters and non-linear regression statistics to model trends using these parameters. Published models that were developed for similar pathosystems, such as apple and pear scab (Gadoury and MacHardy, 1982; MacHardy and Gadoury, 1986; Rossi *et al.*, 1999, 2001, 2009) and citrus greasy spot (Mondal and Timmer, 2002; Mondal *et al.*, 2003, 2004), will be initially used.

2. Quantitative PRA of *Phyllosticta citricarpa*, with special emphasis on the fresh fruit pathway (collaborative project with Florida researchers)

Collaboration in Florida research project will involve a bi-annual workshop where CBS environmental parameters and supply chain survival and establishment probabilities will be debated. At these meetings, knowledge gaps will be identified, which will subsequently be researched. Environmental parameters and probabilities will be incorporated in a stochastic model and Monte Carlo simulation with sensitivity analyses to assess the true risk of *G. citricarpa* moving through all steps in the pathway and becoming established in the termination point of the fresh fruit pathway, i.e., new previously uninfected areas. This study will largely be conducted by Dr Tim Gottwald (USDA-ARS, Florida) with South African researchers providing technical support and conducting gap-fill research where required.

3. New objective: Quantitative detection of *Phyllosticta citricarpa* and *P. capitalensis* ascospores on aerial spore traps in three localities.

The volumetric spore trapping at two localities in the Eastern Cape (Exe 919), one locality in Mpumalanga, and two localities in Limpopo provinces was supplemented with filter paper spore traps (Schweigkofler *et al.*, 2004). Filter paper traps were replaced on a weekly basis, and *Phyllosticta citricarpa* and *P. capitalensis* ascospores will be distinguished and identified using quantitative real-time PCR (as developed by USA collaborator, Prof Megan Dewdney, UC-Florida). In collaboration with Prof Dewdney, we are optimising the protocol for detection and quantification of *Phyllosticta citricarpa* and *P. capitalensis* ascospores on filter spore traps.

4. New objective: Validate and refine *Phyllosticta* pseudothecium maturation and ascospore dispersal models using new data and new modelling approaches.

New and existing data will be analysed using a novel window-pane analysis (Kriss et al. 2010) and epidemiological modelling, which incorporates the *Phyllosticta citricarpa* specific data.

Results and discussion

Objective / Milestone	Achievement
1. Model <i>Phyllosticta</i> pseudothecium maturation and ascospore dispersal based on meso-climatic weather data	Article published in Phytopathology.
2. Quantitative PRA of <i>Phyllosticta citricarpa</i> , with special emphasis on the fresh fruit pathway (collaborative project with Florida researchers)	The last meeting of the collaborative project in Florida was held in November 2014. The project is almost concluded and a scientific article is being written.
3. New objective: Quantitative detection of <i>Phyllosticta citricarpa</i> and <i>P. capitalensis</i> ascospores on aerial spore traps in three localities	Filter paper spore traps and weather stations placed at 5 volumetric spore trap locations from September 2012 to end-March 2015. The real-time PCR protocol is being optimised in the USPP labs and University of Florida labs with US collaborator.
4. New objective: Validate and refine <i>Phyllosticta</i> pseudothecium maturation and ascospore dispersal models using new data and new modelling approaches	New modelling approaches did not yield improved dispersal models.

1. Model *Phyllosticta* pseudothecium maturation and ascospore dispersal based on meso- and micro-climatic weather data

Work was concluded and article was published in the March 2013 edition of Phytopathology;

Reference and Abstract:

Fourie, P. H., Schutte, G. C., Serfontein, S., and Swart, S. H. 2011. Modeling the effect of temperature and wetness on *Guignardia* pseudothecium maturation and ascospore release in citrus orchards. *Phytopathology* 103: 281-292.

Ascospores are the most important inoculum source of citrus black spot (CBS), caused by *Guignardia citricarpa*, but pseudothecium maturation and ascospore release are inadequately studied. *Guignardia* ascospore trapping and concomitant weather data were obtained for three localities over three seasons (July through March, 2006 to 2009) in the Limpopo province of South Africa. Degree-days accumulated until first seasonal ascospore discharge ($> 10^{\circ}\text{C}$ with 1 July as biofix; DDtemp), and DDtemp accumulated on rainy (rainfall > 0.1 mm; DDrain) and moist days (vapour pressure deficit < 5 hPa; DDvdp) were used in two Gompertz models to predict onset of ascospore release: a temperature model [Event = $\exp(-\exp(-(-2.725 + 0.004 \times \text{DDtemp})))$] and a temperature/moisture model [Event = $\exp(-\exp(-(-3.238 + 0.008 \times \text{DDvdp} + 0.004 \times \text{DDtemp} - 0.009 \times \text{DDrain})))$] ($R^2 = 0.608$ and 0.658 , respectively). Both models predicted a delay in pseudothecium maturation in climates with colder winters and springs. A Gompertz equation was also used to predict the proportion of *Guignardia* ascospores trapped (PAT) per season from DDtemp data accumulated on wet or moist days (DDwet2) from the first seasonal ascospore discharge [PAT = $\exp(-4.096 \times \exp(-0.005 \times \text{DDwet2}))$; $R^2 = 0.908$]. The PAT-model predicted lag phases and 7-day peaks in ascospore release patterns with reasonable accuracy. These models can be used to predict the onset and dynamics of ascospore release in climatically diverse regions.

2. Quantitative PRA of *Guignardia citricarpa*, with special emphasis on the fresh fruit pathway (collaborative project with Florida researchers)

The collaborative project with Dr Tim Gottwald (USDA, Florida), entitled “Assess the Viability of Black Spot-blemished Citrus Fruit as a Pathway for Disease Dispersal” was approved by Florida funding agency in February 2011. Collaborator conferences were again held in February 2014 and in November 2014. At each meeting, the model was reviewed, and gapfill research results added. The research outcomes will be invaluable to support market access negotiations with the EU. The project is almost concluded and a scientific article is being written. The article will be included in the final report of this project, to be submitted in 2016.

3. **New objective:** Quantitative detection of *Phyllosticta citricarpa* and *P. capitalensis* ascospores on aerial spore traps in three localities.

In collaboration with CRI-Nelspruit, QMS Agri Science and SRCC, filter paper spore traps were placed out in orchards at volumetric spore traps in Hoedspruit, Letsitele, Nelspruit, Addo and Kirkwood areas from end 2011 to March 2015. These were collected on a weekly basis and are presently cold-stored at USPP until processing. Unfortunately, the DNA extraction protocol could not be optimised and these traps could not yet be processed.

The protocol for detection and quantification of *Phyllosticta citricarpa* (old name is *Guignardia citricarpa*) and *P. capitalensis* ascospores was received from our USA collaborator, Prof Megan Dewdney, UC-Florida in April 2012 only, as she had to change it from the initially planned multiplex PCR to two separate reactions for the two species. The protocol is being optimised in the USPP labs and in collaboration with Prof Dewdney's lab.

DNA was extracted from *P. citricarpa* and *P. capitalensis* freeze dried mycelia with a Qiagen PlantMini kit (Qiagen, Limburg, Netherlands). DNA was diluted to $20 \text{ ng} \cdot \mu\text{L}^{-1}$, and a qPCR standard curve was successfully established for both the *P. citricarpa* and *P. capitalensis* assays from this DNA (Fig. 4.6.6.1). The qPCR reactions consisted of $4.3 \mu\text{L}$ water, primers (forward and reverse 560 nM each), probe (FAM for *P. citricarpa* or Cy5 for *P. capitalensis* 560 nM), Sensimix probe $12.5 \mu\text{L}$ and DNA template $4 \mu\text{L}$ with a final volume of $25 \mu\text{L}$. The cycling conditions were 95°C for 10 min, 95°C for 0.5 min and 59°C for 1 min with 50 cycles. The assay was successfully optimized for mycelial DNA, and the limit of detection (LOD) is 50 fg .

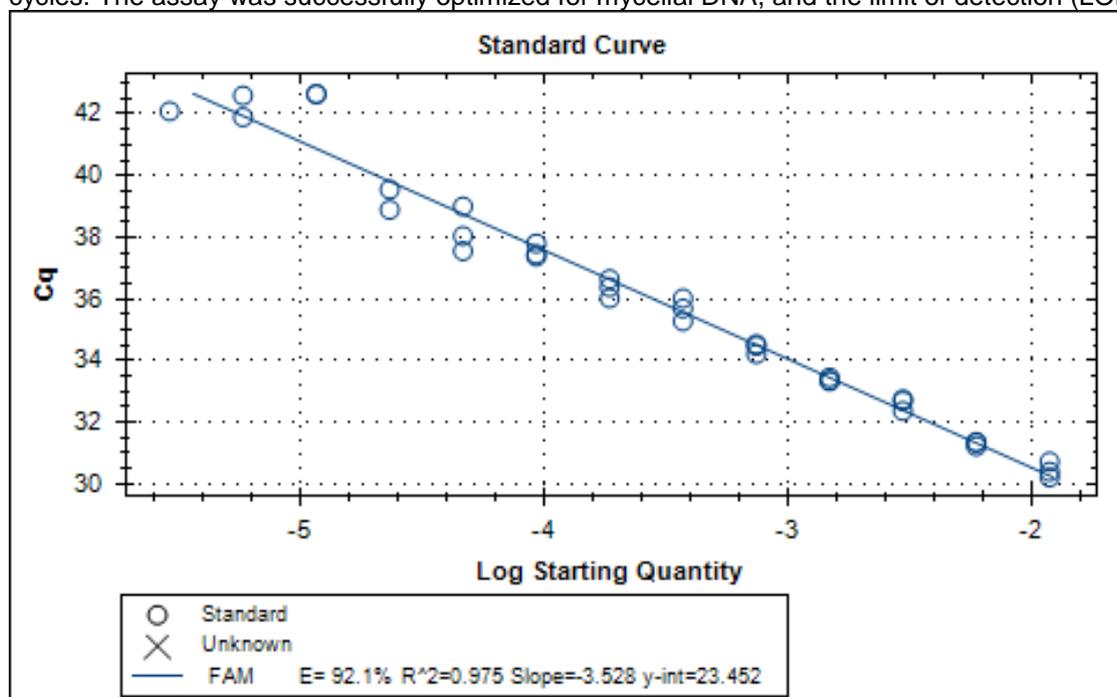


Fig. 4.6.6.1. qPCR standard curve for mycelial DNA of *P. citricarpa* ranging from $20 \text{ ng} \cdot \mu\text{L}^{-1}$ to $50 \text{ fg} \cdot \mu\text{L}^{-1}$

Spore suspensions were made up from *P. citricarpa* cultures grown on PDA. Cirri from pycnidia were observed and pycnidiospores were obtained by removing cirri by washing it from the culture with a pipette and sterile dH_2O , then poured through three layers cheesecloth. The pycnidiospores were placed in a 1.5 mL Eppendorf tube containing 1 mL sterilized water. Spore dilutions were made from this initial 6.33×10^6 or 1.95×10^5 spores. mL^{-1} suspension. Spores were placed onto filter paper sporetraps by pipetting $400 \mu\text{L}$ of spore suspension onto each trap. There were 4 repetitions for each spore concentration. Several DNA extraction methods were tested, including AMPure XP, Qiagen, MO Bio and Promega DNA extraction kits, as well as a Chelex resin method (Carisse *et al.*, 2009). These methods did not yield consistent results between replications, and an accurate quantification assay could not be developed. The most consistent results were observed with the AMPure XP method, and thus this method was used to draw up 'n standard curve (Fig. 4.6.6.2), but sensitivity of the assay was not low enough for modelling purposes.

Analysis of variance of starting quantity of DNA ($\text{ng} \cdot \mu\text{L}^{-1}$) of spiked spore samples showed that there was a significant interaction between experimental repeat and spore concentration ($P < 0.0001$; Table 1) and between qPCR run and experimental repeat ($P < 0.0001$). This indicates that repeatability of the experiment is not satisfactory. Spore concentration had a significant effect ($P < 0.0001$), which indicates that the assay is able to distinguish between different spore concentrations. The coefficient of variation between 4 reps of the

same spore concentration was calculated (Table 2 and 3). A CV of less than 0.25 is considered acceptable. Especially at lower spore concentrations, the CV was much higher than 0.25, which indicates that the assay is not accurate enough for modelling purposes, especially at low spore concentrations. Future work includes using carrier DNA together with the Ampure XP method to improve repeatability.

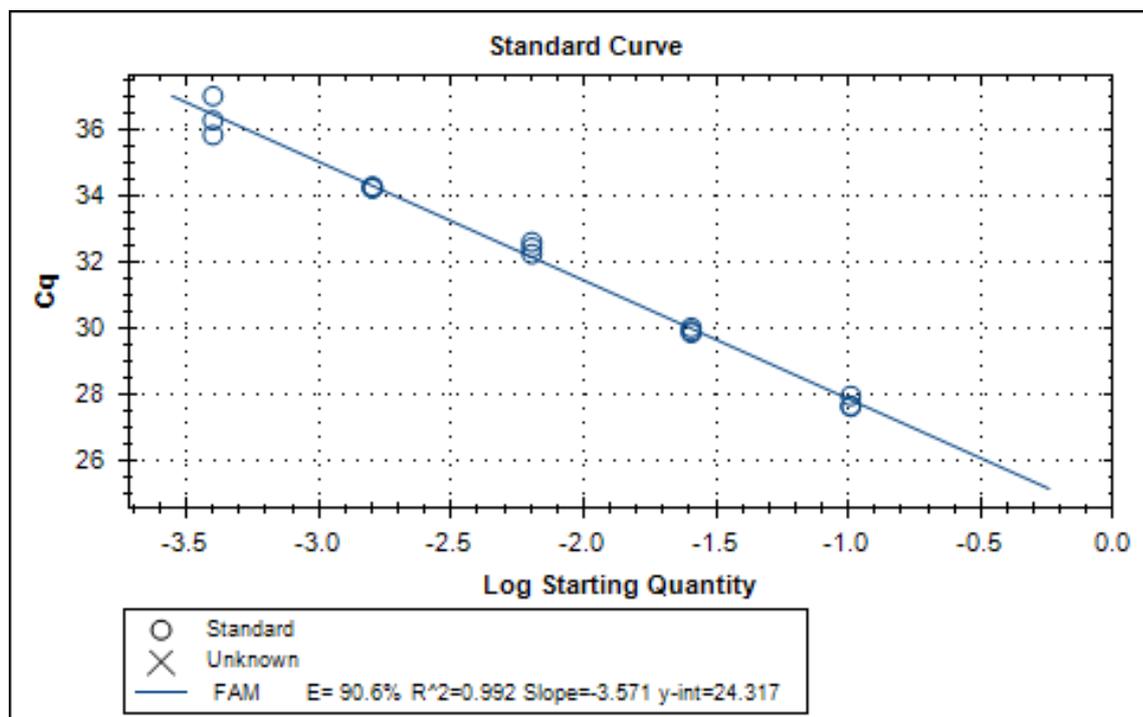


Fig. 4.6.6.2. qPCR standard curve for spore DNA of *P. citricarpa* ranging from 0.1 ng.µL⁻¹ to 0.4 pg.µL⁻¹

Table 4.6.6.1. Analysis of variance of starting quantity (ng.µL⁻¹) as calculated by qPCR of spiked spore traps at 12 different spore concentrations (ranging from 8236 to 195 000 spores) done in 4 repetitions each, with 3 qPCR run technical repeats, and 2 repeats of the experiment.

Source	DF	Mean squares	P-value
Model	71	0.037	< 0.0001
Run	2	0.025	0.007
Repeat	1	0.639	< 0.0001
Spore concentration	11	0.086	< 0.0001
Run*Repeat	2	0.024	0.007
Run*Spore concentration	22	0.002	0.978
Repeat*Spore concentration	11	0.066	< 0.0001
Run*Repeat*Spore concentration	22	0.002	0.981
Error	240	0.005	
Corrected Total	311		

Table 4.6.6.2. Variation coefficient of starting quantity ($\text{ng}\cdot\mu\text{L}^{-1}$) as calculated by qPCR of spiked spore traps spiked with 12 different spore concentrations (ranging from 8236 to 195 000 spores) done in 4 repetitions each, with 3 qPCR run technical repeats, and 2 repeats of the experiment.

Nr of spiked spores	Variation coefficient Repeat 1	Variation coefficient Repeat 2
8236	0.380	0.313
10981	0.313	0.331
14642	0.589	1.011
19522	0.734	0.943
26029	0.398	0.131
34706	0.686	0.192
46274	0.605	0.126
61699	0.569	0.206
82266	0.278	0.245
109688	0.626	0.173
146250	0.440	0.125
195000	0.800	0.186

Table 4.6.6.3. Variation coefficient of starting quantity ($\text{ng}\cdot\mu\text{L}^{-1}$) as calculated by qPCR of spiked spore traps spiked with 12 different spore concentrations (ranging from 47 515 to 632 813 spores) done in 4 repetitions each, with 3 qPCR run technical repeats, and 1 repeat of the experiment.

Nr of spiked spores	Variation coefficient
47515	0.431
63353	0.651
84470	1.376
112627	0.248
150169	0.382
200226	0.399
266968	0.492
355957	0.313
474609	0.260
632813	0.375
843750	0.318
1125000	0.467

Future work involves developing a model from which Ct values from mycelial DNA amplification by qPCR will be correlated with the amount of pycnidiospores from spore DNA amplified by qPCR. This will be used to validate the technique by adding known amounts of spores to filter paper spore traps, which will be subjected to qPCR and quantified by the model. This will be a more robust method, which will address the inconsistent results obtained.

4. New objective: Validate and refine *Phyllosticta* pseudothecium maturation and ascospore dispersal models using new data and new modelling approaches.

Attempts to refine models through collaboration with expert epidemiologists Dr Tim Gottwaldt and his postdoctoral fellow Dr Alissa Kriss have failed. This objective is studied further as part of new RCE funded project, as part of Jacolene Meyer's PhD study.

Technology transfer

- Article published
 - Paul Fourie, Tian Schutte, Suzel Serfontein and Fanus Swart. 2013. Modeling the effect of temperature and wetness on *Guignardia* pseudothecium maturation and ascospore dispersal in citrus orchards. *Phytopathology* 103: 281-292.

- Presentations
 - Paul Fourie, G.C. Schutte, S. Serfontein, S.H. Swart. 2012. Modelling of *Guignardia pseudothecium* maturation and ascospore dispersal in citrus orchards. Oral presentation at the 7th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 19-22 August 2012.
 - Fourie P.H., Schutte G.C., Serfontein S., and Swart S.H. 2012. Modelling of *Guignardia pseudothecium* maturation and ascospore dispersal in citrus orchards. Poster presentation at XII International Citrus Congress, 18-23 November 2012, Valencia, Spain.
 - Paul Fourie, Tian Schutte, Elma Carstens and Vaughan Hattingh. 2014. Is fresh citrus fruit a pathway for Citrus Black Spot into Europe? EFSA's pest risk assessment and technical response by an international expert panel. Oral presentation at 8th CRI Research Symposium, Winterton, 17-20 August 2014.
 - Paul Fourie, Vaughan Hattingh, Elma Carstens, Tian Schutte, Hennie le Roux, Li Hongye, Andrew Miles, Marcel Bellato Spósito, Megan Dewdney. 2014. Citrus Black Spot – a global perspective. Invited presentation at C.L.A.M. General Assembly, Agrotechnical Commission, Madrid, 17 October 2014.
 - Paul Fourie, Vaughan Hattingh, Elma Carstens, Tian Schutte, Hennie le Roux, Lise Korsten, Li Hongye, Andrew Miles, Marcel Bellato Spósito, Megan Dewdney. 2015. Citrus Black Spot: a scientific and political conundrum. Keynote presentation at 49th Congress of the South African Society for Plant Pathology, Bloemfontein, 18-21 January 2015.
- Market Access
 - Research outcomes from this project

Further objectives (milestones) and work plan

CBS epidemiology, and certain of the objectives in this project, will also be studied in new projects:

- Epidemiology, inoculum potential and infection parameters of Citrus Black Spot (PhD study by Mareli Kellerman at Stellenbosch University)
- Epidemiology of CBS in different geographic areas and development of a risk management system for Citrus Black Spot (PhD study by Jacolene Meyer at Free State University)
- The South African Citrus Black Spot Simulation Model; Its Development, Calibration, Verification and Validation on South African and European weather data sets in order to accredit a citrus orchard as being managed to be free of Citrus Black Spot (PhD study by Chris van Ginkel at Free State University)

References cited

- EFSA. 2008. Scientific Opinion of the Panel on Plant Health on a request from the European Commission on *Guignardia citricarpa* Kiely. *The EFSA Journal* 925: 1-108.
- Gadoury, D. M., and MacHardy W. E. 1982. A model to estimate the maturity of ascospores of *Venturia inaequalis*. *Phytopathology* 72:901-904.
- Kriss A. B., Paul P. A., and Madden L. V. 2010. Relationship Between Yearly Fluctuations in Fusarium Head Blight Intensity and Environmental Variables: A Window-Pane Analysis. *Phytopathology* 100: 784-797.
- MacHardy, W. E., D. M. Gadoury (1986): Patterns of ascospore discharge by *Venturia inaequalis*. *Phytopatolgy* 76, 985-990.
- Mondal, S. N., and Timmer, L. W. 2002. Environmental factors affecting pseudothecial development and ascospore production of *Mycosphaerella citri*, the cause of citrus greasy spot. *Phytopathology* 92:1267-1275.
- Mondal, S. N., Gottwald, T. R., and Timmer, L. W. 2003. Environmental factors affecting the release and dispersal of ascospores of *Mycosphaerella citri*. *Phytopathology* 93:1031-1036.
- Mondal, S. N., Howd, D. S., Brlansky, R. H., and Timmer, L. W. 2004. Mating and pseudothecial development in *Mycosphaerella citri*, the cause of citrus greasy spot. *Phytopathology* 94:978-982.
- Rossi, V., Ponti, I., Marinelli, M., Giosuè, S., and Bugiani, R. 1999. Field evaluation of some models estimating the seasonal pattern of airborne ascospores of *Venturia inaequalis*. *J. Phytopathol.* 147:567-575.
- Rossi, V., Ponti, I., Marinelli, M., Giosuè, S., and Bugiani, R. 2001. Environmental Factors Influencing the Dispersal of *Venturia inaequalis* Ascospores in the Orchard Air. *J. Phytopathology* 149, 11-19.
- Rossi, V., Salinari, F., Patteri, E., Giosuè, S., and Bugiani, R. 2009. Predicting the dynamics of ascospore maturation of *Venturia pirina* based on environmental factors. *Phytopathology* 99:453-461.
- Schweigkofler W., O'Donnell K., Garbelotto M. 2004. Detection and Quantification of Airborne Conidia of *Fusarium circinatum*, the Causal Agent of Pine Pitch Canker, from Two California Sites by Using a

4.6.7 **FINAL REPORT: Identifying the fungi that cause CBS-like disease symptoms on citrus fruit**
Project 1088 (2014 – 2017) by GC Schutte, E Basson, C Kotze, Paul Fourie (CRI), T Jensen & S Coertze (SU)

Opsomming

Colletotrichum sp. en geen *Phyllosticta citricarpa* is van letsels soortgelyk aan sitrus swartvlek op pomelos ge-isoleer. Hierdie isolate is toe onderwerp aan molekulere identifikasie deur middel van 'n polimerase ketting reaksie (PKR). Twee spesies, genaamd *Colletotrichum gloeosporioides* en *C. boninense* is hierdeur identifiseer. Olinda Valencia vrugte is toe aan die bome met spoorsuspensies (1×10^7 spore/ml) van beide spesies ge-inokuleer; een stel waar die vrugte gewond is en een stel waar vrugte ongewond gelaat is. Na 4 weke is die vrugte geoes en ondersoek vir letselvorming. Daar is fotos van die letsels geneem en elkeen is gemeet, waarna isolasies vanuit die letsels gemaak is om Koch se postulate te vervul. Daar het slegs letsels op die gewonde vrugte gevorm. Letsels het gevorm op onderskeidelik 65 en 70% van die gewonde vrugte. Duplikaat proewe word in die huidige seisoen uitgevoer waarna 'n finale verslag geskryf sal word.

Summary

Colletotrichum sp., and no *Phyllosticta citricarpa*, were isolated from grapefruit with symptoms similar to that of citrus black spot. These cultures were subjected to molecular identification by means of polymerase chain reaction (PCR). Two predominant species were identified through this process, both belonging to the *Colletotrichum* genus, namely *C. gloeosporioides* and *C. boninense*. Olinda Valencia fruit were inoculated on the trees with a spore suspension (1×10^7 spore/ml) of either species; one set of fruit wounded and the other set left unwounded. After 4 weeks the fruit were harvested and each lesion that developed was photographed, measured and isolations made thereof to fulfil Koch's postulates. Lesions were only observed from the wounded fruit, with 65 and 70% of fruit inoculated with *C. gloeosporioides* and *C. boninense* producing lesions respectively. Duplicate trials will be performed in the current season and a final report will be written.

Introduction

During a recent study, fruit were inspected for citrus black spot (CBS) lesions. Out of the 136 lesions molecularly identified, only 6 were found to be *Phyllosticta citricarpa*, the causal pathogen of CBS. However, from several unidentified lesions *Colletotrichum* spp. was isolated. *Colletotrichum* spp. are known to cause three different anthracnose diseases of citrus: post bloom fruit drop and lime anthracnose both caused by *C. acutatum* and post-harvest anthracnose caused by *C. gloeosporioides*. *C. gloeosporioides* is a primary coloniser of injured and senescent tissue and causes an important post-harvest disease but is incapable of invading healthy tissue.

By definition the term anthracnose is commonly applied to lesions containing acervilli and is in most cases associated with the bruised or injured rind of a citrus fruit. In the case of lime anthracnose the pathogen infects the juvenile tissue of Mexican lime and can in some cases cause localised necrotic lesions of variable sizes. It has further been documented that *Colletotrichum* spp. co-inhabits citrus black spot lesions, but could they be responsible for CBS type lesions? Therefore the reason for the current study, to investigate the ability of *Colletotrichum* to produce CBS like lesions.

Objectives

To collect isolates for identification and to construct pictorial sheets for the identification of CBS-like lesions on citrus fruit confused with CBS.

Materials and methods

Isolation and identification

In a joint CBS project between SA and the USA (project 1026) over two years on Star Ruby grapefruit obtained from GFC at Komatipoort. All the fruit was packed into 20 kg boxes and cold stored at CRI over a period of time. Inspections were done on the day of arrival and two more inspections were done two and four weeks later.

Fruit were inspected for lesions resembled citrus black spot Fig. x1 A-D). All visible lesions were photographed, isolated and identified using PCR to determine the percentage of CBS lesions that came directly from the orchard to the pack house, and lesions that were intercepted in the pack house. Isolations were made from the effected rind onto potato dextrose agar (PDA) and incubated at 25°C for 7 to 14 days. Isolated cultures were sub-cultured and sent to the University of Stellenbosch for identification. Cultures were identified molecularly by internal transcribed spacer region (ITS) sequence analyses. These sequences were subsequently blasted on Genbank for identification.

Inoculation of fruit

Untreated, lesion free fruit were selected from a 27 year old Olinda Valencia orchard at Crocodile Valley Citrus Estate in Nelspruit, Mpumalanga, South Africa. A sealing strip was cut into 1cm x 2cm segments and a hole punched through the centre of each segment, using a cork borer. Holes made had a diameter of ±5mm. Each segment was separately pasted onto 20 randomly selected fruit and inoculated with a 0.25ml droplet containing a 1×10^7 spores/ml spore suspension of either *Colletotrichum gloeosporioides* or *C. boninense*. Of each set of 20, fruit were wounded with an alcohol sterilised dissect needle before inoculation, while the other half was left unwounded. Each set was repeated on four different trees. After inoculation, the sealing strip segments containing the spore concentration was sealed with parafilm® and each fruit enclosed in a plastic bag to replicate a humid chamber (Fig. 4.6.7.2; A-F).

Re-isolation of inoculated fungi

Four weeks (28 days) after inoculation the fruit were collected from the orchard and inspected for lesions in the inoculation area (Fig. 4.6.7.3 A-F). Due to very little lesion formation, the fruit were stored at ambient temperature for an extra two weeks (14 days) where after a second inspection was conducted. Lesions were measured using a calliper, photographed and isolations made. From each lesion four 1mm x 1mm pieces of infected rind were isolated onto PDA and incubated at 25°C for 7 to 14 days. Cultures isolated were then initially identified morphologically to determine if molecular identification will be necessary.

Results and discussion

Objective / Milestone	Achievement
Apr – Jun 2014 2. Collect fruit samples from packing houses in Nelspruit, Karino and Komatipoort with CBS-like symptoms 3. Isolate fungi for Koch postulates, PCR and ITS sequencing	2. Fruit were collected from Vergenoegd packhouse in Komatipoort and inspected for CBS-like symptoms 3. Colletotrichum isolates were identified by the Plant Pathology department of the University of Stellenbosch
Jul – Sept 2014 3. Collect more fruit samples from packing houses in Nelspruit, Karino and Komatipoort with CBS-like symptoms 4. Isolate fungi for Koch postulates, PCR and ITS sequencing	3. Mature fruit were inoculated in an untreated orchard at Crocodile Valley estates in Nelspruit. 4. Fruit were collected from orchard and evaluated.
Oct – Dec 2014 3. Inoculate fruit with different isolates to fulfil Koch's postulates	3. Milestone already achieved.
Jan – Mar 2015 3. Inoculate fruit with different isolates to fulfil Koch's postulates	3. Green fruit were inoculated in an untreated orchard at Crocodile Valley estates in Nelspruit.

Some of the lesions that were identified and subjected to isolations were smaller than 2mm in diameter. Two species of *Colletotrichum* spp. were isolated from these lesions and molecularly identified as *Colletotrichum gloeosporioides* and *C. boninense*. Both are known anthracnose pathogens, but also known to be endophytic in nature on a wide range of host plants. Inoculations induced lesions on the wounded fruit only and resulted in a 65 and 70% lesion forming success rate for *C. gloeosporioides* and *C. boninense* respectively. Moreover, there was no statistical difference in the mean lesion size for each of the species with mean lesion sizes ranging between 3.1 mm and 3.2 mm. This could be a further indication that these

fungi need a rind injury to induce lesions or to colonise a CBS lesions after formation (Wager, 1952; Kotze, 1981). However, the back-isolations produced none of the inoculated fungi, and Koch's postulates could therefore not be fulfilled. The reason for poor isolation success might be the high temperature reached inside of the humid chambers or to the fact that the inoculation process has not been perfected yet. Another reason could be that the fruit were resistant to infection by the time of inoculation and future research would need to focus on inoculations at different maturity stages.

Technology transfer

Data will be presented at the biennial CRI Symposium in August 2016.

Further objectives and work plan

Determine if there is a susceptibility period to the pathogens and if they are only primary wound pathogens. The project will be terminated after the trial is repeated in the 2015/16 season and a final report will be written.

References cited

- Baayen, R.P., P.J.M. Bonants, G. Verkley, G.C. Carroll, H.A Van der Aa, M. De Weerd, I.R. Van Brouwershaven, G.C. Schutte, W. Maccheroni, C. Glienke de Blanco, J.L. Azeved, 2002: Nonpathogenic isolates of the CBS fungus, *Guignardia citricarpa*, identified as a cosmopolitan endophyte of woody plants, *G. mangiferae* (*Phyllosticta capitalensis*). *Phytopathology* 92, 464-477.
- Baldassari, R.B., Wickert, E. De Goes, A. 2008: Pathogenicity, colony morphology and diversity of isolates of *Guignardia citricarpa* and *G. mangiferae* isolated from Citrus spp. *Eur. J. Plant Pathol.* 120, 103-110.
- Glienke, C.O.L., Pereira, D., Stringari, D., Fabris, J. Kava-Cordeiro, V., Gallitereswa, L., Cunnington, J., Shivas, R.G., Groenewald, J.Z., Crous, P.W. 2011: Endophytic and pathogenic *Phyllosticta* species, with reference to those associated with CBS. *Persoonia* 26, 47-56.
- Peres, N.A., Harakava, L., Carroll, G.C, Adaskaveg, J.M., Timmer, L.W., 2007: Comparison of molecular procedures for detection and identification of *Guignardia citricarpa* and *G. mangiferae*. *Plant Dis* 91, 525-531.
- Kotzé, J. M. 1981. Epidemiology and control of citrus black spot in South Africa. *Plant Dis.*65:945-950.
- Timmer, L.W., Garnsey, S.M., Graham, J.H. Compendium of Citrus Diseases, 2nd Edition. APS Press. The American Phytopathological Society. p21-22 and p37-38
- Wager, V.A 1952 The black spot disease of citrus in South Africa. *Sci. Bull. Dep. Agric. For Union S. Afr.* 3030, 52p.

Table 4.6.7.1. Percentage lesions formed and average lesion size on Olinda Valencia fruit inoculated with *C. boninense* and *C. gloeosporioides* and inspected for lesion formation 42 days after inoculation.

Species	Wounded/Unwounded	Lesions formed (%)	Lesion size (mm) ^x
<i>C. boninense</i>	Wounded	70	3.2a
	Unwounded	0	0
<i>C. gloeosporioides</i>	Wounded	65	3.1a
	Unwounded	0	0

^xMeans followed by the same letter are not significantly different at a $P \leq 0.05$ confidence level.

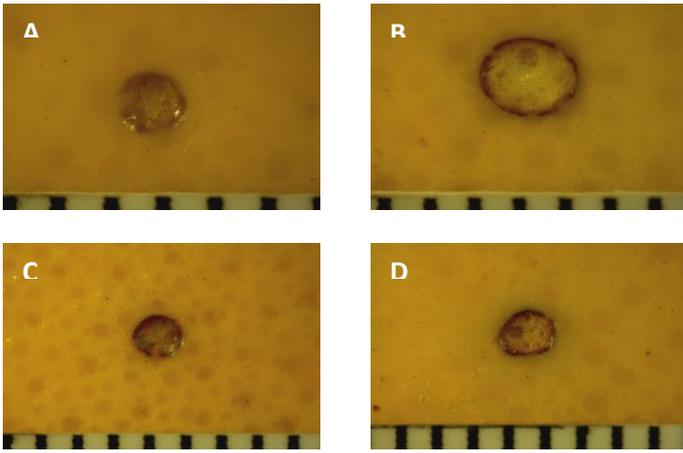


Fig. 4.6.7.1. Citrus black spot like symptoms without pycnidia on grapefruit

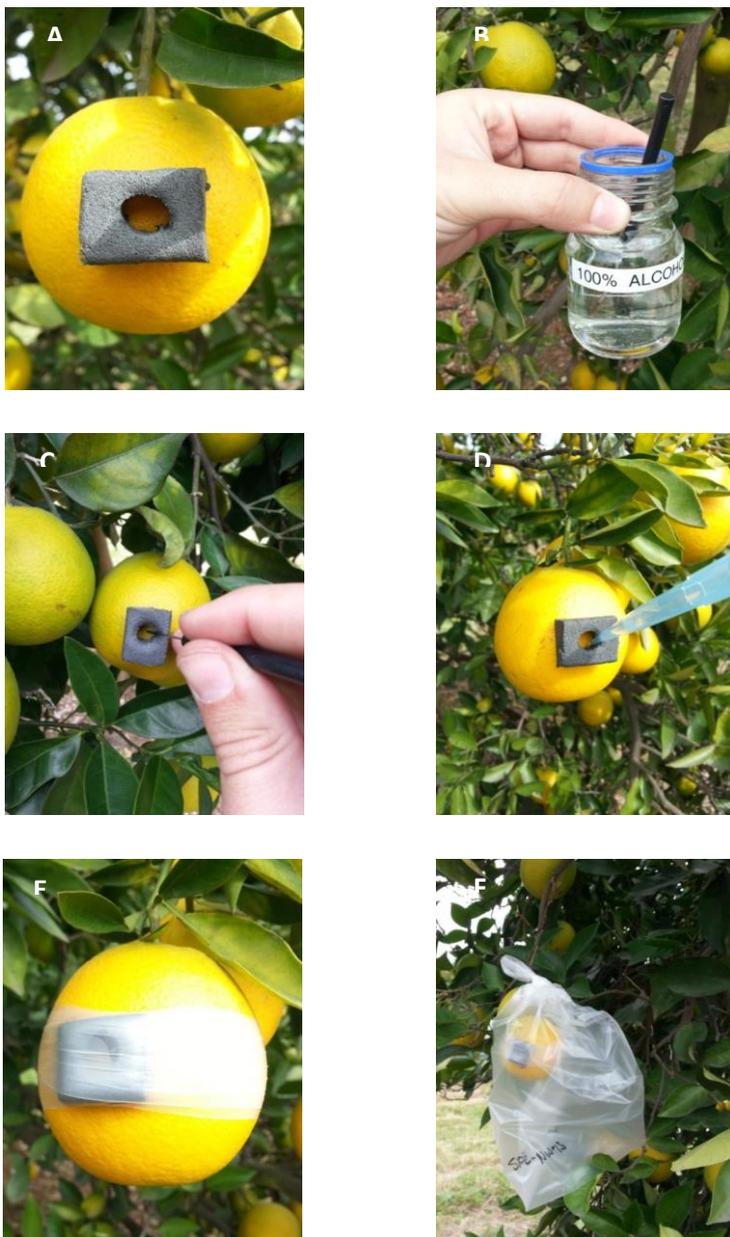


Fig 4.6.7.2. Preparation and inoculation of Valencia oranges with *Colletotrichum* isolates to fulfil Koch's postulates.

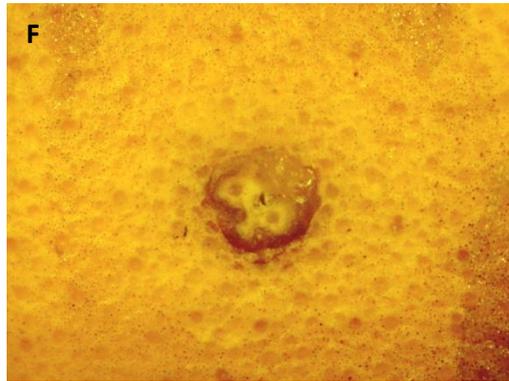
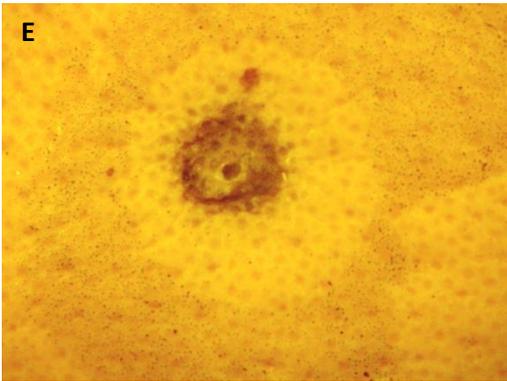
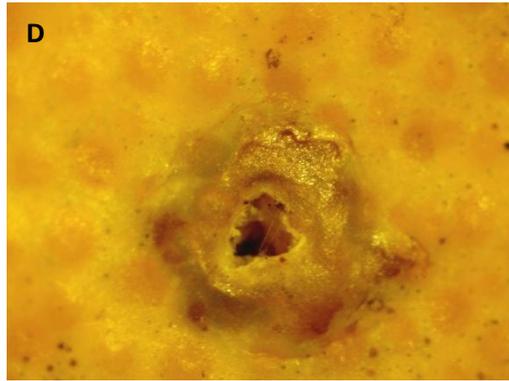
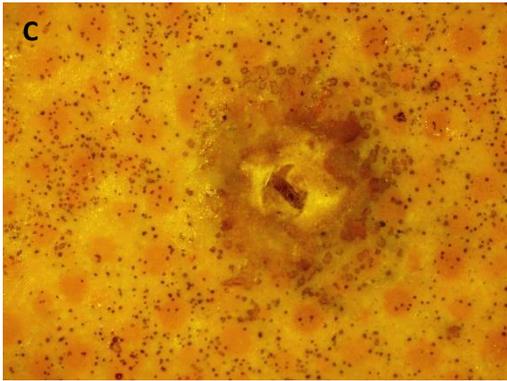
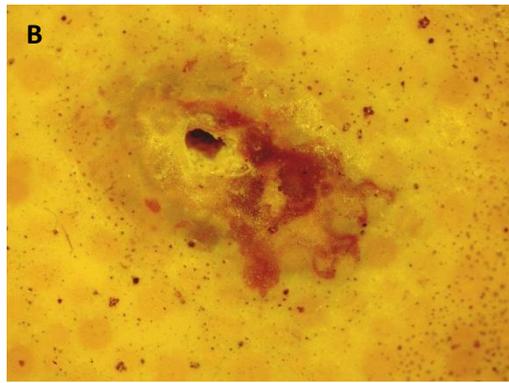
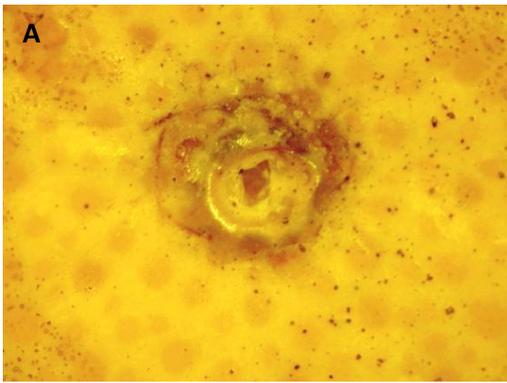


Fig. 4.6.7.3. Artificially infected fruit inoculated with *C. boninense* (A,C,E) and *C. gloeosporioides* (B,D,F) 42 days after inoculation.

4.7 CRI Diagnostic Centre (Elaine Basson, Aubrey Metane, Bhekisisa Cele and Jan van Niekerk - CRI)

Analysis	Citrus nurseries	Commercial samples	Other crops	Research samples
Nematode:Roots	31	441	16	1043
Nematode:Soil	3	22	19	1112
<i>Phytophthora</i>	2593	412	79	570
Water spore trap	160	1	10	0
Black spot identification (PCR)	0	116	0	61
Black spot benzimidazole resistance	0	33	0	0
Citrus greening (PCR)	0	5	0	0
Postharvest Resistance	0	60	0	8
Fruit & Foliar identification	0	2	18	5
Soil dilution plating	0	2	2	30
Internal Fruit Quality	0	16	0	0
TOTAL	2787	1110	144	2829

CIS certified Citrus Nurseries

It is compulsory for all citrus nurseries participating in the Citrus Improvement Scheme (CIS) to send samples for *Phytophthora* analysis on a quarterly basis. The irrigation water must also be tested for *Phytophthora* by making use of the spore trap method. In total, 2593 nursery samples were received by the diagnostic centre for *Phytophthora* analyses. Of these samples, 11.8% tested positive. In addition to soil and water samples, nurseries are required to send root samples once a year to test for the presence of *Tylenchulus semipenetrans*. For the nematode root samples, 0% tested positive and for the nematode soil samples 0% tested positive.

Commercial samples

Samples were received from the following citrus growing areas: Botswana, Eastern Cape, Kwazulu-Natal, Limpopo, Mpumalanga, North West, and Western Cape. Most of the samples received from citrus growers were analysed for *Phytophthora nicotianae* and the citrus nematode, *T. semipenetrans*. Twenty-eight percent of the 441 samples analysed for citrus nematode had counts above the threshold value of 1000 females per 10g of roots, and nematicide treatments were recommended. Fifty-one percent of the 412 samples analysed for *Phytophthora* tested positive.

Other crops

Nematode counts were done on soil or root samples of banana, ginger, grape, macadamia, pomegranate, and vegetables. Nematodes found present on these crops included: *Scutellonema*, *Meloidogyne*, *Pratylenchus*, *Roteltylenchulus*, *Tylenchus*, *Paratylenchus*, *Criconema*, *Hoplolaimus*, *Helicotylenchulus*, *Aphelenchus* and *Tylenchulus semipenetrans*. *Phytophthora* and *Pythium* analyses were done on avocado, macadamia, papaya and pomegranate. The diagnostic centre analysed 54 soil samples from macadamia nurseries and six from avocado nurseries for the presence of *Phytophthora cinnamomi*.

Research samples

Nematode and *Phytophthora* analysis were done on 2725 samples from experimental trials. The Diagnostic Centre assisted in trials to identify possible citrus black spot lesions using PCR protocols.

5 PORTFOLIO: HORTICULTURE

5.1 PORTFOLIO SUMMARY

By Tim G Grout (Manager: Research and Technical)

In 2014/5, no research was conducted in the programme Cold Chain and Packaging, although much of the research conducted in the Rind Condition programme involved problems that are exacerbated by cold chain conditions. Within the Rind Condition programme, research to resolve the problem of rind pitting in various citrus types continued and it was found that in Valencia, various preharvest factors can induce this condition. However, in soft citrus, moisture loss appeared to be important for Nadorcott but not for Nules Clementine. Research on peteca spot confirmed that foliar sprays of Ethephon can reduce the likelihood of this disorder and testing different mandarin selections for susceptibility to chilling injury has shown that M37 was more susceptible than others evaluated. Difficulties with the techniques used to measure sap flow in research on quantifying water usage by citrus trees have been overcome, although the research will take longer than planned. Research requested by growers to determine whether humates and fulvates are beneficial has been concluded and they have been found to improve soil microbial activity and reduce nitrogen leaching. Research on the new practice of erecting nets over citrus trees has confirmed an improvement in fruit size in conditions that have a slightly lower average temperature but higher relative humidity. More extensive, randomised trials with nets are being established with funding assistance from DST. With the increasing popularity of mandarin hybrids, research on flower induction, carbohydrate levels and pruning is also being conducted.

5.2 PROGRAMME: RIND CONDITION

Programme coordinator: Paul Cronjé (CRI-SU)

5.2.1 PROGRAMME SUMMARY

The rind condition programme focuses on firstly understanding factors that contribute to a specific rind disorder in a cultivar. Thereafter the aim is to develop technology to reduce the impact for the producers by testing various possible treatments for efficacy. During the previous season the focus has been on pitting of Valencia orange and mandarins. The results for the 2014 season indicate that the incidence of pitting in Valencia is influenced by preharvest factors, i.e. cultivar, orchard, canopy position, and maturity, as well as postharvest handling, especially the moisture loss before waxing, and TBZ application in the packline (5.2.2, 5.2.4). It has also been shown that not all pitting or rind breakdown type symptoms in mandarins have the same causal mechanism; Nules Clementine is unaffected by dehydration and rehydration prior to packing whereas pitting incidence of Nadorcott increases under these conditions. The reduction of peteca sensitivity of lemon fruit by application of a foliar spray of Ethephon before harvest has been confirmed (5.2.3). These experimental results need to be tested on a larger scale, but could offer a strategy to be employed in order to reduce the impact of this disorder. New mandarin cultivars/selections i.e. Sonet, B17, B24, I22, M37 and Nova, have been tested for chilling susceptibility in cold treatment protocols. During these experiments only 'M37' fruits showed severe chilling symptoms at all storage temperatures (5.2.5). During the 2015 season more in-depth research will be done to further determine aspects relating to sensitivity of fruit to various rind disorders.

PROGRAMOPSOMMING

Hierdie program, wat fokus op sitrusvrugte se skilkwaliteit, probeer eerstens die faktore wat aanleiding gee tot 'n spesifieke skildefek in 'n kultivar identifiseer en verstaan. Daarna word daar gepog om tegnieke te ontwikkel dmv die toets van verskeie behandelings wat die impak van 'n defek kan verminder. Gedurende die afgelope seisoen was daar op Valencia lemoene asook mandaryne en die defekte wat op hulle ontwikkel mee gewerk. Die resultate vir die 2014 seisoen dui aan dat die voorkoms van gepokte skil van Valencias beïnvloed word deur verskeie voor-oes aspekte soos kultivar keuse, tussen boord variasie, vrugposisie in die boom asook vrugrypheid. Na-oes speel voegverlies 'n rol om gepokte skil te vererger maar daarteenoor kan die aanwending van TBZ die voorkoms verminder (5.2.2, 5.2.4). In proefwerk op mandaryne was bevind dat dieselfde toestand wat skilafbraak in een kultivar veroorsaak nie dieselfde gevolg in 'n ander kultivar het nie. Nadorcott is byvoorbeeld sensitief vir dehidrasie gevolg deur rehidrasie maar Nules Clementine reageer nie negatief op die toestande nie. Daar was bevestig dat die voor-oes aanwending van Ethephon die voorkoms van peteca van suurlimoene kan verlaag en kan nou op 'n groter skaal getoets word en kan moontlik lei tot 'n strategie om die defek te beheer (5.2.3). Nuwe mandaryn kultivars (Sonet, B17, B24, I22, M37 and Nova) was getoets vir koue gevoeligheid, maar slegs die M37 het hoë vlakke van koueskade ontwikkel (5.2.5).

Gedurende die 2015 seisoen sal die resultate opgevolg en herhaal word om verder die faktore wat skildefekte beïnvloed te bepaal en probeer manipuleer om sodoende die negatiewe impak te verlaag.

5.2.2 **PROGRESS REPORT: Studies on aspects concerning rind pitting/staining citrus fruit** Project 958 (2009/10 – 2015/6) by PJR Cronje and J North (CRI at SU)

Summary

Postharvest physiological rind disorders, such as staining and pitting, affect most citrus cultivars and have a significantly negative impact on return on investment for producers. Significant cultivar differences exist with Benny Valencia more susceptible than Turkey Valencia. In addition, inside fruit tend to be more susceptible than sun-exposed fruit. By reducing the time between harvest and packing the incidence of pitting in Valencia can be reduced. Optimal application of thiabendazole in the packline can be used to reduce the development of pitting. Pitting of Nadorcott and Nules mandarins differ in causal mechanism, with Nadorcott reacting negatively to dehydration followed by rehydration, but Nules Clementine showing no treatment differences.

Opsomming

Naoes gepokte skil is 'n fisiologiese skildefek wat bykans alle sitrus kultivars negatief kan affekteer en lei tot betekenisvolle finansiële verliese. Gedurende die 2014 seisoene is daar bevind dat daar betekenisvolle verskille tussen Benny (mees gevoelig) en Turkey Valencia bestaan i.t.v. gepokte skil. Daar is ook gevind dat binne vrugte meer vatbaar is as vrugte buit in die blaardak. Daar moet ook gelet word dat daar wel verskille in 'n produksie area bestaan tussen boorde van die selfde kultivar. Deur die tyd tussen pluk en verpakking van Valencia vrugte te verminder kan die voorkoms van gepokteskil verlaag word. So ook het TBZ soos toegedien in die paklyn 'n positiewe uitwerking op die voorkoming van gepokte skil. Daar word vermoed Nadorcott en Nules mandaryne verskil in terme van die meganisme wat lei tot die skilafbraak. Nadorcott toon 'n negatiewe reaksie op die dehidrasie gevolg deur 'n rehidrasie proses. Daarteenoor toon Nules nie dieselfde reaksie wat dui op verskillende onderliggende faktor wat die skildefekte tot gevolg het nie.

5.2.3 **PROGRESS REPORT: Effect of different chemical applications on development of Peteca spot in lemons** Project 833 (2006/7-2015/6) by PJR Cronje and J North (CRI at SU)

Summary

Peteca spot (PS) of lemon is a postharvest physiological disorder resulting in the collapse of the oil gland. Subsequently the oil leaks into the adjacent tissue and causes a darkened depression or sunken area. The occurrence can be severe, resulting in substantial economic losses without any specific pre- or postharvest practices that could be implemented to avoid or significantly reduce the incidence. PS occurs in all production areas of South Africa and is thought to be the result of the immature rind being subjected to postharvest stress associated with high CO₂, the packing line and wax application. Preharvest Ethephon (2-Chloroethyl phosphoric acid) (200 mg/L and 400 mg/L) could be a viable commercial option to reduce the incidence of peteca and will be tested on a semi-commercial scale next season.

Opsomming

Peteka kol (PK) van suurlemoen is 'n na-oes fisiologiese skildefek waar die olieklier in skeur en lek die olie uit in die omliggende weefsel en lei tot 'n donker versonke letsel in die skil. Die voorkoms kan uiters hoog wees en lei tot ernstige finansiële verliese, en daar bestaan tans nie 'n voorkomings of beheer maatreël nie. PK kom voor in alle suurlemoen areas in SA en daar word vermoed dat onvolwasse vrugteskille wat aan na-oes stres (soos hoë CO₂, verpakking en waks aanwending) blootgestel word, lei tot 'n verhoogde voorkoms. Die voor-oes Ethephon (2-Chloroethyl phosphoric suur) (200 mg/L en 400 mg/L) toegedien op vrugte lei tot laer voorkoms van peteka is in alle boorde wat behandel was en kan op 'n semikommersiële basies in probleem boorde getoets word.

5.2.4 FINAL REPORT: The development of a rind disorder prediction model for citrus fruits based on climatic conditions

Project (2014/5 – 2015/6) by N Mathaba (ARC – ITSC)

Summary

In South Africa, loss of citrus fruit through non-chilling physiological rind disorders is increasing and the causal factors are not yet fully known. Therefore, CRI commissioned the ARC-ITSC to review the possible factors causing citrus non-chilling rind disorders. The aim of this review research was to investigate the effect of environmental factors, especially vapour pressure deficit (VPD) on non-chilling rind physiological disorders (pitting). Benny Valencia fruit were harvested from Mahela Estate in Letsitele Limpopo province. Environmental data for June, July and August (harvest months) was obtained from ARC-ISCW and used to calculate VPD. There were no significant non-chilling physiological disorders i.e. pitting found on Benny Valencia from Mahela Estates. However, VPD showed an increasing trend during the August harvest month as compared with June and July. In conclusion, citrus fruit might be highly susceptible to non-chilling rind disorders (pitting) during late harvest, as increased VPD will increase rind water loss.

Opsomming

Die voorkoms van skildefekte in Suid-Afrika sitrusvrugte neem toe alhoewel die onderliggende faktore is nog nie ten volle bekend is nie. CRI het opdrag gegee aan die ARC-ITSC om die moontlike faktore te hersien kan bydrae tot gepokte skil. Die doel van hierdie oorsig navorsing was om die effek van omgewingsfaktore, veral waterdampdruktekort (VPD) op gepokteskil in sitrusvrugte te ondersoek. Benny Valencia vrugte was geoes van Mahale Estate in Letsitele Limpopo provinsie. Klimaat data vir Junie, Julie en Augustus (oes maande) is verkry uit ARC-IGKW en gebruik om VPD te bereken. Daar was geen beduidende gepokteskil gevind in die Benny Valencia van Mahela Estates nie. Die VPD het 'n toenemende neiging in die Augustus-oes maand gehad in vergelyking met Junie en Julie. Ten slotte, kan sitrusvrugte hoogs sensitief wees vir gepokte skil (pitting) gedurende die later oes periode, as die verhoging VPD sal lei tot verhoogde waterverlies.

Introduction

The manifestation of postharvest physiological disorders on South African citrus fruit has been speculated to be affected by season and environmental factors such as rainfall, humidity, temperature, irrigation, etc. Previous work on 'Nules' mandarins (Cronje et al., 2011) and lemons (Mditshwa et al., 2013 and Mathaba and Bertling, 2013) further speculated and proposed the appearance of rind disorders to be affected by orchard environmental factors. South African citrus production occurs mainly in three climatically different production regions, the Mediterranean (Western Cape Citrusdal); warmer coastal (Eastern Cape and KwaZulu-Natal) and tropical/subtropical (Mpumalanga and Limpopo) regions. However, there is no research on the effect of environmental factors and their effect on postharvest storage behaviour of citrus fruit. Therefore, the aim of this proposed research was to study the effect of environment on incidence of citrus rind disorders (chilling injury and pitting) and develop a prediction model in relation to prevailing weather conditions. The model will allow us to advise farmers on potential appearance of physiological disorders before cold storage.

Objectives were changed as requested by the CRI crop and fruit quality research committee, which requested that we only focus on vapour pressure deficit (VPD).

Stated objectives

- To study the effect of environmental conditions (micro-climate and water use) on appearance of non-chilling and chilling disorders on different citrus fruit.
- To investigate the rind physical (fruit weight loss) and physiological parameters (sugars, ascorbic acid, phenolics, beta-carotene and vitamin E) of citrus fruit from different growing regions of Southern Africa.

Materials and methods

Mahela Estate, Letsitele (Limpopo province) planted with Benny Valencia was used for this trial. Benny Valencia fruit were harvested from 10 trees and evaluated for rind pitting (non-chilling pitting). Climatic data were obtained from ARC-ISCW from a weather station nearby Mahela Estate. Data obtained included

temperature, rainfall and relative humidity. Afterwards, the data were used to calculate vapour pressure deficit (VPD) for June, July and August 2014.

Results and discussion

There was minimum non-chilling pitting observed from the 10 randomly chosen trees, and the data could not be statistically analysed. However, VPD data showed an interesting change in the environmental data during harvest time of Benny Valencia fruits. In general, there was an increase in VPD during August when compared with June and July (Table 5.2.4.1). Such an increase in VPD might contribute to rind water loss and therefore, non-chilling rind pitting disorder.

Conclusion

During late harvesting (August), there is an increase in ambient temperature which led to increased VPD in Limpopo province. The increase in VPD might be a factor influencing rind susceptibility via increase rind water loss.

Future research

A full study including more farms in different province is recommended.

Technology transfer

None.

References cited

- Cronje, P.J.R., Barry, G.H. and Hysamer, M. (2011), Postharvest rind breakdown of 'Nules Clementine' mandarins is influenced by ethylene application, storage temperature and storage duration. *Postharvest Biology and Technology*, 60: 192-201.
- Mditshwa, A., Bower, J.P., Bertling, I., Mathaba, N. and Tesfay, S.Z. (2013), The potential of postharvest silicon dips to regulate phenolics in citrus peel as a method to mitigate chilling injury in lemons. *African Journal of Biotechnology*, 12(13):1482-1489.
- Mathaba, N. and Bertling, I. (2013), Hot water and molybdenum dips: The case of antioxidant assay in lemons flavedo during cold storage. *African Journal of Biochemistry Research*, 7(4): 45-54.

Table 5.2.4.1. Vapour pressure deficit of Mahela Estate during citrus harvest months (June, July and August).

Mon	Days in a month																														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	23	24	25	26	27	28	29	30	31	
Jun	0.3	0.5	0.2	0.3	0.7	0.8	0.1	0.1	0.3	0.2	0.2	0.1	0.2	0.1	0.3	0.6	0.1	0.1	0.3	0.4	0.1	0.1	0.2	0.2	0.2	0.1	0.5	0.3	0.1	0.1	
Jul	0.2	0.2	0.2	0.8	0.4	0.7	0.2	0.1	0.1	0.2	0.1	0.2	0.6	0.6	0.3	0.1	0.7	0.8	0.9	0.2	0.4	0.2	0.2	0.5	0.7	1.2	0.3	0.6	0.3	0.2	
Aug	0.5	0.9	0.8	0.6	0.6	1.0	0.5	0.5	0.3	0.2	0.3	0.5	0.4	0.3	0.5	0.6	0.2	0.1	0.6	1.0	1.2	0.8	0.2	1.5	0.6	0.3	1.4	1.5	1.3	0.7	

5.2.5 PROGRESS REPORT: Investigating cold storage potential of new mandarin citrus selections/cultivars and the effect of ethylene degreening on rind disorders
Project PHI64 (Apr 2014 – Mar 2017) by N Mathaba (ARC - ITSC)

Summary

New mandarin cultivars/selections must be tested for quality after cold sterilization, a mandatory requirement for exporting to overseas markets. Degreened and non-degreened mandarin cultivars 'Sonet' (the new ARC-ITSC selection), B17, B24, I22, M37, Nova and Nova ARC were stored at -0.5, 2.0 and 4.0°C for 28 days. After cold sterilization (-0.5°C) only 'M37' fruits showed severe chilling symptoms at all storage temperatures. Furthermore, the chilling sensitivity of 'M37' was associated with significant decrease in fruit firmness, weight loss and increased rind electrolyte leakage. Surprisingly, increased fruit weight loss was associated with an increase in Brix for 'M37' when compared with other cultivars evaluated, irrespective of degreening treatment and storage temperature.

Opsomming

Nuwe mandaryn sitrus kultivars moet getoets word vir gehalte na blootstelling aan koue sterilisasie protokols, 'n verpligte vereiste vir die uitvoer na verskeie oorsese markte. Ontgroende en nie-ontgroende mandaryn kultivars nl. Sonet, B17, B24, I22, M37, Nova en Nova ARC was gestoor teen -0,5, 2.0 en 4.0°C vir 28 dae. Na koue opberging het net M37 vrugte het erge koue simptome getoon. Verder het die koue sensitiviteit van M37 wat verband hou met beduidende afname in vrugfermheid, gewigsverlies en verhoogde skil elektroliet lekkasie. Verbasend, was hoë vrugte gewigsverlies wat verband hou met toename in Brix vir M37 ongeag ontgroening behandeling en opberging temperatuur.

5.3 PROGRAMME: FRUIT PRODUCTION AND QUALITY
Programme coordinator (Acting): Tim G Grout (CRI)

5.3.1 PROGRAMME SUMMARY

Critical research to quantify optimal water use in citrus has been hampered by the techniques used to measure sap flow being compromised by gumming. These challenges have finally been overcome and it was confirmed that these are the best techniques to use, but the research is now behind schedule (5.3.2). Research to evaluate possible benefits of using humates and fulvates has been concluded and it was found that these products increase microbial activity in the soil by increasing both bacteria and fungi and reduced leaching of nitrogen (5.3.3). A new project has started to determine the time and duration of flower induction in mandarins and the possible importance of leaf carbohydrate levels in determining fruit load and quality, but results are not yet available (5.3.4). Early results from the first project on growing citrus under nets show that the average temperature is 0.5°C lower and the relative humidity 3% higher under net and that fruit size did increase (5.3.5). Research on the pruning of late mandarins includes quantifying material removed and measuring leaf carbohydrate levels at three different times in the season. However, resultant trends are not yet discernible (5.3.6).

PROGRAMOPSOMMING

Noodsaaklike navorsing om optimale watergebruik van sitrus te kwantifiseer is bemoeilik deur die tegnieke wat gebruik word om sapsvloei te meet wat deur vergomming beïnvloed word. Hierdie uitdagings is uiteindelik oorkom en dit is bevestig dat hierdie die beste tegnieke is om te gebruik, maar die navorsing is nou agter skedule (5.3.2). Navorsing om moontlike voordele van die gebruik van humates en fulvates te evalueer, is afgehandel en daar is bevind dat hierdie produkte die mikrobiese aktiwiteite in die grond verhoog deur beide bakterieë en swamme te vermeerder en deur die uitloging van stikstof te verminder (5.3.3). 'n Nuwe projek is begin om die tyd en duur van blom-induksie in mandaryne te bepaal en die moontlike belang van blare se koolhidraatvlakke in die bepaling van vruglading en -gehalte, maar resultate is nog nie beskikbaar nie (5.3.4). Vroeë resultate van die eerste projek oor die aanplanting van sitrus onder nette toon dat die gemiddelde temperatuur 0.5°C laer is en die relatiewe humiditeit 3% hoër is onder die nette en dat vruggrootte wel toegeneem het (5.3.5). Navorsing op die snoei van laat mandaryne sluit die kwantifisering van materiaal in wat verwyder is asook die meting van koolhidraatvlakke van blare op drie verskillende tye in die seisoen. Gevolglike tendense is egter nog nie waargeneem nie (5.3.6).

5.3.2 PROGRESS REPORT: A novel approach to water and nutrient management in citrus

Project 986 (Aug 2010 – Mar 2017) by J.T. Vahrmeijer (CRI at UP), N.J. Taylor, S. van der Merwe, M. Sam and M. Banda (UP)

Summary

In previous trials on the validation of sap-flow techniques, the probe sets of the Compensation Heat Pulse method (CHPM), the Heat Ratio Method (HRM) and Thermal Dissipation Method (TDM) were all installed on the same tree. To eliminate the possibility of heat interference between the different methods (that may compromise some of the data) and to test our experimental procedures and equipment, it was decided to install the HRM separately on citrus and *Eucalyptus* trees. Transpiration (water loss) measured with the sap-flow technique was then compared with transpiration measured with a gravimetric method. *Eucalyptus grandis* has been widely used for validating and testing sap-flow techniques such as HRM and CHPM.

Some experimental sites in Citrusdal had to be moved because of excessive gum produced by the citrus trees that influenced sap flow measurements. Due to low yields, trees of the 13 year-old Bahianinha orchard were removed by the owner of the farm and our experimental site was moved to an adjacent 'Washington' navel site. It was also decided to move the experimental site from the 23 year-old Bahianinha orchard to a 'Newhall' navel orchard, because more soil water measurements had to be done to determine ET and the soil of the 23 year-old Bahianinha orchard contains a large number of stones that makes the installation of soil water measuring devices difficult.

Transpiration (g h^{-1}) measured with the HRM on *Eucalyptus grandis* had a clear diurnal trend of the same magnitude and is in close agreement ($R^2 = 0.78$) with the hourly water loss (g h^{-1}) measured with a gravimetric method. At low flow rates ($< 100 \text{ g h}^{-1}$) the gravimetric water losses were slightly over-estimated by the HRM, whilst at high flow rates the gravimetric water losses were underestimated by the HRM. These results are in agreement with findings reported by Bleby et al. (2004), Burgess et al. (2001) and Burgess et al. (2000). Therefore, it was concluded that the equipment and the procedure used in validating the sap-flow techniques for citrus are appropriate and acceptable. For 'Midnight' Valencia the hourly mass loss measured with the HRM had a weak linear correlation ($r^2 = 0.55$) with the hourly mass loss measured with the gravimetric method.

Data collected from the automatic weather station (AWS) in Citrusdal were generally good but problems were experienced with the solar panel responsible for charging the battery. This resulted in a power failure to the logger and data were lost from 15-28 October 2013, 24-27 November 2013 and 5 October to 3 November 2014. Vapour pressure deficit (VPD) and reference evapotranspiration (ET_0) are both important determinants and drivers of water use in plants and are calculated from weather data. Average daily ET_0 for the period (August 2013 - May 2015) was 3.49 mm, with a daily maximum value of 8.47 mm and a minimum of 0.23 mm. VPD varied from 0.12 kPa to 3.77 kPa, with an average of 1.25 kPa for the measurement period. Sap flow data from three of the four new orchards improved and clear diurnal trends are evident in the vast majority of probe sets. This is despite some gum production which was evident during the March 2015 measurement window. For the purposes of assessing the validity of the sap flux density data collected from each orchard, the HPV data from the trees were upscaled to transpiration volumes (ℓ). Transpiration volumes were generally lower in the 4 year-old 'Cambria' navel trees, 6 year-old 'Midnight' Valencia trees and the 8 year-old 'Washington' navel trees than in the 14 year-old 'Midnight' Valencia trees, which is expected as the canopy size and therefore leaf area index (LAI) were much higher in the older 'Midnight' orchard. This once again emphasises the importance of canopy size in determining transpiration volumes and the importance of determining LAI for modelling purposes.

Opsomming

In vorige proewe om sapsvloei-tegnieke te valideer, is die hitte elemente van die kompensasië-hittepolsmetode (CHPM), die hitte-verhoudingsmetode (HRM) en die termiese-dissipasiemetode (TDM) gesamentlik op dieselfde boom geïnstalleer. Om die moontlike invloed van hitte oordraging tussen die verskillende metodes (wat die sapsvloei data nadelig mag beïnvloed) te voorkom en om die eksperimentele prosedures en apparaat te toets, is die HRM afsonderlik op *Eucalyptus* en sitrusbome geïnstalleer. Transpirasie (waterverlies) gemeet met die sapsvloei-tegnieke is vergelyk met transpirasiewaardes soos gemeet met 'n gravimetriese metode. *Eucalyptus grandis* word algemeen vir die toets en validering van sapsvloei-tegnieke, soos HRM en CHPM, gebruik.

Oormatige gom, wat die resultate van die sapvloeimetings kon beïnvloed, is deur van die eksperimentele bome in Citrusdal afgeskei en daarom is die sapvloeiproewe na ander boorde geskuif. Die 13-jaar oue Bahianinha boord is deur die plaaseienaar uitgehaal en die instrumente is na 'n naburige 'Washington' nawel boord geskuif. Die proewe in die 23-jarige Bahianinha boord is ook na die 'Newhall' nawel boord geskuif omdat meer grondwatermetings, om ET te bepaal, beplan word. Die gronde van die 23-jarige Bahianinha boord is klipperig, wat die installering van instrumente om die grondwaterinhoud te bepaal, bemoeilik.

Transpirasie (g h^{-1}) wat met behulp van die HRM in *Eucalyptus grandis* gemeet is, toon 'n duidelike uurlikse patroon en is in ooreenstemming ($R^2 = 0.78$) met die uurlikse waterverlies (g h^{-1}) soos gemeet met 'n gravimetriese metode. By lae sapvloeiensnelhede ($< 100 \text{ g h}^{-1}$) het die HRM die gravimetrie bepaalde transpirasie oorskakel, terwyl die HRM metode dit onderskat by hoë sapvloeiensnelhede. Hierdie resultate is in ooreenstemming met die bevindings van Bleby et al. (2004), Burgess et al. (2001) en Burgess et al. (2000). Die gevolgtrekking was daarom dat die tegnieke en apparaat, om sapvloe in sitrusbome te toets en te valideer, geskik en aanvaarbaar is. In die geval van 'Midnight' Valencias het die uurlikse massaverlies soos gemeet met die HRM swak gekorreleer ($R^2 = 0.55$) met die uurlikse massa verlies soos gemeet met die gravimetriese metode.

Weerdata is met behulp van 'n outomatiese weerstasie versamel. 'n Foutiewe sonpaneel het egter veroorsaak dat data vir die periodes, 15-28 Oktober 2013, 24-27 November 2013 en 5 Oktober – 3 November 2014, nie ingesamel kon word nie omdat die krag toevoer na die instrumentpaneel onderbreek was. Die dampdrukverskil (VPD) en verwysings ET (ET_o), wat belangrike komponente en drywers van watergebruik in plante is, kan vanaf weerdata bereken word. Vir die meetperiode (Augustus 2013 - Mei 2015) was die gemiddelde daaglikse ET_o 3.49 mm, met 'n maksimumwaarde van 8.47 mm en 'n minimum van 0.23 mm. VPD het van 0.12 kPa tot 3.77 kPa gevarieer met 'n gemiddeld van 1.25 kPa. Sapvloeddata van drie van die vier nuwe boorde het wesenlik verbeter met duidelike daaglikse tendense vir meeste van die metingspunte, ten spyte van die teenwoordigheid van boomgom soos waargeneem tydens die veldbesoek in Maart 2015. Om die toepasbaarheid van die sapvloeddigheidsdata wat vir elke boord ingesamel is te evalueer, is die HPV data opgeskaal na transpirasie volumes (ℓ) vir die verskillende bome. Transpirasie volumes was in die algemeen laer vir die 4-jaar oue 'Cambria' nawel bome, 6-jaar oue 'Midnight' Valencia bome en die 8-jaar oue 'Washington' nawel bome as vir die 14-jaar oue 'Midnight' Valencia bome. Hierdie gevolgtrekking was te wagte omdat die blaardagrootte en daarom die blaaroppervlakindeks (LAI) van die ouer 'Midnight' boord heelwat groter is as dié van die ander boorde. Hierdie resultate beklemtoon weereens die belangrikheid om blaardagroottes te meet en om LAI te bereken vir die modellering van transpirasie van sitrusboorde.

5.3.3 FINAL REPORT: Study on the effect of humic and fulvic acids on fertiliser application in citrus

Project 1028 (April 2011 – March 2015) by J T Vahrmeijer (CRI at UP) and A Gatabazi (UP)

Summary

Results from an experiment to determine the influence of humates and fulvate on the microbial activity of soils, showed that humates and fulvate increases the dehydrogenase (microbial activity) in a sandy clay and sandy clay loam soil. Total bacterial counts in both soils, after two weeks of incubation, increased when humates and a fulvate combined with N, P and K were mixed with the soils. After four weeks the bacterial and fungal counts were the highest in the soils treated with humates and fulvate combined with N, P and K fertilisers compared to the soils containing no humates and fulvate. In an experiment with leaching columns it was found that humates and fulvate mixed with N, P and K fertilisers reduced the leaching of N in both soil types, while inconsistent results were found for K and P. The results from pot trials clearly show that humates and fulvates combined with N, P and K fertilisers increased the pH and EC of the leachate and significantly reduced N and P leaching in both soils, but did not reduce K leaching. In general the N, P and K content of the leaf and bark increased when humates and fulvate combined with N, P and K were added to the soil. However, humate and fulvate combined with N, P and K did not increase the N, P and K content of the roots in the sandy clay soil.

Initial results from a long-term field trial, to determine if humates and fulvate can be used to reduce fertiliser application in citrus orchards, showed that irrigation scheduling plays an important role in reducing N leaching at orchard level. When humates and fulvate were mixed with liquid fertilisers, the initial N leaching directly after fertilisation was not reduced. But results from subsequent irrigation events suggest a decrease in N leaching.

Opsomming

Resultate van 'n eksperiment om die invloed van humate en fulvate op die mikrobiologiese aktiwiteit van gronde te bepaal, het aangetoon dat dehidrogenase (mikrobiologiese aktiwiteit) in 'n sandklei- en sandkleileemgrond verhoog. Na twee weke van inkubasie het die totale bakteriese telling in beide gronde, wanneer humate en fulvaat tesame met N-, P- en K-kunsmis met die grond gemeng word, verhoog. Na vier weke was die bakteriese- en swamtellings die hoogste in die gronde wat met humate en fulvaat en N-, P-, K-kunsmis behandel is. In 'n ander eksperiment met loogbuise, is dit gevind dat N loging in beide gronde verminder indien N-, P- en K-kunsmis met humate en fulvate gemeng word, terwyl wisselende resultate vir K en P gevind is. Die resultate van potproewe het duidelik aangetoon dat humate en fulvaat, tesame met N-, P- en K-kunsmis, die pH en EG van loogwater verhoog asook N en P loging verminder, terwyl K loging nie afgeneem het nie. In die algemeen het die N-, P- en K konsentrasies van die blare en bas toegeneem vir die humate en fulvaat, tesame met N-, P- en K-kunsmis, behandeling. Humate en fulvaat, tesame met N-, P- en K-kunsmis, het egter nie die N, P en K konsentrasie van die plantwortels verhoog nie.

Aanvanklike resultate van 'n langtermyn veldproef, om te bepaal of humate en fulvate gebruik kan word om kunsmis toediening in sitrusboorde te verminder, dui daarop dat besproeiing 'n belangrike rol speel in die vermindering van N loging op boordvlak. Humate en fulvate wat met vloeibare kunsmis gemeng is, het geen invloed gehad op die aanvanklike loging van N nie, maar uit die resultate van opvolgende besproeiings wil dit voorkom of die N loging verminder.

Introduction

The use of humic acids (HA) is a promising natural resource to be utilised as an alternative for increase in crop production and reduction in fertiliser application (Sharif, Khattak & Sarir 2002; Selim, El-Neklawy & El-Ashry 2009). The influence of HA on nitrogen application in other crops is well documented. For grapevines it was found that mineral N could be reduced by 50%, with the addition of HA and bio-fertilisers, with an increase in yield and a reduction in NO₃ and NO₂ content of the berry juice (Eman, Saleh & Mostafa 2008). The nitrogen content of lettuce and soil phosphorus availability increased with HA application (Cimrin & Yilmaz 2005), and the fruit quality and yield of watermelons were positively influenced with the addition of HA, although variety played a role in the response to the HA application (Salman, Abou-hussein, El-Nemr *et al.* 2005).

Objectives

- i) To determine the influence of potassium-humates and fulvate on soil microorganisms
- ii) To determine the influence of humic acid and fulvic acid on leaching of plant nutrients
- iii) The long term effect of potassium-humates and fulvates on nitrogen leaching in an commercial orchard

Materials and methods

1. Influence of HA on leaching of plant nutrients

The leaching experiment was conducted in a laboratory using columns consisting of Plexiglas (0.1 m diameter and 0.3 m high). Each column was fitted with five filters of four different sizes that ranged from 5 µm to 2 mm. The leaching studies were conducted on two types of soil (sandy clay loam and sandy clay) and consist of nine treatments and four replications that were arranged in a completely randomised block design (CRBD) on laboratory benches. The soils were mixed prior to filling the leaching columns with 50 mg kg⁻¹ (equivalent to approximately 200 kg ha⁻¹) humates or fulvate. Nitrogen, P and K were then added to the soils at two concentration levels, 100% (220-50-80) and 75% (165-37.5-60) of the fertiliser recommendation for citrus and thoroughly mixed prior to filling the leaching columns (Fertiliser Handbook of South Africa, 2007). The different columns were filled with different soils to a height of 0.17 m at a bulk density of approximately 1498 kg m⁻³. Soils in the leaching columns were left for 14 days to react with N, P, and K fertilisers and the humates and fulvates. The volume of water applied to each column was calculated from bulk density, porosity and the pore space of the soils.

2. Influence of HA on nutrient uptake

The pots were laid out in a completely randomized block design (CRBD) with five treatments and four replicates. The treatments consist of: 1) control 0, containing neither fertiliser nor humates and fulvate; 2) control 75, which represents 75% of the recommended N, P and K-application rate; 3) humate (La) 75, which represents 75% of the recommended N, P and K-application rate with humate low ash (200 kg ha^{-1}); 4) fulvate 75 which represents 75% of the recommended N, P and K-application rate with fulvate (200 kg ha^{-1}); and 5) humate (Ha) 75, which represents 75% of the recommended N, P and K-application rate with humate high ash (200 kg ha^{-1}). Details of the treatments are given in Table 5.3.3.2. The 75% N, P and K-fertiliser application rates are equivalent to 165, 37 and 60 kg ha^{-1} of N, P and K respectively. Humates and fulvate were mixed with the soil at a rate of 200 kg ha^{-1} .

Small 'Delta' Valencia citrus trees were planted in 10 litre pots and left for one month to acclimatise. During this period, each pot was irrigated to field capacity with 3.4 L of distilled water every two days. The quantity of water irrigated was increased to 3.9 L when leaching was performed. The leachate was collected and analysed for N, P and K. At the end of the trial, leaf, bark and root samples were also analysed to determine its concentration for N, P and K.

The trials were done at Letaba Estates. Potassium-humates and fulvates (100 kg ha^{-1}) were mixed with fertilisers and applied to a commercial orchard. Two citrus blocks where no potassium humates and fulvates were applied were used as reference blocks. Leaf and soil samples were taken at regular time intervals and the Ca, Mg-, K-, P-, N-, Fe-, Zn-, Cu-content were determined. Soil water was collected regularly with wetting front detectors and the nitrogen content was determined.

Results and discussion

Objective / Milestone	Achievement
I. The influence of humates and fulvate on the microbial soil community (Bacteria and fungi)	
I.1. Influence of humates and fulvate on bacterial counts.	Total bacterial counts in a sandy clay loam were highest after two weeks of application of humates and fulvate combined with fertilisers. Treatment treated with humate combined with 100% N, P and K was higher than controls (Figure 4.1). After four weeks, the bacterial counts were highest in soil treated with humates and fulvate with N, P and K fertiliser application compared to soils containing no humates and fulvate.
I.2. The influence of humates and fulvate on fungi counts.	The results from (Figure 4.2) show that after two weeks fungal counts were highest in all treatments treated with fulvate and humates combined with N, P and K compared to other treatments. Treatments with humates combined with N, P and K increased fungal counts compared to other treatments treated with fulvate or untreated.
II. To determine the influence of humic acid and fulvic acid on leaching of plant nutrients:	
II.1 Under controlled conditions in leaching columns	The addition of humate or fulvate to soils mixed with fertilisers showed a high significance ($p < 0.01$) in decreasing the N concentration of the leachate of both soil types but did not decrease the concentration of K and P in the leachate.

II.2 Pot trials	In pot trials it was found Humate and fulvate treatments in a sandy clay soil affected N and K contents of citrus leaves and bark but not roots. The humate and fulvate treatments significantly increased the pH and EC of the soils and generally reduced the leaching of N and P for both soils (sandy clay and sandy clay loam). However, no significant effect was observed for K.
III.1 The long term effect of potassium-humates and fulvates on nitrogen leaching	The trial was conducted at Letaba Estates. Results indicate a decrease in nitrogen leaching when humic acids are applied with the fertilisers.

1 Leaching of nutrients

1.1 Nitrogen leaching

Nitrogen concentration of the leachates of the soils mixed with humate, fulvate and fertilisers are presented in Figure 5.3.3.1. These results indicate that humates and fulvate application have a significant ($p < 0.01$) effect on reducing N leaching. For sandy clay soil the N concentration of the leachate of control 0 was 9.7 mg kg^{-1} (37.9 kg ha^{-1}), for control 75 it was 10.56 mg kg^{-1} (42.2 kg ha^{-1}) and for control 100 it was 11.6 mg kg^{-1} (46.7 kg ha^{-1}). Whereas N concentration for humates and fulvate combined with fertilisers varied between 2.3 mg kg^{-1} (9.1 kg ha^{-1}) and 6.3 mg kg^{-1} (25.1 kg ha^{-1}) (Figure 5.3.3.1).

The results for the sandy clay loam are presented in Figure 5.3.3.1. The N concentration of the leachate of for control 0 was 5.75 mg kg^{-1} (22.99 kg ha^{-1}), for control 75 it was 14.50 mg kg^{-1} (57.98 kg ha^{-1}) and for control 100 it was 16.88 mg kg^{-1} (67.50 kg ha^{-1}). The N concentration of the leachate from the humates and fulvate treatments varied between 1.90 mg kg^{-1} (7.59 kg ha^{-1}) for the lowest N and 5.08 mg kg^{-1} (20.31 kg ha^{-1}) for the highest N. Humates and fulvate mixed with N, P and K fertilisers manifested a significant influence ($p < 0.01$) on reducing N leaching compared to the controls. Shaaban *et al.* (2009) reported that the applications of humic acids reduce the leaching of N fertiliser in a silty clay soil and Ortega & Fernandez (2007) also reported that humic and fulvic reduce N due to high stimulation of microbial growth. On the other hand Avnimelech & Raveh (1976) reported that half of the N leached when fertilisers were applied.

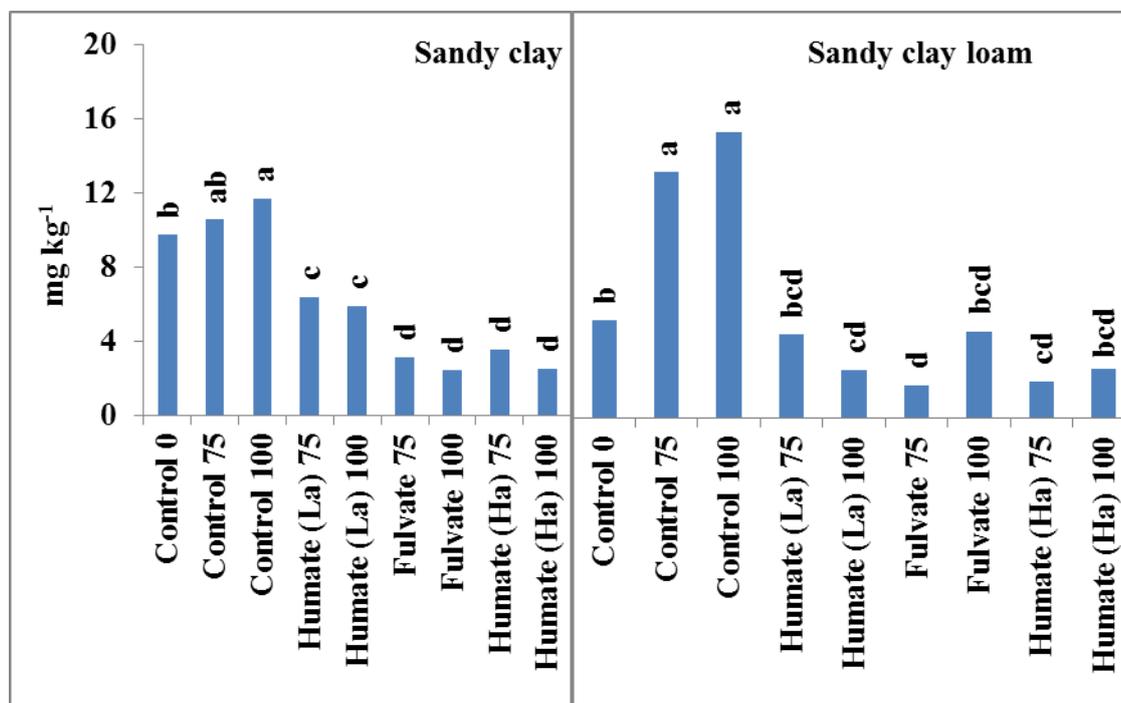


Figure 5.3.3.1. Nitrogen leached (mg kg^{-1}) from sandy clay and sandy clay loam soils.

1.2 Nitrogen mass balance for sandy clay and clay loam soil

The N balance of the sandy clay and sandy clay loam soil was calculated from the following formula:

$$N \text{ mass balance} = N \text{ input (Initial + fertiliser)} - N \text{ output (Leachate + retained)} \quad (5)$$

Where N_{initial} is the in situ N from the mineral and organic complexes of the soils used, $N_{\text{fertiliser}}$ is N added from fertiliser, N_{leachate} is N from the leaching water collected and N_{retained} is N from soil analysed at the end of the trial. On average the N mass balance error for sandy clay soil was 5.2% (Table 5.3.3.1), while for sandy clay loam it was 16.6% (Table 5.3.3.2). This may be due to the N mass balance components such as atmospheric losses, nitrification and denitrification and the mineralisation of N from the humates and fulvates which were not considered in calculating the mass balance error.

In general for both soils, the percentage of the applied N that was leached varied between 2.7-8.3% for humates and fulvate and between 12.9-27.5% for the control treatments. The leaching reduction from humates and fulvate treatments was approximately 300% compared to the controls. Therefore it is can be concluded that humates and fulvate were beneficial in reducing N leaching from the soils, and it is reasonable to expect that this will translate to increased availability to crops.

Table 5.3.3.1. Nitrogen mass balance for the sandy clay soil.

No	Treatments	mg kg ⁻¹								
		Initial	Applied	Total applied	Leached	% of total N leached	Retained	Total leached + Retained	*Mass balance error	*% error
1	Control 0	35.4	0	35.4	9.74	27.51	24.66	34.4	1	2.8
2	Control 75	35.4	41.25	76.65	10.56	13.78	66.26	76.86	-0.17	0.2
3	Control 100	35.4	55.01	90.41	11.69	12.93	73.70	85.39	5.02	5.5
4	Humate (La) 75	35.4	41.25	76.65	6.37	8.31	64.14	70.51	6.14	8.0
5	Humate (La) 100	35.4	55.01	90.41	5.90	6.53	86.60	92.50	-2.09	2.3
6	Fulvate 75	35.4	41.25	76.65	3.12	4.07	86.16	89.28	-12.63	16.4
7	Fulvate 100	35.4	55.01	90.41	2.39	2.64	88.76	91.15	-0.74	0.8
8	Humate (Ha) 75	35.4	41.25	76.65	3.52	4.59	80.70	84.22	-7.57	9.8
9	Humate (Ha) 100	35.4	55.01	90.41	2.48	2.74	88.60	91.08	-0.67	0.7

* Error was calculated by Total applied – Total leached + Retained, * Mass balance error divided by Total leached + Retained x 100.

Table 5.3.3.2. N mass balance for the sandy clay loam soil.

No	Treatments	mg kg ⁻¹								
		Initial	Applied	Total applied	Leached	% of leached compare to applied	Retained	Total leached + Retained	*Mass balance error	%
1	Control 0	20.9	0	20.90	5.75	27.51	15.83	21.58	-0.68	3.2
2	Control 75	20.9	41.25	62.15	16.88	27.16	39.06	55.94	6.21	9.9
3	Control 100	20.9	55.01	75.91	14.50	19.10	40.51	55.01	20.90	27.5
4	Humate (La) 75	20.9	41.25	62.15	2.82	4.53	75.78	78.60	-16.45	26.4
5	Humate (La) 100	20.9	55.01	75.91	4.88	6.42	52.60	57.48	18.43	24.2
6	Fulvate 75	20.9	41.25	62.15	5.08	8.17	63.25	68.33	-6.18	9.9
7	Fulvate 100	20.9	55.01	75.91	1.90	2.50	62.71	64.61	11.30	14.8
8	Humate (Ha) 75	20.9	41.25	62.15	2.82	4.57	71.16	74.00	-11.85	19.0
9	Humate (Ha) 100	20.9	55.01	75.91	2.15	2.83	62.75	64.90	11.01	14.5

* Error was calculated by Total applied – Total leached + Retained

1.3 P concentration of the leachate

The P concentration of the soils mixed with humate, fulvate and fertilisers are presented in Figure 2 for sandy clay and sandy clay loam. The highest significant P concentration in the leachate of the sandy clay soil was found for the fulvate 100 treatment. Higher P concentration was also recorded for the humate (La) 100 treatment. The lowest P concentrations were for the humate (Ha) 100 and control 0 treatments. For sandy clay loam, the results indicated the highest P concentration was for humate (La) 75 treatments, whereas the lowest P leaching was found for the control 0.

In general, the results showed that P concentration in the leachate was the highest for the humate and fulvate combined with fertiliser treatments. Even though P concentration in the leachate varied between 0.01 and 0.89 mg kg⁻¹ across the difference soil types, the range of variation is low. These results are supported by the research conducted by Zhang (2008) who investigated, the effect of soil properties on P subsurface migration in sandy soils in a leaching column, from which he found that P loss by leaching is low when Ca concentration in the soil solution is high. It is also well known that P does not easily leach in the soil due to various factors such as Ca (For the sandy clay the Ca content was 104 mg kg⁻¹ and for sandy clay loam it was 501 mg kg⁻¹) and Fe and this could be the main reason why P movement was lower for all treatments.

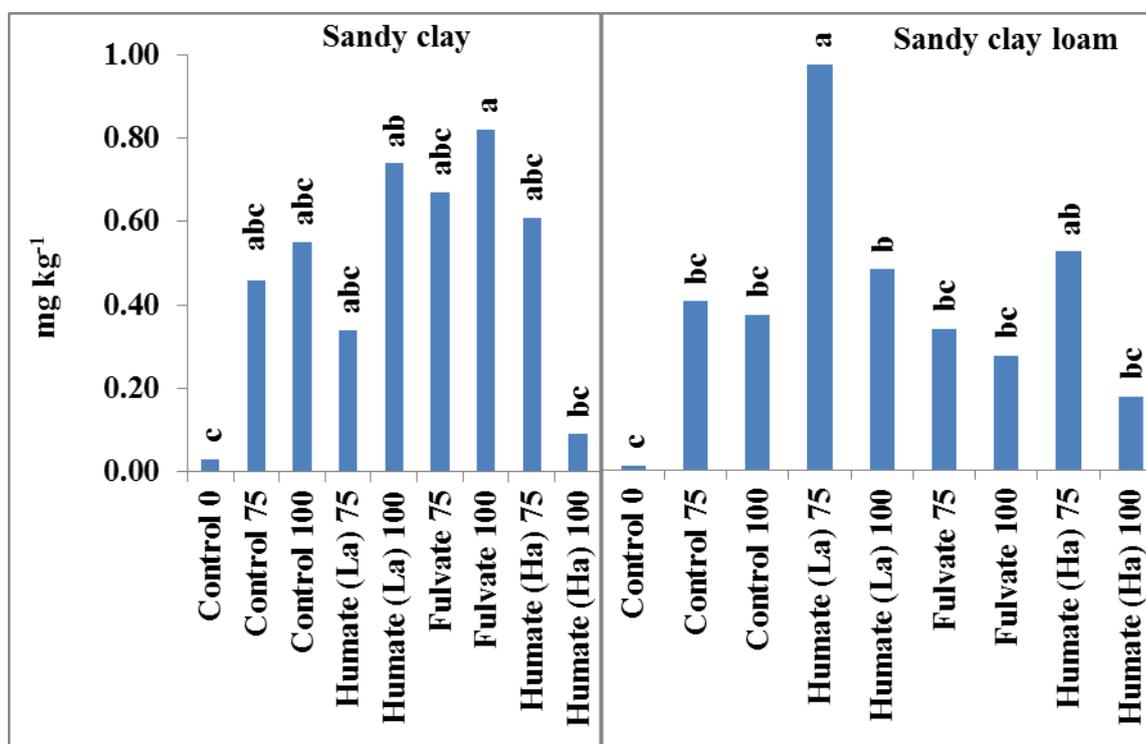


Figure 5.3.3.2. Phosphorus leached (mg kg⁻¹) from sandy clay and sandy clay loam soils.

2 K concentration of the leachate

The K concentration in the leachates of the different soils mixed with humate, fulvate and fertilisers are presented in Figure 5.3.3.3 for sandy clay and for sandy clay loam soils. For sandy clay soil, the leaching of K from control 0 was 2.88 mg kg⁻¹ (11.51 kg ha⁻¹) while for fertilisers and humates or fulvate combined with fertilisers varied between 6.75 mg kg⁻¹ (26.99 kg ha⁻¹) and 9.14 mg kg⁻¹ (36.55 kg ha⁻¹). For sandy clay loam, K leaching for control 0 was 1.15 mg kg⁻¹ (4.59 kg ha⁻¹), for control 75 it was 6.81 mg kg⁻¹ (27.23 kg ha⁻¹) and for control 100 it was 7.60 mg kg⁻¹ (30.39 kg ha⁻¹). These results indicated that K leaching was higher for humate (La) combined with fertiliser and for control 100 compared to the rest of the treatments although not significantly. The maximum leaching of K was for humate (La) 100 and it is clear from Figure 5.3.3.3 that humates and fulvate did not decrease K leaching in both soils.

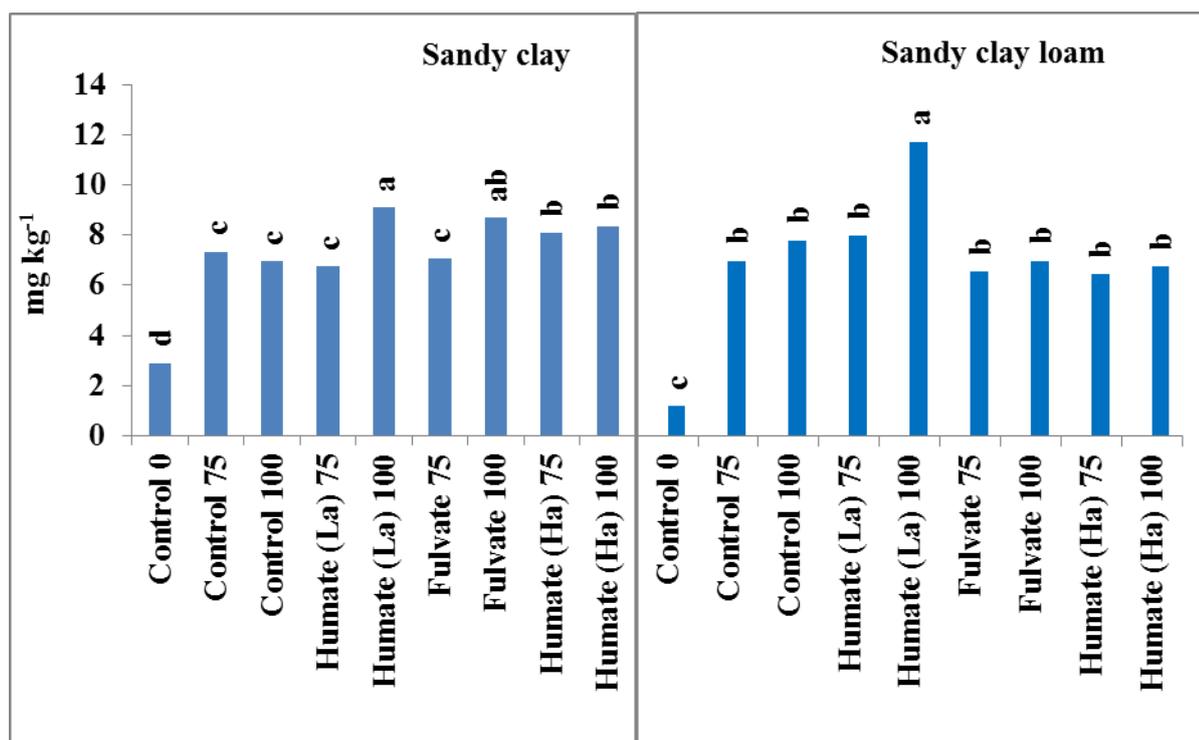


Figure 5.3.3.3. Potassium leached (mg kg⁻¹) from sandy clay and sandy clay loam soils.

3 Conclusions

The addition of humate or fulvate to soils mixed with fertilisers showed a high significance ($p < 0.01$) in decreasing the N concentration of the leachate of both soil types. Humic acids play an important role in the soil and increases nutrient availability and also increase chemical and biological properties of the soils by adding macronutrients. It was reported that humic acids increase carbon content and water holding capacity of the soils that reduces nutrient leaching (Hussein & Hassan, 2012).

Inconsistent results were found for P and K in both soil types and treatments due to the high concentration of P and K in the humates and fulvate. Therefore, humate and fulvate did not reduce P and K. The interaction between humic substances and P increases soil fertility at various soil layers (Selim *et al.*, 2010). Research done on the effect of the application of humic substances on quality and nutrition of potato tubers showed that the application of humic substances to the soil increases soil nutrient content (Ahmed, 2012).

In pot trials it was found Humate and fulvate treatments in a sandy clay soil affected N and K contents of citrus leaves and bark but not roots. P uptake was not significantly affected by the different treatments. For the sandy clay loam soil, N and P in leaves, bark and root were significantly affected by the different treatments. However, humate and fulvate treatments affected K content of the leaves and roots but not the bark. Similar results of N, P and K uptake were previously observed in sugar cane (Hishamo & Mohammad, 2007) maize, potato, spinach (Verlinden *et al.*, 2009) and barley (Ayuso *et al.*, 1996).

Technology transfer

This study is available as an M.Sc. thesis entitled “Nitrogen, phosphorus and potassium availability as influenced by humate and fulvate soil amendment” from the library of the University of Pretoria.

Effects of potassium humates and fulvate on the leaching of N, P and K

AJ Gatabazi¹, PC de Jager¹, JT Vahrmeijer^{1,2}

7th Citrus Research Symposium, Drakensberg, South Africa, August 2012

Influence of potassium humates and fulvate on the culturable soil microbial community

AJ Gatabazi¹, A Van der Merwe¹, PC de Jager¹, JT Vahrmeijer^{1,2}

7th Citrus Research Symposium, Drakensberg, South Africa, August 2012

Effects of potassium humates and fulvate on the leaching of N, P, K and the culturable soil microbial community

AJ Gatabazi¹, PC de Jager¹, A Van der Merwe¹ JT Vahrmeijer^{1,2}

Combined Congress 2013, 21-24 January, Durban

The effect of humate and fulvate amendments on the viable microbial population and nutrient leaching

A Gatabazi, PC de Jager, A vd Merwe. JT Vahrmeijer, NM Asanzi and NJ Taylor

Combined Congress, 20-23 January 2014, Grahamstown, South Africa

References cited

- AHMED, A.M. 2012. Effect of the application of humic substances on yield, quality and nutrient content of potato tubers in Egypt. *Sustainable Potato Production: Global Case Studies*. 471-492.
- AYUSO, M., HERNANDEZ, T., GARCIA, C. & PASCUAL, J.A. 1996. Stimulation of berry growth and nutrient absorption by humic substances originating from various organic materials. *Bioresource Technology*. 57, 251-257.
- CIMRIN, K.M. and YILMAZ, I. 2005. Humic acid applications to lettuce do not improve yield but do improve phosphorus availability. *Acta Agriculturae Scandinavica, Section B - Plant Soil Science* 55, 58-63.
- EMAN, A.A., SALEH, M.M.S. and MOSTAFA, E.A.M. 2008. Minimizing the quantity of mineral nitrogen fertilisers on grapevine by using humic acid, organic and biofertilisers. *Research Journal of Agriculture and Biological Sciences* 4, 46-50.
- HISHAMO, S.O. & MOHAMMAD, S.A. 2007. Utilisation of agricultural residues is an environmental and agricultural necessity; v-production of k-humate from sugar cane bagasse compost. *Africa Crop Science Conference Proceedings*. 8, 1585-1587.
- HUSSEIN, K. & HASSAN, F.A. 2012. Effect of different levels of humic acids on the nutrient content, plant growth, and soil properties under condition of salinity. *Soil and Water Research*. 6, 21- 29.
- SALMAN, S.R., ABOU-HUSSEIN, A.M.R., EL-NEMR, A. and EL-NEMR, M.A. 2005. Fruit yield and quality of watermelon as affected by hybrids and humic acid application. *Journal of Applied Sciences Research* 1, 51-58.
- SELIM, E.M., EL-NEKLAWY, A.S. and EL-ASHRY, S.M. 2009. Beneficial effects of humic substances fertigation on soil fertility to potato grown on sandy soil. *Australian Journal of Basic Applied Sciences* 3, 4351-4358.
- SHARIF, M., KHATTAK, R.A. and SARIR, M.S. 2002. Effect of different levels of lignitic coal derived humic acid on growth of maize plants. *Comunications in Soil Science and Plant Analysis* 33, 3567-3580.
- VERLINDEN, G., PYCKEN, B., MERTENS, J., DEBERSAQUES, F., VERHEYEN, K., BAERT, G., BRIES, J. & HAESAERT, G. 2009. Applications of humic substances result in consistent increases in crop yield and nutrient uptake. *Journal of Plant Nutrient*. 32, 1407-1426.
- ZHANG, M. 2008. Effect of soil properties on phosphorus subsurface migration in sandy soils. *Pedosphere*. 18, 599-610.

5.3.4 PROGRESS REPORT: Determining the time and duration of flower induction in early vs late mandarin cultivars and evaluating the effect of hand thinning, pruning and girdling on leaf and root carbohydrate levels, fruit size, vegetative regrowth and alternate bearing in Nadorcott mandarin

Project 1106 (Apr 2014-Mar 2018) by Jakkie Stander and Paul Cronje (CRI at SU)

Summary

The objective of this project is to pinpoint and compare the time and duration of flower induction in mandarins, by measuring the flowering inhibition response to GA₃ applications at different times throughout the expected flower induction period. Thereafter the project will evaluate manipulations of vegetative and reproductive growth from January to April to change carbohydrate allocation and/or restore carbohydrate levels and reduce the effect of endogenous gibberellins on flower induction in May to August. With this in mind, Nadorcott mandarin trees will be used in experiments to establish whether there is a significant treatment effect on carbohydrate availability in the leaves and possibly correlating it with the following season's fruit load and quality. In addition, treatment effects on problems such as small fruit size and vigorous vegetative regrowth will be quantified throughout.

Opsomming

Die doel van hierdie projek is om die tyd en durasie van blominduksie in mandaryne te bepaal en te vergelyk, deur die blomreaksie op verskillende GA₃ toedienings tydens die verwagde blominduksie periode te meet. Daarna sal verskillende manipulasies van vegetatiewe, sowel as reprodktiewe groei vanaf Januarie tot April evalueer word, met die doel om koolhidraat allokasie tussen sinkorgane te manipuleer en/of om koolhidraatvlakke te herstel en die inhiberende effek van interne gibereliene op blominduksie vanaf Mei tot Augustus te verminder. Nadorcott mandaryn bome sal in eksperimente gebruik word om vas te stel of daar enige betekenisvolle effek van behandelings op blaar- en wortel koolhidraat-vlakke is en dit moontlik korreleer met vruglading en kwaliteit. Behandelingseffekte sal ook addisioneel evalueer word op probleme soos klein vrugte en aggresiewe vegetatiewe groei.

5.3.5 PROGRESS REPORT: Effect of shade net on fruit production of mandarin citrus

Project 522020 (2014/15 – 2016/17) by N.J.R. Roets, R.B. Cronje (ARC-ITSC) and I.F. Ngwamba (UKZN)

Summary

Shade nets in fruit production are used mainly to reduce sunburn and hail damage. Even though shade nets are in use in the South African citrus industry, the effect thereof on production and physiology of mandarins has not been properly established. A trial aiming to determine the effect of white shade net on production, fruit quality and physiology of mandarins and orchard micro-climate has been initiated during 2014/2015. Two trial sites have been laid out on 'Nadorcott' and 'Nova' in the Nelspruit and Ohrigstad areas respectively. Temperature and relative humidity (RH) was compared between orchards under net and open orchards. On average temperature and RH under the net were 0.5°C lower and 3% higher respectively than for open orchards. Daily temperature and RH peaks were also less pronounced under the net compared to open orchards. Transpiration and stomatal conductance were similar for trees under shade nets and open trees in the morning between 9:00 and 10:00, while at midday trees under the nets displayed higher transpiration rates and stomatal conductance. Due to lower light intensity under the net, leaves of trees under the nets had significantly higher chlorophyll content than open trees. During the fruit growth stage, fruit of trees under the shade net grew at the same rate as fruit from open trees, but were significantly larger. During the next season it will be determined if earlier fruit set under the net may explain this result. A student (I.F. Ngwamba) from the University of KwaZulu-Natal has been added to the project and will focus in more detail on the effect of shade nets on post-harvest fruit quality of 'Nadorcott' for her M.Sc. Agric. degree in Horticultural Science. This student is currently busy preparing a comprehensive literature review on the topic.

Opsomming

Skadunette word hoofsaaklik gebruik om sonbrand en haelskade te verminder in vrugproduserende areas. Hoewel skadunette reeds gebruik word in die Suid-Afrikaanse sitrus industrie, ontbreek kennis oor die effek wat skadunette op die produksie en fisiologie van mandaryne het nog grootliks. 'n Eksperiment wat die effek van wit skadunet op die produksie, vrugkwaliteit en fisiologie van mandaryne en boord mikroklimate ondersoek is daarom gedurende die 2014/2015 seisoen geïnisieer. Twee proef persele met die kultivars 'Nadorcott' en 'Nova' word gebruik in die Nelspruit en Ohrigstad areas onderskeidelik. Daar is vasgestel dat temperatuur en relatiewe humiditeit (RH) onder die skadunette onderskeidelik 0.5°C laer en 3% hoër is wanneer vergelyk word met oop persele. Daaglikse temperatuur en RH pieke is ook minder prominent onder die nette as vir oop persele. Die tempo van transpirasie en huidmondjie geleiding is soortgelyk vir bome onder nette en oop bome in die oggend tussen 9:00 en 10:00, maar hoër vir bome onder die nette gedurende die middel van die dag in vergelyking met oop bome. As gevolg van die laer ligintensiteit onder die nette, was die blaar chlorofil inhoud van bome onder die nette hoër as vir oop bome. Gedurende die vruggroei fase, het vrugte van beide bome onder die nette en oop bome teen dieselfde tempo gegroei, maar vrugte van bome onder die nette was betekenisvol groter. Daar sal gedurende die volgende seisoen vasgestel word of dit as gevolg van vroeër vrugset was. 'n Student (I.F. Ngwamba) van die Universiteit van KwaZulu-Natal is bygevoeg op die projek. Vir haar M.Sc. Agric. in Tuinbou Wetenskappe sal sy in meer besonderhede fokus op die effek van die skadunette op na-oes vrugkwaliteit van 'Nadorcott'. Sy is tans besig om 'n omvattende literatuur oorsig oor die onderwerp saam te stel.

5.3.6 **PROGRESS REPORT: Effect of pruning on fruit production of Nadorcott mandarin**
Project 522019 (2014/05 – 2016/17) by R.B. Cronje, C.F. Human and I.M. Ratlapane (ARC-ITSC)

Summary

A trial aiming at developing pruning strategies for 'Nadorcott' mandarin, both young and old trees, was initiated in 2014. The trial on older trees (8 years old) consists of six treatments including selective pruning by hand (light and severe; after harvest or after fruit drop), mechanical pruning after harvest, a combination of hand and mechanical pruning in alternate years and a control (farm practice). The trial on young trees (2.5 years old) includes three treatments, namely two selective hand pruning treatments (pyramid and open vase shape) and a control (untreated until trees touch each other). Pruning was carried out in August 2014, November 2014 (after fruit drop, only one treatment) and January 2015 (shoot control). All removed branches were weighed at all pruning times to determine the amount of plant material removed from each treatment and pruning time. Tree height was measured before and after pruning. Leaf samples were taken at fruit set (Sep), after fruit drop (Nov) and at flower initiation (April) to determine changes in starch levels of the trees.

Tree height in the older trees was reduced on average by 1 m (26.6% of initial height) and was lowest in the selective pruning after fruit drop (14%) and highest in the severe pruning after harvest treatment (33.5%). Removed plant material of all pruning times combined was lowest in the light selective pruning treatment and the control (25 and 27.5 kg/tree, respectively), and the highest in the combination of selective pruning and mechanical pruning in alternate years and the severe selective pruning treatment (43.5 and 40.2 kg/tree, respectively). Starch content varied between 2-4% at fruit set and between 12-14% after the fruit drop period. No tendencies are visible at this stage of the trial. The trials will continue with the first trial harvest data being collected in July 2015 and the second season's pruning to be done in August 2015.

Opsomming

'n Proef vir die ontwikkeling van 'n snoeistrategie vir 'Nadorcott' mandarin op ouer sowel as jong bome is in 2014 begin. Die proef op ouer bome (8 jaar oud) bestaan uit ses behandelings wat selektiewe snoei met die hand (lig en hard, na oes, of na vrugval), meganiese snoei na oes, 'n kombinasie van hand en meganiese snoei in alternatiewe jare en 'n kontrole (plaaspraktyk) insluit. Die proef op jong bome (2.5 jaar oud), sluit drie behandelings in, naamlik twee selektiewe hand snoei behandelings (piramide en oop kelk) en 'n kontrole (onbehandel tot bome aan mekaar raak). Die snoei behandelings is in Augustus 2014, November 2014 (na vrugval, slegs een behandeling) en Januarie 2015 (waterloot beheer) gedoen. Alle afgesnyde takke is geweeg op al die snoeidatums om die hoeveelheid plantmateriaal wat van elke behandeling en snoei tyd verwyder is te bepaal. Boomhoogte is voor en na snoei gemeet. Blaarmonsters is op vrugset (Sept), na vrugval (Nov), en op blominisiasie (April) versamel om veranderings in styselwaardes te bepaal.

Boomhoogte in die ouer bome is gemiddeld met een meter verminder (26.6% van oorspronklike hoogte) en was die laagste in die selektiewe snoei behandeling na vrugval (14%) en die hoogste in die harde selektiewe snoei behandeling na oes (33.5%). Die verwyderde plantmateriaal van alle snoeitye gekombineer was die laagste in die lig selektiewe snoei behandeling en die kontrole (25 en 27.5 kg/boom, respektiewelik) en die hoogste in die kombinasie van selektiewe handsnoei en meganiese snoei in alternatiewe jare en die harde selektiewe snoei behandeling na oes (43.5 en 40.2 kg/boom, respektiewelik). Styselwaardes het gewissel van 2-4% met vrugset en van 12-14% na die vrugvalperiode. Nog geen neigings is sigbaar op hierdie stadium van die proef nie. Die proef gaan voort met die eerste oesdata wat in Julie 2015 versamel word en die tweede seisoen se snoeiaksie wat in Augustus 2015 sal plaasvind.

5.4 **PROGRAMME: CULTIVAR EVALUATION**

Programme coordinator: Johan Joubert (CRI)

5.4.1 **PROGRAMME SUMMARY**

Mandarins and lemons recently became the two mainstream competitors in the citrus industry with regards to consumer demands for variable reasons. Mandarins more specifically for their colour attraction, peelability and flavour, but lemons more for their health benefits. There are more limitations for planting mandarins (5.4.4, 5.4.7, 5.4.8, 5.4.16, 5.4.17, 5.4.18, 5.4.19, 5.4.24) and specific climatic requirements (cool and intermediate) compared to lemons that are suitable for most citrus production areas (5.4.25). The mandarin selection range varies from early to late maturing with numerous new selections still in the pipeline. There were good results with the mandarin selections in specific hot production areas (semi-desert etc.) and future development potential seems promising. The lemon demand is very high and as a result there were high numbers of new plantings. The typical fruit shape and seedlessness of the lemons were crucial in the past, but good fruit quality with some seeds (seed content not a major issue) meets the consumer requirements. The grapefruit (Star Ruby) prices in 2014 were poor and as a result of this scenario, many producers removed their trees or topworked them to either lemons or mandarins. There will be a decrease in grapefruit production (one third) for the next season so prices will recover due to the supply and demand balance. The promising new navel and Valencia selections performed well (5.4.20, 5.4.21, 5.4.23, 5.4.2, 5.4.3, 5.4.5, 5.4.6, 5.4.10, 5.4.22, 5.4.20, 5.4.21, 5.4.23) in the suitable citrus production areas where demand for seedless Valencias with good crop production increased. Producers located in the hot production areas less suitable for mandarin farming, have been investing in the cooler areas for optimal soft citrus production. Future evaluation sites will be located in main citrus production areas with a range of cultivars on suitable rootstocks, to offer the grower the best possible opportunity to determine what they should plant, with the lowest possible risk. Rootstock research is expanding and the importance of optimal rootstock choices for specific scion, climate and soil type as well as water quality are crucial (5.4.9, 5.4.12, 5.4.13). There are a range of new rootstocks in the pipeline that will be more compatible with specific conditions as well as address the needs for smaller tree volumes. The need to distinguish between cultivars using DNA fingerprinting has become crucial. The technique works well with mandarins, but requires fine tuning when it comes to sweet oranges (5.4.26).

PROGRAMOPSOMMING

Die twee hoofrol spelers in die sitrus bedryf huidiglik is mandaryne en suurlemoene wat gewildheid onder verbruikers aanbetref vir verskeie redes. Mandaryne meer vir hulle aantreklike kleur, sklibaarheid en geur waar suurlemoene vir gesondheids redes verbruik word. Mandaryn aanplantings (5.4.4, 5.4.7, 5.4.8, 5.4.16, 5.4.17, 5.4.18, 5.4.19, 5.4.24) word meer beperk deur spesifieke klimaats vereistes (koel en intermediere areas), waar suurlemoene basies in alle sitrus produserende areas aangeplant kan word (5.4.25). Die reeks mandaryn seleksies varieer van vroeg tot laat rypwordend, met baie nuwe seleksies nog steeds in die pyplyn. Die mandaryn seleksies presteer verbasend goed in sekere warm produksie areas (semi-woestyn ens.) met goeie uitbreidings potensiaal vir die toekoms. Die aanvraag na suurlemoene is baie hoog, met die gevolg dat suurlemoen aanplantings die hoogte in geskiet het. Die lang silindriese vrugvorm en saadloosheid wat krities was vir suurlemoen produksie in die verlede het afgeneem en die verbruikers vereis huidiglik goeie kwaliteit vrugte waar saadinhoud nie krities is nie. Met die swakker pomelo (Star Ruby) pryse van 2014 het heelwat produsente hulle bome verwyder of oorgewerk na suurlemoene en mandaryne. Die pomelo aanbod (volume) gaan dus heelwat afneem (een derde) wat goed gaan wees vir die volgende seisoen waar pryse moontlik sal herstel. Die belowende nuwe nawel en Valencia seleksies presteer goed (5.4.20, 5.4.21, 5.4.23, 5.4.2, 5.4.3, 5.4.5, 5.4.6, 5.4.10, 5.4.22, 5.4.20, 5.4.21, 5.4.23) in die geskikte sitrus produksie areas, waar aanvraag vir saadlose Valencia's met goeie opbrengste toeneem. Produsente in die warm sitrus produksie areas wat minder geskik is vir Mandaryn verbouing, infesteer nou in die koeler areas waar sagtesitrus optimaal geproduseer kan word. Toekomstige evaluasie persele sal in die belangrikste sitrus produksie areas gevestig word met die grootste variasie kultivars moontlik op geskikte onderstamme, om vir die sitrus produsent die beste moontlike geleentheid te skep om goed ingeligte besluite te kan neem oor nuwe aanplantings met laagste moontlike risiko. Onderstam navorsing word uitgebrei, die noodsaaklikheid om die optimum onderstam keuse vir 'n spesifieke bostam, klimaat, grond tipe asook waterkwaliteit te bepaal is krities (5.4.9, 5.4.12, 5.4.13). Daar is 'n reeks nuwe onderstamme in die pyplyn wat meer aanpasbaar sal wees vir sekere toestande en om ook die toenemende aanvraag na kleiner boomvolumes aan te spreek. Die behoefte om kultivars van mekaar te onderskei d.m.v DNA vingerafdrukke neem toe. Die tegniek werk relatief goed vir die Mandaryne, maar op die soetlemoene kort daar nog heelwat werk (5.4.26).

5.4.2 **PROGRESS REPORT: Evaluation of Valencia selections in hot humid inland areas (Onderberg)**
Project 75 A by J. Joubert and S.D. Maziya (CRI)

Opsomming

Daar was geen oes hierdie jaar op Weipe wat Limpopo SL as vroegste Valencia seleksie vervang het nie, evaluasies sal voortgaan vir die volgende seisoen. Seleksies wat hierdie seisoen volgens optimum rypheid van vroeg tot laat goed presteer het is soos volg vir hierdie vroege warm produksie area. Weipe seleksie kom op 'n jong boom ouderdom in drag, wat vinniger kontant vloei kan verseker. Turkey sal dan volg, wees net versigtig om nie die seleksie te lank te hang nie. Baie skil probleme kan ontwikkel, want die optimum oes tydperk strek oor gemiddeld vier weke, ses weke maksimum dan moet die vrugte af wees.

Benny 2 kan dan volg wat goeie interne kwaliteit, produksie en vruggrootte lewer. Bend 8A1, Alpha en Midnight, aangevul deur Gusocora en McClean SL verteenwoordig die middel van die Valencia seisoen vir hierdie area, asook Louisa (vroeg ryp op ouer bome) gevolg deur Nouvelle La Cotte en Lavalley 2. Die later seleksies wat kan bydra tot die keuse om die seisoen te verleng, kan bestaan uit Moosrivier Late 1 (eksperimentele seleksie) en Henrietta (lae saadinhoud), en dan laastens opgevolg word deur Skilderkrans, wat 0.5 sade per vrug produseer.

Weipe, Henrietta, Louisa, Skilderkrans en Bend 8A2 is steeds eksperimentele/semi-kommersiele seleksies wat goed presteer. Hierdie seleksies kan in die toekoms ingesluit word soos meer en beter inligting beskikbaar word.

Summary

There was no crop this season on Weipe which replaced Limpopo SL as the earliest maturing experimental Valencia selection; evaluations will continue for the next season. Selections that performed well in this season, according to optimal maturity from early to late in this hot, humid production area, are as follows. Weipe bears fruit precociously and will start cropping good yields on young trees, generating returns for your investment sooner. Turkey will follow, but bear in mind that the selection has a sensitive rind. Do not hang the fruit too long because the optimal picking period is no longer than 4-6 weeks.

Benny 2 would follow, with good internal quality, production and fruit size. Bend 8A1, Alpha and Midnight, with the addition of Gusocora and McClean SL represent the middle of the Valencia season for this area, as well as Louisa (matures earlier on older trees) followed by Nouvelle La Cotte and Lavalley 2. The later selections can broaden the list of choices to extend the season, commencing with Moosrivier Late 1 (experimental selection) and Henrietta (low seeded) followed by Skilderkrans, producing 0.5 seeds per fruit.

Weipe, Henrietta, Louisa, Skilderkrans and Bend 8A2 remain experimental/semi-commercial selections that performed well. These selections should be included in future plantings when more and better information becomes available.

Objective

- To find suitable Valencia selections with superior characteristics for the hot inland citrus production areas.

Materials and methods

Field evaluations and laboratory analyses were conducted on Alpha, Bend 8A 1&2, Benny 2, Gusocora, Henrietta, Lavalley 2, Louisa, McClean SL, Midnight (control), Moosrivier Late 1&2, Nouvelle La Cotte, Ruby, Skilderkrans and Turkey (control) at Esselen Nursery, Malelane, Mpumalanga.

Table 5.4.2.1. Internal fruit quality minimum export requirements for Valencia types.

Variety	Juice %	Brix	Min Acid	Max Acids	Ratio	Colour
Valencia EU	48	8.5	0.6	1.8%	7.5:1	Colour plate 3 of set no. 34
Midknight	52	9.5	0.85	1.8%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

*Interim internal fruit quality standards.

Table 5.4.2.2. List of Valencia selections evaluated at Esselen Nursery (Malelane) during 2014.

Selection	Rootstock	Year Planted	No. of trees
Alpha	CC	1996	1
Bend 8A1	SC	2003	1
Bend 8A2	SC	2003	1
Benny 2	C35	2003	1
Gusocora	SC	2008 (Topwork)	1
Henrietta	MxT	2006	1
Lavalle 2	CC	2010 (Topwork)	2
Louisa	MxT	2006	1
McClellan SL	C35	2001	1
Midknight	C35	2001	1
Moosrivier Late 1	SC	2003	1
Moosrivier Late 2	SC	2003	1
Nouvelle La Cotte	MxT	2006	1
Skilderkrans	MxT	2006	1
Turkey	CC	1996	1
Turkey	C35	1998	1

Results and discussion

This project is ongoing – all evaluations and tasks have been completed to date. Trees were visually evaluated at Esselen Nursery (Malelane) during the 2014 season.

Alpha

Alpha produced similar fruit size this season (compared to 2013) ranging from medium to large (count 72-56) on the trees: optimal fruit size for export Valencias. The trees' condition was very good on Carrizo rootstock, a well known rootstock with good replant qualities. The selection developed less than 0.3 seeds per fruit this season (compared to 0.1 for 2013), resulting in the seedless qualities of the Alpha Valencia. The fruit shape was fairly round with smooth skin texture and thin rind thickness, no navel end was visible and no thorns were on the bearing branches. There was a good crop on the trees, estimated at 100 kg per tree. The season was earlier compared to 2013 with the second evaluation on 15 July 2014, with better external colour (T1 to T2) and exceptional internal quality. Brix was above 10, juice content was 60% and acid levels remained above 1.0%, indicating good shelf life for the fruit (Table 5.4.2.3) and complying with minimum export standards. Based on the internal quality results in Table 5.4.2.3, estimated maturity was end of June to middle of July.

Bend 8A1&2

There was a significant increase in seed content for Bend 8A1 this season with 4.9 seeds per fruit and a decrease for Bend 8A2 from 1.5 to 0.6. The same scenario in regards to fruit size appeared this season, where the fruit size on Bend 8A2 was bigger (between count 64 and 48), and Bend 8A1 smaller, peaking between count 72 and 48. There was a setback this season, where none of the two selections complied with

the export standards. Bend 8A1 developed a juice content of 36.1% and Bend 8A2 a Brix level of 7.6. External colour was similar in both selections between T1 and T3. Due to poor internal quality there was no estimated maturity time for the 2014 season.

Benny 2

The fibre strength (rag) remains soft compared to the other Valencia selections, except for Turkey being softer. The fruit size was erratic this season, measuring from count 88 to 36. Tree size remains one third smaller on Citrange 35 compared to the other combinations, resulting in easier spray and harvesting practices. Benny 2 matures after Turkey and before Midnight or Delta so fits in well in the harvesting and packing programme. Seed count was higher this season and varied from 4.4 to 6.1 seeds per fruit (1.5 to 3.2 for 2013). There was no delay in external colour this season and with the second evaluation, colour development was between T1 and T3. Brix: acid ratio was above 11 and Brix above 11, complying with export standards. Based on the internal quality results in Table 5.4.2.3 maturity was estimated as end of June to middle of July.

Gusocora

The external colour development improved this season compared to 2013. There was still a delay in external colour when compared to the internal quality maturity, between T3 and T5 with average Brix of 8.6 and acid of 1.0%. Swingle as rootstock will delay external colour development on the fruit. Gusocora developed 0.3 seeds per fruit this season and will be regarded as a seedless selection. The internal quality complied with the export standards with low Brix content (8.6), Brix: acid ratio below 10 but external colour T3 to T5. Based on the internal quality results in Table 5.5.2.3 maturity was estimated as mid to end of July.

Henrietta

Fruit shape remained round, rind texture smooth and small thorns were visible on the bearing branches. Rind thickness was fairly thin; fruit peeled easily and contained a medium amount of rind oil. There was an increase in average seed count from 1.2 to 4.9 seeds per fruit, similar to that of the 2012 season. The tree condition on MxT was very good. There was a decrease in fruit size this season and it varied from medium to large (count 72 – 56), compared to last year's bigger fruit size (count 48). This specific size range was more favourable for Valencia exports. Internal quality indicated that Henrietta matures late in the Valencia season, with an acid content of 1.2% and external colour T2-3. Based on the internal quality results in Table 5.4.2.3 maturity was estimated as mid July to end July.

Lavalle 2

Yield remained good to excellent; one of the qualities of the Lavalle 2 selection. Another quality is good fruit size for a Valencia selection, and the fruit size varied from count 88 to 48; excellent for Valencia production. The higher acid level (1.2%) tested with the evaluation, indicated that this selection was late maturing internally. There were 1.8 seeds per fruit present in the fruit evaluated this season; a slight increase from last season. The internal quality complied with the export standards, producing Brix levels of 12 and juice of 59%. Lavalle 2 evaluated was planted on C35 developing a smaller tree size with good internal quality. Based on the internal quality results in Table 5.4.2.3 maturity was estimated as mid to end August.

Louisa

The seed count increased from 0.3 to 0.9 seeds per fruit this season, and at Group 91 the fruit remained completely seedless. The fruit size tended to be bigger (count 56-48), possibly due to the lighter crop. Fruit shape was round, rind texture medium to fairly smooth and the rind was medium to fairly thick. The fruit peeled easily and the internal colour was yellow. There was a lighter crop compared to the other selection of the same age. With the evaluation the acid levels were lower compared to Henrietta, still indicating a late Valencia selection and the external colour was already at T1. Based on the internal quality results in Table 5.4.2.3 maturity was estimated as end of July to mid August.

McClellan SL

The standard McClellan will be included in future trials as a control to compare the SL selection's performance. McClellan SL produced fairly round fruit with soft fibre strength that peeled easily, containing low rind oil levels. All the fruit evaluated remained completely seedless. Many totally seedless selections have fruit set problems and bear less fruit, but this does not appear to be the case with this cultivar. The fruit size peaked at medium-large to large (count 64-48). The internal quality was good with the highest juice levels for the trial site of 64%, Brix 9.5 and acceptable acid levels (1.0%). There was a slight delay in external colour ranging from T3-5. Based on the internal quality results in Table 5.4.2.3 maturity was mid to end July.

Midknight (Control)

C35 in combination with Midknight resulted in a medium sized tree; one third smaller compared to the tree size of Swingle. The bud union looks very good; smooth with no signs of incompatibility. There were a few indications of C35 being incompatible with other selections, Turkey being one of them.

The fruit size increased this season and the tree produced medium to large (count 72-56) fruit size. Fruit shape is fairly round, rind texture medium-coarse, fibre strength fairly soft and the fruit peels easily. Internally the flavour varied from good to very good, with juice levels around 60% and Brix 10. The acid level was higher this season (1.0%), but complied with the export requirements (Max 1.8 to min 0.85). Based on the internal quality results in Table 5.4.2.3 estimated maturity was end of June to middle of July.

Moosrivier Late 1&2

Moos Late 1 developed a very high acid level (1.3%) when the juice (54%) and Brix (8) content were ready for harvesting, and the external colour also developed up to T1-2. Moos Late 1 developed more seeds this season, increasing from 0.8 to 2.1 seeds per fruit. Moos Late 1 had promising performance, developed smooth round fruit with deep yellow internal colour, good flavour, peeled easily and fairly soft rag.

Moos Late 2 performed poorly this season, not meeting export requirements with delayed external colour development between T4-6 and low IQ. Based on the internal quality results in Table 5.4.2.3 estimated maturity for Moos Late 1 was from the end of July to the middle of August.

Nouvelle La Cotte

The acid level dropped lower compared to the 2013 season (1.1%) when the external colour developed to T1-2. Fruit size remained similar and peaked from count 72 to 56, with a light crop on the trees. There were small thorns visible on the bearing branches of the tree. Fruit shape was round, rind smooth and fairly thin, peeled easily and fair flavour. Based on the internal quality results in Table 5.4.2.3, estimated maturity was the end of July to the middle of August.

Ruby

Ruby peaked between count 125 and 88, producing small to medium fruit on the tree. With the evaluation the internal quality complied with the export standards, acid content of 1.3% and external colour was between T3-5. Yield production on the tree was very good, explaining the smaller fruit size. Fruit characteristics consist of good flavour, round fruit shape, smooth skin texture, fairly thin rind, fruit peeling easily and a deep red internal colour. For this season the Esselen trial site compared excellently to the Group 91 trial site with similar results and conclusions. There were some split fruit, no creasing or sunburn visible. Based on the internal quality results in Table 5.4.2.3, estimated maturity was end of July to mid August.

Skilderkrans

Skilderkrans developed medium to large fruit size (count 64-40) on the trees, due to the lighter crop. The internal quality was good, with a higher acid content compared to the previous season of 1.6% at external colour T1, above the export maximum of 1.4% for Middle East and below the 1.8% for Europe by the time of the evaluation. The fruit peeled fairly easily, rind thickness was thin, rag was medium tough (raggy/strong), fruit shape was round and the rind texture medium-rough. The average seed count decreased this season from 1.5 to 0.5 seeds per fruit; compared to 2013. Based on the internal quality results in Table 5.4.2.3 estimated maturity was the end of July to middle August.

Turkey (Control)

Fruit size remained similar to last season from count 88 and 48, medium to large fruit size for this season. Fruit characteristics for Turkey were round fruit shape, smooth rind texture, very good flavour, soft rag, fairly thin rind, fruit peeling easily and higher seed count per fruit of 5.0 on average. The internal colour was light yellow, and externally the fruit remained yellow up to completely over matured fruit. It should be borne in mind that this selection is not a true Valencia and actually has the qualities of a mid-season orange, for instance the exceptionally soft rag of the fruit, and the soft rind that can result in rind problems if managed incorrectly. The Turkey should not be harvested over more than four weeks as extending the harvesting season can lead to rind disorders developing. Based on the internal quality results in Table 5.4.2.3, estimated maturity was the end of May to mid-June.

Weipe

There was no crop on the Weipe trees for the 2014 season; evaluations will continue next season.

Conclusions

Bend 8A1 and Moos Late 2 developed low juice and acid levels and did not comply with the export

standards. The internal quality for this season on the rest of the selections evaluated complied with the export standards, with the exception of the late maturing Skilderkrans, where the acid levels were above 1.4% (EU). Skilderkrans developed into a late maturing Valencia with high acid levels (1.6%) at both Esselen and Group 91's trial sites. These acid levels will decrease towards the end of the season, indicating extended shelf-life of the selections. Where the Brix: acid ratio was below 7:1 it was often associated with later maturing selections having higher acid levels. When the acid levels decrease, the ratio will increase. There was a better colour development present with most of the selections. The average seed count for this season decreased on most of the selections, indicating less cross pollination in the mixed trial block. McClean SL remained completely seedless. Fruit size increased on the trees, between count 88 and increasing up to count 40 on selections with lighter yields, except for Ruby Valencia with count 125 due to heavy crop load.

Table 5.4.2.3. Internal fruit quality data for Valencia and late orange selections at Esselen Nursery (Malelane) during the 2014 season.

Selection	Root-stock	Date harvested	Size mm	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Alpha	CC	2014/07/02	74-80	72-64	56.5	9.6	1.14	8.3	0.0	T3-4
Alpha	CC	2014/07/15	78-85	64-56	59.2	10.0	1.15	9.6	0.3	T1-2
Bend 8A1	SC	2014/07/15	77-87	72-48	36.1	9.0	1.04	8.7	4.9	T1-2
Bend 8A2	SC	2014/07/02	79-86	64-48	57.0	7.6	1.05	7.2	0.6	T1-3
Benny 2	C35	2014/07/02	69-74	88-72	57.3	12.0	1.01	11.9	4.4	T2-3
Benny 2	C35	2014/07/15	72-80	88-64	55.2	11.3	0.93	12.2	6.1	T1-3
Gusocora	SC	2014/07/15	83-90	56-40	54.3	8.6	1.05	8.2	0.3	T3-5
Henrietta	MxT	2014/07/15	77-83	72-56	57.7	9.9	1.20	8.3	4.9	T2-3
Lavalle 2	C35	2014/07/15	68-86	88-48	58.7	11.6	1.15	10.1	1.8	T2-4
Louisa	MxT	2014/07/15	82-87	56-48	51.8	10.7	1.00	10.7	0.9	T1-2
McClean SL	C35	2014/07/15	79-87	64-48	64.0	9.5	0.99	9.6	0.0	T3-5
Midnight	C35	2014/07/02	79-85	64-56	58.2	10.5	0.98	10.7	0.2	T2-3
Midnight	C35	2014/07/15	75-83	72-56	60.1	10.1	0.96	10.5	0.1	T1-2
Moosrivier Late 1	SC	2014/07/15	70-77	88-72	54.2	8.1	1.3	6.2	2.1	T1-2
Moosrivier Late 2	SC	2014/07/15	87-93	56-40	45.9	7.4	0.67	11.0	0.0	T4-6
Nouvelle La Cotte	MxT	2014/07/15	75-85	72-56	53.7	9.7	1.14	8.5	0.4	T1-2
Ruby	SC	2014/07/15	64-70	125-88	58.6	9.6	1.34	7.2	5.7	T3-5
Skilderkrans	MxT	2014/07/15	81-94	64-40	52.3	10.5	1.59	6.6	0.5	T1-3
Turkey	C35	2014/05/15	72-80	88-64	56.2	11.1	1.20	9.3	2.9	T3-6
Turkey	C35	2014/07/02	75-86	72-48	55.4	12.5	0.89	14.0	3.1	T1-2
Turkey	C35	2014/07/15	75-83	72-56	55.8	12.5	0.85	14.7	6.1	T1-2
Turkey	CC	2014/07/15	72-84	88-56	54.9	11.6	0.96	12.1	8.7	T2-3

5.4.3 PROGRESS REPORT: Evaluation of Valencia selections in the hot dry inland areas (Letsitele & Hoedspruit)

Project 75 B by J. Joubert and S.D. Maziya (CRI)

Opsomming

Die seisoen begin met vroeg rypwordende seleksies en duur voort met die laat rypwordende seleksies in die warm droë produksie areas en aanbevelings is soos volg. Weipe SL kan die seisoen begin, hierdie seleksie het Limpopo SL vervang as vroeg rypwordende Valencia. Turkey kan nou volg, wat groot vrugte produseer met goeie interne kwaliteit en sagte vessel. Optimum plukvenster is binne die eerste vier weke van piek rypheid. Benny 1 en 2 volg na Turkey met goeie produksie en medium tot groot vuggrootte. Delta as kontrole pas in voor Gusocora. Gusocora volg dan met totaal saadlose vrugte en goeie Brix: suur verhoudings. Midnight 1 en 2 vul die middel van die Valencia seisoen met goeie interne kwaliteit vrugte, groot vuggrootte, gladde skille en lae saadtellings per vrug. Du Roi is volgende met uitstekende oeste op die bome en medium tot medium/groot vrugte (telling 88 tot 64). Lavalle is huidiglik die laatste rypwordende Valencia seleksie wat semi-kommersieel aangeplant word, wat uitstekende vuggrootte en produksie.

Daar is 'n reeks eksperimentele/semi-kommersiele seleksies wat ook vir die warm produksie areas ingesluit

is. Hier volg die seleksies van vroeg, middle tot laat rypwordend. Die seisoen kan begin word met Bend 8A1&2, hierdie seleksie kan ook as aanvulling saam met Benny 1 en 2 gebruik word. Die middel van die Valencia seisoen word gekomplimenteer deur Jassie en Henrietta, wat goeie produksie en interne kwaliteit vrugte lewer. Louisa word meer aan die einde van die Valencia seisoen ryp, is totaal saadloos met groot vruggroote, gevolg deur Ruby en Skilderkrans. Ruby is die enigste rooi gepigmenteerde Valencia huidiglik beskikbaar met uitstekende produksie op die bome, kleiner vruggroote en drie tot vier sade per vrug. Laat in die seisoen kan aangevul word met Moosrivier Late 1, soos meer inligting beskikbaar word uit verdere evaluasies.

Summary

The season starts with early selections and proceeds to the late maturing selections suitable for this hot-dry production area. Recommendations have therefore been made accordingly. Weipe SL will start the season, replacing Limpopo SL as an early maturing Valencia. Turkey will follow, producing large fruit size with good internal quality and soft fibre. The optimal picking window will be within the first four weeks of peak maturity. Benny 1 and 2 follow after Turkey with good production and medium to large fruit size. Delta as a control fits in before Gusocora. Gusocora follows next with completely seedless fruit and very good Brix: acid ratios. Midnight 1 and 2 cover the middle of the Valencia season with good internal quality fruit, large fruit size, smooth rind and low seed counts per fruit. Du Roi follows with excellent crop on the trees and medium to medium-large fruit size (count 88 to 64). Lavalley is currently the latest maturing Valencia selection that is being planted semi-commercially; developing excellent fruit size and yield.

There is a series of experimental/semi-commercial selections that have also been included in the hot production areas. The selection range will follow from early, mid, to late-maturing options. The season starts with Bend 8A 1&2; this will be additional selections available to fill in with Benny 1 and 2. The middle of the Valencia season will be complimented by Jassie and Henrietta, delivering good production and internal quality fruit. Louisa matures more towards the end of the Valencia season, is completely seedless with large fruit size, followed by Ruby and Skilderkrans. Ruby is the only red pigmented Valencia available with excellent yield production, smaller fruit size and three to four seeds per fruit. Late in the season you could possibly add Moosrivier Late 1 to the options, when more information becomes available from future evaluations.

Objective

- To find suitable Valencia selections with superior characteristics for the hot inland citrus production areas.

Materials and methods

Field evaluations and laboratory analyses were conducted on Alpha, Bend 8A 1&2, Benny 1&2, Delta, Du Roi, Gusocora, Henrietta, Jassie, Lavalley, Louisa, McClean SL, Midnight 1 & 2, Moosrivier Late 1 & 2, Ruby, Skilderkrans, Turkey, Val Late and Weipe at Moriah Citrus (Hoedspruit), Bosveld Citrus (Letsitele) and Group 91 (Letsitele).

Table 5.4.3.1. List of Valencia selections evaluated at Group 91 (Letsitele) during 2014.

Selection	Rootstock	Tree Age	No. of trees
Bend 8A 1	CC/SC	2005	10/10
Bend 8A 2	CC/SC	2005	10/10
Benny 1	CC/SC	2005	10/10
Benny 2	CC/SC	2005	10/10
Gusocora	RL		Semi-Com
Henrietta	CC/RL	2006	Semi-Com
Jassie	CC/SC	2005	10/7
Lavalley	CC	2005	100
Louisa (Letaba Oranje)	CC	2007	Semi-Com
Midnight 1	CC/SC	2005	10/10
Moosrivier Late 1	CC/SC	2005	10/10
Moosrivier Late 2	CC/SC	2005	10/10
Ruby	CC/SC	2005	10/10
Skilderkrans	CC/SC	2005	10/6

Turkey	C35	2005	2
Turkey	CC	2005	4
Turkey	SC	2005	10

Table 5.4.3.2. List of Valencia selections evaluated at Bosveld Citrus (Letsitele) during the 2014 season.

Selection	Rootstock	Planted
Alpha	C35/SC	2009
Benny	C35/SC	2009
Benny 2	C35/SC	2009
Delta (control)	SC	2009
Du Roi	C35/SC	2009
Gusocora	SC	2009
Lavalle	C35/SC	2009
McClellan SL	C35/SC	2009
Midnight 1	C35/SC	2009
Midnight 2	C35/SC	2009
Val Late	C35/SC	2009

Table 5.4.3.3. List of Valencia selections evaluated at Moriah Citrus (Hoedspruit) during the 2014 season.

Selection	Rootstock	Top-worked
Benny 2	MxT	2011
Gusocora	MxT	2011
Lavalle	MxT	2011
Weipe	MxT	2011

Results and discussion

Alpha

Alpha was planted on C35 (cropping 50 kg/tree) and Swingle (cropping 60-70 kg/tree) at the Bosveld trial site. The internal quality was good, juice levels peaked above 55%, Brix was above 9 and acids were fairly high between 1.3 and 1.5%. Fruit size varied from count 88 to 56, excellent for Valencia production and export. External colour peaked from T1 to T4. Maturity seems to be end of June to middle of July (Table 5.4.3.4).

Bend 8A1

Bend 8A1 on Carrizo and Swingle produced a better crop and the tree condition on both combinations improved this season. Fruit size peaked from medium to large, count 88 to 56. The internal quality improved with higher juice levels on both rootstocks (above 53%); Brix ranged from 11.1 up to 11.7, acids were lower (1.4%) after the second evaluation and Brix: acid ratios were above 8. Fruit characteristics of Bend 8A1 were a fairly round fruit shape, fairly smooth rind, fruit peeling easily, the fibre strength is fairly soft and the internal colour light yellow. The average seed count per fruit for Bend 8A1 (2.4 seeds/fruit) was higher compared to 8A2 (1.3 seeds/fruit). Maturity seems to be mid July to beginning of August (Table 5.4.3.4).

Bend 8A2

Bend 8A2 performed well and internally produced slightly lower juice content (52%) than 8A1, similar acids (1.3%) and average Brix: acid ratios (8.5:1). The crop on both rootstock selections was better compared to Bend 8A1. Fruit size varied from count 105 to 64 on both Carrizo and Swingle. Fruit shape was round, fairly smooth rind texture, and deep yellow internal colour; it peeled easily with fairly thin rind. The seed count per fruit was low, avg 1.3 seeds per fruit, lower than 8A1. External colour was advanced compared to Bend 8A1 (T1 with second evaluation). Fruit maturity was estimated at mid July to the end of July (Table 5.4.3.4).

Benny 1 and 2

Benny was evaluated at all three trial sites, Bosveld, Moriah and Group 91. There was a good crop on both selections and fruit size peaked between count 88 and 56 (excellent for Valencia production). The internal colour of the fruit was deep yellow, fruit shape round, rind texture fairly smooth, high rag content and medium rind thickness. Benny 1 and 2 internally produced similar juice levels (avg. 53%), Brix (avg. 10.3) acid (1.3%) and seed counts (avg. 3 seeds per fruit). External colour on both selections by the time of harvest varied between T1 and T2. Based on ratios, Benny 1 and 2 mature end of July to beginning of August (Table 5.4.3.4).

Delta (control)

Delta produced completely seedless fruit as control variety and a good yield on the trees. Fruit size peaked between count 88 and 72 with good internal quality, reaching juice levels of 54%, Brix of 9 and acid content of 1.0%. The external colour of the fruit was between T3 and T4. Maturity was middle to end of June (Table 5.4.3.4).

Du Roi

Du Roi was planted on two rootstocks, C35 and Swingle at the Bosveld trial site as a control selection. There was a good yield on both combinations and fruit size peaked between count 88 and 64. The external colour varied from T1 to T4 and the average seed count was 2.2 seeds per fruit. C35 developed higher juice (58%), Brix (12) and acid (1.44%) levels compared to Swingle with juice content of 57%, Brix of 10 and acids of 1.42%. Maturity was middle to end of July (Table 5.4.3.4).

Gusocora

Gusocora was evaluated at Moriah and Bosveld Citrus this year. The fruit was completely seedless and developed a good internal quality where juice (51%), Brix (9.3) and acid (1.0) were on the lower side, but still complied with export requirements. The external colour varied from T1 to T3, in sequence with the internal quality and Brix: acid ratio of 9.4. Fruit size peaked between counts 88 and 64, optimal fruit size for export Valencias (medium to large). There was a good crop on the trees, bearing in mind that Swingle as well as MxT rootstocks induce good yields and average internal quality. From the ratio on this date it is apparent that Gusocora's maturity is end of June (Table 5.4.3.4).

Henrietta

Fruit size decreased slightly and peaked at count 105 to 64; and count 105 was small for Valencia export. The fruit shape was slightly oblong with a smooth rind texture, deep yellow internal colour and very good flavour. Fibre strength was medium with a medium thick rind, and the fruit peeled easily with fairly low rind oil. The internal quality on Carrizo was good with high juice (57%), Brix (11) and acid (1.3%) levels by the second evaluation. Henrietta on Carrizo (rootstock inducing high internal quality) indicated the shelf life potential of the selection. There were 3.4 seeds per fruit counted on average, higher compared to the 2013 (0.35 seeds per fruit) season. The external colour of the fruit developed into a deep orange, very favourable for export markets. Maturity was end of July to beginning of August (Table 5.4.3.4).

Jassie

Fruit size on Carrizo peaked between count 88 and 56 compared to Swingle with count 105 and 64. Production was good on both rootstock combinations. Internal quality was good with juice levels of 53%, Brix 11.0 and average acid levels of 1.4%. Seed count increased from 3.1 to 4.5 seeds per fruit. Fruit shape was round with a smooth rind texture, internal colour was light yellow, and juice flavour was good. Fibre strength was fairly soft, rind thickness was medium, rind was smooth and the fruit peeled easily. Jassie bore high numbers of fruit inside the tree. Maturity was end of July to beginning of August in this area (Table 5.4.3.4).

Lavalle

The yield on C35 rootstock (trees 3.2 m high) averaged 50 kg per tree and with Swingle (3.8 m high) 60 to 70 kg per tree; very good when you consider the tree height. This season Lavalle produced 0.6 seeds per fruit compared to last year's 0.5. The internal quality complied with export requirements, except for the acid level being on the higher side (1.52%), but keep in mind that Lavalle is a late Valencia selection with good shelf life and the optimal harvest time will be in August/September. The navel end on some fruit seems to develop a button and there were split fruit on some of the trees evaluated, but this varies from season to season (only 2013). From the ratio on this date it is apparent that Lavalle 1 maturity is end of August to end of September (Table 5.4.3.4).

Louisa

The fruit set remained lighter compared to Henrietta and fruit size was optimal for Valencia production between counts 88 and 64. Internal quality complied with the requirements, with juice content of 51% and the acid level of 1.4% indicated that Louisa qualified as one of the later maturing Valencia selections available currently. Louisa remained seedless; the internal colour of the fruit was light yellow, fruit shape round, medium smooth rind texture and fairly thick rind. There were small thorns on the bearing branches and the tree height on Carrizo measured 2.5 m (compact selection). Acid levels and ratios indicate that this cultivar matures end of July to middle of August (Table 5.4.3.4).

McClellan SL

McClellan SL was planted on C35 and Swingle at the Bosveld trial site with a crop production of 80 kg on C35 and 120 kg per tree on Swingle. The Swingle trees were 0.7 metres higher compared to the C35 trees.

McClellan SL remained completely seedless similar to all the other trial sites where the selections was included with a good to very good crop on the trees. Fruit size peaked from count 88 to 72 on C35 and from count 105 to 64 on Swingle to the crop load. External colour varied from T1-5 and Brix: acid ratios were 10:1. Maturity seems to be end of June to middle of July (Table 5.4.3.4).

Midnight 1 & 2

Midnight 1 and 2 bore an average yield of between 70 and 90 kg per tree on the three rootstock combinations where C35 (2.6 m) was the smallest. The fruit size varied between count 105 and 56, juice content was around 54%, Brix levels around 10 and acids at 1.2%. Midnight 1 outperformed Midnight 2 with a better Brix level. The Midnight 2 fruit was completely seedless, compared to Midnight 1 with 0.1 seeds per fruit on average. Fruit shape was round; rind texture was fairly smooth; fruit was raggy with a medium rind thickness and peeled moderately. Midnight 1 developed very good internal quality early in the season (higher Brix compare to Midnight 2) with ratios indicating maturity to be middle of July to beginning of August (Table 5.4.3.4).

Moosrivier Late 1 and 2

This season Moos Late 2 developed a more favourable fruit size (medium size count 72-64) compared to Moos Late 1 (small to large/extra-large size count 105-48) on both rootstocks. Crop production for Moos Late 1 was better compared to Moos Late 2. The tree canopy on Moos Late 2 was very dense with limited light inside the tree for proper fruit set (additional window pruning required). Moos Late 1 performed well, developing internal qualities that met export standards, except for high acids (1.4 to 1.6%) indicating a late maturing Valencia selection. The seed count per fruit varied from 1.8 up to 3.8 (an increase from 2013). Moos Late 2 developed slightly lower Brix and fairly lower acid levels and was completely seedless. When internal quality was taken into consideration, Moosrivier Late 1 this season was the earlier maturing selection, estimated maturity end-July to mid-August. Moosrivier Late 2 with delayed external colour (T2-5) seemed later maturing (Table 5.4.3.4).

Ruby

Ruby performed similarly on both rootstocks with Brix content of 11.4 up to 11.8, juice levels of 56% and acids around 1.4%. External colour on the fruit was between T1 and T2. Fruit size was similar to 2012 (count 105-64) and produced a good to very good crop on the trees (70-80 kg), bearing in mind the relatively small tree size (compact tree). Seeds per fruit were lower this season and varied from 3.0 up to 4.0. Fruit shape was round with fairly smooth rind texture, medium strong fibre internally, medium rind thickness and fruit peeled easily. Internal colour was dark red and well developed. Ruby's estimated maturity time will be end-July to mid-August (Table 5.4.3.4).

Skilderkrans

Skilderkrans at Group 91 cropped lighter this season but still produced a good yield on the trees. Fruit size varied from medium to large (count 88-56); excellent for Valencia production as well as in combination with a good crop on the trees. Internally the Brix content was good (11.3) and the acid level of 1.5 to 1.6% indicated a later maturing Valencia selection. Juice level decreased to average 52%; above the minimum required export figure. There was no delay in external colour on Carrizo or Swingle with T1-2 at all evaluations. The fruit developed a smooth rind, fibre strength was fairly soft and the fruit shape was round. Ratios were lower this season due to the higher acid levels, delaying peak maturity to mid-August on both rootstocks (Table 5.4.3.4).

Turkey

Turkey was planted on three rootstocks, Carrizo, Swingle and C35 to determine the compatibility status. All three combinations performed well; yield was the best in combination with Carrizo relative to tree size. Fruit size distribution ranged from medium to large/very large (count 88-40), high Brix content (above 11.7), fairly high acid levels (1.1 to 1.3%) and better Brix: acid ratio of 9.0 to 10.5:1 and average seed count per tree of 5 seeds. The external colour was similar on all three rootstocks between T2 and T3 at the end of May. Yield production and tree size showed Carrizo to be the best rootstock combination for Turkey. C35 developed the smallest tree size (2.5 m) in combination with Turkey. Based on the ratios, maturity was end-May to mid June (Table 5.4.3.4).

Weipe

The Weipe selection was developed to replace the Limpopo SL as an early maturing Valencia. Weipe was evaluated for the first time at the Moriah trial site and was top-worked on MxT (older trees) as well as planted on C35 (young trees). There was limited fruit on the trees due to the smaller trees with light crop. Fruit size was medium to large (count 72-56), internal quality was good (juice 50%, acid 1.3%) with lower Brix level (7.5). Colour development ranged from T2 to T5. Maturity was estimated to be end of May to the middle of June (Table 5.4.3.4).

Conclusion

Alpha developed a good crop on the tree, the internal quality was good and fruit size peaked between counts 88 to 56.

Bend 8A2 produced fruit with lower seed counts per fruit. The internal quality on Bend 8A 1 was better compared to Bend 8A 2. The fruit size this season was bigger on 8A1 (counts 88-56). Benny 1 and 2 produced similar qualities of fruit this season, as well as yield production and fruit size (peaked from count 88 to 56). Du Roi was planted on C35 and Swingle with fruit size ranging from count 88 to 64. The internal quality on C35 outperformed Swingle this season.

Gusocora performed well on Swingle (average on MxT), meeting the export standards (acid on the lower side). Henrietta decreased on fruit size and peaked between count 105 and 64. The internal quality of the fruit was excellent on Carrizo and developed high Brix and fairly high acids with peak maturity. There was an average seed count in the fruit from 2.7 to 4.0.

Jassie produced an excellent internal quality on Carrizo as well as Swingle; bigger fruit size (count 88-56) optimal for Valencias and very good yields. Lavalley 1 was ultra-late maturing in August/September (acid above 1.3%) on all three rootstocks. The crop production peaked between 80 and 100 kg per tree.

Louisa was completely seedless, developed the ideal fruit size (count 88-64) for Valencias, setting a better crop on the trees, with a more compact tree development (2.5 m on Carrizo).

Fruit quality on Midnight 1 was better with higher Brix than Midnight 2. External colour was delayed on Midnight 2 this season. Normally, Carrizo produces lower acids and develops better external colour compared to Swingle, but with the Midnight 1 selection the opposite seems to be true. With the first evaluations Carrizo's acid levels were higher compared to Swingle, although by the time the second evaluations were completed, acid levels were 1.0% and 1.4%.

Moosrivier Late 2 is later maturing when compared to Moosrivier Late 1, with higher acid percentages on Carrizo and delayed external fruit colour. Moosrivier Late 2 remained completely seedless.

Ruby produced very good quality fruit, excellent yields on the compact tree size and bigger fruit size from count 105 to 64. Skilderkrans performed well this season compared to excellently the previous season, bearing a good crop with good internal quality, and similar seed count (0.2 per fruit).

Turkey performed best in combination with Carrizo when Brix: acid ratio and yield production were considered. Weipe cropped a light yield on the MxT rootstocks at the trial site with good internal quality and high acid levels by the end of May. The external colour ranged from T2 to T5. Future evaluations will determine the value of this cultivar for the citrus industry.

This was the first evaluation of Alpha, Benny 2, Delta (control), Du Roi, Gusocora, Lavalley 1, Midnight 2 and Val Late at the Bosveld trial site, and for Benny 2, Gusocora, Lavalley 1 and Weipe at the Moriah trial site, so information is limited and future evaluations will improve recommendations on these varieties.

Table 5.4.3.4. Internal fruit quality data for Valencia orange selections at Moriah Citrus (Hoedspruit), Groep 91 and Bosveld Citrus (Letsitele) during the 2014 season.

Selection	Root-stock	Date harvested	Site	Size mm	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Alpha	C35	2014/06/24	Bosveld	72-83	88-56	55.3	9.1	1.45	6.3	0.0	T2-4
Alpha	SC	2014/06/24	Bosveld	70-80	88-64	57.5	9.3	1.25	7.4	1.0	T1-2
Bend 8A1	CC	2014/06/24	Group 91	74-79	72-64	64.3	9.4	1.40	6.7	0.9	T1-3
Bend 8A1	SC	2014/06/24	Group 91	69-80	88-64	56.4	10.2	1.51	6.8	4.4	T1-3
Bend 8A1	CC	2014/07/16	Group 91	76-82	72-56	53.2	11.7	1.40	8.4	3.3	T1-2
Bend 8A1	SC	2014/07/16	Group 91	75-80	72-64	52.9	11.1	1.36	8.2	3.5	T1
Bend 8A 2	CC	2014/06/24	Group 91	70-80	88-64	54.2	10.9	1.31	8.3	1.4	T1-3
Bend 8A 2	SC	2014/06/24	Group 91	67-73	105-72	56.6	10.8	1.52	7.1	1.5	T1-2
Bend 8A 2	CC	2014/07/16	Group 91	78-81	64	51.4	11.4	1.27	9.0	0.5	T1
Bend 8A 2	SC	2014/07/16	Group 91	75-80	72-64	52.1	10.7	1.35	7.9	1.7	T1
Benny 1	CC	2014/05/20	Group 91	71-83	88-56	50.4	9.6	1.74	5.5	1.3	T2-4
Benny 1	SC	2014/05/20	Group 91	69-77	88-72	53.3	9.4	1.77	5.3	1.8	T2-4
Benny 1	CC	2014/06/24	Group 91	70-79	88-64	55.9	10.8	1.30	8.3	4.8	T1-2
Benny 1	CC	2014/07/16	Group 91	77-88	72-48	52.4	10.7	1.27	8.4	3.3	T1
Benny 1	SC	2014/07/16	Group 91	75-81	72-64	52.6	11.2	1.38	8.1	3.4	T1-2
Benny 2	C35	2014/06/24	Bosveld	74-80	72-64	55.8	9.3	1.02	9.1	1.3	T1-3
Benny 2	SC	2014/06/24	Bosveld	73-78	72-64	55.5	9.1	1.23	7.4	0.0	T2-4
Benny 2	CC	2014/05/20	Group 91	69-76	88-72	51.9	10.5	2.00	5.3	6.3	T2-4
Benny 2	SC	2014/05/20	Group 91	72-79	88-64	52.0	9.2	2.22	4.1	7.1	T2-4
Benny 2	SC	2014/06/24	Group 91	72-80	88-64	55.0	11.1	1.49	7.4	1.9	T1-2
Benny 2	CC	2014/07/16	Group 91	75-80	72-64	47.3	11.6	1.20	9.7	2.3	T1
Benny 2	SC	2014/07/16	Group 91	78-82	64-56	52.6	11.8	1.52	7.8	2.7	T1
Benny 2	MxT	2014/05/29	Moriah	75-88	72-48	54.9	9.3	1.39	6.7	3.5	T1-3
Benny 2	MxT	2014/06/23	Moriah	76-89	72-48	52.5	10.9	1.30	8.4	5.8	T1-2
Delta	SC	2014/06/24	Bosveld	68-77	88-72	53.8	9.1	0.97	9.4	0.0	T3-4
Du Roi	C35	2014/06/24	Bosveld	69-77	88-72	57.5	11.5	1.44	8.0	2.3	T1-2
Du Roi	SC	2014/06/24	Bosveld	69-78	88-64	56.9	9.6	1.42	6.8	2.1	T1-4
Gusocora	SC	2014/06/24	Bosveld	72-77	88-72	53.1	8.7	1.00	8.7	0.0	T1-2
Gusocora	MxT	2014/06/23	Moriah	74-80	72-64	48.0	9.8	0.98	10.0	0.0	T2-3
Henrietta	CC	2014/06/24	Group 91	66-74	105-72	57.0	10.9	1.62	6.7	4.0	T1-3

Henrietta	CC	2014/07/16	Group 91	69-80	88-64	57.0	10.9	1.33	8.2	2.7	T1-2
Jassie	CC	2014/06/24	Group 91	71-82	88-56	56.3	11.1	1.43	7.8	4.0	T1-3
Jassie	SC	2014/06/24	Group 91	67-77	105-72	55.0	8.7	1.49	5.8	4.4	T1-3
Jassie	CC	2014/07/16	Group 91	77-81	72-56	53.0	11.1	1.34	8.3	4.3	T1
Jassie	SC	2014/07/16	Group 91	75-80	72-64	53.4	10.8	1.35	8.0	4.7	T1-2
Lavalle	C35	2014/06/24	Bosveld	70-80	88-64	58.3	10.1	1.39	7.3	0.5	T1-4
Lavalle	SC	2014/06/24	Bosveld	71-82	88-56	58.2	9.3	1.43	6.5	0.0	T1-4
Lavalle	CC	2014/07/17	Group 91	68-77	88-72	56.6	11.8	1.66	7.1	1.3	T1-2
Lavalle	MxT	2014/06/23	Moriah	78-90	64-40	54.5	8.8	1.36	6.5	0.0	T2-4
Louisa	CC	2014/06/24	Group 91	69-80	88-64	52.7	11.6	1.39	8.3	0.0	T1-2
Louisa	CC	2014/07/17	Group 91	70-78	88-64	50.0	11.7	1.30	9.0	0.0	T1
McClellan SL	C35	2014/06/24	Bosveld	70-76	88-72	57.3	10.7	1.14	9.4	0.0	T1-5
McClellan SL	SC	2014/06/24	Bosveld	66-79	105-64	53.5	9.5	0.95	10.0	0.0	T2-3
Midnight 1	C35	2014/06/24	Bosveld	72-84	88-56	58.0	9.8	1.07	9.2	0.0	T1-2
Midnight 1	SC	2014/06/24	Bosveld	73-80	88-64	56.0	9.2	1.11	8.3	0.0	T1-3
Midnight 1	CC	2014/06/24	Group 91	67-81	105-64	56.7	10.3	1.46	7.1	0.3	T1-2
Midnight 1	SC	2014/06/24	Group 91	68-75	88-72	52.5	11.3	1.26	9.0	0.0	T1
Midnight 1	CC	2014/07/16	Group 91	78-81	64-56	50.6	11.5	1.00	11.5	0.2	T1
Midnight 1	SC	2014/07/16	Group 91	76-82	72-56	48.9	10.8	1.40	7.7	0.3	T1
Midnight 2	C35	2014/06/24	Bosveld	75-81	72-56	56.7	8.8	1.09	8.1	0.0	T1-3
Midnight 2	SC	2014/06/24	Bosveld	69-78	88-64	52.9	8.1	1.20	6.8	0.0	T2-4
Mooslate 1	CC	2014/06/24	Group 91	67-72	105-72	56.1	11.5	1.66	6.9	0.0	T1-2
Mooslate 1	SC	2014/06/24	Group 91	67-78	105-64	53.7	10.7	1.82	5.9	1.8	T1-3
Mooslate 1	CC	2014/07/16	Group 91	72-78	88-64	54.9	11.6	1.41	8.2	3.8	T1
Mooslate 1	SC	2014/07/16	Group 91	75-89	72-48	55.1	11.1	1.59	7.0	2.0	T1
Mooslate 2	CC	2014/07/16	Group 91	77-83	72-64	43.5	11.0	1.64	6.7	0.0	T2-3
Mooslate 2	SC	2014/07/16	Group 91	75-80	72-64	53.3	11.4	1.34	8.5	0.0	T3-5
Ruby	CC	2014/06/24	Group 91	68-71	88	52.9	11.7	1.47	8.0	0.0	T1-3
Ruby	SC	2014/06/24	Group 91	67-78	105-64	57.0	11.5	1.50	7.7	0.0	T1-3
Ruby	CC	2014/07/16	Group 91	75-80	72-64	55.7	11.4	1.38	8.3	4.0	T1-2
Ruby	SC	2014/07/16	Group 91	71-77	88-72	59.4	11.8	1.40	8.4	3.0	T1-2
Skilderkrans	CC	2014/06/24	Group 91	71-84	88-48	52.9	10.1	1.48	6.8	0.0	T1-3
Skilderkrans	SC	2014/06/24	Group 91	74-86	72-48	49.8	10.3	1.57	6.6	0.0	T1-2
Skilderkrans	CC	2014/07/16	Group 91	76-83	72-56	52.3	11.8	1.45	8.1	0.2	T1
Skilderkrans	SC	2014/07/16	Group 91	77-83	72-56	52.3	10.7	1.57	6.8	0.0	T1-2

Turkey	C35	2014/05/20	Group 91	72-77	72-64	53.9	11.5	1.61	7.1	1.0	T2-3
Turkey	CC	2014/05/20	Group 91	68-78	88-64	52.4	11.3	1.54	7.3	3.2	T2-3
Turkey	SC	2014/05/20	Group 91	68-79	88-64	50.0	11.0	1.50	7.3	3.8	T2-3
Turkey	C35	2014/07/16	Group 91	75-93	72-40	52.2	12.3	1.33	9.2	5.6	T1
Turkey	CC	2014/07/16	Group 91	70-91	88-40	49.8	11.7	1.30	9.0	5.8	T1
Turkey	SC	2014/07/16	Group 91	74-87	72-48	50.2	12.0	1.14	10.5	3.8	T1
Val late	SC	2014/06/24	Bosveld	68-81	88-56	53.3	9.3	1.18	7.9	2.3	T1-4
Val late	C35	2014/06/24	Bosveld	70-80	72-56	55.7	9.6	1.43	6.7	2.3	T2-4
Weipe	MxT	2014/05/29	Moriah	75-83	72-56	50.0	7.5	1.32	5.7	0.0	T2-5

5.4.4 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the hot inland areas (Letsitele & Malelane)

Project 75 C by J. Joubert and S.D.Maziya (CRI)

Opsomming

Die resultate van die 2014 seisoen vir hierdie warm produksie area het aangedui dat Tango die vroegste ryp geword het met die kleinste vrug grootte en goeie interne kwaliteit. Daarna het Gold Nugget en Tahoe Gold gevolg, met van die grootste vrugte vir hierdie seisoen. African Sunset, Mor 26 en Shasta Gold was die enigste seleksies wat geen saad in die vrugte ontwikkel het nie. Mor 26 het die beste interne kwaliteit in vergelyking met die ander seleksies (Brix 13) ontwikkel. Yosemite Gold was volgende om ryp te word nader aan die einde van die Mandaryn Hibried reeks, ge-evalueer met 'n goeie interne kwaliteit vrug (Brix: suur verhouding van 12.5: 1), asook goeie eksterne kleur ontwikkeling (T1-2). Shasta Gold was die laaste seleksie gereed vir oes teen einde Julie, wat die Mandaryn Hibried seisoen afsluit vir hierdie proef. Daar word aanbeveel om nie die oesperiode langer as 3 tot 4 weke te verleng nie om goeie interne kwaliteit te verseker met minimum skil probleme.

Summary

The results of the 2014 season indicate that for the warm production areas Tango matures first with the smallest fruit size and good internal quality. Gold Nugget and Tahoe Gold followed, with the biggest fruit size for this season. African Sunset, Mor 26 and Shasta Gold were the only selections that developed no seeds in the fruit. Mor 26 developed the best internal quality compared to the other selections (Brix 13). Yosemite Gold matured next towards the end of the Mandarin Hybrid range evaluated at this trial site with good internal quality (Brix: acid ratio of 12.5: 1) as well as good external colour (T1-2). Shasta Gold was the last selection to mature, at the end of July, ending the Mandarin Hybrid season for this trial. Picking periods should not be longer than 3 - 4 weeks to maintain good internal quality and to avoid rind disorders.

Objectives

- To select Mandarin Hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new Mandarin Hybrid cultivars and to determine the climatic suitability of these cultivars in hot production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from Bosveld Citrus (Letsitele) and Riverside (Malelane) from the Limpopo region. The following varieties were evaluated: African Sunset (B24), Gold Nugget, Mor 26, Shasta Gold, Tahoe Gold, Tango, Valley Gold (B17), Yosemite Gold.

Table 5.4.4.1. List of Mandarin Hybrid selections evaluated at Bosveld Citrus (Letsitele) during the 2014 season.

Selection	Rootstock	Planted
African Sunset (B24)	SC	2009
Gold Nugget	CC	2010
Mor 26	SC	2009
Shasta Gold	CC	2010
Tahoe Gold	CC	2010
Tango	CC	2010
Valley Gold (B17)	SC	2009
Yosemite Gold	CC	2010

Table 5.4.4.2. List of Mandarin Hybrid selections evaluated at Riverside (Malelane) during the 2014 season.

Selection	Rootstock	Planted
Gold Nugget	CC	2011
Shasta Gold	CC	2011
Tahoe Gold	CC	2011
Tango	CC	2011
Yosemite Gold	CC	2011

Results and discussion

All the UCR 5 selections were bearing fruit for the second time this season. The trees at Bosveld are one year older than the trees at Riverside and this had an impact on the quality and quantity of the fruit. This was the first season to evaluate African Sunset, Mor 26 and Valley Gold at the Bosveld trial site.

When the ratio between sugar and acid is 12:1, the fruit is considered to be at peak maturity for Mandarin Hybrids. This ratio is raised as a result of the high sugar levels associated with the new selections. A ratio of 11:1 is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over mature. This process from the start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

African Sunset (B24)

There was only enough fruit on the trees to complete one evaluation at the Bosveld trial site due to a light crop. The large fruit size (count 1XXX) is also a selection quality, but the light crop contributes to this scenario. African Sunset developed a protruding navel-end on most of the fruit, the bigger the fruit size the more visible the navel-end. The internal quality was average due to the one evaluation where the fruit was over matured (acid 0.68%) with delayed external colour (T3-5). Based on the internal quality results in Table 5.4.4.3, estimated maturity will be the end of May to middle of June.

Gold Nugget

Gold Nugget developed a very upright tree shape (V shape) with long aggressive growing shoots in the middle, bearing no crop at all. The crop will be on the other bearing branches towards the middle of the tree, and correctional pruning on this variety is crucial. Remove the long shoots to set the crop lower, and another important reason was to set more smooth textured fruit on the lower branches. Gold Nugget is familiar for its rough textured fruit with coarse rinds, but with the evaluations it came to light that the lower fruit was smoother compared to the fruit on the aggressive long upright branches. The internal quality of the fruit was average (better at Bosveld than Riverside) and developed fair juice (48%), high Brix (9.0) and lower acid (0.9%) levels and a delayed external colour (T4-5). Keep in mind the young tree age, future evaluations will determine the feasibility of this selection in the hot areas. Based on the internal quality results in Table 5.4.4.3, estimated maturity was the middle to end of May.

Mor 26

Mor 26 produced a fair crop on the trees for the 2014 season. The fruit size peaked at count 1-1XX, medium to large fruit. The external colour development was yellow and between T1-2. The internal quality was very good with high juice levels of 56%, Brix above 13 and acceptable acid levels (0.9%). There were no seeds in the fruit at Bosveld. Based on the internal quality results in Table 5.4.4.3, estimated maturity was the middle to end of June.

Shasta Gold

Shasta developed ribbing on most of the fruit, as well as sunburn. The fruit was fairly flat on the trees at both trial sites. Rind texture on the fruit became smoother as the trees matured. Tree size compared to the other selections was medium with only Tahoe Gold developing into a smaller tree, with more compact bearing branches. The fruit quality at the Riverside trial site was better compared to the Bosveld one. The flavour improved with fairly high juice (50%) and rind oil content. Shasta produced fruit with soft fibre strength that peels easily, and all the fruit evaluated were completely seedless. The fruit size peaked from large to very large (count 1X-1XXX). The internal quality was good with juice levels of 50%, Brix above 12 at Riverside and acceptable acid levels (above 1.2%). Based on the internal quality results in Table 5.4.4.3 maturity was end of June to mid July at both Bosveld and Riverside.

Tahoe Gold

This selection developed the smallest tree size when compared to the other UCR 5 varieties (compact tree). Tahoe Gold produced a good crop on the trees compared to the 2013 season. The fruit size peaked from medium to large/very large (count 2-1XXX) and the fruit shape was similar to that of a Minneola tangelo fruit. The external colour improved between T1-3 when the internal quality was optimum. Tahoe produced fruit with soft fibre strength that peels easily, and all the fruit evaluated were completely seedless, except for two evaluations at Riverside with 0.3 seeds per fruit counted. The internal quality was good with juice levels of 58%, Brix averaged 10 and acid levels were acceptable by the time of harvest (Bosveld 0.8 and Riverside 0.9%). Based on the internal quality results in Table 5.5.4.3, estimated maturity was the end of May to middle June.

Tango

There was a good crop on the trees at Bosveld (planted 2010) and a fair crop at Riverside (planted 2011) site. Tango was completely seedless at the Bosveld trial site with 0.2 seeds per fruit at Riverside. The fruit shape was similar to the Nadorcott selection. Rind texture was very smooth with a natural shine (similar to packhouse waxing). The Tango trees were thornless with a upright growth pattern and tree shape. The fruit was firm and the rind thin, fibre was soft and peeled very easily. Internally the fruit was high in juice content (55%), Brix was lower for this selection (8.2), acid levels (0.7) decreased early in the season (shorter shelf life) and deep orange coloured fibre. Fruit size peaked at count 2 to 1XXX (small to medium). Based on the internal quality results in Table 5.4.4.3, estimated maturity was the middle of May.

Yosemite Gold

The fruit set on Yosemite Gold was very light at the Riverside site with no crop on the trees at Bosveld. Additional measurements might be necessary to increase the crop on the trees for example Gibb sprays or girdling. Yosemite Gold developed a very promising soft citrus type fruit shape (similar to Minneola tangelo). The fruit was firm, rind texture was smooth and the fibre was soft, peeled very easily and developed 0.3 seeds per fruit. Yosemite developed the biggest tree size compared to the other UCR 5 selections. This aggressive growth characteristic might be the reason for the poor crop on the trees (vegetative growth), and must be generated into fruit set and crop on the trees. Fruit size varied from medium to large/very large (count 2-1XXX) due to the light crop on the trees, similar to Tahoe Gold. The internal quality improved at Riverside this season with higher juice, Brix and acid levels. External colour developed with the internal quality towards the end of the evaluations (T1-2). Based on the internal quality results in Table 5.4.4.3, estimated maturity was the end of May to middle of June.

Valley Gold (B17)

Valley Gold was evaluated for the first time this season at Bosveld trial site. The internal quality was good with acid levels around 0.78 % and external colour between T1 and 3 when the second evaluation was completed. Fruit size peaked from count 1X to 1XXX due to a fairly light crop on the trees. There were limited fruit split on the trees, unlike the spilling problem in the hot humid production areas. Maturity was estimated to be middle of June for this hot production area.

Conclusion

There was an improvement in the external colour delay in the hot areas that seems to be a problem in the past; future evaluations will clarify the situation. Degreening might be an option for the Gold Nugget and TDEs (fruit colour development was yellow with degreening), but with Tango (W. Murcott selection) and Nadorcott, ethylene reacted slowly. Yosemite Gold might be a possibility to consider for the hot areas due to stronger fruit with optimal fruit size, good internal quality when external colour becomes more intense (T1-2). In the hot areas it will become crucial to cover the mandarin orchards with shade net, to minimise sunburn and improve pack out percentage of the fruit. There was severe sunburn on the Shasta Gold fruit compared to the cooler production areas.

Shasta Gold had the largest fruit size, followed by Yosemite- and Tahoe Gold, then Gold Nugget. The smallest fruit size was produced on Tango. There were more seeds this season in Gold Nugget (0.2), Tahoe- and Yosemite Gold (0.3), Valley Gold (1.0) as well as Tango (0.2): all the other selections were completely seedless.

African Sunset, Mor 26 and Valley Gold was evaluated for the first time this season with limited information, future evaluations will continue to determine suitability for these production areas.

Table 5.4.4.3. Internal fruit quality data for Mandarin hybrid selections at Bosveld (Letsitele) and Riverside (Malelane) during the 2014 season.

Selection	Root-stock	Date harvested	Site	Size mm	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
African Sunset	SC	24/06/2014	Bosveld	85-94	1XXX	45.2	8.9	0.68	13.1	0.0	T3-5
Gold Nugget	CC	17/04/2014	Bosveld	63-77	2-1XX	44.6	7.6	1.70	4.5	0.0	T7-8
Gold Nugget	CC	20/05/2014	Bosveld	69-76	1X-1XX	49.1	8.1	0.92	8.8	0.0	T4-6
Gold Nugget	CC	24/06/2014	Bosveld	67-85	1-1XXX	50.0	10.5	0.75	14.0	0.0	T1-2
Gold Nugget	CC	18/03/2014	Riverside	60-73	2- 1XX	39.3	7.5	1.87	4.0	0.2	T7-8
Gold Nugget	CC	31/03/2014	Riverside	63-72	2- 1XX	53.8	7.8	1.43	5.5	0.0	T7-8
Gold Nugget	CC	15/05/2014	Riverside	70-89	1X-1XXX	47.1	8.6	0.86	10.0	0.0	T3-6
Gold Nugget	CC	11/06/2014	Riverside	72-82	1XX-1XXX	47.7	11.4	0.71	16.1	0.0	T1-4
Gold Nugget	CC	29/07/2014	Riverside	67-83	1-1XXX	45.2	12.3	0.66	18.6	0.0	T1
Mor 26	SC	24/06/2014	Bosveld	64-75	1-1XX	56.1	13.2	0.86	15.3	0.0	T1-2
Shasta Gold	CC	17/04/2014	Bosveld	73-84	1XX-1XXX	47.8	7.1	2.32	3.1	0.0	T7-8
Shasta Gold	CC	20/05/2014	Bosveld	72-88	1XX-1XXX	50.4	7.3	1.59	4.6	0.0	T5-6
Shasta Gold	CC	24/06/2014	Bosveld	78-94	1XX-1XXX	52.2	8.4	1.22	6.9	0.0	T1-4
Shasta Gold	CC	18/03/2014	Riverside	72-78	1XX-1XXX	49.5	7.3	2.47	3.0	0.0	T7-8
Shasta Gold	CC	31/03/2014	Riverside	67-76	1-1XX	52.0	7.2	2.30	3.1	0.0	T7-8
Shasta Gold	CC	15/05/2014	Riverside	74-87	1XX-1XXX	51.5	9.4	1.71	5.5	0.0	T2-5
Shasta Gold	CC	11/06/2014	Riverside	85-97	1XXX	52.5	11.2	1.20	9.3	0.0	T1
Shasta Gold	CC	29/07/2014	Riverside	76-98	1XX-1XXX	46.4	13.1	1.15	11.4	0.0	T1
Tahoe Gold	CC	17/04/2014	Bosveld	65-78	1-1XXX	57.5	7.2	1.42	5.1	0.0	T6-8
Tahoe Gold	CC	20/05/2014	Bosveld	77-85	1XX-1XXX	60.1	7.3	0.91	8.0	0.0	T3-5
Tahoe Gold	CC	24/06/2014	Bosveld	72-83	1XX-1XXX	61.5	9.4	0.82	11.5	0.0	T1-3
Tahoe Gold	CC	18/03/2014	Riverside	62-70	2-1XX	53.9	7.5	2.50	3.0	0.3	T7-8
Tahoe Gold	CC	31/03/2014	Riverside	65-76	1-1XX	62.2	7.8	2.02	3.9	0.3	T7-8
Tahoe Gold	CC	15/05/2014	Riverside	71-78	1X-1XXX	57.1	9.9	1.44	6.9	0.0	T3-5
Tahoe Gold	CC	11/06/2014	Riverside	67-85	1-1XXX	56.6	11.3	0.91	12.4	0.0	T1-3
Tango	CC	17/04/2014	Bosveld	51-69	4-1X	50.7	7.3	1.25	5.8	0.0	T6-8
Tango	CC	20/05/2014	Bosveld	61-70	2-1X	52.5	8.0	1.01	7.9	0.0	T5-6
Tango	CC	24/05/2014	Bosveld	65-70	1-1XX	53.2	8.6	0.70	12.3	0.0	T1-5
Tango	CC	18/03/2014	Riverside	62-72	2-1XX	56.0	7.6	1.05	7.2	0.2	T6-8
Tango	CC	31/03/2014	Riverside	64-80	1-1XXX	59.5	7.6	0.87	8.7	0.0	T6-8
Tango	CC	15/05/2014	Riverside	64-79	1-1XXX	58.9	9.6	0.76	12.6	0.0	T2-4

Tango	CC	11/06/2014	Riverside	64-76	1-1XX	56.3	10.4	1.19	8.7	0.0	T3-5
Tango	CC	29/07/2014	Riverside	64-73	1-1XX	53.8	12.6	1.48	8.5	0.0	T1-3
Valley Gold	SC	29/05/2014	Bosveld	70-78	1X-1XXX	57.8	9.7	0.78	12.4	0.3	T1-5
Valley Gold	SC	24/06/2014	Bosveld	73-82	1XX-1XXX	49.3	10.2	0.78	13.1	1.9	T1-3
Yosemite Gold	CC	18/03/2014	Riverside	61-74	2-1XX	47.1	7.2	1.84	3.9	0.2	T7-8
Yosemite Gold	CC	31/03/2014	Riverside	66-74	1-1XX	56.1	7.3	1.46	5.0	0.0	T7-8
Yosemite Gold	CC	15/05/2014	Riverside	73-85	1XX-1XXX	52.7	8.9	1.08	8.2	0.3	T2-5
Yosemite Gold	CC	11/06/2014	Riverside	73-85	1XX-1XXX	54.7	10.5	0.84	12.5	0.0	T1-2

5.4.5 **PROGRESS REPORT: Evaluation of Valencia selections in the hot inland areas (Swaziland)**
Project 740A by J. Joubert and S.D. Maziya (CRI)

Opsomming

Die produksie area word beskou as warm in kombinasie met vogtige klimaat en maak die verbouing van Valencia en pomelo varieteite baie gunstig. Turkey was eerste gereed om te oes, gevolg deur McClean SL en Portsgate een tot twee weke later. McClean SL was die laatste rypwordende seleksie vir 2013 seisoen, maar het vroeë interne kwaliteit en eksterne kleur ontwikkel vir die 2014 seisoen. McClean saadloos het wel hierdie seisoen baie wisselvallige vruggroottes geproduseer. Turkey het in die kommersiële boorde in hierdie area op driejarige ouderdom reeds swartvlek probleme getoon, hou die jong bome dus goed dop en pas spuitprogramme aan. Turkey op C35 in die koeler produksie areas (Ngonini) ontwikkel te stadig wat 'n te klein boomgrootte tot gevolg het, in vergelyking met die warm areas, maar vir die Tambuti area was die bome gesond en in 'n goeie kondisie, met goeie produksie.

Jassie en Delta kwalifiseer as die mid-rypwordende seleksies van die Valencia seisoen. Jassie, in kombinasie met Carrizo en C35, as eksperimentele seleksie lyk baie belowend. Delta was die kontrole in hierdie proef en het effens vroeër ryp geword hierdie seisoen. Nou het Alpha gevolg as een van die later rypwordende seleksies vir 2014 seisoen by hierdie perseël, met gemiddelde produksie en interne kwaliteit (hoë sure -1.3%), asook feitlik saadlose vrugte (0.2 sade). Daar is verskeie nuwe Alpha aanplantings in die Letsitele omgewing gedoen, met goeie interne kwaliteit en vruggrootte.

Summary

This production area is classified as hot and humid and the establishment of Valencia and grapefruit selections is very favourable. Turkey matured first for harvesting, followed by McClean SL and Portsgate one to two weeks later. McClean SL was the latest maturing selection for the 2013 season, but developed early internal quality and external colour development in the 2014 season. McClean seedless developed erratic fruit size this season from small to large. However, three-year-old Turkey trees in the commercial orchards in this area already have black spot problems, so look out for this on young trees and adapt spray programmes accordingly. Turkey on C35 in the cooler production areas (Ngonini) developed too slowly and the tree size was smaller compared to the hot areas, but for the Tambuti area the trees were healthy and in good condition, bearing a good crop.

Jassie and Delta qualify as the mid-maturing selections of the Valencia season. Jassie, in combination with Carrizo and C35 rootstock, looked promising as an experimental selection. Delta was the control for this trial and matured slightly earlier this season. Now Alpha follows as one of the later maturing selections for 2014 at this trial site, with average production and internal quality (high acids -1.3%), as well as virtually seedless fruit (0.2 seeds). There are numerous new Alpha plantings in the Letsitele area, with good internal quality fruit and fruit size.

Objective

- To find suitable Valencia selections with superior characteristics for the hot inland citrus production areas.

Materials and methods

Field evaluations and laboratory analyses were conducted on Alpha, Delta, Jassie, McClean SL, Portsgate and Turkey at Tambuti Estate, Swaziland.

Table 5.4.5.1. Internal fruit quality data were compared with the minimum export requirements for Valencia types.

Variety	% Juice	Brix °	Min % Acid	Max % Acids	Ratio	Colour
Valencia	48	9.0	0.6	1.6%	8.0:1	Colour plate 3 of set no. 34
Midnight	52	9.5	0.85	1.8%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

Delta SL	52	10.5	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34
----------	----	------	------	------	-------	------------------------------

*Turkey – interim internal fruit quality standards

Table 5.4.5.2. List of Valencia selections evaluated at Tambuti Estate (Swaziland) during 2014.

Selection	Rootstock	Tree Age	No. of trees
Alpha	CC	2003	5
Delta	CC	2003	5
Jassie	C35/CC	2005	10/10
McClellan SL	CC	2003	5
Portsgate	CC	2003	5
Turkey	C35/CC	2005	10/10

Results and discussion

These comments are based on the second evaluation date (31/7/2014). Maturity/estimated maturity was based on a Brix acid ratio of 10.0.

Alpha

Alpha produced small to medium sized fruit (count 88-64), similar to the previous season, due to a lighter crop on the trees. There were 0.2 seeds present in the fruit tested this season, compared to the completely seedless fruit from the previous season. The selection will still be classified as seedless due to the low (virtually seedless) seed count. Tree condition was good, considering that the trees were 12 years old this season on Carrizo rootstock. There was no delay in external colour development (T1-2), but the acid levels were on the high side and Brix remained lower. The internal colour was deep yellow, fibre strength fairly soft, thin, fairly smooth rinds, fruit peeled easily with medium rind oil. Internally the fruit quality complied with the export requirements. The estimated maturity date was middle to end of July.

Delta (control)

The fruit size on the trees remained similar to the previous season from counts 105 and 72 to counts 88 and 64 (small to medium/large). Delta tends to produce small fruit but the advantage of this selection is the fact that it is completely seedless. There was no sunburn, splitting or creasing noticeable on the fruit. The external colour range was delayed this season and peaked at T2 to T4 (2013 season T1-3). Internal quality was good with a Brix level above 10.0 and Brix: acid ratio of 7.2, the ratio will increase later in the season when the Brix improves and the acids (1.5%) decrease. Peak maturity is estimated to be end July.

Jassie

Jassie was planted on Carrizo and C35 at this trial site to determine the impact of the semi-dwarfing C35 rootstock on external fruit colour, production, internal quality, scion/rootstock compatibility and fruit size. The external colour on C35 (T1-3) was delayed compared to Carrizo (T1-2). The yield production was average on both rootstocks. The internal quality related to juice, acid levels and seed count per fruit evaluated, was similar on both rootstocks. Jassie on CC and C35 developed the same Brix (10.9) and juice levels (61.0%). Juice content on both rootstocks peaked at 1.4%. The average seed count of the fruit was higher on C35 (avg 4.1 seeds per fruit) compared to CC (avg 3.7 seeds per fruit) this production season. Once mature (10 years plus), tree size will be approximately one third smaller in height. Maturity is estimated to be middle to end of July.

McClellan SL

McClellan SL remained completely seedless at this trial site and the crop on the trees was good, peaking at 100 kg per tree. Tree condition was excellent, tree height was medium on Carrizo rootstock and the internal fruit colour developed into a deep orange, as well as T1 external colour. This scenario is very favourable for this Valencia selection, due to the fact that the seedless varieties generally do not bear good crops. The fruit size this season remained erratic, and varied from count 105 to count 64. The Brix: acid ratio peaked above 12.0 with acid levels on the lower side, resulting in a good internal quality (Juice 57%, Brix 11.6 and acid 0.9%). The Brix: acid ratio improved relative to that in 2013. There was no sunburn, splitting or creasing problems with the fruit, the fruit peels moderately with high rind oil content. Maturity is estimated to be the middle to the end of July.

Portsgate

The tree condition in combination with Carrizo was very good, developing a medium/large tree size at the trial block in this hot production area. Fruit shape was round with smooth skin texture and no thorns visible

on the bearing branches. Portsgate peaked from count 88 to 72 (medium); bigger compared to the previous season. The internal colour developed to a deep yellow, with good internal quality and Brix: acid ratios above 9, due to acid level (1.0%). There were 0.3 seeds per fruit at the Tambuti trial site this season in the fruit sampled, whereas last season there were 0.1 seeds per fruit. Maturity is estimated to be end of June to middle of July.

Turkey

Turkey was also planted on Carrizo and C35 to compare the impact of the two different rootstocks on the selection, as well as scion/rootstock compatibility on C35. The dwarfing effect on the C35 trees was visible, with a tree size difference between the two combinations, C35 measured 4.0 m high and Carrizo 5.5 m high. C35 produced a better crop on the trees, compared to Carrizo rootstock.

Both rootstock combinations produced good internal quality fruit, and peaked at a Brix reading above 12, juice content above 50.0% and an acid level of above 1.1%; lower and more acceptable than the 2013 season. The external colour improved from T1 and T3 to T1 this season. Future evaluations will determine the extent and effects of C35 dwarfing and compatibility on Turkey. Rind texture remained smooth and internal fibre strength (rag) was very soft. Based on a ratio of 10.0 and due to the high acid levels, maturity will be later towards the middle or end of June.

Conclusions

The acid levels on most of the selections remained on the higher side this year, except for McClean SL, Portsgate and Turkey. Turkey remains a very good, and currently the only, choice as an early maturing commercial Valencia type, but keep in mind that Weipe and Valearly are two new experimental varieties that are being evaluated with possible future value as early maturing Valencia selections.

Jassie proved to be very promising in the other trial sites (Group 91, Letsitele) where the selection was included. The performance of Jassie on Carrizo and C35 for the Tambuti trial site was very similar. Portsgate matures after Turkey and before Benny: when more information becomes available in future this will be a selection to consider for new plantings. Alpha performed well this season and there was an increase in fruit size count: small to medium sized fruit in 2013 to medium sized fruit in 2014 season. McClean SL had a lighter crop on the trees and remained a completely seedless variety. The fruit size remained erratic from small to medium to large.

Table 5.4.5.3. Internal fruit quality data for Valencia orange selections at Tambuti Estate (Swaziland) during the 2014 season.

Selection	Root-stock	Date harvested	Size mm	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Alpha	CC	17/06/2014	70-81	88-64	63.7	8.7	1.55	5.6	0.0	T3-4
Alpha	CC	31/07/2014	70-80	88-64	55.4	9.1	1.34	6.8	0.2	T1-2
Delta	CC	17/06/2014	67-77	105-72	60.9	9.5	1.40	6.8	0.0	T3-5
Delta	CC	31/07/2014	70-78	88-64	52.0	10.8	1.49	7.2	0.0	T2-4
Jassie	C35	17/06/2014	66-74	105-72	65.0	11.2	1.44	7.8	3.8	T2-5
Jassie	CC	17/06/2014	66-76	105-72	65.1	11.4	1.57	7.3	4.4	T2-3
Jassie	C35	31/07/2014	65-81	105-64	58.2	10.6	1.38	7.7	4.4	T1-3
Jassie	CC	31/07/2014	69-78	88-64	56.4	9.7	1.35	7.2	2.9	T1-2
McClean SL	CC	17/06/2014	66-75	105-72	63.7	10.8	1.10	9.8	0.0	T2-3
McClean SL	CC	31/07/2014	67-81	105-64	57.0	11.6	0.90	12.9	0.0	T1
Portsgate	CC	17/06/2014	69-78	88-64	62.5	9.0	1.04	8.7	0.3	T3-5
Portsgate	CC	31/07/2014	70-77	88-72	56.1	9.4	0.96	9.8	0.3	T2-4
Turkey	C35	17/06/2014	68-80	88-64	62.9	13.0	1.38	9.4	5.2	T2
Turkey	CC	17/06/2014	66-76	105-72	63.9	13.0	1.38	9.4	3.8	T2
Turkey	C35	31/07/2014	72-80	88-64	56.5	12.6	1.15	11.0	4.0	T1
Turkey	CC	31/07/2014	72-83	88-64	52.6	12.4	1.18	10.5	8.5	T1

5.4.6 **PROGRESS REPORT: Evaluation of Valencia selections in the intermediate production areas (Tom Burke)**

Project 941 D by J. Joubert and S.D. Maziya (CRI)

Opsomming

Die seisoen word van vroeg tot laat rypwordende seleksies opgedeel in die intermediere produksie area en aanbevelings word daarvolgens gedoen. McClean saadloos sal later in die Valencia seisoen volg, met totaal saadlose vrugte en medium tot groot vruggrootte, tussen telling 72 tot 48. Inligting is op hierdie stadium baie beperk weens die tweede oes op die bome en diefstal vroeg in die seisoen. Opvolg evaluasies sal gedoen word wanneer meer seleksies in produksie kom.

Summary

This is a new trial and meaningful data could only be collected from McClean SL. McClean seedless will follow later in the Valencia season, with completely seedless fruit and medium to large fruit, ranging from counts 72 to 48. Information at this stage was limited due to it being the second crop on the trees and theft of the fruit early in the season. Future evaluations will be conducted when more selections come into production.

Objective

- To find suitable Valencia selections with superior characteristics for the intermediate inland citrus production areas.

Materials and methods

Field evaluations and laboratory analyses were conducted on McClean SL at Klipbokspruit, Tom Burke.

Table 5.4.6.1. List of Valencia selections evaluated at Rolemsha (Tom Burke) during 2014.

Selection	Rootstock	Topwork	No. of trees
McClean SL	RL/SC	2011	1 / 5

Results and discussion

There was limited fruit on the Valencia selections available for the 2014 season. NGB bought Rolemsha (Tom Burke) and the farm name will change back to Klipbokspruit. Most of the fruit on the trees were stolen during the transformation phase and change of ownership. McClean SL was the only Valencia selection evaluated this season due to having a crop on the trees.

***McClean SL**

There was a good crop on the young trees. McClean SL remained completely seedless at this trial site. This scenario is very favourable for this Valencia selection, due to the fact that the seedless varieties generally do not bear good crops. Tree condition was good and the internal fruit colour will develop into a deep orange by the time of peak maturity. Due to the lack of fruit this season (theft) on the trees, only one evaluation was possible with delayed external colour from T5-8 on both rootstocks. The fruit size varied from count 72 to 48 on Rough lemon and count 72 to 56 on Swingle. The Brix: acid ratio was below 6.0, Brix: acid ratio of 7 (low), the ratio will improve later in the season when the acid decreases. There was no sunburn, splitting or creasing problems with the fruit, the fruit peels moderately with high rind oil content. Maturity for this specific production area will be determined when trees grow older.

*This was the second evaluation of McClean SL at this trial site, so information is limited and future evaluations will improve recommendations on these varieties. There were no fruit on Du Roi Valencia this season.

Conclusions

McClean SL will be one of the later maturing selections for this area. Fruit size on McClean SL ranged from medium to large/extra-large. There was an external colour delay on McClean SL with the first evaluation (too early). Hopefully more selections will come into production next season; at this stage information was limited due to young tree age and a limited number of fruit on the trees.

Table 5.4.6.2. Internal fruit quality data for Valencia orange selections at Klipbokspruit (Tom Burke) during the 2014 season.

Selection	Root-stock	Date harvested	Size mm	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
McClearn SL	RL	29/04/2014	75-86	72-48	47.9	7.0	1.24	5.6	0.0	T5-8
McClearn SL	SC	29/04/2014	73-85	72-56	48.2	7.4	1.34	5.5	0.0	T6-8

5.4.7 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the intermediate production areas (Marble Hall & Tom Burke)

Project 941 C by J. Joubert and S.D. Maziya (CRI)

Opsomming

Die kwaliteit van die Mandaryn Hibried vrugte het ooreengestem tussen die twee produksie areas, a.g.v. die klimaats sone en boom ouderdom (2011 teenoor 2012). Die resultate vir die warm produksie area (Tom Burke) het aangedui dat Tango die vroegste ryp geword het, met die kleinste vruggrootte en gemiddelde tot goeie interne kwaliteit. Gold Nugget en Tahoe Gold het daarna gevolg, met groot tot baie groot vrugte vir hierdie seisoen. Daar was geen saad in enige van die seleksies wat hierdie seisoen ge-evalueer was nie. Yosemite Gold het geen oes op die bome gehad en alternatiewe drag patrone moet ondersoek word (Gibb bespuitings krities). Shasta Gold was die laaste seleksie gereed vir oes gewees teen middel tot einde Julie, wat die Mandaryn Hibried seisoen afsluit vir hierdie proef. Daar word aanbeveel om nie die oesperiode langer as 3 tot 4 weke te verleng nie om goeie interne kwaliteit te verseker met minimum skil probleme.

Summary

The quality of the Mandarin Hybrid fruit between the two different production areas was similar, due to the climatic region and tree age (2011 versus 2012). The results indicated that in the warmer (Tom Burke) production area, Tango matures first with the smallest fruit size and fair to good internal quality. Gold Nugget and Tahoe Gold followed, with large to extra large fruit size for this season. There were no seeds in any of the selections evaluated this season. There were no fruit on the Yosemite Gold trees this season and alternative bearing patterns must be investigated (Gibb applications crucial). Shasta Gold was the last selection to mature, the middle to end of July, ending off the Mandarin Hybrid season for this trial. Picking periods should not be longer than 3 - 4 weeks to maintain good internal quality and to avoid rind disorders.

Objectives

- To select Mandarin Hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new Mandarin Hybrid cultivars and to determine the climatic suitability of these cultivars in intermediate, inland production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from Schoonbee Estate (Marble Hall) and Klipbokspruit (Tom Burke) from the Limpopo region. The following varieties were evaluated: Shasta Gold, Tahoe Gold, Yosemite Gold, Gold Nugget and Tango.

Table 5.4.7.1. List of Mandarin Hybrid selections evaluated at Schoonbee Estate (Marble Hall) during the 2014 season.

Selection	Rootstock	Topwork
Shasta Gold	CC	2012
Tahoe Gold	CC & Lina/CC	2012
Gold Nugget	CC & Lina/CC	2012
Tango	CC & Lina/CC	2012

Table 5.4.7.2. List of Mandarin Hybrid selections evaluated at Klipbokspruit (Tom Burke) during the 2014 season.

Selection	Rootstock	Topwork
Shasta Gold	CC/RL/SC	2011
Tahoe Gold	CC/SC/RL	2011
Gold Nugget	CC/RL/SC	2011
Tango	CC/RL/SC	2011

Results and discussion

The trees at both Schoonbee Estate and Klipbokspruit were topworked in 2011; this having an impact on the quality and quantity of the fruit.

When the ratio between sugar and acid is 12:1, the fruit is considered to be at peak maturity for Mandarin Hybrids. This ratio is raised as a result of the high sugar levels associated with the new selections. A ratio of 11:1 is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

There was no crop on the Yosemite Gold trees at Schoonbee Estate or Klipbokspruit this season to evaluate. Crop manipulation measurements will be essential to ensure future evaluations and results. Klipbokspruit changed ownership to the NGB group and in the process most of the fruit on the trees were stolen. Due to the theft only one evaluation was possible because of low fruit numbers.

Lina navel on Carrizo was used as interstock in combination with all the UCR 5 selections (separate trial) during the top-working process, to compare the impact between topworking directly on to the rootstock versus onto Lina navel (interstock) and then Carrizo rootstock. Information was very limited due to a poor crop on the trees and evaluations will continue next season.

Gold Nugget

Gold Nugget developed a very upright tree shape (V shape) with long aggressive growing shoots in the middle, bearing no crop at all. The crop will be on the other lower bearing branches towards the middle of the tree, and correctional pruning on this variety is crucial. Remove the long shoots to set the crop lower on the tree with this variety, and another important reason was to set more smooth textured fruit on the lower branches. Gold Nugget is known for its rough textured fruit with coarse rinds, but with the evaluations it came to light that the lower fruit was smoother compared to the fruit on the aggressive long upright branches. The fruit on all the trees at both trial sites were completely seedless, fruit size was large to extra-large on all rootstock combinations (1-1XXX) except for Swingle with count 2-1XXX (due to better crop). The internal quality of the fruit at Klipbokspruit was average, but Rough Lemon developed low juice (47%), Brix (8.0) and acid levels above 1.0% and a delayed external colour (T5-7). Schoonbee produced low juice levels (44-46%) with both evaluations. Based on the internal quality results in Table 5.4.7.3, estimated maturity was the middle to end of June (external colour delay).

Shasta Gold

The crop on the Shasta Gold trees improved at Klipbokspruit and Schoonbee (light crop due to smaller and younger trees). Shasta Gold developed fairly round fruit (Minneola tangelo type) on the trees at both trial sites. There was less ribbing and sunburn on the fruit, better tree canopy development for protection. There were a lot of thorns on the bearing branches of the trees. Rind texture was smoother on the older trees. Shasta Gold produced fruit with soft fibre strength that peels easily, and all the fruit evaluated was completely seedless. The fruit size peaked at large to very large (count 1-1XXX). The flavour was fair with very high juice (up to 80%) and high rind oil content. The internal quality was average, lower Brix of 8 and fairly high acid levels (one evaluation early in the season due to limited fruit). Based on the internal quality results in Table 5.4.7.3 maturity was end of June to the middle of July depending on rootstock choice.

Tahoe Gold

Tahoe Gold produced a very light crop. This selection developed a small tree size when compared to the other UC5 varieties (compact tree). The tree bears fruit in a similar way to grapefruit (bundles). The fruit size peaked from medium to large/very large (count 3-1XXX) and the fruit shape was similar to that of Minneola tangelo. There was a delay in the external colour development at the Klipbokspruit trial site compared to the Schoonbee site. Tahoe produced fruit with soft fibre strength that peeled fairly easily, and all the fruit

evaluated was completely seedless. The internal quality was poor to average; low juice and Brix: acid ratios except for Rough Lemon (51%) and Carrizo (13) (Table 5.4.7.3). Estimated maturity was middle to end of June.

Tango

Tango remained completely seedless at the Klipbokspruit and Alicedale trial sites. There was a fair crop on the trees and the fruit shape was similar to the Nadorcott selection. Rind texture was very smooth with a natural wax shine on the fruit. The Tango trees were thornless with V-tree shape. The fruit was firm and the rind thin, fibre was soft and peeled very easily. Internally the fruit was high in juice content on Rough lemon (59%), Brix was low for this selection except on Carrizo (11) at Schoonbee and the acid levels (below 1.0) decreased rapidly early in the season. Fruit size peaked at count 3 to 1XXX (small to medium/large) except for one evaluation on Swingle at Klipbokspruit (count 4). Based on the internal quality results in Table 5.4.7.3, estimated maturity was end of May to middle of June.

This was the first evaluation of Shasta- and Tahoe Gold, Gold Nugget and Tango at the Schoonbee site, so information is limited and future evaluations will improve recommendations on these varieties.

Conclusion

The delay in external colour development might be a problem; future evaluation will confirm this. Degreening might be an option for the Gold Nugget and TDEs, but with Tango (W. Murcott selection) and Nadorcott, ethylene reacted slowly or not at all. Shasta Gold might be a possibility to consider for the warmer areas due to higher acid levels late in the season, when external colour becomes more intense (T1-2) due to temperature drop (winter time). The appearance of the Shasta Gold's fruit might be a problem (ribbing and coarse rind). In the warmer areas it will become crucial to cover the mandarin orchards with shade net, to minimise sunburn and improve pack out percentage of the fruit, as well as the possibility of hail damage (Marble Hall area). Gold Nugget improved considerably, smoother fruit size versus poor to fair internal quality). Shasta Gold had the largest fruit size, followed by Gold Nugget, and then Tahoe Gold. The smallest fruit size was produced on Tango. There were no incidences of seed in the fruit at the two trial sites (all selections were completely seedless).

Table 5.4.7.3. Internal fruit quality data for Mandarin hybrid selections at Schoonbee Estate (Marble Hall) and Klipbokspruit (Tom Burke) during the 2014 season.

Selection	Root-stock	Date harvested	Site	Size mm	Count	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
Gold Nugget	CC	2014/04/29	Klipbokspruit	68-77	1X-1XX	54.0	9.0	1.27	7.1	0.0	T3-7
Gold Nugget	RL	2014/04/29	Klipbokspruit	68-80	1X-1XXX	46.6	7.9	1.11	7.1	0.0	T5-7
Gold Nugget	SC	2014/04/29	Klipbokspruit	62-72	2-1XX	53.2	9.2	1.37	6.7	0.0	T3-6
Gold Nugget	CC	2014/05/27	Schoonbee	64-80	1-1XXX	46.2	9.0	1.29	7.0	0.0	T2-4
Gold Nugget	CC	2014/06/26	Schoonbee	69-80	1X-1XXX	44.3	11.7	0.96	12.2	0.0	T1
Gold Nugget/Lina	CC	2014/05/27	Schoonbee	70-85	1X-1XXX	41.3	7.7	1.02	7.5	0.0	T4-6
Gold Nugget/Lina	CC	2014/06/26	Schoonbee	65-84	1-1XXX	39.7	10.5	0.99	10.6	0.0	T1-3
Shasta Gold	CC	2014/04/29	Klipbokspruit	65-98	1-1XXX	70.3	8.0	2.03	3.9	0.0	T5-7
Shasta Gold	RL	2014/04/29	Klipbokspruit	81-97	1X-1XXX	66.6	7.5	1.54	4.9	0.0	T4-6
Shasta Gold	SC	2014/04/29	Klipbokspruit	77-92	1XX-1XXX	80.2	7.4	1.18	6.3	0.0	T5-8
Shasta Gold	CC	2014/05/27	Schoonbee	69-85	1X-1XXX	55.7	7.4	1.95	3.8	0.0	T3-5
Tahoe Gold	CC	2014/04/29	Klipbokspruit	57-92	3-1XXX	32.2	7.9	1.53	5.2	0.0	T5-7
Tahoe Gold	SC	2014/04/29	Klipbokspruit	73-85	1XX-1XXX	24.1	8.5	1.49	5.7	0.0	T2-6
Tahoe Gold	RL	2014/04/29	Klipbokspruit	64-106	1-1XXX	51.4	7.6	1.53	5.0	0.0	T5-7
Tahoe Gold	CC	2014/05/27	Schoonbee	74-95	1XX-1XXX	49.6	8.2	1.26	6.5	0.0	T1-2
Tahoe Gold/Lina	CC	2014/06/26	Schoonbee	75-93	1XX-1XXX	44.1	10.2	0.79	12.9	0.0	T1-3
Tango	CC	2014/04/29	Klipbokspruit	58-76	3-1XX	47.0	7.5	0.87	8.6	0.0	T4-6
Tango	SC	2014/04/29	Klipbokspruit	52-75	4-1XX	40.9	8.2	1.14	7.2	0.0	T5-6
Tango	RL	2014/04/29	Klipbokspruit	58-81	3-1XXX	59.3	7.7	0.94	8.2	0.0	T3-6
Tango	CC	2014/05/27	Schoonbee	64-69	1-1X	51.0	8.6	1.01	8.5	0.0	T3-4
Tango	CC	2014/06/26	Schoonbee	69-73	1X-1XX	47.0	10.6	0.73	14.5	0.0	T1-3
Tango/Lina	CC	2014/06/26	Schoonbee	67-80	1-1XXX	31.5	9.5	0.78	12.2	0.0	T1-2

5.4.8 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the hot dry inland areas (Tshipise)

Project 899 B by J. Joubert and S.D. Maziya (CRI)

Opsomming

Die kwaliteit van die Mandaryn Hibried vrugte het aansienlik verskil tussen die verskillende produksie areas, wat 'n baie belangrike punt uitlig wanneer dit gaan kom by die keuse van kultivars vir aanplantings, sowel as die onderstam wat gebruik gaan word. Die resultate van die 2014 seisoen vir hierdie warm produksie areas het aangedui dat Tango die vroegste ryp geword het met die kleinste vruggrootte en goeie interne kwaliteit. Daarna het Gold Nugget en Tahoe Gold gevolg, met beter eksterne vrug kleur. All die seleksie was totaal saadloos gewees hierdie seisoen. Yosemite Gold was volgende om ryp te word, nader aan die einde van die Mandaryn Hibried reeks, met 'n gemiddelde interne kwaliteit (lae Brix), asook goeie eksterne kleur ontwikkeling (T1). Shasta Gold was die laatste seleksie gereed vir oes gewees met die grootste vrugte vir hierdie seisoen, einde Junie tot middel Julie, wat die Mandaryn Hibried seisoen afsluit vir hierdie proef. Daar word aanbeveel om nie die oesperiode langer as 3 tot 4 weke te verleng nie om goeie interne kwaliteit te verseker met minimum skil probleme.

Summary

The quality of the Mandarin Hybrid fruit between the different production areas was very different, indicating how important it is to decide what variety to plant where, as well as the suitable rootstock for that area. The results of the 2014 season indicate that for the warm production areas Tango matures first with the smallest fruit size and good internal quality. Gold Nugget and Tahoe Gold followed; with improved external colour. All the selections evaluated were completely seedless this season. Yosemite Gold matured next towards the end of the Mandarin Hybrid range evaluated at this trial site with average internal quality (low Brix), as well as good external colour development (T1). Shasta Gold was the last selection to mature with the biggest fruit size for this season at the end of June to middle of July, ending off the Mandarin Hybrid season for this trial. Picking periods should not be longer than 3 - 4 weeks to maintain good internal quality and to avoid rind disorders.

Objectives

- To select Mandarin Hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new Mandarin Hybrid cultivars and to determine the climatic suitability of these cultivars in hot, dry production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from Alicedale (Tshipise) from the Limpopo region. The following varieties were evaluated: Shasta Gold, Tahoe Gold, Yosemite Gold, Gold Nugget and Tango.

Table 5.4.8.1. List of Mandarin Hybrid selections evaluated at Alicedale (Tshipise) during the 2014 season.

Selection	Rootstock	Topworked
Shasta Gold	RL/X639	2010
Tahoe Gold	RL/X639	2010
Yosemite Gold	RL	2010
Gold Nugget	RL/X639	2010
Tango	RL/X639	2010

Results and discussion

When the ratio between sugar and acid is 12:1, the fruit is considered to be at peak maturity for Mandarin Hybrids. This ratio is raised as a result of the high sugar levels associated with the new selections. A ratio of 11:1 is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

Shasta Gold

Shasta Gold developed fairly round fruit (Minneola tangelo type) on the trees at the trial site. There was ribbing on most of the fruit, as well as sunburn. The crop on the Shasta Gold trees was good to excellent (80 kg/tree) this season. The tree size remained on the smaller and compact side. There were a lot of thorns on the bearing branches of the trees. Rind texture was rough (scale 4-5). The flavour was fair with average to low juice (44-52%) and high rind oil content. Shasta produced fruit with soft fibre strength that peels easily, and all the fruit evaluated was completely seedless. The fruit size peaked from large to very large (count 1X-1XXX). The internal quality was fair with low juice (avg 49%) and Brix (9) levels, but high acid levels (above 1.1% final evaluation). Based on the internal quality results in Table 5.4.8.2 maturity was end of June.

Tahoe Gold

Tahoe Gold produced a good crop on the trees at Alicedale (rough lemon). This selection developed a small tree size when compared to the other UC5 varieties (compact tree). The tree bears fruit in a similar way to grapefruit (bundles). The fruit size peaked from medium to large/very large (count 2-1XXX) and the fruit shape was similar to that of Minneola tangelo. There was a slight delay in the external colour when the internal quality was optimal. Rough lemon developed better acid levels this season, improving fruit quality, flavour and shelf life. Tahoe Gold produced fruit with soft fibre strength that peeled fairly easily, and all the fruit evaluated were completely seedless. The internal quality on rough lemon was good with juice levels reaching 60% and higher, lower Brix averaging 7 and acid levels were acceptable (0.8% and higher). Based on the internal quality results in Table 5.4.8.2, estimated maturity was the middle to end of May.

Yosemite Gold

Yosemite Gold cropped a light yield on Rough lemon with no fruit on X639 and additional measures might be necessary to increase the crop on the trees (Gibb sprays or girdling). Yosemite developed a very promising soft citrus fruit shape. The fruit was firm, rind texture was smooth and the fibre was soft. It peeled very easily and was completely seedless. Yosemite Gold developed the biggest tree size compared to the other TDE selections at Alicedale. This aggressive growth characteristic might be the reason for the poor crop on the trees (vegetative growth), and must be channelled into fruit set and crop. Fruit size varied from large to very large (count 1X-1XXX), similar to Shasta- and Tahoe Gold. The internal quality was average to good developing higher juice and acid levels, but low Brix. External colour developed with the internal quality towards the end of the evaluations. Based on the internal quality results in Table 5.4.8.2, estimated maturity was the end of May to middle of June.

Gold Nugget

Gold Nugget developed a very upright tree shape (V shape) with long aggressive growing shoots in the middle, bearing no crop at all. The crop will be on the other lower bearing branches towards the middle of the tree, and correctional pruning on this variety is crucial. Remove the long shoots to set the crop lower on the tree with this variety, and another important reason was to set more smooth textured fruit on the lower branches. Gold Nugget is known for its rough textured fruit with coarse rinds, but with the evaluations it came to light that the lower fruit was smoother compared to the fruit on the aggressive long upright branches. Fruit was smoother compared to 2013 with a 60:40 ratio between smooth and coarse. Fruit size at Alicedale was medium to large/extra large (count 2-1XXX) and the fruit on all the trees were completely seedless. The internal quality of the fruit improved from fair to good and developed juice (avg 53%), Brix (9.5) and acid levels above 1.0% avg and an external colour from T1-2. Future evaluations will determine the feasibility of Gold Nugget in the hot areas. Based on the internal quality results in Table 5.4.8.2, estimated maturity was the middle to end of June.

Tango

Tango remained completely seedless at Alicedale where there was a better crop (30-40 kg/tree) on the trees. The fruit shape was similar to the Nadorcott selection. Rind texture was very smooth with a natural wax shine on the fruit. The Tango trees were thornless and upright V-shape. The fruit was firm and the rind thin, fibre was soft and peeled very easy. Internally the fruit was high in juice content (55 to 66%), Brix improved for this selection (10 with second evaluation), acid levels (below 1.0) decreased rapidly early in the season (short shelf life) and deep orange coloured fibre. Fruit size peaked at count 4 to 1XXX (small to medium/large). Based on the internal quality results in Table 5.4.8.2, estimated maturity was end of April to middle of May.

Conclusion

The external colour delay (internal quality improved with more mature trees) in the hotter areas remained a problem; future evaluations will confirm this. Degreening might be an option for the Gold Nugget and TDEs, but with Tango (W. Murcott selection) and Nadorcott, ethylene reacted slowly or not at all. Shasta Gold might be a possibility to consider for the hot areas due to higher acid levels late in the season, when external colour becomes more intense (T1-2) due to temperature drop (winter time). The appearance of Shasta Gold's fruit in the Tshipise area (hot) might be a problem. In the hot areas it will become crucial to cover the mandarin orchards with shade net, to minimise sunburn and improve pack-out percentage (Shasta had severe sunburn at Alicedale). Gold Nugget improved considerably with good internal quality, better production and medium to large fruit size. Shasta Gold had the largest fruit size, followed by Tahoe- and Yosemite Gold, then Gold Nugget. The smallest fruit size was produced on Tango. All the selections were completely seedless this season.

Table 5.4.8.2. Internal fruit quality data for Mandarin hybrid selections at Alicedale (Tshipise) during the 2014 season.

Selection	Root-stock	Date harvested	Size mm	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Gold Nugget	RL	2014/04/15	60-78	2-1XXX	53.2	7.6	0.99	7.7	0.0	T5-7
Gold Nugget	X639	2014/04/15	59-70	2-1X	53.2	8.0	1.31	6.1	0.0	T5-8
Gold Nugget	X639	2014/06/25	77-80	1XXX	51.7	9.5	0.8	12.3	0.0	T1-2
Shasta Gold	RL	2014/04/15	73-85	1XX-1XXX	51.9	7.2	1.48	4.9	0.0	T4-6
Shasta Gold	X639	2014/04/16	70-83	1X-1XXX	52.2	7.3	1.58	4.6	0.0	T5-7
Shasta Gold	RL	2014/06/25	79-90	1XXX	48.6	9.0	1.11	8.1	0.0	T1-2
Shasta Gold	X639	2014/06/25	80-90	1XXX	44.3	8.3	1.09	7.6	0.0	T1-3
Tahoe Gold	RL	2014/04/15	63-70	2-1X	61.7	7.6	1.40	5.4	0.0	T5-7
Tahoe Gold	X639	2014/04/16	60-78	2-1XXX	60.1	7.4	1.39	5.3	0.0	T6-8
Tahoe Gold	RL	2014/06/25	71-81	1X-1XXX	64.3	7.7	0.80	9.6	0.0	T1
Tahoe Gold	X639	2014/06/25	73-89	1XX-1XXX	40.6	7.1	0.89	8.0	0.0	T1-3
Tango	RL	2014/04/16	58-79	3-1XXX	55.8	7.3	0.74	9.9	0.0	T5-6
Tango	X639	2014/04/16	52-63	4-2	66.1	7.5	0.82	9.1	0.0	T5-8
Tango	X639	2014/06/25	55-75	3-1XX	54.5	10.2	1.02	10.0	0.0	T2-4
Yosemite	RL	2014/04/15	70-83	1X-1XXX	63.9	7.5	1.12	6.7	0.0	T3-7
Yosemite	RL	2014/06/25	79-95	1XXX	45.1	7.6	0.86	8.8	0.0	T1

5.4.9 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the cool inland areas (Burgersfort)

Project 990 by J. Joubert and S.D. Maziya (CRI)

Opsomming

Goeie eksterne kleur ontwikkeling tesame met goeie interne vrug kwaliteit was een van die sterkpunte van die koeler produksie areas. Die resultate van die 2014 seisoen vir hierdie koel binnelandse produksie area het aangedui dat Tango die vroegste ryp geword het met medium tot groot vrugte, asook baie goeie interne kwaliteit en smaak. Daarna het Gold Nugget met die beste interne kwaliteit vir die seisoen, hoë Brix en goeie suur vlakke wat belowende rakleef tyd sal verseker. Die sap inhoud moet noukeurig met opvolg evaluasies gekontroleer word, dit kan effens aan die laer kant wees. Daar was geen saad in enige van die Mandaryn hibried vrugte hierdie seisoen gewees nie. Tahoe Gold was volgende om ryp te word, nader aan die einde van die laat Mandaryn hibried seisoen met goeie interne kwaliteit vrugte wat van die hoogste sap vlakke opgelewer het. Shasta Gold was die laatste seleskie gereed vir oes, wat die Mandaryn hibried seisoen afsluit vir hierdie proef. Daar word aanbeveel om nie die oesperiode langer as 3 tot 4 weke te verleng nie om goeie interne kwaliteit te verseker met minimum skil probleme.

Summary

Good external colour development was one of the strong points in the cooler production areas together with good internal fruit quality. The results of the 2014 season indicated that Tango matures first for the cool inland production area with medium to large fruit size, very good internal quality and flavour. Gold Nugget followed with the best internal quality for the season with high Brix and good acid levels extending the shelf life of the fruit. Monitor the juice levels closely on this selection, because they tend to be on the low side. There were no seeds present in any of the Mandarin Hybrid fruit this season. Tahoe Gold matured next towards the end of the Mandarin Hybrid range, evaluated at this trial site with good internal quality resulting in high juice levels in the fruit. Shasta Gold was the last selection to mature, ending of the Mandarin Hybrid season for this trial. Picking periods should not be longer than 3 - 4 weeks to maintain good internal quality and to avoid rind disorders.

Objectives

- To select Mandarin Hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new Mandarin Hybrid cultivars and to determine the climatic suitability of these cultivars in hot production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from Viljoen Farm (Burgersfort) from the Limpopo region. The following varieties were evaluated: Shasta Gold, Tahoe Gold, Yosemite Gold, Gold Nugget and Tango.

Table 5.4.9.1. List of Mandarin Hybrid selections evaluated at Viljoen (Burgersfort) during the 2014 season.

Selection	Rootstock	Topwork
Shasta Gold	CC	2011
Tahoe Gold	CC	2011
Yosemite Gold	CC	2011
Gold Nugget	CC	2011
Tango	CC	2011

Results and discussion

All the selections were bearing fruit for the first time this season.

When the ratio between sugar and acid is 12:1, the fruit is considered to be at peak maturity for Mandarin Hybrids. This ratio is raised as a result of the high sugar levels associated with the new selections. A ratio of 11:1 is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

Shasta Gold

There was less ribbing compared to the hot production trial sites (Tshipise, Tom Burke, Letsitele and Malelane) on most of the fruit, and barely any sunburn. Shasta Gold developed fairly flat fruit on the trees at the trial sites. The tree size was similar to Yosemite Gold and Tango, between 1.5 and 1.7 m high. Rind texture was smoother this season. The fruit size was slightly smaller and peaked from large to very large (avg. count 1-1XX), similar to hot production areas. The internal quality was good with juice levels of 50%, Brix as high as 12 and good acid levels of 1.2% when the colour peaked at T1-2. The higher acid might delay harvest time (Brix: acid ratio of 9.9), but improve shelf life of the fruit. Shasta produced fruit with soft fibre strength that peels easily, and all the fruit evaluated was completely seedless (0.1 seeds 2013). Based on the internal quality results in Table 5.4.9.2 maturity was middle to end of August (ultra late).

Tahoe Gold

Tahoe Gold developed the smallest tree size when compared to the other UC5 varieties (compact round tree shape). The fruit size peaked from medium to large/very large (count 2-1XXX) and the fruit shape was similar to that of a Minneola tree. The external colour was optimum (T1) when the internal quality reached peaked maturity with acid level above 1.2%. Tahoe produced fruit with soft fibre strength that peels easily, and all the

fruit evaluated was completely seedless. The internal quality on Tahoe was not outstanding compared to 2013 (Brix: acid ratio of 8.0). With the second last evaluation the juice levels were above 50% with high acid levels of 1.4%. Based on the internal quality results in Table 5.4.9.2, estimated maturity was the middle to end of July.

Yosemite Gold

There was no fruit on the trees to evaluate due to poor fruit set. Yosemite tends to have severe alternative cropping patterns. Future evaluations will determine the magnitude of the problem.

Gold Nugget

Gold Nugget developed a very upright tree shape (V shape) with long aggressive growing shoots in the middle, bearing no crop at all. The crop will be on the other bearing branches towards the middle of the tree, and correctional pruning on this variety is crucial. Remove the long shoots to set the crop lower, and another important reason is to set more smooth textured fruit on the lower branches. This unique selection is familiar for its rough textured fruit with coarse rinds. After completing the evaluations it came to light that the lower fruit was smoother compared to the fruit on the aggressive long upright branches. Gold Nugget developed good internal quality (Brix: acid ratio 10), Brix content of 12 and high acid (1.2%) levels for longer shelf life at optimal external colour (T1). Juice levels throughout all the evaluations at the Burgersfort site remained in the higher 40s (range from 46 to 49.0%), although low to compete in the export market demands. Keep in mind the young tree age, future evaluations will determine the optimal internal quality of this selection in the cooler areas. The fruit was completely seedless during all the evaluation. Based on the internal quality results in Table 5.4.9.2, estimated maturity was the end of July to middle of August.

Tango

The fruit shape was similar to the Nadorcott selection. Tango was completely seedless at the Viljoen trial site in Burgersfort and the trees developed a good crop (50 to 70 kg/tree). Rind texture was very smooth with a natural shine (similar to packhouse waxing). The Tango trees were thornless and V-shaped with medium growth rate on Carrizo rootstock. The fruit was firm and the rind thin, fibre was soft and peeled very easily. Internally the fruit was one of the top 2 selections evaluated in this trial, high in juice content (52%), Brix was very good for this selection (11.7) and excellent acid level (1.1%) at external colour ranging from T1-4. Brix: acid ratio peaked between 10.6 and 10.7 and the fruit displayed a deep orange fibre colour. Fruit size improved and peaked from count 2 to 1XX (medium to large). Based on the internal quality results in Table 5.4.9.2, estimated maturity was the middle end of June to the middle of July.

Conclusion

There was no external colour delay in the cooler area and the optimal colour development was in sequence with a good to very good internal quality; future evaluation will support this statement. In the cooler areas it might be necessary to cover the mandarin orchards with shade net, to minimise possible hail damage and sunburn to improve packout percentage of the fruit.

The best internal quality fruit was between Tango (Brix: acid ratio 12) and Gold Nugget, developing Brix content of up to 12, Shasta Gold will mature late in the Mandarin hybrid season due to high acid levels (1.2% with last evaluation). Shasta Gold had the largest fruit size, followed by Tahoe Gold, then Gold Nugget. Tango developed bigger fruit size and increased from count 5 to count 2 on the smaller side. All the selections were completely seedless this season.

Table 5.4.9.2. Internal fruit quality data for Mandarin hybrid selections on Carrizo citrange at Viljoen Farm (Burgersfort) during the 2014 season.

Selection	Date	Size	Count	Juice	Brix °	Acid	Ratio	Avg.	Colour
	harvested	mm		(%)		(%)		seed	
Gold Nugget	2014/05/19	58-75	3-1XX	48.5	8.6	1.57	5.5	0.0	T4-6
Gold Nugget	2014/06/05	67-80	1-1XXX	47.8	10.5	1.16	9.1	0.0	T2-4
Gold Nugget	2014/07/01	64-76	1-1XX	49.0	12.0	1.21	9.9	0.0	T1-2
Shasta Gold	2014/05/19	75-100	1XX-1XXX	54.6	8.2	1.75	4.7	0.0	T4-6
Shasta Gold	2014/06/05	75-84	1XX-1XXX	50.4	10.4	1.60	6.5	0.0	T1-4
Shasta Gold	2014/07/01	67-82	1-1XXX	54.5	11.5	1.18	9.7	0.0	T1
Tahoe Gold	2014/05/19	63-97	2-1XXX	54.6	8.7	1.39	6.3	0.0	T4-5
Tahoe Gold	2014/06/05	77-90	1XX-1XXX	54.9	9.9	1.26	7.9	0.0	T1-4

Tahoe Gold	2014/07/01	79-95	1XXX	49.7	11.8	1.42	8.3	0.0	T1-2
Tango	2014/05/19	60-75	2-1XX	58.0	9.2	1.07	8.6	0.0	T4-6
Tango	2014/06/05	67-73	1-1XX	48.8	9.7	0.91	10.7	0.0	T3-4
Tango	2014/07/01	60-68	2-1X	51.9	11.7	1.10	10.6	0.0	T2-4

5.4.10 PROGRESS REPORT: Evaluation of Delta Valencia on new imported rootstocks in the Marble Hall area

Project 94 by J. Joubert and S.D. Maziya (CRI)

Opsomming

Die prestasie van Delta Valencia op 42 verskillende onderstamme in herplant gronde was oor 'n agt jaar termyn in die Marble Hall omgewing geëvalueer, tot in 2007. Daarna is elk tweede boom afgesaag om meer spasie in die rye te verseker. Die bome het stadig herstel, maar was in 2014 weer gereed om geëvalueer te word (opvold evaluasie). Geen tekens van onverenigbaarheid was sigbaar op enige van die bostam: onderstam kombinasies nie. Die bome se interne kwaliteit het baie verbeter met indrukwekkende toename in produksie. Vruggrootte het gepiek by telling 72/88, groter vruggootte vir Delta Valencia. Flying dragon x Sunki 1113, gevolg deur US 812 (Sunki x Beneke) en Flying dragon x Sunki 1112 het die beste oes produksie op die bome verseker. Die tradisionele onderstamme wat in die sitrus bedryf gebruik word, het heelwat swakker gevaar (laagste oes was op Carrizo gewees). Die Sunki hibried onderstamme vorm 'n heelwat kleiner boomgrootte, maar die boomvorm (oop kroon gedeelte met take wat afhang) kan moontlik probleme inhou (sonbrand, takke breek ens).

Summary

The performance of Delta Valencia on 42 different rootstocks in replant soils was evaluated over an eight-year production cycle in the Marble Hall production area up to 2007. Every second tree was removed in 2008 to allow better tree spacing in the rows. The trees recovered slowly and a follow-up evaluation was only possible in 2014. No signs of incompatibility were visible on any of the scion: rootstock combinations. The internal quality of the trees improved markedly with impressive crop production. Fruit size peaked at count 72/88, bigger fruit size for Delta Valencia. Flying dragon x Sunki 1112, followed by US 812 (Sunki x Beneke) and Flying dragon x Sunki 1112 produced excellent yields on the trees. The traditional rootstocks being used in the citrus industry performed moderately (lowest crop was on Carrizo). The Sunki hybrid rootstocks developed smaller trees (dwarfing effect) but the tree shape (more open with long bearing branches) might be problematic (sunburn and weaker branches).

Objectives

- To investigate the performance of Delta Valencia on 42 new, imported rootstocks on replant soils over an eight-year production cycle.
- To improve production, internal quality, rind colour and fruit size count distributions.

Materials and methods

A randomised block design was used for 22 rootstocks with two replicates of five trees each; the other 20 rootstocks were planted in a non-randomised design comprising 10 trees per rootstock. All 42 rootstocks were selected and evaluated in the 2013 season, after only 30 had been evaluated the previous time in the 2007 season (best combinations selected).

Every second tree had been removed from the orchard in 2008, due to limited tree spacing in the rows. Trees recovered slowly; this been the reason for harvesting the trees only in 2013.

Delta Valencia on the following rootstocks are being evaluated: F80/8, F80/3, C32, C35, X639, RL-C, RL-S, RL-W, PT, US 812 (Sunki x Beneke), RxT, Sunki 1113, CM, CC, TC, Volk, KC, TB, ML, RC, JT, RT, BC, Sunki 1112, ST, SC, RP, SM, SFS, Sunki 1116, AT, K, C, SCS, GT, OT, CA, JT, ChM, N, CLM, CO, RT, JC. The trees were planted in 1998. Trees were evaluated at Moosrivier Estates (Marble Hall), in Mpumalanga during the 2007 (final evaluation) season. Full names for these abbreviations appear below.

Table 5.4.10.1. Number of trees per rootstock in the Delta Valencia trial at Marble Hall.

No	Abbreviation	Rootstock	No of trees
1	F80/8	F80 citrumelo 8	5
2	PT	Pomeroy trifoliolate	5
3	C32	Citrange 32	5
4	AT	Australian trifoliolate	5
5	HRS 812	US-812 (Sunki x Beneke)	5
6	K	Konejime	5
7	CM	C. macrophylla	5
8	C35	Citrange 35	5
9	C	Calamandarin	5
10	SCS	Sun chu sha	5
11	X639	Cleopatra x Trifoliolate	5
12	GT	Gou Tou	5
13	ML	Milan Lemon	5
14	OT	Orlando tangelo	5
15	CA	C. amblycarpa	5
16	RC	Rusk citrange	5
17	JT	Jacobsen trifoliolate	2
18	RL-S	Rough lemon schaub	5
19	SC	Swingle citrumelo	5
20	RP	Rangpur lime	5
21	SM	Shekwasha mandarin	5
22	ChM	Changsa mandarin	5
23	N	Natsudaidai	5
24	RxT	Rangpur x Troyer	5
25	RL-C	Rough lemon cairn	5
26	CLM	Cleopatra mandarin	5
27	Sunki 1113	Flying dragon x Sunki(1113)	5
28	CO	C.obovoideae	5
29	CC	Carrizo citrange	5
30	TC	Troyer citrange	5
31	Volk	Volkameriana	5
32	KC	Koethen citrange	5
33	TB	Terrabella citrumelo	5
34	RT	Rubidoux trifoliolate	5
35	JC	Japanese citron	5
36	BC	Benton citrange	5
37	F80/3	F80 citrumelo 3	5
38	Sunki 1112	Flying dragon x Sunki(1112)	5
39	ST	Sampson tangelo	5
40	SFS	Smooth flat seville	5
41	Sunki 1116	Flying dragon x Sunki(1116)	5
42	RL-W	Rough lemon wallace	5

Results and discussion

Internal fruit quality analysis (Table 5.4.10.2)

- Juice%: The highest juice content was produced by C35 (56.3%), followed by Koethen (56.2%) and RxT with 56.0%. Five of the rootstocks evaluated (Calamandarin, Milan Lemon, Sunki 1113, Benton and Rough lemon Wallace) tested below 52%, not complying with the minimum export standards for Delta Valencia. Benton had the lowest juice content of 51.3%.

- Brix°: This season the Brix content decreased, with 37 of the 42 rootstock combinations evaluated producing a Brix higher than 9.5 (export minimum requirement for Delta), except for Konejime, Rough lemon schaub, Rough lemon cairn, Volkameriana and Japanese citron. Rusk citrange produced the highest Brix content of 12.5, followed by Rough lemon wallace with 12.2 and Sunki 1112 with 12.1.
- Acid: Sampson tangelo provided the highest acid content (1.32%) for this season, followed by Rubidoux trifoliolate with 1.29% and Rusk citrange with 1.27%. The lowest acid content was produced on Rough lemon schaub (0.7%), followed by Rough lemon cairn (0.81%) and Volkameriana (0.82%), not complying with the minimum export standards (above 0.85%).

Fruit size distribution (Table 5.4.10.3)

- The fruit size increased this season and Delta in combination with 19 of the 42 rootstocks peaked at count 72, followed by 16 rootstocks that peaked at count 88, 4 peaked at count 56 and the remaining 3 at count 105/125.

Production per tree (Table 5.4.10.4)

- Sunki 1113 (Flying dragon x Sunki 1113) produced the best crop on the trees with an excellent yield of 163.1 kg per tree. US-812 (Sunki x Beneke) bore the second highest crop on the trees, peaking at 158.7 kg per tree. Sunki 1112 (Flying dragon x Sunki 1112) was next with 150.6 kg per tree (2013 cropping 205.7 kg per tree), outperforming all the prominent rootstocks used currently in the citrus industry (C35, X639, RL-C, CC, TC, VOLK, TB, and BC). The lowest crop production was in combination with Carrizo bearing 65.4 kg per tree.

Conclusions

The internal quality of the fruit in general was very good to excellent for the 2013 season; and only five of the rootstock combinations were below the minimum level required for juice content of 50.0% (Delta), as well as below the 9.5 requirement for Brix levels on Delta. Rough lemon schaub developed the lowest acid level for this trial (0.7%). Two of the Sunki cross selections (Sunki 1112 and 1113) developed high acid levels with maturity, this will be valuable information for the lower acid varieties as well as areas where low acid levels in the fruit occur (Marble Hall and Groblersdal area).

Fruit size peaked at count 72 on 19 of the scion: rootstock combinations, followed by count 88 (16 combinations): better fruit size for Delta Valencia (typical smaller fruit size). Crop production on the trees decreased compared to 2013 with 158.7 kg per tree on Flying dragon x Sunki 1113, followed by US 812 (Sunki x Beneke) cropping 158.7 kg per tree and Flying dragon x Sunki 1112 with 150.6 kg per tree. The lowest crop was on Carrizo bearing 65.4 kg per tree.

Table 5.4.10.2. Internal fruit quality of Delta Valencia on different rootstocks at Moosrivier Estate (Marble Hall) on 26 August 2014.

Root-stock	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
F80/8	54.4	11.3	1.07	10.56	0.0	T1
PT	55.5	11.7	1.05	11.14	0.0	T1
C32	53.3	11.3	1.05	10.76	0.0	T1
AT	54.9	11.7	1.15	10.17	0.0	T1
US-812	54.8	11.0	1.04	10.58	0.0	T1
K	55.4	8.9	0.91	9.78	0.0	T1
CM	50.6	10.9	1.02	10.69	0.0	T1
C35	56.3	11.6	1.07	10.84	0.0	T1
C	51.7	11.8	1.05	11.24	0.0	T1-2
SCS	53.8	10.6	1.06	10.00	0.0	T1
X639	54.4	10.5	0.98	10.71	0.0	T1-2
GT	55.4	9.9	0.94	10.53	0.0	T1
ML	51.5	7.9	0.77	10.26	0.0	T1
OT	54.6	9.9	0.98	10.10	0.0	T1

CA	55.6	9.5	0.97	9.79	0.0	T1
RC	54.8	12.5	1.27	9.84	0.0	T1
JT	52.9	11.5	0.86	13.37	0.0	T1
RL-S	52.1	8.2	0.7	11.71	0.0	T1
SC	53.4	10.7	0.95	11.26	0.0	T1
RP	53.1	9.8	0.89	11.01	0.0	T1-2
SM	53.0	10.3	1.03	10.00	0.0	T1
ChM	52.4	10.4	1.03	10.10	0.0	T1
N	55.3	9.9	0.92	10.76	0.0	T1
RxT	56.0	11.6	1.03	11.26	0.0	T1
RL-C	53.1	8.9	0.81	10.99	0.0	T1-2
CLM	55.2	10.0	0.96	10.42	0.0	T1
Sunki 1113	51.5	11.9	1.17	10.17	0.0	T1-2
CO	55.9	10.6	1.05	10.10	0.0	T1-2
CC	53.7	11.0	0.91	12.09	0.0	T1
TC	52.4	10.6	0.96	11.04	0.0	T1
Volk	54.2	8.2	0.82	10.00	0.0	T1
KC	56.2	10.6	1.18	8.98	0.0	T1
TB	54.6	11.2	1.06	10.57	0.0	T1
RT	54.0	11.9	1.29	9.22	0.0	T1
JC	53.7	9.2	0.86	10.70	0.0	T1
BC	51.3	11.7	1.07	10.93	0.0	T1-2
F80/3	54.1	9.9	1.12	8.84	0.0	T1
Sunki 1112	54.8	12.1	1.18	10.25	0.0	T1
ST	55.2	11.0	1.32	8.33	0.0	T1
SFS	54.2	11.3	1.03	10.97	0.0	T1
Sunki 1116	54.2	11.4	1.02	11.18	0.0	T1-2
RL-W	51.5	12.2	1.11	10.99	0.0	T1

Table 5.4.10.3. Fruit size distribution of Delta Valencia on different rootstocks at Moosrivier Estate (Marble Hall) during the 2014 season.

Rootstock	Count	% Fruit	Rootstock	Count	% Fruit	Rootstock	Count	% Fruit
F80/8	48	2.3	CA	48	1.3	CC	48	4.3
F80/8	56	15.6	CA	56	14.9	CC	56	27.4
F80/8	72	32.3	CA	72	28.5	CC	72	34.9
F80/8	88	26.8	CA	88	29.7	CC	88	21.2
F80/8	105/125	20.0	CA	105/125	22.7	CC	105/125	10.5
F80/8	144	3.1	CA	144	3.1	CC	144	1.7
Rootstock	Count	% Fruit	Rootstock	Count	% Fruit	Rootstock	Count	% Fruit
PT	48	8.4	RC	48	2.5	TC	48	3.9
PT	56	37.2	RC	56	11.9	TC	56	21.3
PT	72	32.4	RC	72	22.6	TC	72	29.7
PT	88	15.0	RC	88	30.7	TC	88	23.7
PT	105/125	5.8	RC	105/125	26.8	TC	105/125	19.3
PT	144	1.2	RC	144	5.5	TC	144	2.1
Rootstock	Count	% Fruit	Rootstock	Count	% Fruit	Rootstock	Count	% Fruit
C32	48	2.3	JT	48	2.3	Volk	48	1.6
C32	56	12.0	JT	56	12.9	Volk	56	17.9
C32	72	25.2	JT	72	22.9	Volk	72	32.2

C32	88	28.7	JT	88	26.4	Volk	88	26.3
C32	105/125	27.7	JT	105/125	30.4	Volk	105/125	19.3
C32	144	4.2	JT	144	5.1	Volk	144	2.7
Rootstock	Count	% Fruit	Rootstock	Count	% Fruit	Rootstock	Count	% Fruit
AT	48	0.2	RL-S	48	0.2	KC	48	0.3
AT	56	8.6	RL-S	56	8.5	KC	56	5.3
AT	72	27.3	RL-S	72	24.2	KC	72	17.3
AT	88	33.2	RL-S	88	29.1	KC	88	39.7
AT	105/125	26.9	RL-S	105/125	31.9	KC	105/125	39.4
AT	144	3.8	RL-S	144	6.1	KC	144	8.0
Rootstock	Count	% Fruit	Rootstock	Count	% Fruit	Rootstock	Count	% Fruit
HRS812	48	3.1	SC	48	4.3	TB	48	7.9
HRS812	56	15.8	SC	56	30.6	TB	56	30.8
HRS812	72	27.3	SC	72	33.5	TB	72	28.6
HRS812	88	26.1	SC	88	19.8	TB	88	17.9
HRS812	105/125	22.6	SC	105/125	9.8	TB	105/125	12.9
HRS812	144	5.1	SC	144	1.9	TB	144	1.9
Rootstock	Count	% Fruit	Rootstock	Count	% Fruit	Rootstock	Count	% Fruit
K	48	3.1	RP	48	1.8	RT	48	0.9
K	56	18.2	RP	56	16.6	RT	56	17.5
K	72	29.8	RP	72	31.5	RT	72	37.6
K	88	27.0	RP	88	26.9	RT	88	28.2
K	105/125	18.8	RP	105/125	20.2	RT	105/125	12.9
K	144	3.1	RP	144	3.1	RT	144	2.9
Rootstock	Count	% Fruit	Rootstock	Count	% Fruit	Rootstock	Count	% Fruit
CM	48	16.4	SM	48	2.2	JC	48	1.0
CM	56	28.0	SM	56	12.4	JC	56	12.2
CM	72	21.9	SM	72	33.8	JC	72	28.1
CM	88	14.4	SM	88	29.1	JC	88	31.9
CM	105/125	15.8	SM	105/125	19.8	JC	105/125	23.1
CM	144	3.4	SM	144	2.8	JC	144	3.7
Rootstock	Count	% Fruit	Rootstock	Count	% Fruit	Rootstock	Count	% Fruit
C35	48	1.8	ChM	48	1.3	BC	48	15.3
C35	56	11.0	ChM	56	11.6	BC	56	34.2
C35	72	23.9	ChM	72	31.8	BC	72	26.6
C35	88	30.0	ChM	88	31.3	BC	88	13.3
C35	105/125	28.7	ChM	105/125	21.2	BC	105/125	8.1
C35	144	4.7	ChM	144	2.8	BC	144	2.5
Rootstock	Count	% Fruit	Rootstock	Count	% Fruit	Rootstock	Count	% Fruit
C	48	2.8	N	48	1.3	F80/3	48	8.5
C	56	15.2	N	56	16.3	F80/3	56	26.9
C	72	24.7	N	72	32.3	F80/3	72	30.1
C	88	26.8	N	88	30.3	F80/3	88	18.2
C	105/125	26.0	N	105/125	17.3	F80/3	105/125	13.5
C	144	4.5	N	144	2.6	F80/3	144	2.8

Rootstock	Count	% Fruit	Rootstock	Count	% Fruit	Rootstock	Count	% Fruit
SCS	48	0.4	RxT	48	1.2	Sunki 1112	48	2.0
SCS	56	6.3	RxT	56	16.0	Sunki 1112	56	14.9
SCS	72	18.0	RxT	72	32.7	Sunki 1112	72	31.2
SCS	88	28.6	RxT	88	27.2	Sunki 1112	88	29.9
SCS	105/125	38.7	RxT	105/125	19.2	Sunki 1112	105/125	19.2
SCS	144	8.1	RxT	144	3.7	Sunki 1112	144	2.7
Rootstock	Count	% Fruit	Rootstock	Count	% Fruit	Rootstock	Count	% Fruit
X639	48	1.7	RL-C	48	0.5	ST	48	1.6
X639	56	12.1	RL-C	56	7.9	ST	56	15.0
X639	72	25.5	RL-C	72	22.0	ST	72	33.2
X639	88	27.7	RL-C	88	30.2	ST	88	29.6
X639	105/125	27.4	RL-C	105/125	34.1	ST	105/125	16.9
X639	144	5.6	RL-C	144	5.3	ST	144	3.8
Rootstock	Count	% Fruit	Rootstock	Count	% Fruit	Rootstock	Count	% Fruit
GT	48	3.1	CLM	48	1.7	SFS	48	2.3
GT	56	13.1	CLM	56	10.8	SFS	56	19.1
GT	72	25.6	CLM	72	29.2	SFS	72	34.0
GT	88	29.8	CLM	88	28.8	SFS	88	26.8
GT	105/125	24.0	CLM	105/125	24.6	SFS	105/125	15.0
GT	144	4.4	CLM	144	5.0	SFS	144	2.8
Rootstock	Count	% Fruit	Rootstock	Count	% Fruit	Rootstock	Count	% Fruit
ML	48	1.6	Sunki 1113	48	1.7	Sunki 1116	48	1.5
ML	56	12.5	Sunki 1113	56	11.1	Sunki 1116	56	12.7
ML	72	25.0	Sunki 1113	72	27.1	Sunki 1116	72	27.6
ML	88	27.5	Sunki 1113	88	30.9	Sunki 1116	88	30.8
ML	105/125	26.8	Sunki 1113	105/125	25.6	Sunki 1116	105/125	23.6
ML	144	6.6	Sunki 1113	144	3.6	Sunki 1116	144	3.8
Rootstock	Count	% Fruit	Rootstock	Count	% Fruit	Rootstock	Count	% Fruit
OT	48	0.7	CO	48	1.3	RL-W	48	0.9
OT	56	10.8	CO	56	14.3	RL-W	56	7.2
OT	72	27.9	CO	72	32.5	RL-W	72	21.7
OT	88	31.8	CO	88	29.1	RL-W	88	32.8
OT	105/125	25.1	CO	105/125	20.0	RL-W	105/125	32.1
OT	144	3.8	CO	144	2.8	RL-W	144	5.3

Table 5.4.10.4. Production of Delta Valencia on different rootstocks at Moosrivier Estate (Marble Hall) during the 2014 season.

Rootstock	Kg/tree		
	2007	2013	2014
F80/8	75.5	142.0	112.7
PT	66.9	135.9	69.0
C32	61.0	310.0	141.3
AT	0.0	133.0	111.5
HRS 812	75.5	175.1	158.7
K	0.0	135.9	108.7
CM	104.6	129.0	96.7

C35	61.1	144.4	132.7
C	0.0	120.1	94.6
SCS	0.0	127.8	105.0
X639	72.5	157.7	109.0
GT	0.0	139.7	115.7
ML	87.3	135.2	106.5
OT	0.0	107.3	93.4
CA	0.0	84.1	79.3
RC	72.9	103.7	111.1
JT	35.8	136.8	97.4
RL-S	75.2	140.5	81.7
SC	79.3	160.9	71.7
RP	89.0	151.1	76.1
SM	86.3	130.2	94.8
ChM	0.0	170.7	116.2
N	0.0	134.7	135.9
RxT	57.0	100.8	81.6
RL-C	77.6	165.9	105.3
CLM	0.0	112.7	72.5
Sunki 1113	78.5	149.1	163.1
CO	77.6	124.3	78.1
CC	77.6	159.0	65.4
TC	101.1	146.0	72.4
VOLK	49.5	155.9	74.0
KC	72.7	136.8	122.5
TB	56.9	168.3	102.5
RT	0.0	144.3	107.5
JC	0.0	172.1	116.4
BC	80.1	188.5	79.2
F80/3	65.0	204.0	119.8
Sunki 1112	88.0	205.7	150.6
ST	73.7	128.3	107.9
SFS	115.4	135.5	113.0
Sunki1116	92.4	169.2	144.9
RL-W	44.8	172.2	145.3

5.4.11 PROGRESS REPORT: Evaluation of Valencias on new imported rootstocks in the Malelane area

Project 416 A by J. Joubert and S.D. Maziya (CRI)

Opsomming

Midnight, met 'n gesonde entlas verbinding, het bewys dis verenigbaar met US 812, 'n hibried onderstam kruising tussen Sunki mandaryn en Beneke trifoliaat (Sunki 812). Die boomgrootte van hierdie kombinasie word as medium beskou (vergelyk met Carrizo boomgrootte en groeikragtigheid), alhoewel US 812 onderstam as boom op sy eie baie groeikragtig is en 'n groot boom oplewer. Die produksie hierdie seisoen het met 37 kg per boom toegeneem, met 'n ooreenstemmende afname in vruggrootte met pieke by telling 72.

Delta toon vereenigbaarheid met US 812, HRS 802 en FF-6 onderstamme vir hierdie proef perseel. Die entlas tussen die onderstam en bostam was glad met geen tekens van onverenigbaarheid (geen groeipunte by entlas) nie. Daar was 'n uitstekende toename in oes produksie op die bome gewees hierdie seisoen, met FF6 wat met meer as 25% verbeter het. Vruggrootte het effens afgeneem by al drie onderstam kombinasies en het by telling 105/125, 88 en 72 gepiek.

Evaluasies tot op datum toon aan dat hierdie onderstamme waardevol kan wees vir die sitrus produsente, meer spesifiek US 812, waar hoë pH vlakke en kalkagtige gronde voorkom. US 812 was vir sy hoë

verdraagsaamheid teen Phytophthora, citrus aalwurms en tristeza, asook beter weerstand vir hoër pH en kalkagtige gronde geselekteer.

Summary

Visual evaluations of the Midnight: US 812 bud-union, indicated that the union was in good condition and the combination compatible. US 812 is a hybrid rootstock cross between a Sunki mandarin and Beneke trifoliolate (Sunki 812). The tree size of this combination was described as medium (similar to Carrizo tree size and growth rate), although US 812 rootstock as a tree on its own is aggressive and develops into a fairly large tree. Yield production this season was excellent and increased by with 37 kg per tree, with a corresponding decrease in fruit size, peaking at count 72.

Delta seems to be compatible with US 812, HRS 802 and FF-6 rootstocks at this trial site. The bud-union between the rootstock and scion was fairly smooth without any signs of incompatibility (no growth tips at bud-union). There was a good increase on crop production this season with FF-6 producing more than 25% additional fruit on the trees. Fruit size on all three rootstock combinations decreased slightly and peaked at count 105/125, 88 and 72.

Evaluations to date show that these rootstocks could be of value to citrus producers, particularly US 812, should high pH levels and calcareous soils be a problem. US 812 was selected for its high tolerance to Phytophthora, citrus nematodes and tristeza, as well as better tolerance of high pH and calcareous soils.

Objectives

- To investigate the performance of Midnight and Delta Valencias on new, imported rootstocks on replant soils.
- To improve production, internal quality, rind colour and fruit size count distributions.

Materials and methods

Seeds of HRS 802, US 812 (Sunki x Beneke), HRS 809 and FF-6 were imported and propagated in 1996 by Esselen Nursery, a CIS accredited nursery in the Malelane region of Mpumalanga.

Delta Valencia was budded onto the following three newly imported rootstock hybrids at Esselen nursery in 1997: HRS 802 (Siamese pummelo x Gotha Road trifoliolate), US 812 (Sunki mandarin x Beneke trifoliolate) and FF-6 (Sunki x MTO trifoliolate orange). Midnight Valencia was budded onto US 812 (Sunki x Beneke). The trees were planted at Esselen Nursery in March 1999.

Table 5.4.11.1. Number of trees per rootstock in the Delta and Midnight Valencia trial at Malelane.

Selection	Rootstock	No. of trees
Midnight	US 812	4
Delta	US 812	4
Delta	HRS 802	4
Delta	FF-6	5

Results and discussion

Midnight Valencia

This is the first trial of Midnights planted on US 812 (Sunki x Beneke) in South Africa. There was another significant increase in crop production for the 2014 season from 72.0 to 108.9 kg/tree in comparison with the previous season (Table 5.4.11.4), and the 8 year mean was 63.0 kg/tree (Table 5.4.11.4). Internally fruit quality was good with Brix (10.2) and juice levels slightly lower compared to 2013 (61.7%) with 57.2% (Table 5.4.11.2).

The acid content decreased even more this season (1.0%) compared to the 2013 season (1.4%), improving the Brix: acid ratio closer to 10 (9.9). The crop production between Midnight and Delta on US 812 (Sunki x Beneke) was similar for 2014 with an average of 107 kg/tree. The Midnight trees were slightly smaller compared to the Delta trees, but the difference was not significant enough in this case and the difference in yields cannot be attributed to vigour and tree size. Fruit size peaked at count 72 (34%), followed by count 56 (23%) and count 88 (22.8%), producing a smaller fruit size on the trees for this season, due to the higher crop (excellent fruit size distribution).

Delta Valencia

Delta on FF6 produced the best juice content (55.8%), as well as the highest Brix:acid ratio of 11:1, followed by US 812 (Sunki x Beneke) with the highest Brix level (11.1) and acid of 1.2%, and 802 the juice (52.0%) content (Table 5.1.11.2). There was a delay on the external colour development on all three rootstock combinations (T2/3-4/5). Fruit size decreased on all three rootstocks and peaked at count 105/125), followed by count 88 and count 72. There was a slight drop in crop production this season on US 812 (Sunki x Beneke) (from 114 to 105.5 kg/tree) and 803 (from 112.0 to 107.3 kg/tree), but FF-6 improved with more than 25% from 106.1 kg per tree to 134.8 kg per tree.

Conclusions

The crop production remained impressive on all the combinations this season due to excellent fruitset, less theft (better security) as well as trees in peak production at this trial site.

Midnight on US 812 (Sunki x Beneke) performed well, producing an excellent crop compared to the previous season (from 72 to 109 kg per tree), with slightly smaller fruit size due to the better yield, and good internal qualities. The acid levels (1.0%) decreased this season compared to 2013 (1.4%). The Brix: acid ratio was higher due to the lower acid level, slightly delaying the maturity of the selection with external colour at T1-3.

Delta was evaluated on three hybrid rootstocks, US 812 (Sunki x Beneke), HRS 802 and FF-6. The more important combination of the above mentioned was US 812 (Sunki x Beneke). US 812 (Sunki x Beneke) was selected for replant conditions, very specific high pH and calcareous soils. Delta performed well on all three combinations this season, producing fruit with good internal quality and slightly smaller fruit size. There was a good crop increase on the FF-6 combination, producing well over 25% more fruit on the trees. The other two combinations decreased in crop production. FF-6 internally performed well with the best internal quality (Brix: acid ratio 10.8). The fruit size peaked at counts 105/125 for all three selections for this trial site.

The FF-6 rootstock was also selected for high pH soils, future trials in the Musina and Kakamas areas must be conducted to determine these characteristics.

Table 5.4.11.2. Internal fruit quality of Midnight and Delta Valencias on different rootstocks at Esselen Nursery (Malelane) on 6 August 2014.

Selection	Root-stock	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Delta	FF-6	55.8	9.2	0.85	10.82	0.3	T2-4
Delta	802	52.0	9.6	0.92	10.43	0.1	T3-5
Delta	US 812	54.4	11.1	1.20	9.25	0.2	T3-5
Midnight	US 812	57.2	10.2	1.03	9.90	0.4	T1-3

Table 5.4.11.3. Fruit size distribution at Esselen nursery during the 2014 season.

Cultivar	Rootstock	Count	% Fruit	Cultivar	Rootstock	Count	% Fruit
Midnight	US 812	48	5.21	Delta	802	48	0.28
Midnight	US 812	56	23.02	Delta	802	56	6.82
Midnight	US 812	72	34.03	Delta	802	72	22.04
Midnight	US 812	88	22.87	Delta	802	88	30.44
Midnight	US 812	105/125	13.56	Delta	802	105/125	35.88
Midnight	US 812	144	1.30	Delta	802	144	4.55
Cultivar	Rootstock	Count	% Fruit	Cultivar	Rootstock	Count	% Fruit
Delta	US 812	48	2.19	Delta	FF-6	48	0.39
Delta	US 812	56	12.25	Delta	FF-6	56	4.98
Delta	US 812	72	21.75	Delta	FF-6	72	18.46
Delta	US 812	88	23.50	Delta	FF-6	88	26.58
Delta	US 812	105/125	32.04	Delta	FF-6	105/125	42.42
Delta	US 812	144	8.27	Delta	FF-6	144	7.18

Table 5.4.11.4. Production per tree of Midnight and Delta Valencia trees on different rootstocks at Esselen Nursery (Malelane) during the 2014 season.

Cultivar	Root-stock	Kg/tree (2007)	Kg/tree (2008)	Kg/tree (2009)	Kg/tree* (2010)	Kg/tree* (2011)	Kg/tree* (2012)	Kg/tree (2013)	Kg/tree (2014)	8 Year Total	8 Year Mean
Midnight	US 812	98.7	50.4	51.7	40.2	34.1	47.6	72.0	108.9	503.6	63.0
Delta	US 812	120.5	66.4	78.6	58.7	42.7	56.5	114.0	105.4	642.8	80.4
Delta	HRS 802	120.6	102.3	102.4	81.4	74.7	51.1	112.0	107.3	751.8	94.0
Delta	FF-6	134.1	97.2	80.7	79.8	61.4	33.3	106.1	134.8	727.4	90.9

*Note: Heat wave experienced during fruit set period (fruit also stolen from this specific section)

5.4.12 PROGRESS REPORT: Evaluation of various Navel selections on different rootstocks in the Burgersfort and Marble Hall area

Project 590 A by J. Joubert and S.D. Maziya (CRI)

Opsomming

Daar was geen oes op die bome by die Moosrivier proef perseel hierdie seisoen gewees om te oes en evalueer nie, a.g.v. 'n verwoestende hael storm in die omgewing. Evaluasies asl voortgaan in die 2014 seisoen.

Glen Ora Late op die Burgersfort was vir die tweede keer tydens die 2014 season ge-evalueer. Glen Ora Late het die beste sap op C35 (50.5%) en beste Brix vlakke op Swingle (12.2) ontwikkel. Die hoogste suur was op X639 (1.22%) en die laagste op Terrabella (0.86%) gewees, en daarom ook die hoogste Brix: suur verhouding van 13.8. Die eksterne kleur ontwikkeling het tussen T1 en T4 gepiek en Swingle het die eksterne kleur ontwikkeling met twee tot drie weke vertraag. Glen Ora Laat in kombinasie met Carrizo onderstam het die beste oes op die bome geproduseer (93.1 kg per boom). Vruggrootte by vyf van die sewe onderstamme het by telling 56 gepiek, gevolg deur telling 72, baie gunstig vir Valencia uitvoer potensiaal.

Summary

There was no crop on the trees at the Moosrivier trial site this season to harvest and evaluate due to a severe hail storm in the area, evaluations will continue 2014.

Glen Ora Late navel at the Burgersfort site was evaluated for the second time during the 2014 season. Glen Ora Late developed the best juice on C35 (50.5%) and best Brix levels on Swingle (12.2). The highest acid was on X639 (1.22%) and the lowest on Terrabella (0.86%), therefore the highest Brix: acid ratio of 13.8. The external colour development peaked between T1 and T4 and Swingle delaying the external colour two to three weeks. Glen Ora Late in combination with Carrizo rootstock produced the best crop on the trees (93.1 kg per tree). Fruit size peaked at count 56 on five of the seven rootstocks, followed by count 72, very favourable for Valencia export potential.

Objectives

- Evaluate and assess the horticultural performance and capability of various new Navel selections on different rootstocks.
- Determine the superior rootstock combinations for these new selections.
- Be able to make credible commercial recommendations.

Materials and methods

Trees were planted at Moosrivier in 2005, and the additional trees established at BBE Boerdery late in 2004. Trees were evaluated visually to determine production per tree, trueness to type and compatibility with scion and each tree was harvested with the sizer to determine production per tree as well as fruit size distribution per tree. Samples were taken and internal quality tested and analysed. Fruit colour was also evaluated and analysed.

Table 5.4.12.1. List of cultivar and rootstock combinations in the Navel trial at Moosrivier in the Marble Hall area.

Selection	Rootstock	Qty Trees
Fukumoto	C35	4
Fukumoto	CC	5
Fukumoto	MxT	4
Fukumoto	SC	5
Fukumoto	Terrabella	3
Fukumoto	X639	5
Newhall	C35	5
Newhall	CC	3
Newhall	MxT	2
Newhall	SC	5
Newhall	Terrabella	2
Newhall	X639	5
Glen Ora Late	C35	5
Glen Ora Late	CC	3
Glen Ora Late	MxT	2
Glen Ora Late	SC	5
Glen Ora Late	Terrabella	4
Glen Ora Late	X639	5
Cal Lane Late	C35	5
Cal Lane Late	CC	5
Cal Lane Late	MxT	4
Cal Lane Late	SC	5
Cal Lane Late	Terrabella	4
Cal Lane Late	X639	5

Table 5.4.12.2. List of cultivar and rootstock combinations in the Navel trial at BBE Boerdery in the Burgersfort area.

Selection	Rootstock	Qty Trees
Glen Ora Late	C35	2
Glen Ora Late	CC	9
Glen Ora Late	KC	4
Glen Ora Late	MxT	11
Glen Ora Late	SC	14
Glen Ora Late	Terrabella	7
Glen Ora Late	X639	4

Results and discussion

This was the second evaluations for the trial site at BBE Boerdery (Burgersfort); the trial site was included recently to represent the production information of Glen Ora Late navel in the cooler production areas.

Moosrivier had no crop on the trees this year, due to a severe hail storm in the area, removing most of the fruit on the trees. Fruit that was left on the trees after the hail dropped later because of the damage. Evaluations at this trial site will continue next season.

Glen Ora Late Navel

Glen Ora on C35 rootstock developed the best juice (50.5%) levels, followed by Terrabella (49.4%) and MxT (49.0%). X639 was next with a high Brix of 12.3, excellent quality for this navel. Terrabella was below 1.0% acid, although Brix: acid ratio was excellent above 13. The external colour matured from T1 to T4 with Swingle delaying the colour by 2 to 3 weeks (Table 5.4.12.3).

Fruit size peaked at count 56 on five of the seven rootstocks, except for Koethen and X639 at count 72, exactly the same fruit size distribution compared to 2013. Excellent fruit size for good quality export navel with medium to large fruit on the trees (Table 5.4.12.4).

This year Carrizo was the optimum rootstock choice for maximum crop production on the trees, bearing 93.1 kg fruit per tree. Terrabella was second with 85.6 kg per tree, followed by MxT and X639 with 82.4 and 72.4 kg per tree.

Conclusions

The late maturing Glen Ora Late navel was evaluated at Burgersfort trial sites. The internal quality was good and C35 performed well with high Brix, acid and Brix: acid ratios. Fruit size peaked at counts 57 and 72, excellent for navel production. Yield production on the trees at Burgersfort was lower this season on five of the seven combinations, except for Carrizo and Terrabella (89.4 to 93.1 kg per tree 49.1 to 85.6 kg per tree).

Table 5.4.12.3. Internal fruit quality data for Glen Ora Navel on different rootstocks at BBE Boerdery, Burgersfort on the 1st of July 2014.

Root-Stock	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
C35	50.5	11.9	0.95	12.53	0.0	T1-2
CC	48.7	12.1	1.02	11.86	0.0	T1-2
KC	47.8	12.1	1.04	11.63	0.0	T1-2
MxT	49.0	12.0	1.16	10.34	0.0	T1-3
SC	46.3	12.2	1.05	11.62	0.0	T1-4
TB	49.4	11.9	0.86	13.84	0.5	T1-3
X639	46.8	12.3	1.22	10.08	0.0	T1-3

Table 5.4.12.4. Fruit size distribution of Glen Ora Navel per rootstock at BBE Boerdery, Burgersfort during the 2014 season.

Rootstock	Count	% Fruit	Rootstock	Count	% Fruit
C35	48	6.02	SC	48	7.81
C35	56	31.63	SC	56	28.88
C35	72	30.87	SC	72	28.75
C35	88	19.13	SC	88	19.71
C35	105/125	11.45	SC	105/125	13.64
C35	144	0.90	SC	144	1.21
Rootstock	Count	% Fruit	Rootstock	Count	% Fruit
CC	48	7.18	TB	48	17.06
CC	56	30.89	TB	56	41.33
CC	72	29.07	TB	72	25.22
CC	88	18.08	TB	88	11.44
CC	105/125	13.15	TB	105/125	4.08
CC	144	1.63	TB	144	0.87
Rootstock	Count	% Fruit	Rootstock	Count	% Fruit
KC	48	5.28	X639	48	1.48
KC	56	27.76	X639	56	15.86
KC	72	29.24	X639	72	29.74
KC	88	21.71	X639	88	25.59
KC	105/125	14.77	X639	105/125	24.99
KC	144	1.25	X639	144	2.35
Rootstock	Count	% Fruit			
MxT	48	8.31			
MxT	56	30.85			
MxT	72	30.36			
MxT	88	18.22			
MxT	105/125	11.32			

MxT	144	0.94
-----	-----	------

Table 5.4.12.5. Production per tree of Glen Ora Navel on different rootstocks at BBE Boerdery, Burgersfort during the 2014 season.

Rootstock	Kg/tree	Kg/tree
	2013	2014
C35	75.2	58.3
CC	89.4	93.1
KC	92.7	60.9
MxT	101.4	82.4
SC	91.2	54.8
TB	49.1	85.6
X639	91.4	72.4

5.4.13 PROGRESS REPORT: Evaluation of various Valencia selections on different rootstocks in the Komatipoort area

Project 590 B by J. Joubert and S.D. Maziya (CRI)

Opsomming

Delta op Koethen citrange het 'n Brix : suur verhouding bo 15 opgelewer, die hoogste vir die totale proef. Carrizo, Koethen en Swingle het lae sap en suur vlakke teen die oestyd ontwikkel en het nie aan die uitvoer standaard voldoen nie. Delta se vruggrootte op al die onderstam kombinasies het by telling 56 gepiek, waarna telling 72 gevolg het (goeie toename). Die oes produksie op al die kombinasies het afgeneem behalwe by MxT met 83 kg/boom. Geen onverenigbaarheids tekens was by enige van die kombinasies sigbaar nie.

McClellan SL het ook op al die onderstamme aan die uitvoer standaard voldoen, die interne kwaliteit het heelwat verbeter op die vrugte. MxT het die hoogste sap inhoud (57%) gelewer met Koethen citrange die hoogste Brix: suur verhouding van 13:1. Vruggrootte was uitstekend vir Valencias gewees, en die tellings het gepiek tussen 56, 72 en 88. McClellan SL op vyf van die sewe bostam: onderstam kombinasie se produksie het afgeneem, behalwe vir X639 met die hoogste opbrens (182.2 kg/boom), gevolg deur Swingle met 61.1 kg per boom.

Midnight op C35 en X639 het van die hoogste sap vlakke vir hierdie proef geproduseer (bo 54.5%). Die hoogste suurvlak was op X639 gewees, wat dan ook die laagste Brix: suur vlak van 8.4 tot gevolg gehad het. Hierdie bostam: onderstam kombinasie kan later in die seisoen geoes word om verhouding te verbeter, hou wel die eksterne kleur ontwikkeling (T1) goed dop. Midnight was die enigste seleksie met saad tellings in die vrugte (reeks van 0.1 tot 0.6 sade per vrug). Die vruggroottes het tussen telling 56 en 72 gepiek op al sewe onderstamme. Oes produksie op twee van die kombinasies (Carrizo en MxT) was beter, met C35 die hoogste (69.4 kg per boom), maar die res het ongeveer 'n derde van hulle oes afgestaan. Warm temperature tydens blomset in hierdie produksie area het 'n groot invloed op die prestasie.

Portsgate het hierdie seisoen goed gevaar. Die interne kwaliteit van die vrugte op al die onderstam kombinasies het aan die uitvoer standaard voldoen. Hierdie seleksie word vroeg ryp wanneer die hoë Brix: suur verhouding in ag geneem word (oorryp). Al die vrugte op die bome was saadloos gewees. Vruggroottes het gepiek by telling 56 vir al die seleksies behalwe Carrizo met telling 72 (toename in grootte vir 2014). Die hoogste oes obrenge in kombinasie met Portsgate was op MxT geproduseer (65.7 kg/boom), gevolg deur Swingle (62.7 kg/boom). Nie een van die kombinasies met Portsgate het 'n beter oes hierdie seisoen geproduseer nie.

Summary

Delta on Koethen citrange developed a Brix: acid ratio above 15, the highest ratio for this trial. Carrizo, Koethen and Swingle developed low juice and acid levels by the time of harvest and did not comply with the export requirements. Delta peaked on all the rootstock combinations with fruit size at count 56, followed by count 72 (good increase). Yield production decreased on all rootstock selections except for MxT with 83 kg/tree). There were no incompatibility problems on the rootstock combinations visible.

McClean SL on all the rootstock combinations complied with the minimum export standards due to a considerable improvement in the internal quality of the fruit. MxT developed the best juice level (57%) with Koethen Citrange the highest Brix: acid ratio of 13:1. Fruit size for Valencias was excellent, fruit counts peaked between count 56, 72 and 88. McClean SL on five of the seven scion: rootstock combinations decreased their crop, except for X639 with the highest yield (71.4 kg/tree), followed by Swingle with 61.1 kg per tree.

Midnight on C35 and X639 developed the best juice levels for this trial; above 54.5%. Midnight on X639 produced the highest acid level, causing the lowest Brix: acid ratio of 8.4. Harvest this scion: rootstock combination later to improve ratio, but keep external colour development (T1) in mind. Midnight was the only selection to develop seeds in the fruit for this trial (ranging from 0.1 to 0.6 seeds per fruit). The fruit sizes peaked between count 56 and 72 on all seven rootstocks. Yield production on two of the combinations (Carrizo and MxT) was better, with C35 the best (69.4 kg per tree), the rest dropped nearly a third in production. High temperature during the flower set period in this production area played a role.

Portsgate performed well this season. The internal quality of the fruit on all the rootstocks complied with the export requirements. This selection was early maturing when taking the high Brix: acid ratios into consideration (over matured). All the fruit on the trees were seedless. Fruit size peaked at count 56 for all selections except Carrizo with count 72 (increase in size for 2014). The highest yield production in combination with Portsgate was produced on MxT (65.7 kg/tree), followed by Swingle (62.7 kg/tree). Not one of the seven scion: rootstock combinations produced a better crop this season.

Objectives

- Evaluate and assess the horticultural performance and capability of various new Valencia selections on different rootstocks.
- Determine the superior rootstock combinations for these new selections.
- Be able to make credible commercial recommendations.

Materials and methods

Five trees of each cultivar rootstock combination were planted in 2002. These were evaluated visually to determine production per tree, trueness to type and compatibility with scion. Each tree was harvested and the fruit sized to determine production per tree as well as fruit size distribution per tree. Samples were taken for internal quality testing and analysis. Fruit colour was also evaluated.

Table 5.4.13.1. List of cultivar and rootstock combinations in the Valencia trial at Golden Frontiers Citrus, Hectorspruit in the Komatipoort area.

Selection	Rootstock	Qty Trees
Delta (Control)	C35	5
Delta (Control)	CC	5
Delta (Control)	KC	5
Delta (Control)	MxT	5
Delta (Control)	SC	5
Delta (Control)	Terrabella	5
Delta (Control)	X639	5
McClean SL	C35	5
McClean SL	CC	5
McClean SL	KC	5
McClean SL	MxT	5
McClean SL	SC	5
McClean SL	Terrabella	5
McClean SL	X639	5
Midnight	C35	5
Midnight	CC	5
Midnight	KC	4
Midnight	MxT	5
Midnight	SC	5
Midnight	Terrabella	5

Midknight	X639	4
Portsgate	C35	5
Portsgate	CC	5
Portsgate	KC	5
Portsgate	MxT	5
Portsgate	SC	5
Portsgate	Terrabella	5
Portsgate	X639	5

Results and discussion

Delta Valencia

Delta on C35 produced the best Brix (13.1) and highest acid level (1.12%). The best Brix: acid ratio of 15.1 was developed by Koethen, followed by X639 with the highest juice level (58.4%) and Swingle the lowest acid (0.77). The external colour on all the selections was T1. Carrizo, Koethen and Swingle did not comply with the export (Table 5.4.13.2). Fruit size on C35, Swingle, Terrabella and X639 rootstocks peaked at count 56, followed by Carrizo, Koethen and MxT at count 72 (Table 5.4.13.3). Six of the seven scion: rootstock combinations decreased crop production this season except for MxT which increased from 73.8 to 82.8 kg per tree. The lowest production was on Koethen with 25.7 kg per tree. The second highest production was in combination with Swingle cropping 60.8 kg of fruit per tree (Table 5.4.13.4).

McClellan SL Valencia

MxT in combination with McClellan SL developed the highest juice content of 57.3%, in combination with 0.88% acid resulting in a Brix: acid ratio of 12. The lowest acid levels for this trial were produced on Koethen and X639 (0.86 & 0.88%). C35, similar to 2013, outperformed the other scion: rootstock combinations with the best Brix (13.1) and Koethen with the Brix: acid ratio (13.03). The external colour on all the selections was T1 and McClellan SL on all rootstocks was completely seedless (Table 5.4.13.2). All the rootstocks peaked at count 56 except for C35 with count 88 (smaller fruit) (Table 5.4.12.3). There was a decrease on all seven scion: rootstock combinations in crop production this season. Highest yield production this season was on X639 (71.4 kg/tree), followed by Swingle with 61.1 kg per tree. The lowest crop was on Koethen with 19.4 kg per tree (Table 5.4.13.4).

Midknight Valencia

C35 in combination with Midknight peaked with juice and Brix levels of 55.3% and 12.4, followed by CC developing the best Brix: acid ratio of 11.5 and the lowest acid level of 1.1%. The external colour on all the selections was T1 (Table 5.4.13.2). C35, Carrizo, Swingle and Terrabella peaked at count 56, followed by Koethen, MxT and X639 at count 72 (Table 5.4.13.3). Most of the seven scion: rootstock combinations decreased crop production this season except for Carrizo (increase from 28.6 to 42.6 kg per tree) and MxT (increase from 20.3 kg per tree to 46.7 kg per tree). The highest 7 year total was on C35 with 406.6 kg and 67.8 kg per tree 7 year mean (Table 5.4.13.4).

Portsgate Valencia

Portsgate on MxT produced the best juice (55.8%) levels, followed by Carrizo with the best Brix level (13.1) and MxT the highest Brix: acid ratio above 16 (indicating over mature fruit) as well as lowest acid level of 0.77% (early maturing selection). The external colour on all the selections was T1 (Table 5.4.13.2). Six of the seven rootstocks peaked at count 56, except for Carrizo with count 72 (Table 5.4.13.3). All seven scion: rootstock combinations decreased crop production this season. MxT outperformed the other combinations with 65.7 kg per tree. The second best was Swingle with 62.7 kg per tree, although 26.5 kg lower than in 2013. The lowest crop was on Koethen with 26.1 kg per tree (Table 5.4.13.4).

Conclusions

Delta on all the rootstocks produced seedless fruit with very good internal quality. The highest Brix: acid ratio was in combination with Koethen, peaking at 14.6:1 for this trial. The fruit size production for Delta peaked between count 56 and 72 on all the rootstock combinations. Production was lower this season; most of the combinations decreased their yield on the trees except for MxT (82.8 kg per tree).

McClellan seedless on MxT produced the highest juice levels (57.3%) for this trial. C35 outperformed the other rootstocks with the highest Brix level (13.1) for the third time this season. The best Brix: acid ratio average was 13.1 on Koethen, very good for export quality and meets the requirements. Six of the rootstocks peaked at count 56, except for C35 at count 72. McClellan seedless produces on average a good fruit size for a Valencia selection. There was a decrease in crop production on all the rootstocks combinations this, X639

bore a crop of 71.4 kg per tree (182.2 kg/tree 2013), followed by Swingle with 61.1 kg per tree. McClean seedless in combination with Koethen and Carrizo performed poorly this season, producing a yield of only 19.4 and 21.6 kg per tree.

Midnight's performance was better this season and the internal quality improved. The juice and Brix levels increased; C35 with 55.3% juice and 12.4 Brix. Acids on X639 were high (1.42%), but still acceptable and below the export maximum; lowest was on Carrizo (1.1%). Midnight always tends to have larger fruit and this season was the norm, with four of the rootstocks peaking at count 56 (C35, CC, SC, TB). Koethen, MxT and X639 peaked at count 72. Crop set on the trees were lower and only two of the seven combinations increased, Carrizo with 42.6 kg per tree and MxT 46.7 kg per tree.

Portsgate performed well internally with juice levels on six of the combinations above 52%, except for Carrizo with 50.8%. The highest juice was on MxT (55.8%), Brix on Carrizo (13.1) and Brix: acid ratio on MxT (16.6%), also developing the lowest acid (0.77%). Portsgate produced bigger fruit on the trees this season, peaking at count 56 on six rootstocks, and count 72 on Carrizo. MxT in combination with Portsgate was the only rootstock that produced a better crop this year (65.7 kg per tree). The lowest yield was on Koethen with 26.1 kg per tree.

Table 5.4.13.2. Internal fruit quality data for Valencias on different rootstocks at Golden Frontiers Citrus, Hectorspruit during the 2014 season.

Selection	Root-stock	Date harvested	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Midnight	C35	28/07/2014	55.3	12.4	1.25	9.9	0.3	T1
Midnight	CC	28/07/2014	51.7	12.4	1.08	11.5	0.1	T1
Midnight	KC	28/07/2014	53.2	12.2	1.31	9.3	0.5	T1
Midnight	MxT	28/07/2014	53.3	12.0	1.28	9.4	0.3	T1
Midnight	SC	28/07/2014	53.5	12.4	1.27	9.8	0.2	T1
Midnight	TB	28/07/2014	54.2	12.5	1.14	11.0	0.6	T1
Midnight	X639	28/07/2014	54.7	11.9	1.42	8.4	0.2	T1
Delta	C35	02/09/2014	53.7	13.1	1.12	11.7	0.0	T1
Delta	CC	02/09/2014	51.2	12.8	0.88	14.5	0.0	T1
Delta	KC	02/09/2014	51.0	11.8	0.78	15.1	0.0	T1
Delta	MxT	02/09/2014	56.3	11.4	0.85	13.4	0.0	T1
Delta	SC	02/09/2014	56.5	11.3	0.77	14.7	0.0	T1
Delta	TB	02/09/2014	55.5	12.2	0.88	13.9	0.0	T1
Delta	X639	02/09/2014	58.4	12.5	0.96	13.0	0.0	T1
McClean SL	C35	02/09/2014	52.8	13.1	1.24	10.6	0.0	T1
McClean SL	CC	02/09/2014	53.0	12.2	0.97	12.6	0.0	T1
McClean SL	KC	02/09/2014	54.1	11.4	0.86	13.3	0.0	T1
McClean SL	MxT	02/09/2014	57.3	11.7	0.94	12.4	0.0	T1
McClean SL	SC	02/09/2014	55.8	11.6	0.89	13.0	0.0	T1
McClean SL	TB	02/09/2014	55.5	11.7	0.99	11.8	0.0	T1
McClean SL	X639	02/09/2014	56.6	10.6	0.88	12.0	0.0	T1
Portsgate	C35	02/09/2014	52.4	12.9	1.14	11.3	0.0	T1
Portsgate	CC	02/09/2014	50.8	13.1	0.85	15.4	0.0	T1
Portsgate	KC	02/09/2014	52.7	13.0	0.80	16.3	0.0	T1
Portsgate	MxT	02/09/2014	55.8	12.8	0.77	16.6	0.0	T1
Portsgate	SC	02/09/2014	54.5	12.2	0.81	15.1	0.0	T1
Portsgate	TB	02/09/2014	53.4	12.3	0.81	15.2	0.0	T1
Portsgate	X639	02/09/2014	52.3	12.2	0.87	14.0	0.0	T1

Table 5.4.13.3. Fruit size distribution per rootstock at Golden Frontiers Citrus, Hectorspruit during the 2014 season.

Cultivar	Rootstock	Count	% Fruit	Cultivar	Rootstock	Count	% Fruit
Delta	C35	48	10.62	Midnight	C35	48.0	19.3
Delta	C35	56	35.10	Midnight	C35	56.0	41.9
Delta	C35	72	25.64	Midnight	C35	72.0	23.3
Delta	C35	88	13.63	Midnight	C35	88.0	9.8
Delta	C35	105/125	13.16	Midnight	C35	105/125	4.5
Delta	C35	144	1.85	Midnight	C35	144.0	1.2
Cultivar	Rootstock	Count	% Fruit	Cultivar	Rootstock	Count	% Fruit
Delta	CC	48	5.86	Midnight	CC	48.0	8.7
Delta	CC	56	26.17	Midnight	CC	56.0	35.1
Delta	CC	72	26.30	Midnight	CC	72.0	29.6
Delta	CC	88	19.40	Midnight	CC	88.0	15.7
Delta	CC	105/125	17.97	Midnight	CC	105/125	8.3
Delta	CC	144	4.30	Midnight	CC	144.0	2.5
Cultivar	Rootstock	Count	% Fruit	Cultivar	Rootstock	Count	% Fruit
Delta	KC	48	9.70	Midnight	KC	48.0	8.9
Delta	KC	56	27.51	Midnight	KC	56.0	24.8
Delta	KC	72	29.45	Midnight	KC	72.0	30.1
Delta	KC	88	18.34	Midnight	KC	88.0	21.9
Delta	KC	105/125	14.11	Midnight	KC	105/125	12.4
Delta	KC	144	0.88	Midnight	KC	144.0	1.9
Cultivar	Rootstock	Count	% Fruit	Cultivar	Rootstock	Count	% Fruit
Delta	MxT	48	4.14	Midnight	MxT	48.0	5.1
Delta	MxT	56	29.70	Midnight	MxT	56.0	26.6
Delta	MxT	72	36.51	Midnight	MxT	72.0	32.6
Delta	MxT	88	20.22	Midnight	MxT	88.0	19.5
Delta	MxT	105/125	8.17	Midnight	MxT	105/125	13.4
Delta	MxT	144	1.25	Midnight	MxT	144.0	2.8
Cultivar	Rootstock	Count	% Fruit	Cultivar	Rootstock	Count	% Fruit
Delta	SC	48	12.02	Midnight	SC	48.0	17.2
Delta	SC	56	50.08	Midnight	SC	56.0	38.3
Delta	SC	72	24.96	Midnight	SC	72.0	29.0
Delta	SC	88	9.87	Midnight	SC	88.0	11.1
Delta	SC	105/125	1.66	Midnight	SC	105/125	3.8
Delta	SC	144	1.41	Midnight	SC	144.0	0.6
Cultivar	Rootstock	Count	% Fruit	Cultivar	Rootstock	Count	% Fruit
Delta	TB	48	9.35	Midnight	TB	48.0	14.4
Delta	TB	56	41.59	Midnight	TB	56.0	29.3
Delta	TB	72	25.35	Midnight	TB	72.0	27.2
Delta	TB	88	11.68	Midnight	TB	88.0	15.7
Delta	TB	105/125	9.23	Midnight	TB	105/125	9.6
Delta	TB	144	2.80	Midnight	TB	144.0	3.8
Cultivar	Rootstock	Count	% Fruit	Cultivar	Rootstock	Count	% Fruit

Delta	X639	48	6.80	Midnight	X639	48.0	6.9
Delta	X639	56	43.94	Midnight	X639	56.0	26.2
Delta	X639	72	28.87	Midnight	X639	72.0	26.9
Delta	X639	88	13.99	Midnight	X639	88.0	18.9
Delta	X639	105/125	6.01	Midnight	X639	105/125	17.9
Delta	X639	144	0.39	Midnight	X639	144.0	3.3
Cultivar	Rootstock	Count	% Fruit	Cultivar	Rootstock	Count	% Fruit
McClean SL	C35	48	7.80	Portsgate	C35	48.0	12.6
McClean SL	C35	56	23.22	Portsgate	C35	56.0	35.2
McClean SL	C35	72	24.75	Portsgate	C35	72.0	22.0
McClean SL	C35	88	35.93	Portsgate	C35	88.0	15.1
McClean SL	C35	105/125	7.97	Portsgate	C35	105/125	13.1
McClean SL	C35	144	0.34	Portsgate	C35	144.0	2.0
Cultivar	Rootstock	Count	% Fruit	Cultivar	Rootstock	Count	% Fruit
McClean SL	CC	48	20.14	Portsgate	CC	48.0	6.3
McClean SL	CC	56	41.22	Portsgate	CC	56.0	32.5
McClean SL	CC	72	22.25	Portsgate	CC	72.0	32.7
McClean SL	CC	88	9.60	Portsgate	CC	88.0	15.7
McClean SL	CC	105/125	5.85	Portsgate	CC	105/125	10.3
McClean SL	CC	144	0.94	Portsgate	CC	144.0	2.5
Cultivar	Rootstock	Count	% Fruit	Cultivar	Rootstock	Count	% Fruit
McClean SL	KC	48	21.75	Portsgate	KC	48.0	9.9
McClean SL	KC	56	41.91	Portsgate	KC	56.0	36.0
McClean SL	KC	72	24.14	Portsgate	KC	72.0	24.3
McClean SL	KC	88	7.43	Portsgate	KC	88.0	15.1
McClean SL	KC	105/125	3.98	Portsgate	KC	105/125	10.5
McClean SL	KC	144	0.80	Portsgate	KC	144.0	4.3
Cultivar	Rootstock	Count	% Fruit	Cultivar	Rootstock	Count	% Fruit
McClean SL	MxT	48	15.44	Portsgate	MxT	48.0	17.4
McClean SL	MxT	56	35.46	Portsgate	MxT	56.0	50.1
McClean SL	MxT	72	29.18	Portsgate	MxT	72.0	4.0
McClean SL	MxT	88	12.65	Portsgate	MxT	88.0	18.2
McClean SL	MxT	105/125	6.37	Portsgate	MxT	105/125	8.5
McClean SL	MxT	144	0.90	Portsgate	MxT	144.0	1.9
Cultivar	Rootstock	Count	% Fruit	Cultivar	Rootstock	Count	% Fruit
McClean SL	SC	48	20.59	Portsgate	SC	48.0	5.1
McClean SL	SC	56	54.47	Portsgate	SC	56.0	40.8
McClean SL	SC	72	18.59	Portsgate	SC	72.0	38.5
McClean SL	SC	88	4.69	Portsgate	SC	88.0	11.4
McClean SL	SC	105/125	1.65	Portsgate	SC	105/125	2.8
McClean SL	SC	144	0.00	Portsgate	SC	144.0	1.5
Cultivar	Rootstock	Count	% Fruit	Cultivar	Rootstock	Count	% Fruit
McClean SL	TB	48	17.55	Portsgate	TB	48.0	22.6
McClean SL	TB	56	52.66	Portsgate	TB	56.0	44.7
McClean SL	TB	72	18.71	Portsgate	TB	72.0	19.0
McClean SL	TB	88	6.62	Portsgate	TB	88.0	6.7

McClellan SL	TB	105/125	4.03	Portsgate	TB	105/125	5.0
McClellan SL	TB	144	0.43	Portsgate	TB	144.0	1.9
Cultivar	Rootstock	Count	% Fruit	Cultivar	Rootstock	Count	% Fruit
McClellan SL	X639	48	17.64	Portsgate	X639	48.0	12.6
McClellan SL	X639	56	41.18	Portsgate	X639	56.0	42.0
McClellan SL	X639	72	26.19	Portsgate	X639	72.0	26.3
McClellan SL	X639	88	10.67	Portsgate	X639	88.0	11.2
McClellan SL	X639	105/125	4.14	Portsgate	X639	105/125	6.4
McClellan SL	X639	144	0.18	Portsgate	X639	144.0	1.5

Table 5.4.13.4. Production per tree of Valencia selections on different rootstocks at Golden Frontiers Citrus, Hectorspruit during the 2014 season.

Cultivar	Rootstock	Kg/tree							7 Year Total	7 Year Mean
		2008	2009	2010	2011	2012	2013	2014		
Delta	C35	60.1	45.6	51.7	37.6	50.5	53.8	40.2	339.5	48.5
Delta	CC	33.4	25.6	21.5	38.4	58.3	60.2	33.0	270.4	38.6
Delta	KC	38.5	26.2	10.4	32.6	49.0	30.6	25.7	213.0	30.4
Delta	MxT	39.4	40.0	6.4	13.8	48.4	73.8	82.8	304.6	43.5
Delta	SC	73.9	76.8	65.7	41.7	70.1	140.4	60.8	529.4	75.6
Delta	TB	34.9	30.5	32.3	31.0	44.4	87.8	40.6	301.5	43.1
Delta	X639	35.1	51.8	44.7	51.1	69.1	107.7	48.7	408.2	58.3
McClellan SL	C35	81.0	64.3	32.5	46.3	46.1	69.0	26.0	365.2	52.2
McClellan SL	CC	29.2	54.6	22.6	27.5	32.4	83.7	21.6	271.6	38.8
McClellan SL	KC	24.7	58.3	21.6	38.8	46.7	90.3	19.4	299.8	42.8
McClellan SL	MxT	19.4	26.6	10.1	4.5	16.1	71.8	49.0	197.5	28.2
McClellan SL	SC	35.7	98.1	49.7	47.5	74.0	170.8	61.1	536.9	76.7
McClellan SL	TB	46.9	39.4	49.6	37.2	55.5	57.6	36.0	322.2	46.0
McClellan SL	X639	29.3	96.2	49.9	80.6	49.2	182.2	71.4	558.8	79.8
Midnight	C35	33.9	83.4	54.8	39.0	49.7	76.4	69.4	406.6	58.1
Midnight	CC	20.9	32.1	3.6	12.3	41.8	28.6	42.6	181.9	26.0
Midnight	KC	11.3	29.7	15.2	16.9	44.0	81.4	41.7	240.2	34.3
Midnight	MxT	8.2	27.0	1.2	8.6	30.3	20.3	46.7	142.3	20.3
Midnight	SC	13.8	57.7	8.1	30.7	53.0	95.9	53.3	312.5	44.6
Midnight	TB	19.6	53.7	14.3	15.5	36.6	56.0	52.7	248.4	35.5
Midnight	X639	5.2	17.9	3.3	13.0	36.3	68.7	34.5	178.9	25.6
Portsgate	C35	33.6	53.5	22.3	26.2	68.4	60.4	39.3	303.7	43.4
Portsgate	CC	26.6	31.3	9.6	4.2	50.9	35.0	32.7	190.3	27.2
Portsgate	KC	19.0	31.3	4.9	11.8	58.2	47.4	26.1	198.7	28.4
Portsgate	MxT	30.5	19.4	3.3	7.5	25.1	76.0	65.7	227.5	32.5
Portsgate	SC	55.2	44.0	19.9	15.3	106.0	89.2	62.7	392.3	56.0
Portsgate	TB	48.0	40.8	30.4	31.8	55.8	77.7	40.0	324.5	46.4
Portsgate	X639	35.6	73.0	37.1	73.1	91.7	96.9	53.4	460.8	65.8

5.4.14 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region (Western Cape)
Project 57D by S. Meeding (CRI)

Opsomming

Hierdie spesifieke Satsuma proef word bestempel as een van die beste Satsuma proewe in die land. Die proef se ligging is goed geskik vir Satsuma produksie. Die bome is ongeveer 10 jaar oud (geplant in 2006).

Die bome is volwasse met groot boom volume. Imamura het ontwikkel in die grootste boom in vergelyking met die ander seleksies. Owari en Dobashi Beni was meer kompakte bome wat kleiner was in boomgrootte. , Dobashi Beni en Imamura het 'n alternatiewe drag patroon met Imamura wat die groter probleem het. Die skil tekstuur van die buite vrugte was redelik grof teenoor die gladder skil van die binne vrugte. Die orde van rypwording was as volg: Ueno, Owari, Aoshima op Carrizo Citrange met Ohtsu, Imamura en Aoshima op Swingle Citrange wat die seisoen eindig.

Pluk periodes vir Satsumas sal strek van 2-3 weke aangesien vrugte se sure vinnig daal en die skil powerig raak. Vrugte se kleur is laat teenoor die interne kwaliteit en ontgroening sal moet gedoen word.

Summary

This specific Satsuma trial was mentioned to be one of the best Satsuma trials in the country. The trial location is in a good area well suited for Satsuma production. The trees are 10 years old (planted 2006). The trees are mature with large tree canopies. Imamura developed into the biggest tree compared to the other selections. Owari and Dobashi Beni were more compact trees with smaller tree size. Two of the selections, Dobashi Beni and Imamura had alternative bearing patterns with Imamura having the more serious problem. The rind texture on the outside fruit was fairly rough compared to the smoother texture of the inside fruit. The order of maturity was as follows; Ueno, Owari, Aoshima on Carrizo Citrange, Ohtsu, Imamura, and Aoshima on Swingle Citrange ended the season.

Picking periods for Satsumas should be limited to 2-3 weeks to ensure good internal quality and avoid puffiness. Satsuma selections need degreening after harvest as the internal quality is ahead of the colour development.

Objectives

- To select Satsuma cultivars with improved and consistent productivity, fruit size, rind colour, and internal fruit quality (Brix, acidity and ratio).
- To extend the harvest period (both earlier and later maturity).
- To describe the characteristics of new Satsuma cultivars and determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Late Satsuma selections from the Paarl region of the Western Cape. The following selections were evaluated: Aoshima, Dobashi Beni, Imamura, Ohtsu, Owari and Ueno.

Table 5.4.14.1 List of Satsuma selections evaluated at Lustigaan (Paarl) during 2014.

Selection	Rootstock	Planted
Aoshima	Carrizo/Swingle	2006
Dobashi Beni	Carrizo	2006
Imamura	Carrizo	2006
Ohtsu	Carrizo	2006
Owari	Carrizo	2006
Ueno	Carrizo	2006

Results and discussion

Aoshima

Aoshima was planted on two rootstocks at this trial site, Carrizo- and Swingle Citrange. The difference between the two rootstocks was clearly visible from a distance (tree size and vigor). Both rootstocks developed a good fruit size on the trees; Aoshima on Carrizo peaked at count 1x and on Swingle at count 1x. Aoshima in combination with Swingle matured 4 weeks later compared to Carrizo and the rind texture was rough. Aoshima in combination with Carrizo was third to mature and on Swingle ended the Satsuma season, maturing after Imamura, the latest selection to mature.

Dobashi Beni

There was no crop on the trees this season.

Imamura

Imamura had a serious alternative bearing problem, cropping a good to excellent yield in an on year to less than 5% or in some cases no fruit in an off year. In 2013 there were no fruit on the trees and in 2014 there was a good crop. Imamura developed the largest tree size of all the selections evaluated. The rind texture of the fruit was smooth and the fruit developed a deep orange internal colour. Imamura was completely seedless compared to the other selections.

Ohtsu

Ohtsu was the fourth Satsuma selections to mature at the Lustigaan trial site. Ohtsu had a good fruit size this season and peaked from count 1-1xx. The internal quality was good with one of the highest juice percentages (58.2%), only Imamura outperformed Ohtsu with 59.1%.

Owari

Owari is one of the older Satsuma selections and was used as control for the trial site. Owari was only evaluated and tested once; by the second evaluation it was already over mature and excessive fruit drop occurred. Dobashi – Beni and Owari's fruit tends to get puffy more quickly. Owari developed smaller fruit size and peaked with count 1. The fruit has a pronounced neck. Owari had the best colour development closer to peak maturity (T3).

Ueno

Ueno was the first selection to mature this season. There tree canopy was large with a heavy crop on the trees. Fruit size development remained above average ranging from count 1x-1xx, despite the heavy crop on the trees. Colour development was slightly delayed. Ueno had a low seed count with only 0.08 seeds per fruit.

When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. A ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

Conclusion

Aoshima on Carizzo Citrange, Ohtsu and Ueno had the best fruit size (count 1xx) with Imamaru and Ohtsu having the best juice percentages 59.1% and 58.2%. Imamura was the only selection that was completely seedless of all the selections in the trial site. Owari had the smallest fruit size with a count 1. Dobashi Beni was the only selection with no yield on the tree. Imamura developed the smoothest rind texture and Aoshima on Swingle the roughest rind texture, possibly due to the rootstock choice.

Table 5.4.14.2 Internal fruit quality data for Satsuma selections in the Paarl region (Lustigaan) of the Western Cape during the 2014 season.

Date	Selection	Rootstock	Count	Juice %	Brix°	Acid %	Ratio	Avg. Seed	Colour
2014/04/29	Aoshima	CC	1xx	51.0%	11.1	0.85	13.0	0.2	T5
2014/05/15	Aoshima	CC	1xx	52.3%	11.2	0.90	12.5	0.4	T4
2014/04/29	Aoshima	SC	1x	48.0%	10.4	1.10	9.4	0.3	T6
2014/05/15	Aoshima	SC	1x	51.9%	10.8	1.01	10.7	0.1	T5
2014/04/29	Imamura	CC	1x	43.0%	10.9	1.07	10.2	0	T6
2014/05/15	Imamura	CC	1x	59.1%	10.7	0.95	11.3	0	T4
2014/04/29	Ohtsu	CC	1	46.0%	10.8	0.91	11.9	0.4	T6
2014/05/15	Ohtsu	CC	1xx	58.2%	11.0	0.84	13.1	0.3	T3
2014/04/29	Owari	CC	1	49.0%	11.8	1.12	10.5	0.1	T3
2014/04/29	Ueno	CC	1xx	42.0%	11.5	0.79	14.6	0.1	T4
2014/05/15	Ueno	CC	1x	53.6%	11.5	0.88	13.1	0.1	T5

5.4.15 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Clementine mandarins in a cold production region (Western Cape)

Project 1000D by S. Meeding (CRI)

Opsomming

Die seisoen het begin met Clemenpons, gevolg deur Nules, Marisol en het geeindig met Bonanules. Bonanules en Clemenpons is oorgewerk in 'n kommersiële Marisol boord. In 2014 is dieselfde probleme met kleurontwikkeling ondervind as in die 2013 seisoen. Nules het voor Marisol ryp geword wat ook die geval was in die 2013 seisoen.

Summary

The Clementine season started with Clemenpons, followed by Nules, Marisol and ended with Bonanules. Bonanules and Clemenpons were top worked in a commercial Marisol orchard. In 2014 the same problems with colour development were seen as in the 2013 season. Nules matured before Marisol, which was also noted in the 2013 season.

Objectives

- To select Clementine cultivars with improved and consistent productivity, fruit size, rind colour, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the cultivar characteristics of new Clementine cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Clementine selections from the Wellington region of the Western Cape; the planting age is unknown. The following varieties were evaluated: Bonanules, Clemenpons, Marisol and Nules.

Table 5.4.15.1. List of Clementine selections evaluated at Bonathaba (Wellington) during 2014.

Selection	Rootstock	Planted
Bonanules	Troyer	Unknown
Clemenpons	Carrizo	Unknown
Marisol	Troyer	Unknown
Nules	Troyer	Unknown

Results and discussion

Bonanules

Bonanules was third to mature of the 4 selections. The fruit was a bit smaller than in the 2013 season and peaked with fruit size at count 1 in the 2014 season. The colour development of Bonanules was delayed with a colour plate T5-6 at full maturity. Some fruit was granulated, the same as in the 2013 season. The trees are very close to a windbreak that may compromise fruit quality and tree performance.

Clemenpons

Clemenpons was the first Clementine selection for this trial to reach maturity with one of the higher Brix values (10.9). The selection was seedless with very poor colour development; colour plate T6-7 at full maturity. Granulation also occurred.

Marisol and Nules

Marisol and Nules are two older selections used as controls for Clementine trials. Both selections were seedless with Nules having the highest Brix content (12.0).

Conclusion

All the selections evaluated were seedless at this trial with a much delayed colour development. Degreening practices will be essential after harvesting to ensure optimal colour development. Bonanules and Marisol had the highest juice percentages with Bonanules 56% and Marisol 55.9%. Bonanules and Clemenpons were

planted next to a windbreak, affecting the overall quality of the fruit on the trees. Bonanules and Clemenpons both showed signs of granulation.

Table 5.4.15.2. Internal fruit quality data for Clementine selections in the Wellington region (Bonathaba) of the Western Cape during the 2014 season.

Date	Site	Selection	Root-stock	Count	Juice %	Brix°	Acid %	Ratio	Colour	Avg. Seed
2014/04/04	Wellington	Bonanules	TC	1	56.0%	10.5	1.12	9.4	T5-6	0
2014/04/22	Wellington	Bonanules	TC	2	44.0%	10.2	0.86	11.8	T5-6	0
2014/04/22	Wellington	Clemenpons	TC	1	49.9%	10.9	0.73	14.9	T6-7	0
2014/04/04	Wellington	Marisol	TC	1	51.0%	9.3	1.15	8.1	T4-5	0
2014/04/22	Wellington	Marisol	TC	2	55.9%	10.0	0.89	11.2	T5-6	0
2014/04/22	Wellington	Nules	TC	2	49.1%	12.0	0.86	13.9	T6-7	0

5.4.16 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (East Cape Midlands)

Project 997A by S. Meeding and Z. Zondi (CRI)

Opsomming

Al 5 van die UCR 5 seleksies het 'n ongelooflike goeie drag in die 2014 seisoen gehad. Dit was een van 2 proef persele wat as goeie voorbeeld kan dien om die seleksies se volle potensiaal te wys. Die bome van al hierdie seleksies is groter in die Oos-Kaap as in beide die Wes- en Noord-Kaap. Van die seleksies het tekens gewys dat dit wel gevoelig is vir koue skade. Tango en Gold Nugget was meer verdraagsaam teen die koue as Tahoe-, Shasta- en Yosemite Gold. Met Tahoe-, Shasta- en Yosemite Gold was daar baie nuwe groei wat terug gesterf het van die koue. Daar moet ook in gedagte gehou word dat die Oos-Kaap se Middellande (Cookhouse) van die koudste sitrus produserende areas in die land is.

Summary

All 5 of the UCR 5 selections had exceptional yields in the 2014 season. This trial site is one of 2 sites in the country that can be used as a reference to indicate the full potential of these 5 varieties. The trees of these 5 varieties are larger in size in the Eastern Cape compared to both the Western and Northern Cape. Some of the selections showed signs of cold damage. Tango and Gold Nugget were more tolerant to the cold than Tahoe, Shasta and Yosemite Gold which had new growth that died back because of the severe cold. Bear in mind that the Eastern Cape Midlands (Cookhouse) is one of the coldest citrus production areas in the country.

Objectives

- To select mandarin hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new mandarin hybrid cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections from the Cookhouse and Fort Beaufort region of the East Cape Midlands. A range of new mandarin hybrids have been added to this area and should be bearing fruit in the 2014 season. The following varieties were evaluated: Gold Nugget, Nadorcott, Shasta Gold, Tahoe Gold, Tango and Yosemite Gold.

When the ratio between sugar and acid is 12:1, the fruit is considered to be at peak maturity for mandarin hybrids. This ratio is raised as a result of the high sugar levels associated with the new selections. A ratio of 11:1 is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

Table 5.4.16.1. List of mandarin hybrid selections in the Cookhouse (J&B) region of the East Cape Midlands during the 2014 season.

Selection	Rootstock	Topwork
Gold Nugget	CC	2010
Nadorcott	CC	2010
Shasta Gold	CC	2010
Tahoe Gold	CC	2010
Tango	CC	2010
Yosemite Gold	CC	2010

Results and discussion

Gold Nugget

Gold Nugget started the season with a high Brix° and high Acid (%), a good sign that the variety can hang longer on the trees and indicate a longer shelf life. The selection sets a good number of fruit every year with good fruit size (in 2014 season count 1xx). The tree has a very upright growth habit and tends to set a lot of fruit on the long bearing shoots that often break due to the heavy weight.

Nadorcott

Nadorcott is used as a control for the mandarin varieties, and more specifically Tango. It was second to mature and developed the second smallest fruit size (count 1) for this trial site. The selection was seedless with good colour development (T1) at peak maturity.

Shasta Gold

Shasta Gold had a good fruit size (count 1xx) and the fruit had a smooth rind with very good internal colour (deep orange). The Brix° and Acid content remained low, even lower than in the other production areas. The selection had the highest seed count with 0.7 seed per fruit.

Tahoe Gold

Tahoe Gold had a different maturity period to the other production areas. Tahoe Gold was supposed to mature in middle July, the same time as Tango and Nadorcott. At this trial site Tahoe Gold ended the season and came in after Yosemite Gold and Shasta Gold that matures middle August to early September. The selection had the smallest fruit size with count 3-1xx.

Tango

Tango was completely seedless this season and produced the same fruit size as Nadorcott (count 1). The selection has a smooth rind texture with a natural shine and very good juice percentage (57%). The external colour of Tango was delayed compared to Nadorcott (T3-4 compared to T1), but internally Nadorcott had a higher Brix: acid ratio of 13.5 with the second evaluation.

Yosemite Gold

Yosemite Gold struggled with the same problem as Shasta Gold. Both selections had a delayed external colour development that was not a problem in the Western Cape or the rest of the Eastern Cape (Gamtoos and Sundays River Valley). The selection had large fruit of count 1xx. The seed count was the second highest for this trial with 0.5 seeds per fruit, as well as the second highest juice percentages (57.7%).

Conclusion

Yosemite Gold, Shasta Gold and Gold Nugget developed the best fruit size with count 1xx. Tahoe Gold, Yosemite Gold and Shasta Gold showed signs of severe cold damage with young shoots dying back. All 5 selections were top worked in a commercial Nadorcott orchard, explaining the number of seeds present in the fruit. In trial sites where the selections were top worked in an orchard where cross pollination wasn't a problem, all the selections were completely seedless. Tahoe Gold and Yosemite Gold produced the higher juice percentages (close to 60%).

Table 5.4.16.2. Internal fruit quality data for Mandarin hybrid selections from the Cookhouse (J&B) region of the East Cape Midlands during the 2014 season.

Date	Selection	Count	Juice %	Brix°	Acid %	Ratio	Colour	Avg. Seed
2014/06/24	Gold Nugget	1xx	50.3%	11.8	1.29	11.8	T5-6	0
2014/06/24	Nadorcott	1	57.9%	10.6	0.97	11.3	T4	0
2014/07/15	Nadorcott	1	52.4%	11.1	0.82	13.5	T1	0
2014/06/24	Shasta Gold	1xx	56.9%	9.9	1.47	6.7	T5	0.7
2014/07/15	Shasta Gold	1xx	52.0%	9.0	1.13	8.7	T4	0.6
2014/08/15	Shasta Gold	1xx	52.5%	9.6	0.82	11.3	T3	0
2014/06/24	Tahoe Gold	1x	61.7%	10.1	1.15	8.8	T5-6	0.2
2014/07/15	Tahoe Gold	3	48.0%	10.3	1.20	8.6	T1	0.4
2014/08/15	Tahoe Gold	1xx	58.9%	11.4	1.12	10.2	T2-3	0
2014/06/24	Tango	1	55.8%	10.0	1.05	9.5	T3-4	0
2014/07/15	Tango	1	57.0%	10.8	0.85	12.7	T3-4	0
2014/08/15	Tango	1x	50.9%	11.1	0.87	12.8	T2-3	0
2014/06/24	Yosemite Gold	1xx	57.7%	10.2	1.43	7.1	T5-6	0.3
2014/07/15	Yosemite Gold	1xx	51.9%	9.8	0.98	10.0	T4	0
2014/08/15	Yosemite Gold	1xx	52.1%	11.0	1.06	10.4	T4-5	0.5

5.4.17 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Sundays River Valley)

Project 997B by S. Meeding and Z. Zondi (CRI)

Opsomming

In die 2014 seisoen was die UCR 5 seleksies se tweede drag. Die Yosemite- en Tahoe Gold bome het hulle geherplant om spasio te maak vir 'n nuwe pakhuis. Van die bome het nie die verskuiwing oorleef nie. Gold Nugget en Clemcott het die beste interne kwaliteit en smaak gehad, met 'n hoë Brix en Suur verhouding wat aanleiding kan gee tot langer pluk periods, asook beter rak leeftyd.

Summary

In the 2014 season the UCR 5 trees produced their second crop at this trial site. The Yosemite- and Tahoe Gold trees were relocated due to the packhouse being extended. Some of the trees did not survive the transplant. Gold Nugget and Clemcott had the best internal quality and good flavour with a high Brix° and high acid ratio, indicating the longer picking periods as well as extended shelf life of these two selections.

Objectives

- To select mandarin hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new mandarin hybrid cultivars and to determine the climatic suitability of these cultivars in cold production regions

Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections from the Sundays River Valley. A range of new mandarin hybrids had been added to this area and were bearing fruit in the 2014 season. The following varieties were evaluated: Clemcott, Gold Nugget, Shasta Gold, Tahoe Gold, Tango and Yosemite Gold.

Table 5.4.17.1. List of Mandarin hybrid selections evaluated in the Sundays River Valley region during the 2014 season.

Selection	Rootstock	Planted
Furr (Clemcott)	Carrizo	2004

Selection	Rootstock	Topwork
Gold Nugget	Carrizo	2011
Shasta Gold	Carrizo	2011
Tahoe Gold	Carrizo	2011
Yosemite Gold	Carrizo	2011
Tango	Carrizo	2011

Results and discussion

Furr (Clemcott)

Clemcott is a Murcott x Clementine selection and was used as control for the mandarin trial site. Clemcott tends to have large fruit size ranging from count 1 to 1xxx. The seed count was high this season with up to 5.5 seeds per fruit. The selection developed a high Brix° content and good Brix: acid ratio, as well as fairly high acid levels that indicate a later maturing fruit with delayed external colour development. The fruit will have a good shelf life. The fruit is very firm with a smooth rind and developed a very good internal and external colour. The tree size is large (aggressive growth pattern) and cropped a good yield consistently every production year.

Gold Nugget

Gold Nugget cropped a good yield this year for the tree age and size. The fruit rind was coarse, but will get smoother as the tree matures. There was light ribbing present on some of the fruit. The tree has a very upright growth habit with long aggressive shoots that bear no fruit. Good pruning practices must be implemented to remove these shoots and stop the active vegetative growth. Some of the fruit (less than 5%) started splitting on the stylar-end. There were signs of light sunburn present on some of the fruit.

Shasta Gold

Shasta Gold produced a good yield and fruit size for the young tree age. The fruit developed some ribbing but will become smoother as the trees mature. The Shasta fruit shape was fairly flat with a fruit size of count 1xxx. Shasta Gold has a very good external colour development (deep orange). Colour development of the fruit was at colour plate (T1) long before peak maturity.

Tahoe Gold

Tahoe Gold developed a smaller tree volume compared to the other 5 selections evaluated. Trees were transplanted at 3 years old and some did not survive. Tahoe Gold tends to have larger fruit in the first 2 years of production. Thereafter the fruit stabilize to a smaller but still very good size, due to the heavier crop. Tahoe Gold produced the highest juice percentages (over 60%) at this trial site in comparison to the remaining UCR selections. Colour development was good and peaked at colour plate T1 with maturity. Tahoe Gold has a short picking window; fruit becomes puffy soon after peak maturity.

Tango

Tango was the first selection to mature with very good fruit size (count 1xx). The fruit was mostly seedless. Tango produced a flat fruit shape with excellent internal and external colour, deep orange. Fruit peels easily and the albedo has a pinkish colour.

Yosemite Gold

This selection had no fruit this season.

Conclusion

Tango was the first selection to mature, followed by Clemcott, Tahoe Gold, Gold Nugget and the season ended off with Shasta Gold. Yosemite Gold had no fruit on the trees. Clemcott developed a Brix: acid ratio of 11.3:1 with an acid content remaining above 1%. Tahoe Gold and Clemcott have the highest juice percentages (over 60%). Gold Nugget reached the highest Brix: acid ratio of 17.7:1 as well as Brix content of 14.3. Clemcott developed the highest seed count of 5.5 seeds per fruit. Gold Nugget and Shasta Gold were completely seedless. All the selections were fully coloured at peak maturity.

Table 5.4.17.2. Internal fruit quality data for Mandarin hybrid selections from various regions of the Sundays River Valley during the 2014 season.

Date	Selection	Count	Juice %	Brix°	Acid %	Ratio	Colour	Avg. Seed
2014/06/10	Clemcott	1xxx	61.9%	12.5	1.11	11.3	T2-3	4.8
2014/06/25	Clemcott	1	56.1%	12.8	1.16	11.0	T1	5.5
2014/06/10	Gold Nugget	1x	56.4%	10.3	1.14	9.0	T6	0
2014/07/09	Gold Nugget	1xxx	54.4%	11.5	1.00	11.5	T3-4	0
2014/07/24	Gold Nugget	1xxx	51.6%	12.2	0.96	12.7	T1	0
2014/08/14	Gold Nugget	1xx	43.7%	13.5	0.93	14.5	T1	0
2014/08/27	Gold Nugget	1xxx	50.4%	14.3	0.81	17.7	T1	0
2015/06/25	Gold Nugget	1xxx	54.3%	10.5	1.01	10.4	T4	0
2014/06/10	Shasta Gold	1x	58.2%	9.9	1.44	6.9	T6	0
2014/06/25	Shasta Gold	1xxx	56.5%	10.4	1.26	8.3	T3	0
2014/07/09	Shasta Gold	1xxx	56.3%	11.0	1.24	8.9	T2-3	0
2014/07/24	Shasta Gold	1xxx	52.3%	10.5	1.10	9.5	T1	0
2014/08/14	Shasta Gold	1xxx	54.6%	11.9	1.04	11.4	T1	0
2014/08/27	Shasta Gold	1xxx	54.6%	11.5	0.99	11.6	T1	0
2014/06/10	Tahoe Gold	1	63.2%	10.2	10.00	10.2	T6	0
2014/06/25	Tahoe Gold	1xxx	64.9%	10.6	0.95	11.2	T2-3	0
2014/07/24	Tahoe Gold	1xxx	59.2%	12.5	1.10	11.4	T1	0.2
2014/06/10	Tango	1x	52.5%	10.5	0.95	11.1	T4-5	0
2014/06/25	Tango	1xx	51.1%	10.9	0.91	12.0	T2-3	0
2014/07/09	Tango	1xx	45.3%	11.8	0.88	13.4	T2	0
2014/07/24	Tango	1xx	57.6%	12.3	0.85	14.5	T1	0
2014/08/14	Tango	1xx	55.3%	10.6	1.31	8.1	T1	0.4
2014/08/27	Tango	2	50.6%	13.3	0.86	17.2	T1	0

5.4.18 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Gamtoos River Valley)

Project 997C by S. Meeding and Z. Zondi (CRI)

Opsomming

Die 2 proef persele wat bespreek word verskil in ligging. Daar is 'n hoof perseël in die Hankey area en 'n hoof perseël in die Patensie area. In beide proef persele is al die UCR 5 seleksies se interne en eksterne eienskappe ooreenstemmend. Al wat verskil het was die periodes van rypwording a.g.v. klimaats verskille. In Patensie het die seisoen begin met Tango, gevolg deur Tahoe Gold, Nadorcott, Gold Nugget, Shasta Gold en die seisoen het geeëindig met Yosemite Gold. In Hankey het die seisoen begin met Nadorcott, gevolg deur Gold Nugget, Tahoe Gold, Yosemite Gold en die seisoen het geeëindig met Shasta Gold. Die aantal sade per vrug in die Hankey proef perseël is aansienlik hoër as in die Patensie proef perseël. Die rede hiervoor kan wees dat in Hankey is die UCR 5 seleksies in 'n boord geplant saam met Empress mandaryn wat 'n sterk kruisbestuier is. Die 2014 seisoen was die eerste drag vir die Hankey perseël se bome, die dra takke moes gestut word om te keer dat dit nie breek nie.

Summary

The 2 trial sites in the discussion are in different locations. There is a main trial site in the Hankey area, as well as in the Patensie area. In both sites all the UCR 5 selections have the same internal and external features. The only thing that differs is the maturing periods due to temperature differences. In Patensie the season started with Tango followed by Tahoe Gold, Nadorcott, Gold Nugget, Shasta Gold and the season ended with Yosemite Gold. In Hankey the season started with Nadorcott followed by Gold Nugget, Tahoe Gold, Yosemite Gold and the season ended with Shasta Gold. The number of seeds per fruit in Hankey is higher compared to the Patensie trial site. The UCR 5 selections are planted in an orchard with Empress mandarin which is a strong cross pollinator. The 2014 season was the first year for the trees to bear fruit, but the tree branches had to be supported to prevent any damage to the trees.

Objectives

- To select mandarin hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new mandarin hybrid cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections from the Gamtoos River Valley (Hankey and Patensie). A range of new mandarin hybrids had been added to this area and were bearing fruit in the 2014 season. The following varieties were evaluated: Shasta-, Tahoe- and Yosemite Gold, as well as Gold Nugget and Tango.

Table 5.4.18.1 List of experimental mandarin hybrid selections evaluated in the Patensie (L.Ferreira) region of the Gamtoos River Valley during the 2014 season.

Selection	Rootstock	Topwork
Gold Nugget	Carrizo	2011
Shasta Gold	Carrizo	2011
Tahoe Gold	Carrizo	2011
Tango	Carrizo	2011
Yosemite Gold	Carrizo	2011

Table 5.4.18.2 List of experimental mandarin hybrid selections evaluated in the Hankey region (M. Kleyn) of the Gamtoos River Valley during the 2014 season.

Selection	Rootstock	Planted
Gold Nugget	Carrizo	2011
Shasta Gold	Carrizo	2011
Tahoe Gold	Carrizo	2011
Tango	Carrizo	2011
Yosemite Gold	Carrizo	2011

Table 5.4.18.3 List of experimental mandarin hybrid selections evaluated in the Hankey region (Spitzbak) of the Gamtoos River Valley during the 2014 season.

Selection	Rootstock	Planted
Gold Nugget	Carrizo	2011
Shasta Gold	Carrizo	2011
Tahoe Gold	Carrizo	2011
Tango	Carrizo	2011
Yosemite Gold	Carrizo	2011

Results and discussion

Gold Nugget

Gold Nugget performed similarly to the other production areas. The trees have a very upright growth habit, the fruit higher up in the trees tends to be coarser than fruit lower down. Some fruit is showing signs of ribbing. Trees produced a fair to good yield although tree manipulation (pruning) is necessary to prevent aggressive growth patterns. Gold Nugget was fourth to mature (end of July to end of August) after Tango, Tahoe Gold and Nadorcott. The selection was completely seedless with high Brix° and good colour development.

Shasta Gold

Shasta Gold came in second last of all the selections, before Yosemite Gold that ended off the mandarin season. The fruit was completely seedless with excellent colour development, colour plate T1 6 weeks before peak maturity. Trees with a heavier crop had smaller fruit with a smoother rind texture compared to the trees with a lighter crop. There was ribbing on most of the fruit. Shasta Gold developed a good juice percentage, well over 50% this season.

Tahoe Gold

The Tahoe Gold developed into a smaller, more compact tree. The trees bore a heavy crop and the grower had to support the branches to prevent any damage to bearing branches. With the heavy crop on the trees, fruit size was still good ranging from count 1x – 1xxx. External colour development was very good this season (deep orange).

Tango

Tango, similar to Gold Nugget developed a very upright growth habit (V-shape tree). Tango sets a heavy crop on the trees with good fruit size ranging from count 1 -1xx. The rind texture was smooth with a deep orange external colour (natural wax shine).

Yosemite Gold

Yosemite Gold grows vigorously and developed the largest tree volume of the UCR 5 varieties. There was a problem with fruit set on the trees this season and alternative bearing patterns must be investigated for on and off-production years. The fruit is very firm with a good fruit size peaking at count 1xxx. The fruit rind was thin and peels easily as well as cleanly. The seed count on this selection was higher with up to 0.9 seeds per fruit.

Conclusion

The colour development of all 5 selections was very good and peaked at colour plate T1 when mature. At the Patensie trial site only Tahoe Gold had seeds of all the varieties evaluated, peaking with 0.2 seeds per fruit. In the Hankey trial sites all the selections had seeds due to the strong cross pollination (Empress Mandarin). All 5 selections had good internal quality with Gold Nugget being the best, developing good flavour with high Brix in both areas.

Table 5.4.18.4. Internal fruit quality data for experimental mandarin hybrid selections from the Patensie (L. Ferreira) region of the Gamtoos River Valley region during the 2014 season.

Date	Selection	Count	Juice %	Brix°	Acid %	Ratio	Colour	Avg. Seed
2014/06/11	Gold Nugget	1xx	57.2%	10.2	1.11	9.2	T5-6	0
2014/06/24	Gold Nugget	1xx	52.9%	11.3	1.04	10.9	T5	0
2014/07/07	Gold Nugget	1xx	48.3%	11.6	1.00	11.6	T3-4	0
2014/07/23	Gold Nugget	1xx	48.1%	11.4	0.98	11.6	T2-3	0
2014/08/13	Gold Nugget	1xxx	48.8%	12.5	0.88	14.2	T1	0
2014/08/26	Gold Nugget	1xxx	46.1%	12.5	0.80	15.6	T1	0
2014/09/08	Gold Nugget	1xx	50.5%	13.3	0.75	17.7	T1	0
2014/09/17	Gold Nugget	1xx	47.1%	13.3	0.83	16.0	T1	0
2014/06/11	Nadorcott	1	56.8%	10.7	1.03	10.4	T4-5	0
2014/06/24	Nadorcott	1x	51.8%	9.6	1.17	9.6	T3-4	0
2014/07/23	Nadorcott	1xx	49.5%	11.0	0.86	12.8	T1	0
2014/06/11	Shasta Gold	1xxx	58.4%	10.1	1.32	7.7	T5	0
2014/06/24	Shasta Gold	1xxx	53.4%	10.5	1.27	8.3	T3-4	0
2014/07/07	Shasta Gold	1xxx	53.6%	10.4	1.20	8.9	T1-2	0
2014/07/23	Shasta Gold	1xxx	55.7%	10.4	1.12	9.3	T1	0
2014/08/13	Shasta Gold	1xx	56.6%	11.5	1.06	10.8	T1	0
2014/08/26	Shasta Gold	1xxx	47.1%	11.6	0.92	12.6	T1	0
2014/09/08	Shasta Gold	1xx	49.5%	11.6	0.85	13.6	T1	0
2014/09/17	Shasta Gold	1xx	54.8%	12.3	0.91	13.5	T1	0
2014/06/11	Tahoe Gold	1x	60.4%	10.0	1.01	9.9	T6	0
2014/06/24	Tahoe Gold	1xx	61.8%	10.2	1.01	10.2	T3	0
2014/07/07	Tahoe Gold	1xxx	55.1%	10.6	0.72	14.7	T1-2	0
2014/07/23	Tahoe Gold	1xxx	52.3%	10.2	0.76	13.4	1	0
2014/08/13	Tahoe Gold	1xx	52.7%	10.7	0.76	14.1	T1	0
2014/08/26	Tahoe Gold	1x	53.0%	10.6	0.66	16.1	T1	0.2
2014/09/08	Tahoe Gold	1x	48.6%	11.4	0.60	19.0	T1	0.2
2014/06/11	Tango	1	50.2%	9.3	0.75	12.4	T4-5	0
2014/06/24	Tango	1xx	53.4%	9.0	0.79	12.5	T3	0
2014/07/07	Tango	1xx	52.0%	10.4	0.73	14.3	T1-2	0
2014/07/23	Tango	1xx	46.6%	10.7	0.70	15.3	T1	0
2014/06/11	Yosemite Gold	1xxx	54.0%	8.8	1.10	8.0	T6-7	0
2014/06/24	Yosemite Gold	1xxx	50.70%	9.7	1.01	9.6	T4-5	0
2014/07/07	Yosemite Gold	1xxx	53.4%	10.2	1.00	10.2	T3-4	0
2014/07/23	Yosemite Gold	1xxx	48.6%	10.3	0.98	10.5	T1-2	0
2014/08/13	Yosemite Gold	1xxx	51.8%	11.2	0.84	13.3	T1	0
2014/08/26	Yosemite Gold	1xxx	50.8%	10.7	0.86	12.4	T1	0
2014/09/08	Yosemite Gold	1xxx	50.8%	10.2	0.76	13.4	T1	0
2014/09/17	Yosemite Gold	1xxx	53.6%	11.6	0.81	14.3	T1	0.2

Table 5.4.18.5 Internal fruit quality data for experimental mandarin hybrid selections from the Hankey region (M. Kleyn) of the Gamtoos River Valley region during the 2014 season.

Date	Selection	Count	Juice %	Brix°	Acid %	Ratio	Colour	Avg. Seed
2014/06/11	Gold Nugget	1x	52.0%	9.9	1.30	7.6	T6	0
2014/06/24	Gold Nugget	1xx	52.1%	10.1	1.11	9.1	T5	0.2
2014/07/07	Gold Nugget	1xx	46.2%	10.9	1.01	7.1	T3-4	0.3
2014/07/23	Gold Nugget	1x	47.6%	11.6	1.00	11.6	T1-2	0
2014/08/13	Gold Nugget	1x	45.5%	12.2	0.88	13.9	T1	0.2
2014/08/26	Gold Nugget	1x	42.6%	12.6	0.80	15.8	T1	0
2014/09/08	Gold Nugget	1x	43.9%	12.7	0.72	17.6	T1	0
2014/06/11	Nadorcott	1	57.2%	9.8	1.21	8.1	T5	0
2014/06/24	Nadorcott	1	55.7%	10.9	1.13	9.6	T3-4	0.5
2014/06/11	Shasta Gold	1xxx	59.4%	10.4	1.78	5.8	T5-6	0.8
2014/06/24	Shasta Gold	1xxx	55.4%	10.0	1.76	5.7	T5	0.3
2014/07/07	Shasta Gold	1xxx	54.1%	10.5	1.50	8.9	T4-5	0
2014/07/23	Shasta Gold	1xx	57.1%	10.2	1.46	7.0	T4-5	0.3
2014/08/13	Shasta Gold	1xx	54.3%	11.2	1.16	9.7	T1	0
2014/08/26	Shasta Gold	1xx	57.7%	10.2	1.27	8.0	T1	0.4
2014/09/08	Shasta Gold	1xxx	55.4%	12.2	0.99	12.3	T1	0.5
2014/06/11	Tahoe Gold	1	60.5%	10.8	1.39	7.8	T6	0.8
2014/06/24	Tahoe Gold	1xxx	60.0%	10.7	1.29	8.3	T5	0.8
2014/07/07	Tahoe Gold	1xx	57.6%	10.8	1.20	6.9	T5-6	0.5
2014/07/23	Tahoe Gold	1xx	57.2%	10.8	1.05	10.3	T4	0.5
2014/08/13	Tahoe Gold	1x	58.2%	10.7	1.00	10.7	T2-3	0.7
2014/08/26	Tahoe Gold	1xx	53.3%	10.3	0.88	11.7	T1	0.8
2014/09/08	Tahoe Gold	1xx	51.6%	11.8	0.79	14.9	T1	0.6
2014/06/11	Yosemite Gold	1xxx	56.0%	9.4	1.75	5.4	T7	1.1
2014/06/24	Yosemite Gold	1xxx	54.8%	9.5	1.46	6.5	T6-7	0.9
2014/07/07	Yosemite Gold	1xxx	53.6%	9.6	1.40	6.9	T6-7	0.3
2014/07/23	Yosemite Gold	1xxx	55.7%	10.5	1.30	8.1	T4-5	0
2014/08/13	Yosemite Gold	1xxx	53.0%	11.5	1.08	10.6	T1	0.5
2014/08/26	Yosemite Gold	1xxx	53.2%	10.5	0.91	11.5	T1	0.3
2014/09/08	Yosemite Gold	1xxx	56.9%	11.9	0.90	13.2	T1	0.5

Table 5.4.18.6 Internal fruit quality data for experimental mandarin hybrid selections from the Hankey (Spitzbak) region of the Gamtoos River Valley region during the 2014 season.

Date	Selection	Count	Juice %	Brix°	Acid %	Ratio	Colour	Avg. Seed
2014/06/11	Gold Nugget	1x	55.9%	10.6	1.12	9.5	T6	0
2014/06/24	Gold Nugget	1xx	53.6%	11.8	1.00	11.8	T4-5	0
2014/07/23	Gold Nugget	1xx	55.0%	13.1	0.99	13.2	T1-2	0
2014/08/13	Gold Nugget	1xx	51.9%	10.1	0.97	14.5	T1	0
2014/09/08	Gold Nugget	1xx	58.7%	14.0	0.50	17.5	T1	0
2014/06/11	Shasta Gold	1x	58.2%	11.2	1.69	6.6	T5-6	0
2014/06/24	Shasta Gold	1xxx	59.4%	11.9	1.67	7.1	T2-3	0.3
2014/07/23	Shasta Gold	1xx	62.3%	13.5	1.50	9.0	T1-2	0

2014/08/13	Shasta Gold	1xxx	55.5%	14.1	1.29	10.9	T1	0
2014/09/08	Shasta Gold	1xxx	59.8%	14.1	1.07	13.2	T1	0.1
2014/06/11	Tahoe Gold	1x	64.0%	11.8	1.44	8.2	T5-6	0.8
2014/06/24	Tahoe Gold	1xxx	63.1%	12.1	1.06	11.4	T3	0.5
2014/07/23	Tahoe Gold	1xx	61.5%	13.2	0.96	13.8	T1	0
2014/08/13	Tahoe Gold	1x	58.1%	13.0	0.83	15.7	T1	0.9
2014/09/08	Tahoe Gold	1xxx	52.8%	12.6	0.63	20.0	T1	0.4
2014/06/11	Yosemite Gold	1xxx	63.6%	10.3	1.17	8.8	T6-7	0.4
2014/07/23	Yosemite Gold	1xxx	55.9%	10.2	1.25	8.2	T3	0

5.4.19 PROGRESS REPORT: Cultivar characteristics and climatic suitability of mandarin hybrids in a cold production region (Western Cape)

Project 997D by S. Meeding (CRI)

Opsomming

Die proef perseël in die Paarl wat hier bespreek word, is een van die oudste persele in die land wat Tahoe Gold, Gold Nugget, Shasta Gold en Yosemite Gold ingesluit het. Die bome is volwasse en groeikragtig, produseer goeie kwaliteit vrugte asook goeie vruggroottes. In die Wes-Kaap se proef persele het Tahoe Gold die kleinste boom met 'n baie swaar drag gelewer. Yosemite Gold het die grootste boom van die 4 seleksies, maar het 'n baie ernstige alternatiewe drag probleem. Soos die Shasta Gold bome volwasse raak verminder die persentasie ribbing op die vrugte. Gold Nugget set 'n groot aantal vrugte en die lang raamtakke breek sonder ondersteuning. Tahoe Gold se skil raak powwerig kort na optimal rypheid wat dan vrugval veroorsaak.

In die Piketberg area is die seleksies oorgewerk op Growweskil onderstam. Die drag was swaarder op Growweskil in vergelyking met Carizzo Citrange. Die eet gehalte van die vrugte was beter op Carizzo Citrange as op Growweskil, met goeie smaak. Die vrugte was gesteel gedurende die seisoen en evaluasies kon nie voltooi word tot by optimale rypheid nie.

Summary

The Paarl trial site being mentioned in this discussion is one of the oldest sites in the country with Gold Nugget, Tahoe-, Shasta- and Yosemite Gold. The trees are mature and vigorous, producing very good fruit quality and fruit size. In the Western Cape trial sites Tahoe Gold developed the smallest tree with a very heavy crop load. Yosemite Gold developed the largest tree of the 4 selections with a serious alternative bearing problem. On the mature Shasta Gold trees the ribbing on the fruit tends to decrease. Gold Nugget sets a heavy crop; breaking the long bearing branches due to heavy crop. Tahoe Gold becomes puffy soon after peak maturity and fruit starts to drop from the trees.

In the Piketberg area the selections are top worked onto Rough Lemon rootstock. The crop load was heavier on Rough Lemon compared to Carizzo Citrange rootstock. The fruit quality was better on Carizzo Citrange than on Rough Lemon, with very good flavour. Fruit were stolen at the trial site and evaluations terminated before peak maturity.

Objectives

- To select mandarin hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new mandarin hybrid cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections from Citrusdal, Piketberg, Porterville and Paarl region of the Western Cape.

Table 5.4.19.1 List of experimental mandarin hybrid selections evaluated in the Paarl region of the Western Cape during the 2014 season.

Selection	Rootstock	Planted
Tahoe Gold	CC	2008
Shasta Gold	CC	2008
Yosemite Gold	CC	2008
Gold Nugget	CC	2008

Table 5.4.19.2 List of experimental mandarin hybrid selections evaluated in the Piketberg region of the Western Cape during the 2014 season.

Selection	Rootstock	Topwork
Tahoe Gold	RL	2010
Shasta Gold	RL	2010
Yosemite Gold	RL	2010
Gold Nugget	RL	2010
Tango	RL	2010

Table 5.4.19.3 List of experimental mandarin hybrid selections evaluated in the Porterville region of the Western Cape during the 2014 season.

Selection	Rootstock	Topwork
Tahoe Gold	CC	2010
Shasta Gold	CC	2010
Yosemite Gold	CC	2010
Gold Nugget	CC	2010
Tango	CC	2010

Results and discussion

Gold Nugget

In the first 2 years Gold Nugget tends to have a coarse rind. Fruit higher up in the trees tends to develop a coarser rind texture compared to fruit lower down. After year 2 the fruit rind gets smoother, but will never be as smooth as Tango or Nadorcott. Tree manipulation is necessary to control the strong vegetative- and upright growth habit. Gold Nugget developed the best tasting fruit with a high Brix: acid ratio. The fruit peaked internally with Brix of 14.6. Due to the good quality of the fruit it will be possible to hang the fruit longer on the trees with extended shelf life. The fruit was seedless at all the trial sites except for Citrusdal where the selection was planted in a variety block with high cross pollination potential. Gold Nugget sets a heavy crop on the trees every year. The fruit develops a deep yellow external colour.

Shasta Gold

Shasta Gold ended the mandarin season off in both Paarl and Piketberg area. The fruit has severe ribbing problems when the trees are still in their youth phase. The fruit shape was flat with large to very large fruit size (1xx-1xxx). Shasta Gold developed a very good external fruit colour (deep orange at peak maturity). The fruit rind was thin, peeled easily and cleanly. Seed content ranged from 0 - 0.5 seeds per fruit. Shasta Gold had a problem with high acid levels fairly late in the season (1.2%). The Brix content remained on the low side.

Tahoe Gold

Tahoe Gold struggled with high acids in the 2014 season. The acid levels never dropped low enough for the fruit to reach the peak maturity ratio of 12:1. The fruit had a very good colour development and peaked at colour plate T1 before fruit was fully matured. Due the high acids the fruit becomes puffy before it reaches peak maturity. Tahoe Gold developed seeds at all the trial sites, up to 1 seed per fruit. The selection has the smallest tree size (compact) of the UCR 5 selections with a rounder shape and dense canopy.

Tango

Tango started the season off in the Piketberg (1st week July) area and was second to mature in the Porterville area. At the Piketberg site, Tango was top worked onto Rough Lemon rootstock. The combination cropped a god yield on the trees this season. Tango developed a very smooth rind texture (similar to

Nadorcott) with a natural shine. The fruit had a very good colour development in the cooler areas, colour plate T1 with peak maturity.

Yosemite Gold

Yosemite Gold had a serious alternative bearing problem. At the Porterville and Citrusdal sites, Yosemite Gold was not bearing a crop for the second year in a row. At the Paarl site, Yosemite Gold outperformed the other two sites, with large fruit size (1xxx). The fruit had an excellent colour development with a deep orange rind colour. Yosemite Gold is a very firm fruit with thin rinds that peels easily and cleanly. The juice percentages were high this season with over 50%. Yosemite Gold had the second highest seed content of all the selections evaluated with 0.4 seeds per fruit.

Conclusion

Yosemite- and Shasta Gold developed the largest fruit size compared to the other UCR 5 selections with count 1xxx. All 5 selections had a good colour development, being at colour plate T1 at peak maturity. Tango, Yosemite- and Shasta Gold had the best external colour, with Yosemite- and Shasta Gold having a deep orange/red colour. Tango matured with a deep orange rind colour and natural wax shine on the fruit. Tango, Gold Nugget and Tahoe Gold had the best yields, followed by Tahoe Gold where crop manipulation will be essential to prevent heavy yields on the trees and small fruit size. Tahoe Gold bears a fair number of inside fruit with delayed colour development compared to the outside fruit on the trees. Tango and Gold Nugget outperformed the other selections with the best eating quality fruit (very good flavour).

Table 5.4.19.4. Internal fruit quality data for experimental mandarin hybrid selections from the Paarl region of the Western Cape during the 2014 season.

Date	Selection	Count	Juice %	Brix°	Acid %	Ratio	Avg. Seed	Colour
2014/06/18	Gold Nugget	1	56.6%	11.2	1.22	9.2	0	T3
2014/07/08	Gold Nugget	1x	56.1%	11.4	0.96	11.9	0	T2
2014/08/05	Gold Nugget	1x	54.9%	12.1	0.87	13.9	0	T2
2014/06/18	Shasta Gold	1xx	61.7%	10.2	1.36	7.5	0.5	T1
2014/07/08	Shasta Gold	1xx	58.4%	10.0	1.70	5.9	0	T2
2014/08/05	Shasta Gold	1xxx	56.5%	10.1	1.49	6.8	0	T1
2014/06/18	Tahoe Gold	1xx	59.5%	9.6	2.03	4.7	0	T3
2014/07/08	Tahoe Gold	1x	62.4%	10.6	1.31	8.1	0.7	T1
2014/08/05	Tahoe Gold	1	64.1%	10.5	1.13	9.3	0.6	T1
2014/06/18	Yosemite Gold	1xxx	53.9%	9.9	1.54	6.4	0.3	T3
2014/07/08	Yosemite Gold	1xxx	53.8%	10.2	1.34	7.6	0.2	T1
2014/08/05	Yosemite Gold	1xxx	52.4%	11.5	1.19	9.7	0	T1

Table 5.4.19.5. Internal fruit quality data for experimental mandarin hybrid selections from the Piketberg region of the Western Cape during the 2014 season.

Date	Selection	Count	Juice %	Brix°	Acid %	Ratio	Avg. Seed	Colour
2014/05/15	Gold Nugget	2	52.6%	9.7	1.44	6.8	0	T6
2014/06/17	Gold Nugget	2	54.0%	10.0	0.99	10.1	0	T4
2014/07/02	Gold Nugget	1	53.8%	10.4	0.92	11.3	0	T3
2014/05/15	Shasta Gold	1xxx	57.1%	9.3	2.24	4.2	0	T5
2014/06/17	Shasta Gold	1xx	55.5%	9.9	1.57	6.3	0.1	T2
2014/07/02	Shasta Gold	1xx	55.8%	9.5	1.36	7.0	0	T2
2014/05/15	Tahoe Gold	2	63.3%	8.1	1.34	6.0	1	T6
2014/06/17	Tahoe Gold	1	61.8%	8.9	1.01	8.8	0	T2
2014/07/02	Tahoe Gold	1xx	58.8%	9.0	0.84	10.7	0.3	T3
2014/05/15	Tango	1	55.4%	8.7	1.06	8.2	0	T6
2014/06/17	Tango	1	56.5%	10.6	1.05	10.1	0	T1
2014/07/02	Tango	1	55.6%	10.6	0.88	12.0	0	T2
2014/05/15	Yosemite Gold	1x	44.3%	9.1	1.55	5.9	0	T7
2014/06/17	Yosemite Gold	1xx	52.2%	9.5	1.23	7.7	0.1	T2
2014/07/02	Yosemite Gold	1xx	54.5%	10.3	1.28	8.0	0.4	T2

Table 5.4.19.6. Internal fruit quality data for experimental mandarin hybrid selections from the Porterville region of the Western Cape during the 2014 season.

Date	Selection	Count	Juice %	Brix°	Acid %	Ratio	Colour	Avg. Seed
2014/05/15	Gold Nugget	2	50.10	12.2	2.24	5.4	6	0
2014/06/17	Gold Nugget	2	53.90	13.9	1.78	7.8	3	0
2014/07/02	Gold Nugget	2	51.20	14.6	1.53	9.5	2	0
2014/06/17	Tahoe Gold	1x	59.20	12.4	1.82	6.8	4	0.2
2014/07/02	Tahoe Gold	1xx	55.30	12.0	1.58	7.6	3	0.3
2014/05/15	Tango	3	54.60	13.3	2.37	5.6	5	0
2014/06/17	Tango	1x	56.50	13.6	1.81	7.5	2	0
2014/07/02	Tango	1	52.10	13.3	1.50	8.8	2	0.4

Table 5.4.19.7. Internal fruit quality data for experimental mandarin hybrid selections from the Citrusdal region of the Western Cape during the 2014 season.

Date	Selection	Count	Juice %	Brix°	Acid %	Ratio	Avg. Seed	Colour
2014/05/15	Gold Nugget	1	51.9%	10.5	1.41	7.4	1	T6
2014/06/19	Gold Nugget	2	54.3%	11.4	1.21	9.5	0.3	T3
2014/07/02	Gold Nugget	1xx	55.4%	11.3	1.00	11.4	0.2	T2
2014/08/04	Gold Nugget	1x	50.7%	12.9	0.89	14.6	0	T2
2014/05/15	Shasta Gold	1	57.8%	9.6	2.05	4.7	0	T7
2014/06/19	Shasta Gold	1xx	59.0%	10.2	1.59	6.4	0	T4
2014/07/02	Shasta Gold	1xx	57.2%	10.7	1.45	7.4	0	T3
2014/08/04	Shasta Gold	1xx	57.3%	11.5	1.18	9.7	0.2	T1
2014/05/15	Tahoe Gold	1x	59.4%	11.0	1.11	9.9	1	T5
2014/06/19	Tahoe Gold	1	62.5%	11.1	1.34	8.3	0.2	T2

2014/07/02	Tahoe Gold	1xx	61.4%	11.5	1.30	8.9	0.5	T2
2014/08/04	Tahoe Gold	1x	60.1%	11.4	0.99	11.5	0	T1
2014/05/15	Tango	3	54.8%	10.6	1.54	6.9	0	T6
2014/06/19	Tango	2	58.5%	10.8	1.11	9.7	1	T1
2014/07/02	Tango	1	57.7%	11.8	1.12	10.5	0	T1
2014/08/04	Tango	2	55.2%	11.1	1.14	9.8	0	T1

5.4.20 PROGRESS REPORT: Cultivar characteristics and climatic suitability of navel oranges in a cold production region (Sundays River Valley)
Project 998B by S. Meeding and Z. Zondi (CRI)

Opsomming

Die vroeë navel proef is gevestig in die Addo area van die SondagsRivierVallei. E-Navel is 'n mutasie wat gevind is en word direk vergelyk en ge-evalueer met 'n paar vroeë Navel seleksies. Al hierdie seleksies is gedurende die 2007 seisoen getoets. Swingle- en Troyer Citrange word as onderstamme vir die proef gebruik. Die E-Navel seleksie wat ge-evalueer word is dogter bome en word direk vergelyk met Fukumoto-, Lina-, Newhall-, Palmer-, Tulegold-, en Washington navel.

Summary

The early maturing navel trial is based in the Addo area of the Sundays River Valley. E-Navel (new mutation) was included at this trial site and evaluated with a few early navel selections. All selections were top worked in the 2007 season. Rootstocks Swingle- and Troyer Citrange were used for the trial. The E-Navel selection evaluated in this trial are daughter trees and was compared with Fukumoto, Lina, Newhall, Palmer, Tulegold and Washington navels.

Objectives

- To select navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new navel cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on navel selections from the Sundays River Valley region of the Eastern Cape. The following early to mid maturing selections were evaluated: Fukumoto, Lina, Newhall, Palmer, Tulegold, E-Navel and Washington.

When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. A ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which it is considered overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instance of quality and rind issues.

Table 5.4.20.1. List of navel selections evaluated at Sundays River Valley (Penhill) during 2014.

Selection	Rootstock	Planted
E Navel	Swingle	2007
Fukumoto	Troyer	2007
Lina	Troyer	2007
Newhall	Troyer	2007
Palmer	Swingle	2007
Tulegold	Troyer	2007
Washington	Swingle	2007

Results and discussion

E-Navel

The selection had a good size and peaked at count 56. Internal quality was good with a high juice percentage of 52%. E-Navel developed a good external colour up to colour plate T1 at peak maturity. The selection was completely seedless and produced a good crop on the trees. The leaves had a dark green colour and the tree canopy was fairly dense.

Fukumoto

Fukumoto was the selection with the second highest Brix: acid ratio of 12 and was completely seedless. The colour development was delayed with colour plate T4-5 at peak maturity. At peak maturity the Brix was 10.8 and the acid still acceptable at 0.9%. Fukumoto produced a good fruit size and peaked at count 56. The navel-end on the fruit was fairly open and protruding.

Lina

Lina had a delayed colour development with a colour plate T5-6 at peak maturity. The selection had a very good fruit size and peaked at count 56; Lina Navel is a selection that has a problem with smaller fruit size in general. The fruit shape was more elongated, as well as large navel-end (fairly open). Lina developed a high juice content (51%) with Brix of 11.6 and complied with the export standards.

Newhall

The fruit peaked at count 56 for this season; very favorable for navel production. Newhall had a delayed colour development (colour plate T5-6) when the fruit was at peak maturity. The Brix remained low (9.8) with a fairly high acid percentage of 1.0. The selection's juice percentage was low, being less than 50%.

Palmer

Palmer was the selection with the highest juice content for this trial with up to 57%. The external colour development of the selection was very good (colour plate T1) at peak maturity. The selection had a good fruit size and peaked at count 56. Palmer had a high Brix: acid ratio of 12.3. The acids dropped slowly, indicating that the selection can hang on the tree for slightly longer periods.

Tulegold

The selection had a delayed colour development with a colour plate T5 just before peak maturity. The juice content was high (over 55%) before peak maturity. Most of the fruit on the trees developed a round fruit shape, but some did tend to be more elongated. The selection will remain experimental and there are no commercial plantings to date.

Washington

Washington and Palmer were used as controls at this trial site and are well-known selections. The fruit had a round shape with small-medium navel end openings. Fruit size was good and peaked at count 56. The selection's colour development was uniform, but was delayed well after peak maturity internally. The selection's juice content was the 2nd highest of all the selections evaluated at this trial site (54%).

Conclusion

The Addo area is well suited for navel production. All the selections had a very good size and peaked at count 56. The delayed external colour development as well as high acid percentages in some of the selections will be due to the Swingle rootstock that is 2-3 weeks later in maturity compared to Carizzo and Troyer Citrange with delayed external colour development. Washington, Palmer and Lina had the best juice content with well over 50%. Fukumoto and Washington had the highest Brix with 11. The order of maturity was as follow; Fukumoto started the season followed by Lina, E-Navel, Newhall, Palmer, Tulegold and the season ended with Washington.

Table 5.4.20.2. Internal fruit quality data for early and mid Navel selections from the Addo (Penhill) region of the Sundays River Valley during the 2014 season.

Date	Selection	Count	Juice %	Brix°	Acid %	Ratio	Avg. Seed	Colour
2014/04/15	E Navel	56	48.0%	9.4	0.98	9.6	0	T6-7
2014/05/19	ENavel	56	52.0%	10.0	0.88	11.0	0	T1
2014/04/15	Fukumoto	56	50.3%	10.8	0.90	12.0	0	T4-5

2014/05/19	Fukumoto	56	49.1%	11.0	1.00	10.7	0	T1
2014/04/15	Lina Navel	56	51.0%	10.0	0.86	11.6	0	T5-6
2014/04/15	Newhall Navel	56	47.6%	9.8	1.03	9.5	0	T5-6
2014/04/15	Palmer Navel	56	52.9%	9.8	1.02	9.6	0	T7
2014/05/19	Palmer Navel	56	51.4%	10.2	0.98	10.1	0	T2
2014/06/10	Palmer Navel	56	57.4%	10.7	0.87	12.3	0	T1
2014/04/15	Tulegold	56	55.4%	9.6	1.03	9.3	0	T5
2014/04/15	Washington Navel	56	45.5%	10.0	1.14	8.8	0	T7
2014/06/10	Washington Navel	56	54.4%	11.0	0.68	16.2	0	T2-3

5.4.21 PROGRESS REPORT: Cultivar characteristics and climatic suitability of experimental navel oranges in a cold production region (Gamtoos River Valley)

Project 1001B by S. Meeding and Z. Zondi (CRI)

Opsomming

Die proef wat hier bespreek word bestaan uit 4 eksperimentele navel seleksies waar die die oorspronklike mutasie of dogterbome ge-evalueer is. Skoon materiaal van die seleksies is in verskillende proefblokke gevestig. Cambria is as kontrole gebruik in die proefperseël. De Wet 1 is 'n mid-rypwordende navel met 'n ronde vrugvorm. Die vrugte het 'n toe navel-ent met geen manipulasie nodig nie. Lazyboy, KS navel en Suitangi is laat navel seleksies met goeie eksterne kleur ontwikkeling; Lazyboy kan vir 'n langer periode aan die boom hang. KS navel was geselekteer uit Cambria as 'n natuurlike mutasie wat ronder vrugte geproduseer het as Cambria.

Summary

The trial consists of four experimental navel selections where the original mutation or daughter trees were evaluated. In addition clean material was added in numerous new trial blocks. Cambria was used as a control at the trial site. De Wet 1 is a mid-maturing navel producing a round fruit shape. The fruit developed a closed navel end without any manipulation necessary. Lazyboy, KS Navel and Suitangi are all late maturing navel selections with good external colour development; Lazyboy indicated that it can hang on the trees for an extended period of time (matures well). KS Navel was selected from Cambria because of a natural mutation that produced fruit with a rounder fruit shape compared to Cambria.

Objectives

- To select navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new navel cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on navel selections from various regions of the Gamtoos River Valley. The following selections were evaluated: De Wet 1, KS navel, Suitangi, Lazyboy with Cambria as a control.

A ratio of 9:1 is considered to be the build-up towards peak maturity. When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. After reaching the peak, the ratio increases to 11:1, after which it is considered overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

Table 5.4.21.1 List of navel selections evaluated at various sites in the Gamtoos River Valley, Eastern Cape during the 2014 season.

Selection	Rootstock	Planted
De Wet 1	Rough Lemon	Young

Cambria	Rough Lemon	Old
KS Navel	Rough Lemon	Young
Suitangi	Rough Lemon	Old
Lazyboy	Rough Lemon	Young

Results and discussion

Cambria

Cambria is a well-known late navel selection with very good internal quality. The selection was used as control for late maturing navels in the Gamtoos River Valley. The fruit shape was more elongated compared to other navel selections (Palmer etc.). Cambria had a good fruit size and peaked from count 56 to 64. The selection had a delayed colour development, being at colour plate T2-4 at peak maturity. The internal quality of the fruit was good with high Brix (11.2) and good acid percentage (0.8%), as well as fairly high juice percentages (51.9%).

De Wet 1

De Wet 1 is a mid-maturing navel that produced a good crop consistently every year. Manipulation is necessary to control fruit size because over cropping resulting in smaller fruit size on the trees. The selection developed a fairly soft rind, one of the characteristics of the De Wet selection. De Wet 1 had a closed navel end on the fruit without having to spray 2,4-D, as well as a small internal navel. On some of the fruit there was a small opening at the navel end. The selection had good fruit size and peaked from count 56 to 64. Fruit developed a round fruit shape and the colour development was better on the mother tree compared to the daughter trees. The internal quality was good with juice content as high as 55%. At peak maturity the external colour peaked at colour plate T2-3. The Brix remained on the low side at 8.8.

KS navel

KS navel was selected as a branch mutation on a Cambria tree. The fruit shape appeared more round compared to the standard Cambria selection. Daughter trees were planted in a Cambria orchard and fruit tends to be more round in shape compared to Cambria. The selection had good internal quality with high Brix (11.4) and acceptable acid percentages of 0.78%. The juice percentages remained higher than Cambria with 52.5%. The selection peaked from count 56 to 64.

Lazyboy

Lazyboy developed round fruit on the trees with good internal quality. The tree had a round “bushy” shape and bore most of the fruit on the outside of the tree. The fruit remained firm and can hang on the tree for an extended period. The selection had the highest Brix: acid ratio of all the selections evaluated. Lazyboy reached Brix levels of 14.8 in combination with acid levels of 0.81%. The Brix: acid ratio at that time was 18.3; fairly high due to the lower acid content and high Brix. The juice percentage peaked over 50%.

Suitangi

Suitangi was one of the late maturing navel selections evaluated with very good external colour development. The selection had a deep orange rind colour with fairly small navel end. There was a good crop on the trees this season. Suitangi peaked from count 56 to count 64. Internally Suitangi produced good quality fruit with high juice content; every sample tested was over 52% juice. High Brix levels of over 11 with acids of 0.8 assured good tasting fruit with good flavour. The Brix: acid ratio reached levels of over 17.4:1.

Conclusion

The season started with De Wet 1 navel (mid to end of June); the selection developed the lowest Brix content this season. After De Wet 1 navel, Lazyboy matured with high Brix: acid ratios. The navel season for this trial ended off with Cambria, followed by KS Navel and Suitangi, (these 3 selections matured at the same period). All the navel selections produced a good fruit size and peaked between count 56 and 64. Suitangi and De Wet 1 developed the highest juice content with over 55%.

Table 5.4.21.2. Internal fruit quality data for Experimental Navel selections from the Gamtoos River Valley region of the Eastern Cape during the 2014 season.

Date	Selection	Count	Juice %	Brix°	Acid %	Ratio	Colour	Avg. Seed
2014/07/07	Cambria	64	50.4%	9.4	0.70	13.4	T4-5	0
2014/07/23	Cambria	56	49.5%	11.2	0.80	14.0	T2-3	0
2014/08/13	Cambria	56	49.8%	11.1	0.61	18.2	T3-4	0
2014/08/26	Cambria	64	51.9%	11.2	0.61	18.4	T1	0
2014/09/17	Cambria	64	49.2%	10.6	0.53	20.0	T1	0
2014/06/11	De Wet Navel	56	55.8%	8.8	0.68	12.9	T2-3	0
2014/07/07	De Wet Navel	64	53.9%	8.7	0.62	14.1	T2-3	0
2014/07/07	KS Navel	64	52.5%	10.3	0.78	13.2	T4-5	0
2014/07/23	KS Navel	56	49.1%	11.4	0.78	14.6	T2-3	0
2014/08/13	KS Navel	64	45.0%	11.2	0.63	17.8	T1	0
2014/08/26	KS Navel	56	48.5%	10.8	0.60	18.0	T1	0
2014/09/17	KS Navel	56	47.6%	11.0	0.53	20.8	T1	0
2014/07/07	Lazyboy	56	47.9%	13.0	0.84	15.5	T5-6	0
2014/07/23	Lazyboy	56	54.6%	13.6	1.62	13.4	T4	0
2014/08/13	Lazyboy	56	50.7%	14.4	0.93	15.5	T3	0
2014/09/08	Lazyboy	64	43.4%	14.8	0.81	18.3	T1	0
2014/07/07	Lazyboy daughter	56	48.9%	13.2	1.01	13.1	T5-6	0
2014/07/23	Lazyboy daughter	56	54.0%	12.9	0.78	16.5	T3-4	0
2014/08/13	Lazyboy daughter	56	47.7%	12.4	0.66	18.8	T1	0
2014/09/08	Lazyboy daughter	56	49.4%	13.6	0.79	17.2	T1	0
2014/07/07	Suitangi Navel	56	52.5%	11.1	0.80	13.9	T4-5	0
2014/07/28	Suitangi Navel	64	54.1%	11.0	0.80	13.8	T1	0
2014/08/13	Suitangi Navel	56	55.3%	11.5	0.66	17.4	T1	0

5.4.22 PROGRESS REPORT: Evaluation of Valencia selections in a semi-desert production area (Kakamas)

Project 964 B by S. Meeding (CRI)

Opsomming

Die Valencia's wat bespreek word in hierdie proef was in die 2010 seisoen getopwerk. Die bome het hulle eerste drag in die 2014 seisoen gehad. Daar was 'n groot probleem met vrugtevlug en meeste van die vrugte was gesteeek en baie vrugval het voorgekom. Slegs een evaluasie was moontlik hierdie seisoen as gevolg van die beperking op die aantal vrugte.

Summary

The Valencia's discussed in this trial were top worked in the 2010 season. The trees produced their first crop in the 2014 season. There was a big problem with fruit fly and most of the fruit were stung and massive fruit drop occurred. Only one evaluation was possible this season due to fruit quantity limitations.

Objective

- To find suitable Valencia selections with superior characteristics for the semi-desert production area (Kakamas).

Materials and methods

Field evaluations and laboratory analyses were conducted on Benny 2, Gusocora, Henrietta, Jassie, Lavalley 2, Louisa, McClean SL, Midnight 1, Moosrivier Late and Ruby Red.

Table 5.4.22.1. List of Valencia selections evaluated at Mosplaas (Kakamas) during 2014.

Selection	Rootstock	Topwork
Benny 2	X639	2010
Gusocora	X639	2010
Henrietta	X639	2010
Jassie	X639	2010
Lavalle 2	X639	2010
Louisa	X639	2010
McClean SL	X639	2010
Midnight 1	X639	2010
Moosrivier Late	X639	2010
Ruby Red	X639	2010

Results and discussion

Midnight 1 was the first selection to mature for the Valencia trial, followed by Gusocora with good Brix and acid levels above 1.0%. Lavalle 2 was the last Valencia selection to mature (mid July) with high Brix (10.7°) and high acid levels (1.74%). Lavalle 2 is the latest maturing Valencia selection currently available in South Africa with the largest fruit size peaking between count 72 and 56 (very even fruit size distribution). Louisa and Mclean SL had the second largest fruit size and peaked from count 88 to count 64. Benny 2 had the lowest Brix levels at 9.9°. Colour development on all the selections was very good. Most of the selections developed a colour plate between T1 and T3 long before peak maturity. Gusocora, Lavalle, Midnight 1 and McClean SL were the only selections that were completely seedless. Henrietta had the highest seed count with 6.3 seeds per fruit. Lavalle had the highest juice percentage at 56.7%.

Conclusion

The 2014 season was the first year for the trees to produce a crop at this trial site. Due to a fruit fly problem there was a severe fruit drop on the trees. The fruit that was left on the tree was sampled and evaluated to determine the potential of these Valencia's in a semi-desert production area. The 2015 season will provide better crops on the trees for future evaluations and more accurate data.

Table 5.4.22.2. Internal fruit quality data for Valencia selections at Mosplaas (Kakamas) during the 2014 season.

Date	Selection	Rootstock	Count	Juice %	Brix°	Acid %	Ratio	Colour	Avg. Seed
2014/07/09	Benny 2	X639	105-88	52.7%	9.9	1.39	7.1	T1-3	3.8
2014/07/09	Gusocora	X639	88-72	52.1%	10.8	1.07	10.1	T1-2	0.0
2014/07/09	Henrietta	X639	88-72	50.0%	10.1	1.54	6.6	T1-2	6.3
2014/07/09	Jassie	X639	88-72	49.1%	11.2	1.47	7.6	T1-2	4.8
2014/07/09	Lavalle	X639	72-56	56.7%	10.7	1.74	6.1	T2-3	0.0
2014/07/09	Louisa	X639	88-64	44.6%	10.1	1.24	8.1	T1-3	0.6

2014/07/09	McClellan SL	X639	88-64	47.2%	10.8	1.17	9.2	T1-3	0.0
2014/07/09	Midknight 1	X639	125-64	41.7%	10.6	1.01	10.5	T1-2	0.0
2014/07/09	Moosrivier Late	X639	105-64	51.5%	11.1	1.50	7.4	T1-3	4.4
2014/07/09	Ruby Red	X639	88-72	51.0%	11.3	1.56	7.2	T1-3	2.5

5.4.23 PROGRESS REPORT: Cultivar characteristics and climatic suitability of experimental Navel oranges in a semi-desert region (Kakamas)
Project 964 C by S. Meeding (CRI)

Opsomming

Die 2014 produksie jaar was die bome se eerste vrugset. 'n Hoë populasie vrugtevlug in die proefblok het veroorsaak dat daar 'n groot aantal vrugval was. Selgs een evaluasie was moontlik as gevolg van 'n beperking op die aantal vrugte. Vrugtevlug beheer sal toegepas word in die 2015 seisoen om 'n beter drag en evaluasie te verseker. Die 2014 evaluasie data verskaf 'n goeie indukasie van sekere nawel seleksies wat moontlik kan werk in die semi-woestyn produksie area.

Summary

The 2014 production season was the first year for fruit on the trees. Fruit drop occurred due to a high population of fruit fly in the trial orchard. Only one evaluation was possible this season as a result of limited fruit numbers. Fruit fly control will be implemented for the 2015 season to ensure a better crop on the trees and to provide adequate evaluation data. The 2014 evaluation data provide a good indication of certain navel selections that could work in this semi-desert production area.

Objectives

- To select navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new navel cultivars and to determine the climatic suitability of these cultivars in a semi-desert region.

Materials and methods

A ratio of 9:1 is considered to be the build-up towards peak maturity. When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. After reaching the peak, the ratio increases to 11:1, after which it is considered overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

Field evaluations and laboratory analyses were conducted on navel selections from a semi-desert region of the Northern Cape. The following selections were evaluated.

Selection	Rootstock	Topwork
Barnfield	X639	2010
Carninka	X639	2010
Coetzee Late	X639	2010
De Wet 1	X639	2010
Gloudie	X639	2010
Lane Late	X639	2010
Powell Summer	X639	2010
Robyn 2	X639	2010
Santa Catarina	X639	2010
XGreenwash	X639	2010

Results and discussion

Barnfield, Robyn 2 and XGreenwash were the only three navel selections that had seed in the fruit. The cross pollination pressure in the trial orchard would have been very high to develop seeds in the navel fruit. All the navel selections produced a good fruit size and peaked from medium to large (count 88 to 56). Carninka was the only navel selection to reach a colour plate level of T1 by the time of peak maturity. Santa Catarina developed the highest juice percentage and averaged over 54% juice content. Lane Late had the lowest juice levels of 36% for this trial site, as well as lowest Brix content (8.5°). Barnfield and Carninka produced the highest Brix levels for this semi-desert production area (over 11°).

Conclusion

The late maturing navels will be a better choice compared to the early maturing navels in the semi-desert production area due to external colour development and better internal quality. The late maturing navels tend to set more fruit on the trees than the early selections and acid levels seems to drop quickly because of high temperatures early in the production season.

Table 5.4.23.2. Internal fruit quality data for experimental navel selections from the semi-desert (Kakamas) region of the Northern Cape during the 2014 season.

Date	Selection	Rootstock	Count	Juice %	Brix°	Acid %	Ratio	Colour	Avg. Seed
2014/06/03	Barnfield	X639	72	49.5%	11.1	0.60	18.4	T3	0.1
2014/07/09	Carninka	X639	88-56	50.0%	11.1	0.76	14.6	T1	0
2014/06/03	Coetzee Late	X639	64	50.8%	9.7	1.09	8.9	T5	0
2014/05/19	De Wet 1	X639	88-64	46.7%	9.7	0.82	11.8	T2-4	0
2014/07/09	Gloudie	X639	88-64	51.9%	11.6	0.78	14.9	T2-3	0
2014/05/19	Lane late	X639	64	36.7%	8.5	0.85	10.0	T3-5	0
2014/06/03	Lane late	X639	64	48.9%	11.0	0.75	14.7	T5	0
2014/06/03	Powell summer	X639	64	49.0%	10.7	0.79	13.5	T4	0
2014/06/03	Robyn 2	X639	72	52.6%	10.7	0.85	12.6	T3	0.3
2014/06/03	Santa Catarina	X639	72	54.2%	9.5	0.85	11.2	T5	0
2014/06/03	XGreenwash	X639	72	51.7%	9.5	0.88	10.8	T4	1.3

5.4.24 PROGRESS REPORT: Cultivar characteristics and climatic suitability of mandarin hybrids in a semi-desert production region (Kakamas) Project 964D by S. Meeding (CRI)

Opsomming

Die 5 UCR laat mandaryn seleksies was getopwerk op X639 in die 2010 seisoen. Daar was 'n ernstige probleem met vrugtevlug en vrugval het plaasgevind lank voor optimale rypheid. Slegs 2 evaluasies was moontlik. Die bome was minder kompak en groeikragtig as in die Oos- en Weskaap se proefblokke. Die blaar kleur op die bome was redelik vaal in vergelyking met die ander proef persele.

Summary

The 5 UCR late maturing mandarin selections were top worked onto X639 rootstock in 2010. There was a serious problem with fruit fly at the trial site this season and fruit started dropping from the trees long before it reached peak maturity. Only 2 evaluations could be completed. The trees were less dense and vigorous compared to the Western- and Eastern Cape trial site. The leaf colour on the trees was fairly pale compared to the other trial sites.

Objectives

- To select mandarin hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new mandarin hybrid cultivars and to determine the climatic suitability of these cultivars in semi-desert regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections from the semi-desert (Kakamas) region of the Northern Cape. A range of new mandarin hybrids have been added to this area and should be bearing fruit in the 2014 season. The following varieties were evaluated: Gold Nugget, Shasta Gold, Tahoe Gold, Tango and Yosemite Gold.

A ratio of 11:1 is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

Table 5.4.24.1 List of mandarin hybrid selections in the semi-desert region (Kakamas) during the 2014 season.

Selection	Rootstock	Topwork
Gold Nugget	X 639	2010
Shasta Gold	X 639	2010
Tahoe Gold	X 639	2010
Tango	X 639	2010
Yosemite Gold	X 639	2010

Results and discussion

Gold Nugget

Gold Nugget was the only selection that was completely seedless. The selection developed the largest tree size in this area compared to the other 4 selections with an upright growth habit. Colour development on the trees was good with colour plate T2 before peak maturity. The tree had a good crop production as well as good fruit size (peaked from count 3 to 1XXX). Internal quality was good and juice percentages were high, especially in a semi-desert area with over 50%. The Brix were on the lower side in comparison to the Western- and Eastern Cape.

Shasta Gold

Shasta Gold had a small tree size with a very poor yield on the trees. The fruit size was very large (1X-1XXX) due to the light crop with a very coarse rind and ribbing on the fruit, typical characteristics on younger Shasta trees. Trees were thorny on the vigorous main branches and the thorns will disappear when the trees mature, as well as when the crop load increase. The fruit did not develop to a deep orange or red external colour. The average seed count per fruit was much higher in this trial (high cross pollination pressure) compared to the Western- and Eastern Cape with 1.5 seeds per fruit.

Tahoe Gold

Tahoe Gold produced a good yield on the trees, taking the small tree size into consideration. Tahoe Gold developed into a fairly compact tree compared to the other two TDE selections with good fruit size distribution (count 1-1XX). External colour development was good with a colour plate T2 before peak maturity. The internal quality was good with high juice percentages, peaking at 57%.

Tango

Tango had good colour development for this semi-desert production area; the selection was at colour plate T1 before peak maturity. The selection had the highest juice percentages of 57.4% for this trial site. Tango had the highest seed count (3 seeds per fruit), higher than both the Western- and Eastern Cape trial sites due to possible cross pollination pressure (mixed trial blocks). The acid levels remained high during the season and the Brix content reached 12.4°. Fruit size was on the smaller side and peaked between count 3 and 1).

Yosemite Gold

No crop on the trees to evaluate.

Conclusion

2014 was the first year to evaluate this trial site in the semi-desert production area and due to fruit fly problems there was severe fruit drop limiting the evaluation potential this season. Colour development was good on all the selections. Shasta Gold did not develop a deep orange to red rind colour, compared to the other mandarin selections. The trees are less dense in comparison to the Western- and Eastern Cape trial blocks. For a semi-desert production area; the fruit quality was good with juice percentages well over 50%. Gold Nugget was the only selection evaluated that was completely seedless. Tango had the highest seed count of all the selections with up to 3 seeds per fruit.

Table 5.4.24.2. Internal fruit quality data for Mandarin hybrid selections from the semi-desert region (Kakamas) during the 2014 season.

Date	Selection	Rootstock	Count	Juice %	Brix°	Acid %	Ratio	Colour	Avg-Seed
2014/05/19	Gold Nugget	X639	3-1x	50.0%	9.3	2.14	4.3	T1-4	0
2014/06/03	Gold Nugget	X639	1xxx	55.2%	11.5	1.28	9.0	T2	0
2014/05/19	Shasta Gold	X639	1x-1xxx	47.4%	9.0	2.27	4.0	T2-4	0
2014/06/03	Shasta Gold	X639	1xxx	51.7%	10.3	1.81	5.7	T3	1.5
2014/05/19	Tahoe Gold	X639	1-1xx	54.5%	9.1	1.97	4.6	T-2-4	1.7
2014/06/03	Tahoe Gold	X639	1xx	57.0%	10.6	1.48	7.2	T2	0.6
2014/05/19	Tango	X639	3-1	56.4%	10.7	1.66	6.4	T1-3	3
2014/06/03	Tango	X639	2	57.4%	12.4	1.60	7.8	T1	2.4

5.4.25 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of lemons in a semi-desert production region (Kakamas)** Project 964 E by S. Meeding (CRI)

Opsomming

Die 2014 seisoen was die eerste drag vir die suurlemoen proef. Dit was Willowtree Long en Plaat B se eerste vrugset in die land as gevolg van die hoë temperature en suurlemoene se groeikragtigheid. Die blare op al die seleksies was lig geel en addisionele bemesting sal moontlik nodig wees in die toekoms. Lisbon en Eureka het 'n beter vrugset gehad as die res van die suurlemoen seleksies. Hoë temperature gedurende blom periodes het swak vrugset tot gevolg gehad vir sekere van die seleksies. Al die seleksies was getopwerk aan die einde van die 2010 seisoen op X639.

Summary

The 2014 season was the first crop on the trees for the Lemon trial. Willowtree Long and Plaat B bore their first fruit at this trial site in the country due to semi-desert temperatures and lemon growth rate. The leaves were yellowish on all the trees and additional fertilizers might be necessary in the future. Lisbon and Eureka had a better fruit set compared to the rest of the lemon selections. High temperatures during the flower periods induced poor fruit set on some of the selections. All the selections were top worked at the end of the 2010 season on X639 rootstock.

Objectives

- To find Lemon selections suitable for the semi-desert production area.

- To produce lemon selections with Eureka like fruit shape, high juice content, everbearing characteristics, low seed content and high rind oil for processing purposes.

Materials and methods

Field evaluations were conducted on Eureka, Genoa, Lisbon, Lisbon Yen Ben, Plaat B (Roos) and Willowtree Long.

Table 5.4.25.1 List of Lemon selections evaluated at Kakamas during 2014.

Selection	Rootstock	Topworked
Eureka	X639	2010
Genoa	X639	2010
Lisbon	X639	2010
Lisbon Yen Ben	X639	2010
Plaat B (Roos)	X639	2010
Willowtree Long	X639	2010

Results and discussion

Lisbon Yen Ben (27%) and Plaat B (32%) developed the lowest juice percentages for the season, but Eureka had the highest juice percentage of 49%. Genoa and Lisbon produced the highest juice percentages which remained constant during the two evaluations completed more than a month apart (44% juice). The highest seed content per fruit was Lisbon (9 seeds per fruit), followed by Genoa (7 seeds per fruit).

Conclusion

For the first season there was a reasonable crop produced on the trees. The lemon selections were not that vigorous and tree canopy was less dense. High temperatures can affect the fruit set and as well as the juice percentages. The three commercial Lemon selections, Eureka, Lisbon and Genoa, performed well and more suitable for the semi-desert production area compared to the rest of the experimental selections.

Table 5.4.25.2. Internal fruit quality data for Lemon selections from the semi-desert region (Kakamas) during the 2014 season.

Date	Selection	Rootstock	Count	Juice %	Colour	Avg. Seed
2014/05/19	Eureka	X639	216-100	39.3	T2-4	3.5
2014/07/09	Eureka	X639	138-100	49.4	T1-2	6.8
2014/05/19	Genoa	X639	138-64	44.1	T2-4	0.0
2014/07/09	Genoa	X639	113-64	44.6	T1-2	7.8
2014/05/19	Lisbon	X639	138-75	44.4	T2-4	5.4
2014/07/09	Lisbon	X639	113-64	44.6	T1-2	9.9
2014/05/19	Lisbon Yen Ben	X639	216-113	27.5	T2-4	5.9
2014/07/09	Lisbon Yen Ben	X639	189-75	43.5	T1-2	4.8
2014/05/19	Plaat B	X639	216-100	47.1	T2-4	3.7
2014/07/09	Plaat B	X639	216-100	32.4	T1-3	0.4
2014/05/19	Willowtree long	X639	138-75	39.6	T2-4	6.2
2014/07/09	Willowtree long	X639	113-88	45.7	T1-2	5.5

5.4.26 **FINAL REPORT: Establishment of a molecular citrus genotype reference database for citrus cultivar verification within the Citrus Improvement Scheme**

Project TS 514022 (April 2012 – March 2015) by A Severn-Ellis, E Hajari, A Sippel, D Nonyane, N Combrink (ARC-ITSC), G Cook, Thys du Toit (CRI)

Summary

Citrus (*Citrus reticulata*) accessions entering the Virus Free Nucleus Blocks at the Agricultural Research Councils' Institute for Tropical and Subtropical Crops (ARC-ITSC) and at Citrus Research International (CRI) have been characterised based on important morphological or agronomical features. These defining morphological or agricultural characteristics are not continuously expressed within the potted greenhouse environment of the Virus Free Nucleus Blocks and the Pre-immunised Foundation Block at Uitenhage. Therefore, it is not always possible to check or verify the trueness-to-type of an accession, which may prevent the detection of misidentifications or duplications. Thus, to ensure correct cultivar identity, a procedure for accurate identification using molecular data is urgently needed.

Twenty three of the previously selected 26 microsatellite (SSR) markers were finally included and used to differentiate amongst all citrus accessions included in the Citrus Improvement Scheme (CIS). To date, PCR amplification, visualisation and documentation of DNA fragments generated for the Mandarin hybrid-, Clementine-, Satsuma-, lemon-, lime-, grapefruit-, pumello-, rootstock-, sweet orange and diverse citrus cultivars as per the available CIS list has been completed. The PCR results were verified and captured on an Excel spreadsheet which will form part of the genotype reference database.

Although genetic differences were detected between most of the cultivars using the selected SSR markers, limited genetic variation was detected between the sweet orange cultivars. It was hypothesised that additional marker systems such as Sequence-Related Amplified Polymorphism (SRAP markers) may provide the supplementary genetic information required to distinguish between these closely related cultivars. The results indicated that the difficulties encountered in discriminating amongst the sweet oranges could not be adequately addressed by the application of SRAP markers due to limitations with the technique. At present, the ability to confidently discriminate amongst *Citrus* cultivars is limited, until further refinements can be made.

Opsomming

Sitrus (*Citrus reticulata*) kultivars and seleksies in die Virusvrye Kernblokke van die Landbounavorsingsraad se Instituut vir Tropiese en Subtropiese Gewasse (LNR-ITSG) en die Citrus Research International (CRI), word tradisioneel beskryf op grond van belangrike morfologiese of landboukundige eienskappe. Hierdie morfologiese of landboukundige eienskappe kom nie altyd tot uiting in die potplant kweekhuis omgewing van die Virusvrye Kernblokke en die Sitrus Grondvesblok te Uitenhage nie, wat dit moeilik maak om tipegtheid te kontroleer of te bevestig. Dit kan lei tot duplisering en/of die introduksie en vrystelling van verkeerde kultivars. Om die rede is dit noodsaaklik dat 'n metode van akkurate identifisering van kultivars deur die gebruik van molekulêre merkers ontwikkel word.

Drie en twintig van die voorheen gekose 26 mikrosatelliet (SSR) merkers is uiteindelik ingesluit en gebruik om te onderskei tussen al die sitrus kultivars en seleksies in die Sitrus Verbetering Skema (SVS). Tot datum is PCR versterking, visualisering en dokumentasie van DNA fragmente gegeneer en voltooi vir al die Mandaryn hibriede-, Clementine-, Satsuma-, suurlemoen-, lemmetjie-, pomelo-, pompelmoes-, onderstam-, soet lemoen en diverse sitrus kultivars soos op die aanvangs SVS kultivar lys. Die PCR resultate is geverifieer en vasgelê op 'n Excel spreistaat wat deel van die genotipe verwysing databasis vorm.

Alhoewel genetiese verskille waargeneem is tussen die meeste van die kultivars met behulp van die gekose SSR merkers, is beperkte genetiese variasie bespeur tussen die soet lemoen kultivars. Die hipotese was dat bykomende merker stelsels soos Volgorde-verwante Versterkte Polimorfisme (SRAP merkers), die aanvullende genetiese inligting wat nodig is om te onderskei tussen hierdie nou verwant kultivars kan voorsien. Die resultate dui egter daarop dat soet lemoene nie voldoende onderskei kan word van mekaar deur die gebruik van SRAP merkers nie weens beperkinge met die tegniek. Op die oomblik is die vermoë om met selfvertroue te onderskei tussen sitrus kultivars beperk. Die probleem kan egter aangespreek word deur verdere verfyning van die beskikbare tegnieke.

Introduction

The characterisation of germplasm upon entering gene banks is essential to provide information on the

traits of accessions to enable end users to make maximal use of the available germplasm collection. Currently, *Citrus* cultivar accessions entering the Citrus Improvement Scheme (CIS) via the two Virus Free Nucleus Blocks, i.e., the one at the Agricultural Research Councils' Institute for Tropical and Subtropical Crops (ARC-ITSC) and the one at Citrus Research International (CRI), have been characterised based on important morphological or agronomical features. These defining morphological or agricultural characteristics are not always expressed within the potted greenhouse environment of the Virus Free Nucleus Blocks and Pre-immunised Foundation Block at Uitenhage. Therefore, it is not always possible to check or verify the trueness-to-type of selected accessions within this environment. Consequently, the detection of potential errors incurred during processing for routine genebank operations such as misidentifications or duplications are impossible and/or difficult to identify and correct. This severely hampers the operational efficiency of genebanks and can lead to the inadvertent, and costly, distribution of misidentified material to the industry.

The last several decades have seen the evolution of molecular markers as tools with great potential application to the challenges of germplasm characterisation. These markers have a distinct advantage over morphologically based phenotypic characterisation, as they are generally unaffected by the host of factors able to influence plant or organ characteristics. This allows comparisons between accessions within a collection or amongst collections at different locations at any time of year, while phenotypic characteristics can be masked by environmental or cultural affects. Molecular characterisation has a number of applications in the management of germplasm collections. These include the identification of gaps and redundancies in the collection, correction of misidentified accessions, and assessment of the genetic diversity present within the collection, development of core subsets and characterisation of newly acquired germplasm (Bretting and Widrechner, 1995; Krueger and Roose, 2003).

To date, genetic diversity and phylogenetic studies on *Citrus* has been performed using a number of available marker systems, viz. Restriction Fragment Length Polymorphism (RFLP, Frederici *et al.*, 1998), Random Amplified Polymorphic DNA (RAPD, Nicolosi *et al.*, 2000), Amplified Fragment Length Polymorphism (AFLP, Pang *et al.*, 2007), chloroplast DNA sequence (Morton *et al.*, 2003), Simple Sequence Repeats (SSR, Cristofani-Yaly *et al.*, 2011) and Sequence-Related Amplified Polymorphism (SRAP, Uzun *et al.*, 2009). There are advantages and limitations in each marker system, and the selection of a particular technique will depend on the application required. Simple Sequence Repeat (SSR) markers have been used in various *Citrus* genetic studies (Luro *et al.*, 2001; 2008; Gulsen and Roose 2001; Barkley *et al.*, 2006; Ollitrault *et al.*, 2010) as they are highly polymorphic, co-dominant, generally locus specific, randomly dispersed throughout the plant genome and have the potential to unravel the genetic diversity in *Citrus* at the interspecific, intraspecific and intra-population level (Froelicher *et al.*, 2008).

The aim of the present study was to use molecular markers to establish a *Citrus* genotype reference database for cultivar verification within the CIS. Towards this end, microsatellite (SSR) markers were used. Difficulties were experienced in detecting differences between accessions within the sweet orange group using SSR markers. This was likely due to the limited genetic variation within this group (Ahmad *et al.*, 2003). Considering this, it was suggested that SRAP markers be tested to discriminate amongst sweet orange accessions as it was shown to be effective in other studies with *Citrus*, e.g. Uzun *et al.* (2009; 2011); Amar *et al.* (2011); Gulsen *et al.* (2011); Amar (2012) and Kacar *et al.* (2013). The SRAP markers are reported to be simple and efficient and have several advantages over other marker systems, viz. that it targets open reading frames, reveals co-dominant markers, allows for easy isolation of bands and has a reasonable throughput rate (Li and Quiros, 2001). Furthermore, each SRAP primer can combine with any number of other primers and the special PCR running conditions combined with the larger size of the SRAP primers ensures improved reproducibility compared with other methods such as RAPDs (Li *et al.*, 2013). Therefore, the objectives were amended to include the investigation of SRAP markers to distinguish amongst sweet orange cultivars.

Stated objectives

1. Molecular characterisation of citrus cultivars within the Virus Free Nucleus Blocks and the Pre- Immunised Foundation Block using microsatellites (SSR) and Expressed Sequence Tag (EST) markers.
2. Establishment of a reference genotype database.

Materials and methods

DNA Extraction

- Plant leaf material was collected from all *Citrus* cultivars housed within the virus free CIS collection. Plant material was obtained from the Virus Free Nucleus Blocks at the ARC-ITSC and the

CRI as per the list of cultivars provided by the CIS in Uitenhage.

- A list of the plant material tested is provided in Table 5.4.26.1.
- DNA was extracted by grinding 0.4 g of leaf tissue in 5 ml extraction buffer in plastic envelopes. Homogenised samples were pipetted into microcentrifuge tubes. The DNA was extracted according to a modified CTAB DNA extraction procedure (Risterucci *et al.*, 2005). The DNA concentration was determined after precipitation using a spectrophotometer.

PCR Amplification

SSR

- The PCR reaction consisted of 37.5 ng of template DNA, 12.5 ul EmeraldAmpMax HS (Takara) master mix, 0.025 uM forward and reverse primer to a final volume of 25 ul.
- PCR amplification of samples was performed using a G-Storm thermocycler. Cycling conditions consisted of an initial hot start at 98°C for 1 min; 35 cycles of 98°C for 15 sec, 50-60°C for 30 sec, and 72°C for 1 min, and a final extension step at 72°C for 5 min.
- Amplified PCR products were resolved and scored on 4% agarose gels.
- GeneTools (Syngene) software was used to visualise gel images and analyse and determine length of PCR products.
- Each group of 24 or 48 samples contained two standard cultivars for quality control purposes to ensure continuity and also serve as positive reference between runs.

SRAP

- PCR amplification of samples was performed using a G-Storm thermocycler. Cycling conditions consisted of an initial hot start at 98°C for 1 min; 5 cycles of 94°C for 1 min, 35°C for 1 min, 72°C for 1 min, 35 cycles of 94°C for 30 sec, 50°C for 30 sec, 72°C for 1 min and a final extension step at 72°C for 5 min.

Primer selection

- The SSR primers used in the current work were selected based on the results of a pilot study and those described by Barkley *et al.* (2006); Novelli *et al.* (2006); Froelicher *et al.* (2008); Ollitrault *et al.* (2010; 2011), as well those used in the genetic characterisation of rootstock collections of the *Citrus* breeding for efficient water and nutrient use project (Project No. 015453 – CIBEWU). The list of SSR primers are provided in Table 5.4.26.2.
- The SRAP primers tested were ME1 – EM4 (refer to Table 5.5.27.3) as per Li and Quiros (2001).

Visualisation of PCR products

- PCR products were separated with the QIAxcel capillary electrophoresis system using the DNA High Resolution Kit and OM800 method to distinguish between fragment sizes of 100-500 bp with a resolution quality of 3-5 bp.

Excel Data base

- Amplification results were visualised, and fragment sizes determined, using the QIAxcel ScreenGel Software.
- Reports of results were generated in jpg, pdf and Excel format.
- An Excel genotype database based on fragment size was compiled from the reports using individual electropherograms and gel images for each cultivar and primer.

Phylogenetic Analysis

- GenAIEx 6.3 was used to create a genetic distance matrix.
- MEGA was used to construct an UPGMA tree.

Results and discussion

Establishment of PCR conditions

It is generally recognised that there are three true *Citrus* species, viz. *C. medica* (citron), *C. reticulata* (mandarin) and *C. maxima* (pummelo). The other cultivated species within *Citrus* were derived by hybridisation and natural mutations (Barrett and Rhodes, 1976) between *Citrus* and other closely related genera. *Citrus* is primarily propagated by grafting to ensure retention of desirable horticultural characteristics. This, combined with the inherent ability for adventitious nucellar embryony that is common in mandarins, grapefruit and sweet oranges, contributes towards the stabilisation and perpetuation of hybrids that might otherwise be eliminated in nature (Ramadugu *et al.*, 2013). The principal taxonomic groups that are of commercial significance are sweet oranges (*C. sinensis*), lemon (*C. limon*), lime (*C. aurantifolia* and *C. latifolia*), grapefruit (*C. paradisi*), pummelo (*C. maxima*) and mandarin (*C. reticulata*).

In the present study, the protocol was first tested to distinguish between mandarin hybrid, Clementine and Satsuma cultivars from the Virus Free Nucleus Block at ARC-ITSC. Parameters for the protocol as well as the SSR primers were selected and tested to distinguish between the above-mentioned accessions. The products for PCR amplification were visualised using capillary electrophoresis. The outputs from electrophoresis (i.e. gel images and electropherograms) were used to determine the sizes of the amplified fragments. This data was used to create an Excel database and the experiments were repeated in order to verify the results. Subsequently, the same method was used to distinguish between lemon, lime, grapefruit, pummelo and diverse *Citrus* cultivars of the CIS, rootstock cultivars of the Virus Free Nucleus Block (ARC-ITSC) and amongst sweet orange, Navel, Midseason and Valencia cultivars of the ARC-ITSC. In addition, SRAP markers were also evaluated to discriminate amongst the sweet orange cultivars.

The results from the SSR analysis indicated that genetic differences were detected between most of the tested cultivars. The SSR-PCR amplification of the sweet oranges was completed but it was decided not to verify the results due to the limited genetic variation detected. Additional marker systems such as SRAP markers may provide the supplementary genetic information required to distinguish between these closely related cultivars. Such SRAP markers (Li and Quiros, 2001) have been described as a simple and efficient marker system that can be adapted for a variety of purposes in different crops, including map construction, gene tagging, genomic and cDNA fingerprinting and was used by Uzun *et al.* (2009) to study genetic diversity and relationships within *Citrus* and related genera. Therefore, SRAP markers were tested to distinguish between the sweet orange cultivars, in addition to the SSR markers tested.

Rootstocks

As mentioned previously, *Citrus* is usually vegetatively propagated by grafting scions onto a rootstock, enabling retention of mutations, tree uniformity, early tree production and tolerance to pathogens (Snoussi *et al.*, 2012). In the present study, the rootstocks were assessed using 22 SSR primers. The results were processed and used to construct dendrograms using the unweighted pair-group method with the arithmetic averages (UPGMA) clustering algorithm. In order to verify the goodness of fit between a cluster analysis and the associated similarity matrix, the cophenetic correlation coefficient (CCC) is calculated using different methods. The CCC provides an indication of the correlation of the cophenetic values matrix with the values of the similarity matrix on which the clustering was based. The clustering is further validated using a bootstrap analysis. In the present study, three methods were used to calculate the similarity coefficients for SSR markers, viz. MSD, Pearson and RMSD. These three methods produced CCC values of 0.880, 0.877 and 0.910, respectively. Throughout this report, where visual inspection of the dendrograms revealed similar clustering structures for the methods tested, only one dendrogram with the highest CCC value is presented. Therefore, for the rootstocks, the dendrogram for the RMSD method is presented (Fig. 5.4.26.1). The cluster analysis suggested two main groups, one with C MACROPHYLLA PAPEDA, Macrophylla and Kiyomi and the second group containing all the other rootstocks. Five additional sub-groups could be discriminated. The first sub-group contained the sour orange hybrids and all the rough lemons (except for the Sweet and Vangasy Rough Lemons). The second sub-group contained a mixture of lemon and mandarin rootstocks while the Citranges were spread across three sub-groups (3, 4 and 5). The hybrid trifoliates also appeared across three separate groups. It was apparent that the cluster analysis for the rootstocks revealed some rootstock types that were dispersed across a number of sub-groups. This is in contrast to the analysis generated by El-Mouei *et al.* (2011) where all similar types of rootstocks separated perfectly into distinct clusters. However, in an analysis by Snoussi *et al.* (2012), the cluster analysis revealed imperfect groupings amongst rootstocks, similar to that found in the present study.

Lemons, limes, grapefruits and pummelos

This group encompassed 46 samples comprising lemons, limes, grapefruits and pummelos. These samples were assessed using SSR markers. As for the rootstocks, high CCC values were obtained for this group. This is indicative of a good fit between the data and the method of analysis, thereby lending confidence to the results. The cluster analysis (Fig. 5.4.26.2) indicated that Kiyomi (a Satsuma selection) was the most distant from all of the tested cultivars. From the dendrogram, four groups can be seen. The first group contained all of the grapefruit selections, along with two pummelos (Oroblanco and Kukemoes) and a lemon (Feminello SL). As expected the two Star Ruby cultivars grouped closely together within the first cluster, as did all three of the Jackson cultivars, indicating a high degree of genetic similarity, which was supported by a high bootstrap value (90). The second cluster contained mostly lemons along with a kumquat (Calamondin Green) and pummelo (Cocktail pummelo). All three Eureka cultivars occurred together, as did the Nagamis and Lisbons. The third cluster comprised the rest of the kumquats, a lime and lemon. The last cluster contained a mixture of limes and pummelos. For the Pomelits, two of them shared a close genetic similarity while the Ruby Pomelit occurred in a separate clade. Other researchers have published work on the genetic variability present in grapefruits and pummelos (Corazza- Nunes *et al.*, 2002) using SSR and RAPD markers. Those authors also reported a sub-group which contained all the grapefruit accessions (similar to the present study). They also suggested that a narrow genetic base exists amongst grapefruit accessions thereby indicating that the expressed phenotypic polymorphisms (e.g. seeded vs. seedless fruit) might be associated with somatic mutations.

Mandarins

The mandarins are considered one of the true *Citrus* species, belonging to the *C. reticulata* group (Rahman *et al.*, 1994). Mandarins represent a diverse group, comprising numerous intergeneric species and interspecific hybrids (Colletta Filho *et al.*, 2000; Koehler-Santos *et al.*, 2003). The genetic variability present within the mandarin group has been attributed to a number of factors including a large percentage of zygotic twins, intergeneric cross compatibility, high heterozygosity, nucellar embryony and a long history of cultivation and world-wide distribution (Das *et al.*, 2007). Despite the numerous cultivars and hybrids present within the mandarin group, they are generally separated into five principle types, viz. King (*C. nobilis*), Mediterranean (*C. deliciosa*), small-fruited (*C. indica*, *C. tachibana* and *C. reshni*), Satsuma (*C. unshiu*) and common mandarin (*C. reticulata*). In addition, various hybrids are recognised, e.g. tangor (*C. reticulata* x *C. sinensis*) and tangelo (*C. reticulata* x *C. paradisi*). Clementines (*C. clementina*) are also considered a unique sub-group (Tanaka, 1977; Aleza *et al.*, 2009).

A large number of mandarins were investigated in the present study, i.e. approximately 115 cultivars. These were separated into five groups, viz. M1 – M5. For the genetic analysis, each of the groups were analysed separately to generate a dendrogram (Figs 5.4.26.3 – 7). When attempts were made to produce a dendrogram with all the mandarin cultivars together, this was found to be impossible since the UPGMA program has a restriction that allows for a maximum of 100 samples per dendrogram. This was a system setting that could not be altered. Considering this, combinations of the mandarin groups were analysed together in order to identify relationships within and between groups. This restriction hampered data analysis and interpretation of the results in the present study as all the mandarin cultivars could not be evaluated simultaneously. This limitation may be overcome by investigating alternative software programs for analysis or further exploring existing software with the assistance of technical specialists. However, this was beyond the scope of the present study and may be suggested for future work.

The M1 group contained mandarins predominantly classified as *C. reticulata*, a few Clementines and a Satsuma (Fig. 5.4.26.3). Four sub-groups could be distinguished from the dendrogram. Of these, clusters 1 and 2 occurred within the same clade while clusters 3 and 4 occurred in a separate clade. Of note was the observation that the Nova and its seedless counterpart, demonstrated a high degree of genetic similarity, as did the Mor cultivars. In addition, the Mor cultivars were closely related to 2PH. This was not surprising as the Mor cultivars were generated via an induced mutation of a Murcott tangor. It was also apparent that when the same genotype was represented in two samples that were named differently, for example B17 and Valley Gold, they proved to be genetically similar in the cluster analysis. Some of the cultivars with common parents were observed to group within the same cluster, e.g. Tahoe gold and Yosemite. It appeared that two of the Clementines, viz. Esbal and Hernandina, were genetically similar as evidenced by their position on the dendrogram. This might be related to the fact that both these cultivars were derived from a similar parent, i.e. *C. clementina* via bud mutation. Nules Clementine and Serilla appeared to be the most genetically divergent of the tested cultivars.

The M2 group contained a diverse mixture of mandarins. The cluster analysis indicated that Steynsburg occurred on its own with all of the other mandarins in a sub-group (Fig. 5.4.26.4). It was clear that the two lemons (Eureka and Limoneira) and grapefruits (Marsh and Nel Ruby) were genetically similar to each other with high bootstrap values (100). The Satsumas (Dobashi, Miyagawa Wase and Kuno) also

occurred within a group as similarly reported by Kacar *et al.* (2013). The Nadorcott and its seedless counterpart were also genetically similar.

The dendrogram for the M3 group (Fig. 5.4.26.5) indicated two main clusters. Within the first cluster, all of the Clementines grouped together, viz. Marisol, Saratoga Clementine, Orogrande and Clemenpons. There was also a suggestion of close genetic similarity between B17 and B18. Cluster 2 contained a mixture of Satsumas, *C. reticulata* and a tangelo. The Ellendales Leng and Herps were genetically similar with a high bootstrap value (100). Other studies investigating the genetic diversity of mandarin have also reported that the Clementines occurred together in a subgroup (Barkley *et al.*, 2006; Kacar *et al.*, 2013).

The M4 group contained predominantly Clementines and Satsumas with a few *C. reticulata* (Fig. 5.4.26.6). In a similar manner to that found with the M3 group, the Clementines in the M4 group displayed a similar genetic background. In an analogous manner, the Satsumas also occurred together in the same cluster. The only misnomer was that the Clementine Corsica 2 was found within the same clade as the Satsumas rather than with the other Clementines. The few *C. reticulata* present occurred in a group between the Clementines and Satsumas.

The M5 group contained only *C. reticulata* which could be separated into two main clusters (Fig. 5.4.26.7). The Micals were found to be genetically similar, as were the Mmans. However, the two Meravs were found to occur in different clusters. Steynsburg was the most genetically distant cultivar, an observation that was also apparent for Nules Clementine in Figs 5.4.26.3 and 5.4.26.5.

As mentioned above, combinations of the mandarin groups were investigated, the first being M1 and M2 (Fig. 5.4.26.8). Four groups could be distinguished with the M1 and M2 selections interspersed between the groups. As found with the cluster analysis for each group, the Clementines (except for Cami and SRA 63) occurred in close association within the first cluster. Similarly, the Satsumas displayed genetic relatedness by occurring within the second cluster. Within the third cluster, the two grapefruits showed a high degree of genetic similarity, as did the Novas. The fourth cluster contained most of the mandarins from the M1 group in addition to two lemons.

The dendrogram for the combination of M1 and M3 (Fig. 5.4.26.9) could be separated into two large clusters (1 and 2). Within the first cluster, the Clementines showed a high degree of genetic similarity as they occurred within the same sub-group. The rest of cluster 1 comprised the mandarins from M1. Cluster 2 contained the Satsumas and its hybrids, as well as two of the Ellendales. Valley gold, B17 and B18 occurred in a sub-group within the second cluster.

The combination of M1 and M4 (Fig. 5.4.26.10) showed separation into three clusters, with a number of genetically divergent outliers at the bottom of the dendrogram. Cluster 1 contained most of the Clementines while the second cluster contained the mandarin and Satsuma hybrids (except for the Clementines, Guillermina and SRA 63). The third cluster contained cultivars from the M1 group. The outliers were comprised of Clementines (Corsica 2 and Alkantara), Satsuma mandarins (Sugiyama, Ueno and Kiyomi) and a pigmented mandarin (Tacle).

The M1 and M5 combination (Fig. 5.4.26.11) could be separated into three clusters. Cluster 1 contained the three Clementines (except for SRA 63) as well as a mixture of mandarin hybrids and those selections made from progeny of Dancy (e.g. Tahoe gold and Tami). The second cluster contained the Satsuma Ayoshima and other mandarin hybrids while the third cluster comprised selections from M1.

The dendrogram for the M2 and M3 combination (Fig. 5.4.26.12) suggested two main groups, with most of the mandarin hybrids, Clementines, an Ellendale and the two grapefruit in the first group. In the second group were the two lemons, the remaining two Ellendales, most of the Satsumas and some mandarin hybrids. There appeared to be no clear genetic distinction between the groups.

In contrast, the combination of M2 and M4 suggested distinct separations (Fig. 5.4.26.13). In this regard, the mandarins and its hybrids occurred in the first cluster, the second cluster contained exclusively Clementines and the third cluster had predominantly Satsumas and the two lemons.

The M2 and M5 grouping is presented in Fig. 5.4.26.14. The first group contained selections predominantly from M2. The second cluster contained a mixture of mandarins and hybrids and the third cluster the two lemons and grapefruits along with selections derived from Dancy and a Satsuma.

The cluster analysis for the M3 and M4 groups showed that most of the Clementines (excluding Corsica 2 and Alkantara) occurred in cluster 1. The Satsumas were dispersed in a subgroup within cluster 1 and

in cluster 2. The dendrogram for the M3 and M5 grouping illustrated the genetic similarity amongst the Clementines in cluster 1. Cluster 2 was comprised predominantly of M5 mandarins while most of the Satsumas occurred in cluster 3.

The final grouping of M4 and M5 is presented in Fig 5.4.26.17. As noted previously, the Clementines occurred as a group in cluster 1, the Satsumas and some mandarin hybrids occurred in cluster 2 while clusters 3 and 4 contained selections predominantly from M5.

The analyses performed indicate a few interesting patterns, *viz.* that certain selections consistently occurred within groups, i.e. the Clementines and Satsumas. However, this observation cannot be thoroughly investigated due to the limitations encountered, i.e. the inability to generate a single dendrogram with all the mandarin cultivars. This is a significant drawback and requires further investigation.

Oranges

The oranges were separated into three groups containing 117 cultivars. The first group (O1) contained Navel selections. Results from preliminary studies indicated that the SSR markers tested could not efficiently discriminate amongst the cultivars. For this reason, SRAP markers were tested in addition to the SSR markers. The results for both marker types are presented. Fig. 5.4.26.18 illustrates the dendrogram for the O1 group using SSR markers, while Fig. 5.4.26.19 shows SRAP markers for the same tested samples. A common pattern observed for the oranges was that the CCC values for the SSR markers were consistently higher than that obtained with the SRAP markers (e.g. 0.972 with RMSD and MSD methods with SSR markers compared with 0.183 and 0.220 for the Jaccard and Dice methods with SRAP markers for the O1 samples). Furthermore, it was apparent that the clustering of cultivars was different with the SRAP and SSR markers. This observation is not uncommon as Meyer *et al.* (2004) also reported differences in the structures of dendrograms when different marker systems were used.

In the present study with the SSR cluster analysis (Fig. 5.4.26.18), the late season selections were interspersed throughout the dendrogram. However, with the SRAP analysis, a number of late selections occurred within the first group. In addition, the subgroup within the fourth cluster displayed predominantly early and/or midseason selections. It was apparent that the two B17 samples did not occur in close association with either method. This was unexpected and the reason for this needs to be resolved with further investigation. In addition, the low CCC values obtained with the SRAP markers needs to be addressed. A low CCC value is indicative of a poor fit between the original matrix and the cluster analysis method. This might indicate inconsistencies amongst groupings and is also suggestive of a complex pattern of many similarities between individuals, which was difficult for the clustering algorithm to resolve (Barnesky and Lammers, 1997).

The observation of the low CCC value proved to be a feature throughout the analyses performed with the SRAP markers. The value of SRAP markers to distinguish amongst *Citrus* cultivars is evident from the number of published reports on the successful implementation of the technology, e.g. Uzun *et al.* (2009; 2011), Amar *et al.* (2011), Gulsen *et al.* (2011), Amar (2012) and Kacar *et al.* (2013). Further research is needed to overcome the limitations imposed by the low CCC values obtained with the SRAP markers in the present study. An additional difficulty encountered with the SRAP markers was the inability to replicate results consistently. This represents a significant drawback to the implementation of SRAP marker technology in *Citrus* breeding. This requires further investigation as the true value of the technology to the *Citrus* industry can only be evaluated if results are replicable and verifiable. Therefore, the results presented need to be interpreted with caution for the abovementioned reasons.

A further factor that complicates the phylogeny of *Citrus* is that while high heterozygosity is evident, certain groups including sweet oranges, lemons, limes and grapefruit share similar genetic organisation with limited sequence variation (Novelli *et al.*, 2006). This is particularly evident amongst cultivars that are known to have arisen via mutations, e.g. sweet oranges that mutated from the original sweet orange domesticate.

The O2 group was comprised predominantly of Valencia oranges. Of note for both the methods, is that the various Midnight selections were dispersed throughout the dendrograms (Figs 5.4.26.20 and 21). The third group of oranges (O3) represented the pigmented or blood orange selections. This group comprised Tarocco selections, a Sunstar and 1528, the latter of which represents a lemon.

The combination of O1 and O3 produced dendrograms that distinguished the two groups into separate clades, irrespective of the marker system used (Fig. 5.4.26.24 and 25). Overall, the navels could be distinguished from the blood oranges with either marker system. This is in contrast to work reported by

Debbabi *et al.* (2014) that showed an inability to distinguish blood oranges from navel and blonde oranges using AFLP markers. Figures 5.4.26.26 and 27 illustrate the dendrograms for the O2 and O3 combination. With the SSR markers (Fig. 5.4.26.26), most of the O2 selections were present in the first cluster, except for Alpha Rietspruit Valencia, Jincheng, Judas and Midnight 3F17.

The O3 group also presented in a single cluster except for Tarocco Ippolito. The cluster analysis for the SRAP markers were slightly different (Fig. 5.4.26.27) as most of the O2 and O3 selections separated into distinct groups, excluding Jassie, Weipe and Pera22 which grouped with the blood oranges. Overall, the results suggest the ability to distinguish between the groups of oranges but discrimination within groups remains uncertain. However, the inability to represent all of the oranges on a single dendrogram limits the abovementioned suggestion.

Conclusion

The present work used molecular tools (SSR and SRAP markers) to establish a molecular verification database for *Citrus*. The use of EST markers as per one of the objectives was not explored as it is a much more complicated method than the SSR method. To date, a molecular database has been established, but with some limitations that will hinder application of the technology. In this regard, the SSR markers appeared to be promising to distinguish between groups of *Citrus*, however, difficulties were encountered in distinguishing between the sweet oranges. The application of an additional marker system (i.e. SRAP marker) did not resolve this problem due to limitations in the method and an inability to replicate results. Further refinement of the SRAP system is necessary in order to fully evaluate the potential application of this technology to the *Citrus* industry. At present, the ability to confidently discriminate amongst *Citrus* cultivars is limited, until further refinements can be made.

Future research

The results from the present study identified a number of avenues for future research. Further research is required to optimise the SRAP protocol to improve CCC values and determine the potential of this method to discriminate between the sweet orange accessions. Alternatively, if SRAP marker technology proves inadequate, then other methods could be investigated. In addition, inconsistencies in the dataset need to be resolved, for example, the observation of two B17 samples occurring in different clusters on the same dendrogram. It is also imperative to generate dendrograms that can accommodate all the selections within the specified groups. Furthermore, it would be very informative to produce a single dendrogram with all the *Citrus* groups to establish the genetic diversity represented within the germplasm collection. Clearly, further investigations are required to realise the potential of molecular marker technology to the *Citrus* industry.

Table 5.4.26.1. List of CIS Citrus cultivars included in the genotyping verification database.

Document Ref	Citrus Type/Species	Cultivar/Variety Group	Cultivar/Variety	Status of Release to the CFB	Development Status				CFB Budwood Production Category
					Experimental	Potential	Semi-commercial Semi-kommersteel	Commercial	
1	Mandarin	Clementine	Alkantara (C 2191)	Final	Y				4
2	Mandarin	Clementine	Andes 1 - Clemenluz	Provisional	Y				4
3	Mandarin	Clementine	Basol	Final	Y				4
4	Mandarin	Clementine	C 1867	Final	Y				4
5	Mandarin	Clementine	Cami	Final	Y				4
6	Mandarin	Clementine	Clemenpons 2	Final	Y				4
8	Mandarin	Clementine	Corsica 2	Final	Y				4
9	Mandarin	Clementine	Early	Final	Y				4
10	Mandarin	Clementine	Esbal	Final				Y	3
11	Mandarin	Clementine	Guillermina	Final	Y				5
12	Mandarin	Clementine	Hernandina	Final	Y				5
13	Mandarin	Clementine	LL	Final	Y				5
14	Mandarin	Clementine	Mandared (C1732)	Final	Y				4
15	Mandarin	Clementine	Marisol	Final				Y	4
16	Mandarin	Clementine	Nour	Final	Y				4
17	Mandarin	Clementine	Nules	Final				Y	2
18	Mandarin	Clementine	Orogrande	Final	Y				4
19	Mandarin	Clementine	Oronules	Final			Y		4
20	Mandarin	Clementine	Oroval	Final				Y	4
21	Mandarin	Clementine	Saratoga	Final	Y				4
22	Mandarin	Clementine	SRA 63	Final			Y		3
23	Mandarin	Clementine	Tardif de de Mars (LL)	Final	Y				5
24	Mandarin	Clementine	Tardif de Janvier I	Final	Y				4
25	Diverse	Diverse	Bergamot Addo	Final	Y				4
26	Diverse	Diverse	Bergamot Messina	Final	Y				4
27	Diverse	Diverse	Calamondin Green	Provisional	Y				4
28	Diverse	Diverse	Calamondin Variegated	Provisional		Y			4
29	Diverse	Diverse	Chinotto	Provisional		Y			4
30	Diverse	Diverse	Citrus Junos	Provisional	Y				4
31	Diverse	Diverse	Fingered Citron (Buddha's Hand)	Final	Y				4
32	Diverse	Diverse	Fukusan (Seleksie A)	Provisional	Y				4
33	Diverse	Diverse	Messina Bitter Seville	Provisional		Y			4
34	Diverse	Diverse	Thai Lime	Provisional		Y			4
35	Mandarin	Ellendale	Herps	Final		Y			5
36	Mandarin	Ellendale	Leng	Final		Y			5
37	Mandarin	Ellendale	Nouvelle	Final			Y		4

38	Grapefruit	Grapefruit	Flame	Final				Y	5
39	Grapefruit	Grapefruit	Henderson 17 (RedHeart)	Final				Y	2
40	Grapefruit	Grapefruit	Jackson 1	Provisional		Y			4
41	Grapefruit	Grapefruit	Jackson 2	Provisional	Y				4
42	Grapefruit	Grapefruit	Jackson Seedless	Final	Y				4
43	Grapefruit	Grapefruit	Marsh	Final				Y	3
44	Grapefruit	Grapefruit	Marsh (Bolton Estates)	Final	Y				5
45	Grapefruit	Grapefruit	Nartia	Final		Y			3
46	Grapefruit	Grapefruit	Nelruby	Final				Y	3
47	Grapefruit	Grapefruit	Rio Red	Final		Y			5
48	Grapefruit	Grapefruit	Rosé (Redblush)	Final				Y	4
49	Grapefruit	Grapefruit	Star Ruby	Final				Y	1
50	Grapefruit	Grapefruit	Star Ruby Late	Final	Y				4
51	Grapefruit	Grapefruit	XTG 999	Final	Y				5
52	Diverse	Kumquat	Nagami	Final				Y	3
53	Lemon	Lemon	2PH SL Eureka (QDPI #291)	Final	Y				3
54	Lemon	Lemon	Elongated Eureka	Final	Y				4
55	Lemon	Lemon	Eureka	Final				Y	1
56	Lemon	Lemon	Eureka SL	Final				Y	3
57	Lemon	Lemon	Eureka SL Israel	Final	Y				5
58	Lemon	Lemon	Femminello SL	Provisional	Y				4
59	Lemon	Lemon	Genoa	Final				Y	2
60	Lemon	Lemon	Lemox (Triploid)	Final	Y				4
61	Lemon	Lemon	Limoneira 8A	Final				Y	1
62	Lemon	Lemon	Lisbon	Final				Y	1
63	Lemon	Lemon	Lisbon Yen Ben	Final	Y				4
64	Lemon	Lemon	Messina	Final	Y				5
65	Lemon	Lemon	Roos	Provisional	Y				4
66	Lemon	Lemon	Willow Tree Long	Final	Y				4
67	Lemon	Lime	Bearss	Final				Y	3
68	Lemon	Lime	ITSG West Indian (CSFRI)	Final	Y				5
69	Lemon	Lime	Limequat	Final		Y			5
70	Lemon	Lime	West Indian Key	Final				Y	4
71	Mandarin	Mandarin Hybrid	2PH Low Seed Murcott	Final	Y				3
72	Mandarin	Mandarin Hybrid	A3	Final	Y				4
73	Mandarin	Mandarin Hybrid	ALG Early Minneola	Final	Y				4
74	Mandarin	Mandarin Hybrid	Ambersweet	Final	Y				5
75	Mandarin	Mandarin Hybrid	B17 (Valley Gold) (GFMS12)	Final				Y	1
76	Mandarin	Mandarin Hybrid	B18	Final	Y				4
77	Mandarin	Mandarin Hybrid	B24 (African Sunset)	Final				Y	2
78	Mandarin	Mandarin Hybrid	Baygold	Final	Y				4
79	Mandarin	Mandarin Hybrid	C27	Final	Y				4
80	Mandarin	Mandarin Hybrid	Clara	Final	Y				5
81	Mandarin	Mandarin Hybrid	Clem x Murcott	Final		Y			4
82	Mandarin	Mandarin Hybrid	Edit x Nova	Final	Y				4
83	Mandarin	Mandarin Hybrid	Empress Mandarin	Final				Y	2
84	Mandarin	Mandarin Hybrid	Encore	Final	Y				5
85	Mandarin	Mandarin Hybrid	Etna	Final	Y				4

86	Mandarin	Mandarin Hybrid	Fairchild	Provisional	Y	4
87	Mandarin	Mandarin Hybrid	Gold Nugget	Final	Y	4
88	Mandarin	Mandarin Hybrid	Hadas	Final	Y	4
89	Mandarin	Mandarin Hybrid	Honey Gold (I22)	Final	Y	4
90	Mandarin	Mandarin Hybrid	IR M1 (QDPI #237)	Final	Y	4
91	Mandarin	Mandarin Hybrid	IR M2 (QDPI #283)	Final	Y	4
92	Mandarin	Mandarin Hybrid	Irradiated I22	Final	Y	4
93	Mandarin	Mandarin Hybrid	Kedem	Final	Y	4
94	Mandarin	Mandarin Hybrid	Mandalate (D8811)	Final	Y	4
95	Mandarin	Mandarin Hybrid	Merav 119	Final	Y	4
96	Mandarin	Mandarin Hybrid	Merav 63	Final	Y	4
97	Mandarin	Mandarin Hybrid	MH7	Final	Y	4
98	Mandarin	Mandarin Hybrid	Michal 6/47	Final	Y	4
99	Mandarin	Mandarin Hybrid	Michal 69/64	Final	Y	4
100	Mandarin	Mandarin Hybrid	Minneola x Temple	Final	Y	5
101	Mandarin	Mandarin Hybrid	Mman 1	Provisional	Y	4
102	Mandarin	Mandarin Hybrid	Mman 2	Provisional	Y	4
103	Mandarin	Mandarin Hybrid	Monica	Final	Y	4
104	Mandarin	Mandarin Hybrid	Mor 15	Final	Y	5
105	Mandarin	Mandarin Hybrid	Mor 2	Final	Y	4
106	Mandarin	Mandarin Hybrid	Mor 22	Final	Y	5
107	Mandarin	Mandarin Hybrid	Mor 25	Final	Y	4
108	Mandarin	Mandarin Hybrid	Mor 26	Final	Y	2
109	Mandarin	Mandarin Hybrid	Murcott Seedless	Final	Y	4
110	Mandarin	Mandarin Hybrid	Nadorcott 1	Final	Y	1
111	Mandarin	Mandarin Hybrid	Nadorcott ARC	Final	Y	4
112	Mandarin	Mandarin Hybrid	Nadorcott Seedless	Final	Y	4
113	Mandarin	Mandarin Hybrid	Nova	Final	Y	2
114	Mandarin	Mandarin Hybrid	Nova ARC	Final	Y	2
115	Mandarin	Mandarin Hybrid	Or 4	Final	Y	3
116	Mandarin	Mandarin Hybrid	Oranique Tangor	Final	Y	3
117	Mandarin	Mandarin Hybrid	Red Tangerine	Provisional	Y	4
118	Mandarin	Mandarin Hybrid	Reina	Final	Y	4
119	Mandarin	Mandarin Hybrid	Roma	Final	Y	5
120	Mandarin	Mandarin Hybrid	Shani Seedless	Final	Y	4
121	Mandarin	Mandarin Hybrid	Shasta Gold	Final	Y	4
122	Mandarin	Mandarin Hybrid	Sirio	Final	Y	4
123	Mandarin	Mandarin Hybrid	Sunset	Final	Y	4
124	Mandarin	Mandarin Hybrid	Sweet Spring	Final	Y	4
125	Mandarin	Mandarin Hybrid	Tacle	Final	Y	4
126	Mandarin	Mandarin Hybrid	Tahoe Gold	Final	Y	4
127	Mandarin	Mandarin Hybrid	Tami 2/65	Final	Y	4
128	Mandarin	Mandarin Hybrid	Tango	Final	Y	4
129	Mandarin	Mandarin Hybrid	Tasty 1	Provisional	Y	4
130	Mandarin	Mandarin Hybrid	Tasty 2	Provisional	Y	4
131	Mandarin	Mandarin Hybrid	Thoro Temple 2	Provisional	Y	4
132	Mandarin	Mandarin Hybrid	Winola	Final	Y	4
133	Mandarin	Mandarin Hybrid	Worcester	Provisional	Y	4

134	Mandarin	Mandarin Hybrid	Yosemite Gold	Final	Y	4
135	Orange	Midseason	Clanor	Final	Y	5
136	Orange	Midseason	Hamlin	Provisional	Y	4
137	Orange	Midseason	Mallorca	Final	Y	4
138	Orange	Midseason	Pera 222	Provisional	Y	4
139	Orange	Midseason	Premier	Provisional	Y	3
140	Orange	Midseason	Salustiana	Final	Y	5
141	Orange	Midseason	Shamouti 2	Provisional	Y	4
142	Orange	Midseason	Sunstar	Final	Y	4
143	Orange	Midseason	Tarocco	Final	Y	4
144	Orange	Midseason	Tarocco #1	Final	Y	4
145	Orange	Midseason	Tarocco #2	Final	Y	4
146	Orange	Midseason	Tarocco #3	Final	Y	4
147	Orange	Midseason	Tarocco #4	Provisional	Y	4
148	Orange	Midseason	Tarocco #5	Final	Y	4
149	Orange	Midseason	Tarocco #6	Final	Y	4
150	Orange	Midseason	Tarocco 57/1E/1	Final	Y	4
151	Orange	Midseason	Tarocco Gabella	Final	Y	4
152	Orange	Midseason	Tarocco Gallo	Final	Y	4
153	Orange	Midseason	Tarocco Ippolito	Final	Y	4
154	Orange	Midseason	Tarocco Meli Nuc. C8158	Provisional	Y	4
155	Orange	Midseason	Tarocco Messina Nuc. C1635	Provisional	Y	4
156	Orange	Midseason	Tarocco Rosso VCR	Provisional	Y	4
157	Orange	Midseason	Tarocco Sant' Alfio	Final	Y	4
158	Orange	Midseason	Tarocco Scire	Final	Y	4
159	Orange	Midseason	Tarocco Scire (Nuc)	Final	Y	4
160	Orange	Midseason	Tarocco Tapi	Final	Y	4
161	Orange	Midseason	Tarocco TDV Nuc.	Provisional	Y	4
162	Orange	Navel	1203	Provisional	Y	4
163	Orange	Navel	6/1997	Provisional	Y	4
164	Orange	Navel	ALG	Final	Y	5
165	Orange	Navel	Atwood	Final	Y	4
166	Orange	Navel	Autumn Gold	Final	Y	2
167	Orange	Navel	Bahianinha	Final	Y	1
168	Orange	Navel	Barnfield Summer	Final	Y	4
169	Orange	Navel	Cambria 3	Final	Y	1
170	Orange	Navel	Cambria 4 (K-Tak)	Provisional	Y	3
171	Orange	Navel	Cambria R2	Final	Y	3
172	Orange	Navel	Cara Cara	Final	Y	3
173	Orange	Navel	Carninka	Final	Y	3
174	Orange	Navel	Chislett M7	Provisional	Y	1
175	Orange	Navel	Chislett Summer	Final	Y	3
176	Orange	Navel	Clarke	Final	Y	4
177	Orange	Navel	Coetzee Late	Final	Y	4
179	Orange	Navel	Dairy Lina	Provisional	Y	4
180	Orange	Navel	Davey	Provisional	Y	4
181	Orange	Navel	Dream	Final	Y	4
182	Orange	Navel	Early Lina	Provisional	Y	4

183	Orange	Navel	EDP1 (5022)	Provisional	Y	4
184	Orange	Navel	EDP2 (5056)	Provisional	Y	4
185	Orange	Navel	Fischer	Final	Y	3
186	Orange	Navel	Fukumoto	Final	Y	3
187	Orange	Navel	Fukumoto 2	Provisional	Y	4
188	Orange	Navel	Glenora Late	Final	Y	3
189	Orange	Navel	Gloudie	Final	Y	3
190	Orange	Navel	Golden Buckeye	Final	Y	4
191	Orange	Navel	Hutton	Final	Y	4
192	Orange	Navel	Kakamas Laat	Provisional	Y	4
193	Orange	Navel	Kirkwood Red	Final	Y	3
194	Orange	Navel	Kloof Bo	Provisional	Y	4
195	Orange	Navel	Krajewski Early	Final	Y	4
196	Orange	Navel	Lane Late Cal.	Final	Y	2
197	Orange	Navel	Lazyboy 1	Provisional	Y	4
198	Orange	Navel	Leng	Final	Y	4
199	Orange	Navel	Letaba Early	Final	Y	4
200	Orange	Navel	Lina	Final	Y	2
201	Orange	Navel	Navelina	Final	Y	4
202	Orange	Navel	Newhall	Final	Y	2
203	Orange	Navel	Painter Early	Provisional	Y	4
204	Orange	Navel	Palmer	Final	Y	1
205	Orange	Navel	Patensie (Sel A) (5008)	Provisional	Y	4
206	Orange	Navel	Powell Summer	Final	Y	3
207	Orange	Navel	Rayno Early	Final	Y	4
208	Orange	Navel	Robyn 2	Provisional	Y	4
209	Orange	Navel	Santa Catarina # 1	Final	Y	5
210	Orange	Navel	Santa Catarina # 3	Final	Y	5
211	Orange	Navel	Summer Gold	Final	Y	4
212	Orange	Navel	Tulegold	Final	Y	5
213	Orange	Navel	Washington	Final	Y	1
214	Orange	Navel	Waterwel	Provisional	Y	4
215	Orange	Navel	Witkrans 3	Final	Y	1
216	Orange	Navel	Xgreenwash	Final	Y	5
217	Pummelo	Pummelo	Kukemoes	Final	Y	4
218	Pummelo	Pummelo	Pomelit-2	Final	Y	4
219	Rootstock	Rootstock	1386	Final	Y	5
220	Rootstock	Rootstock	1394	Final	Y	5
221	Rootstock	Rootstock	1207 (Sour orange hybrid NTO (1002))	Final	Y	5
222	Rootstock	Rootstock	1390 (Sour orange x Djeroek keprok) x Cal rough lemon)	Final	Y	5
223	Rootstock	Rootstock	1391 (Trifoliolate 39 x Marsh)	Final	Y	5
224	Rootstock	Rootstock	1392 (Triumph 95 x Corsican 150)	Final	Y	5
225	Rootstock	Rootstock	1431 (Rough Lemon unknown)	Final	Y	5
226	Rootstock	Rootstock	530 A (Ruby grapefruit)	Final	Y	5
227	Rootstock	Rootstock	598 (Rough lemon B)	Final	Y	5
228	Rootstock	Rootstock	AFRICAN SHADDOCK x RUBIDOUX TRIF	Final	Y	5

229	Rootstock	Rootstock	Benton Citrange	Final	Y	2
230	Rootstock	Rootstock	C. MACROPHYLLA PAPEDA	Final	Y	5
231	Rootstock	Rootstock	C35 Citrange	Final	Y	1
232	Rootstock	Rootstock	Carrizo Citrange 669	Final	Y	4
233	Rootstock	Rootstock	Carrizo Citrange	Final	Y	1
234	Rootstock	Rootstock	Changsa Mandarin	Final	Y	5
235	Rootstock	Rootstock	Cleopatra Mandarin	Final	Y	3
236	Rootstock	Rootstock	F80/3 Citrange	Final	Y	4
237	Rootstock	Rootstock	F80/9 Citrange	Final	Y	5
238	Rootstock	Rootstock	Flying Dragon	Final	Y	5
239	Rootstock	Rootstock	GRIFIN 09	Final	Y	5
240	Rootstock	Rootstock	Koetha Citrange	Final	Y	4
241	Rootstock	Rootstock	Macrophylla	Provisional	Y	4
242	Rootstock	Rootstock	Minneola x Trifoliata	Final	Y	3
243	Rootstock	Rootstock	MORTON CITRANGE	Final	Y	5
244	Rootstock	Rootstock	Poorman x Trifoliata	Final	Y	5
245	Rootstock	Rootstock	Rangpur Lime	Final	Y	5
246	Rootstock	Rootstock	Red Tangerine			
247	Rootstock	Rootstock	Rough Lemon (Cairn)	Final	Y	1
248	Rootstock	Rootstock	Rough Lemon (Schaub)	Final	Y	4
249	Rootstock	Rootstock	Rubidoux Trifoliata	Final	Y	5
250	Rootstock	Rootstock	Sour Orange Hybrid	Final	Y	4
251	Rootstock	Rootstock	Sour orange Hybrid 1206	Final	Y	5
252	Rootstock	Rootstock	Sun Chu Sha	Final	Y	5
253	Rootstock	Rootstock	Sunki x Beneke	Final	Y	2
254	Rootstock	Rootstock	Sweet Rough Lemon 72	Final	Y	5
255	Rootstock	Rootstock	Sweet Rough Lemon	Final	Y	4
256	Rootstock	Rootstock	Swingle Citrumelo	Final	Y	2
257	Rootstock	Rootstock	Tabtha 109 T	Final	Y	5
258	Rootstock	Rootstock	Troyer Citrange	Final	Y	3
259	Rootstock	Rootstock	Troyer Citrange 609	Final	Y	4
260	Rootstock	Rootstock	Troyer Citrange 717	Final	Y	5
261	Rootstock	Rootstock	Vangasay Rough Lemon	Final	Y	5
262	Rootstock	Rootstock	Volkameriana	Final	Y	3
263	Rootstock	Rootstock	X639	Final	Y	1
264	Rootstock	Rootstock	Yuma Citrange	Final	Y	3
265	Rootstock	Rootstock	Yuma Ponderosa Lemon	Final	Y	5
266	Mandarin	Satsuma	Aoshima	Final	Y	4
267	Mandarin	Satsuma	Belalate	Provisional	Y	4
268	Mandarin	Satsuma	Dobashi Beni	Final	Y	4
269	Mandarin	Satsuma	Imamura	Final	Y	4
270	Mandarin	Satsuma	Kuno	Provisional	Y	4
271	Mandarin	Satsuma	Miho Wase	Provisional	Y	1
272	Mandarin	Satsuma	Miyagawa Wase	Final	Y	4
273	Mandarin	Satsuma	Okitsu Wase	Final	Y	5
274	Mandarin	Satsuma	Owari	Final	Y	3
275	Mandarin	Satsuma	Primosole	Final	Y	4
276	Mandarin	Satsuma	Sonet	Final	Y	2

277	Mandarin	Satsuma	Sonet 2	Provisional	Y	4
278	Mandarin	Satsuma	Sugiyama	Final	Y	4
279	Mandarin	Satsuma	Ueno	Final	Y	4
280	Mandarin	Satsuma	WH Satopus	Final	Y	4
281	Orange	Valencia	AB Seedless	Provisional	Y	4
282	Orange	Valencia	Alpha	Final	Y	3
283	Orange	Valencia	Bend 8A 2	Final	Y	5
284	Orange	Valencia	Benny 2	Final	Y	1
285	Orange	Valencia	Delicia	Final	Y	4
286	Orange	Valencia	Delta	Final	Y	2
287	Orange	Valencia	Du Roi 2	Final	Y	2
288	Orange	Valencia	G5	Final	Y	2
289	Orange	Valencia	Henrietta	Final	Y	3
290	Orange	Valencia	Jassie	Final	Y	3
291	Rootstock	Valencia	Jincheng			
292	Orange	Valencia	Judas	Final	Y	4
293	Orange	Valencia	Kobus du Toit Late	Provisional	Y	4
294	Orange	Valencia	Late	Final	Y	1
295	Orange	Valencia	Lavalle	Final	Y	1
296	Orange	Valencia	Lavalle 2	Final	Y	3
297	Orange	Valencia	Louisa (Letaba oranje)	Final	Y	3
298	Orange	Valencia	Marss	Provisional	Y	4
299	Orange	Valencia	McClellan	Final	Y	3
300	Orange	Valencia	McClellan Seedless	Final	Y	3
301	Orange	Valencia	Midnight	Final	Y	1
302	Orange	Valencia	Midnight 1 (I15)	Final	Y	2
303	Orange	Valencia	Midnight 2 (H14)	Final	Y	4
304	Orange	Valencia	Midnight 3 (F17)	Final	Y	4
305	Orange	Valencia	Midnight Friedenheim 1	Final	Y	4
306	Orange	Valencia	Midnight Friedenheim 2	Provisional	Y	4
307	Orange	Valencia	Millennium 24	Removed	Y	4
308	Orange	Valencia	Moosrivier Late 1	Final	Y	4
309	Orange	Valencia	Moosrivier Late 2	Final	Y	4
310	Orange	Valencia	Nouvelle la Cotte	Final	Y	4
311	Orange	Valencia	Rhode Red	Final	Y	5
312	Orange	Valencia	Ruby	Final	Y	4
313	Orange	Valencia	Skilderkrans	Final	Y	4
314	Orange	Valencia	Swartvlei	Final	Y	4
315	Orange	Valencia	Turkey	Final	Y	2
316	Orange	Valencia	Valearly (Mouton Early)	Provisional	Y	4
317	Orange	Valencia	Weipe	Provisional	Y	4

Table 5.4.26.2. List of SSR primers tested.

No.	Code	Primer name	Forward	Reverse
1	TAA1	TAA 1	5' GAC AAC ATC AAC AAC AGC AAG AGC 3'	5' AAG AAG AAG AGC CCC ATT AGC 3'
2	TAA15	TAA 15	F -5' GAA AGG GTT ACT TGA CCA GGC 3'	R -5' CTT CCC AGC TGC ACA AGC 3'
3	TAA 41	TAA 41	F -5' AGG TCT ACA TTG GCA TTG TC 3'	R -5' ACA TGC AGT GCT ATA ATG AAT G 3'
4	GT 03	GT 03	F -5' GCC TTC TTG ATT TAC CGG AC 3'	R -5' TGC TCC GAA CTT CAT CAT TG 3'
5	CiB	Ci01C07	F -5' GTC ACT CAC TCT CGC TCT TG 3'	R -5' TTG CTA GCT GCT TTA ACT TT 3'
6	CiC	Ci01C09	F -5' GAC AGA ATG GGA GAG GAG A 3'	R -5' TTG TCC CTT CCC TTT GTA 3'
7	CiI	Ci07C07	F -5' TAT CCA GTT TGT AAA TGA G 3'	R -5' TGA TAT TTG ATT AGT TTG G 3'
8	CiL	Ci02F07	F -5' GCA GCG TTT GTT TTC T 3'	R -5' TGC TGG TTT TCA GAT ACT T 3'
9	CiM	Ci06A05b	F -5' TCT CTG GTT GGT TTT TGT GA 3'	R -5' ATG ATG AAA AGC AAG GGG 3'
10	CiO	mCrCIR07D06	F -5' CCT TTT CAC AGT TTG CTA T 3'	R -5' TCA ATT CCT CTA GTG TGT GT 3'
11	CiP	Ci02B07	F -5' CAG CTC AAC ATG AAA GG 3'	R -5' TTG GAG AAC AGG ATG G 3'
12	CiR	Ci07E06	F -5' AAT AAA CGC CCA CCT GAG AC	R -5' CAG TTG TTA AAA GGG AAG AAT GAA
13	CiU	Ci08C05	F -5' TCC ACA GAT TGC CCA TTA 3'	R -5' CCC TAA AAA CCA AGT GAC A 3'
14	mCA	mCrCIR01B02	F -5' TCA ACT TCT CTG GTC TCT C 3'	R -5' TTA GCA ATA TCA ACA TCA T 3'
15	mCE	mCrCIR01F04a	F -5' AAG CAT TTA GGG AGG GTC ACT 3'	R -5' TGC TGC TGC TGT TGT TGT TCT 3'
16	mCI	mCrCIR06A08	F -5' TTT TTG TTA TGG TGT TCG TTG TT 3'	R -5' TGG TAT TAT TTT GTC ATT CAT TTG 3'
17	mCF	mCrCIR01F08a	F -5' ATG AGC TAA AGA GAA GAG G 3'	R -5' GGA CTC AAC ACA ACA CAA 3'
18	mCK	mCrCIR06B05	F -5' GAA CGA TGG AAT GAA GTG 3'	R -5' ATG TTG ATT ACG AGA CCT T 3'
19	mCN	mCrCIR07B05	F -5' TTT GTT CTT TTT GGT CTT TT 3'	R -5' CTT TTC TTT CCT AGT TTC CC 3'
20	mCQ/SR12	mCrCIR07E12	F -5' TGAGTCAAAGCATCAC 3'	R -5' TCTATGATTCTGACTTTA 3'
21	mCU	mCrCIR08B08	F -5' TTC CGT AGA TTC CAA AGT G 3'	R -5' GTC CAA GGT CAA CAA CAA G
22	SR 6	mCrCIR07D06	F -5' CCT TTT CAC AGT TTG CTA T 3'	R -5' TCA ATT CCT CTA GTG TGT GT 3'
23	SR 9	mCrCIR03D12a	F -5' GCC ATA AGC CCT TTC T 3'	R -5' CCC ACA ACC TCA CC 3'
24	SR 17	Mest 132	F -5' TTA TTT CCT TTG ACG GTG GG 3'	R -5' TTC TTT GGA GCC GAA CAA CT 3'
25	SCM 02	SCM 02	5'GAA TGG CTT AGA TGA CAA A 3'	5' ATT CAC AAA CGA AAC ACT-3'
26	SCM 05	SCM 05	5'CAG CTA CTA TCA GAA AAA TAA TCA G 3'	5'-GCA CAA AGA GAA AAA GGC-3'
Additional Sweet Orange primers				
27	CCSM13	CCSM13	F -5' CTAGAGCCGAATTCACC 3'	R -5' AACAGTACCAAGACACC 3'
28	CCSM17	CCSM17	F -5' ACATGGACAGGACAACAACTAAG 3'	R -5' GTTATGATACGTCTGTGTCC 3'
29	CCSM18	CCSM18	F -5' AA CAG TTG ATG AAG AGG AAG 3'	R -5' GTG ATT GCT GGT GTC GTT 3'
30	CCSM147	CCSM147	F -5' AGA CTC ACG TAA CCT ACT TC 3'	R -5' GCT ATG TTA TGA TAC GTC TG 3'
Additional Rootstock Primers				
31	Nem1	7A4(1407)	F -5' GTT GGA AAG ACG ACT CTG TTA 3'	R -5' CAA ACA GAA CAT TAC TTT TCA ATC 3'
32	Nem 2	4L17R	F -5' GAT GTA GGG GTC TTT ATG TAA 3'	R -5' GTC GCT TTG CAT GTT GTA 3'
33	Nem 3	4L17L	F -5' TAG AAA CCC CAA TTC AAA AG 3'	R -5' GAC TTG AGA AGC AGA GAA TGT C 3'
34	RP1	Ci03C08	F -5' CAGAGACAGCCAAGAGA 3'	R -5' GCTTCTTACATTCTCAAA 3'
35	RP2	Ci02D09	F -5' AATGATGAGGGTAAAGATG 3'	R -5' ACCCATCACAAAACAGA 3'
36	RP3	Ci02D04B	F -5' CTCTCTTTCCCATAGTA 3'	R -5' AGCAAACCCACAAAC 3'
37	RP4	Ci02G12	F -5' AAACCGAAATACAAGAGTG 3'	R -5' TCCACAAACAATACAACG 3'
38	RP5	Ci02A09	F -5' ACAGAAGGTAGTATTTTAGGG 3'	R -5' TTGTTTGGATGGGAAG 3'
39	RP6	Ci03D12a	F -5' GCCATAAGCCCTTCT 3'	R -5' CCCACAACCATCACC 3'
40	RP7	Ci03G05	F -5' CCACACAGGCAGACA 3'	R -5' CTTGGAGGAGCTTTAC 3'
41	RP8	MEST458	F -5' CCCCTCTTTTCTCTTCCA 3'	R -5' TTCTGGGCTGGTAGGTTTCCAG 3'
42	RP9	MEST121	F -5' TCCTATCATCGCAACTTC 3'	R -5' CAATAATGTTAGGCTGGATGGA 3'
43	RP10	MEST 431	F -5' GAGCTCAAACAATAGCCGC 3'	R -5' CATACTCCCGTCCATCTA 3'

Table 5.4.26.3. List of SRAP primers tested.

Primer	Forward primers	Primer	Reverse primers
ME1	TGAGTCCAAACCGGATA	EM1	GACTGCGTACGAATTAAT
ME2	TGAGTCCAAACCGGAGC	EM2	GACTGCGTACGAATTTGC
ME3	TGAGTCCAAACCGGAAT	EM3	GACTGCGTACGAATTGAC
ME4	TGAGTCCAAACCGGACC	EM4	GACTGCGTACGAATTTGA



Figure 5.4.26.2. UPGMA dendrogram for lemons (CCC = 0.996, MSD).

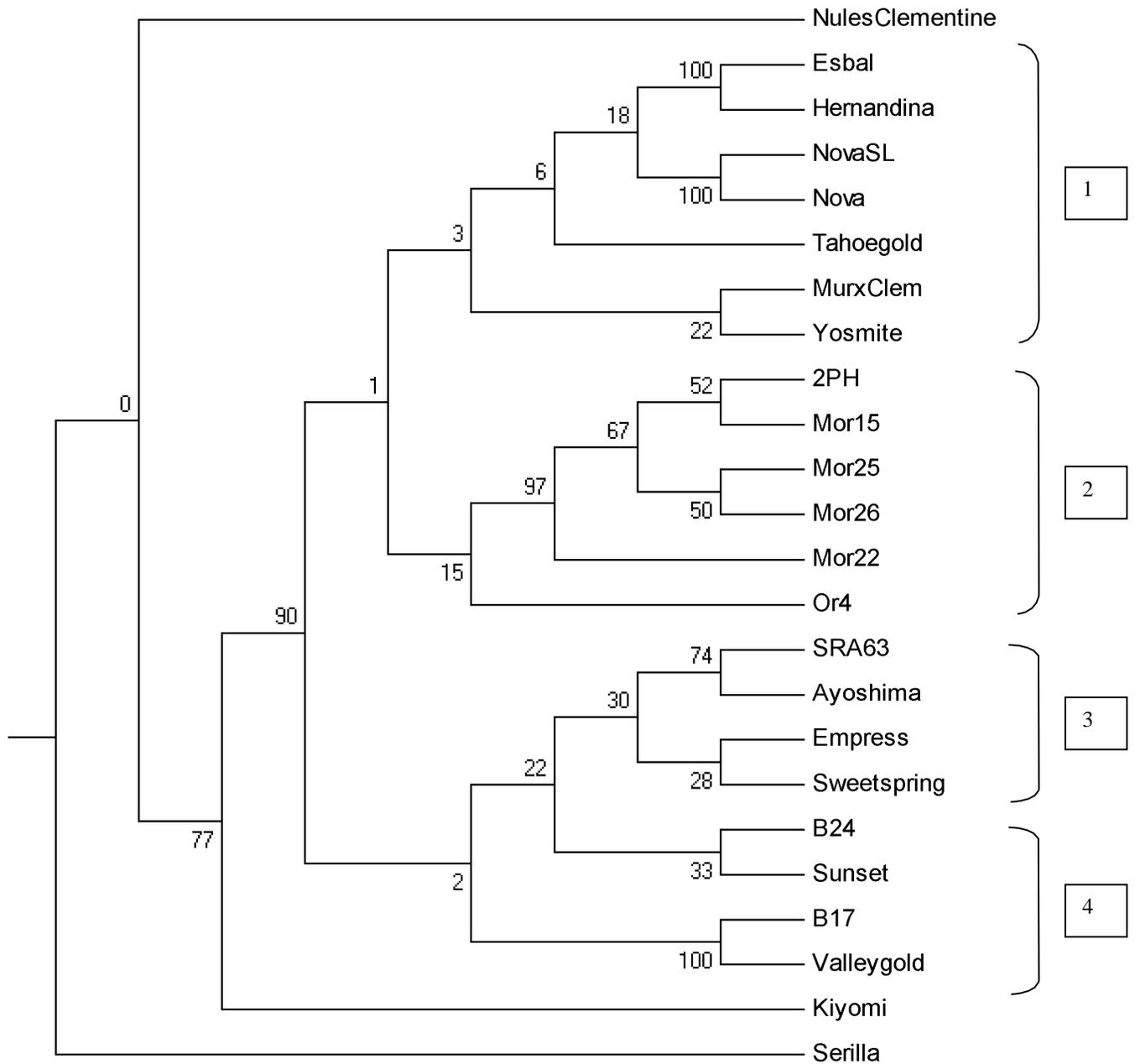


Figure 5.4.26.3. UPGMA dendrogram for mandarins group M1 (CCC = 0.988, MSD).

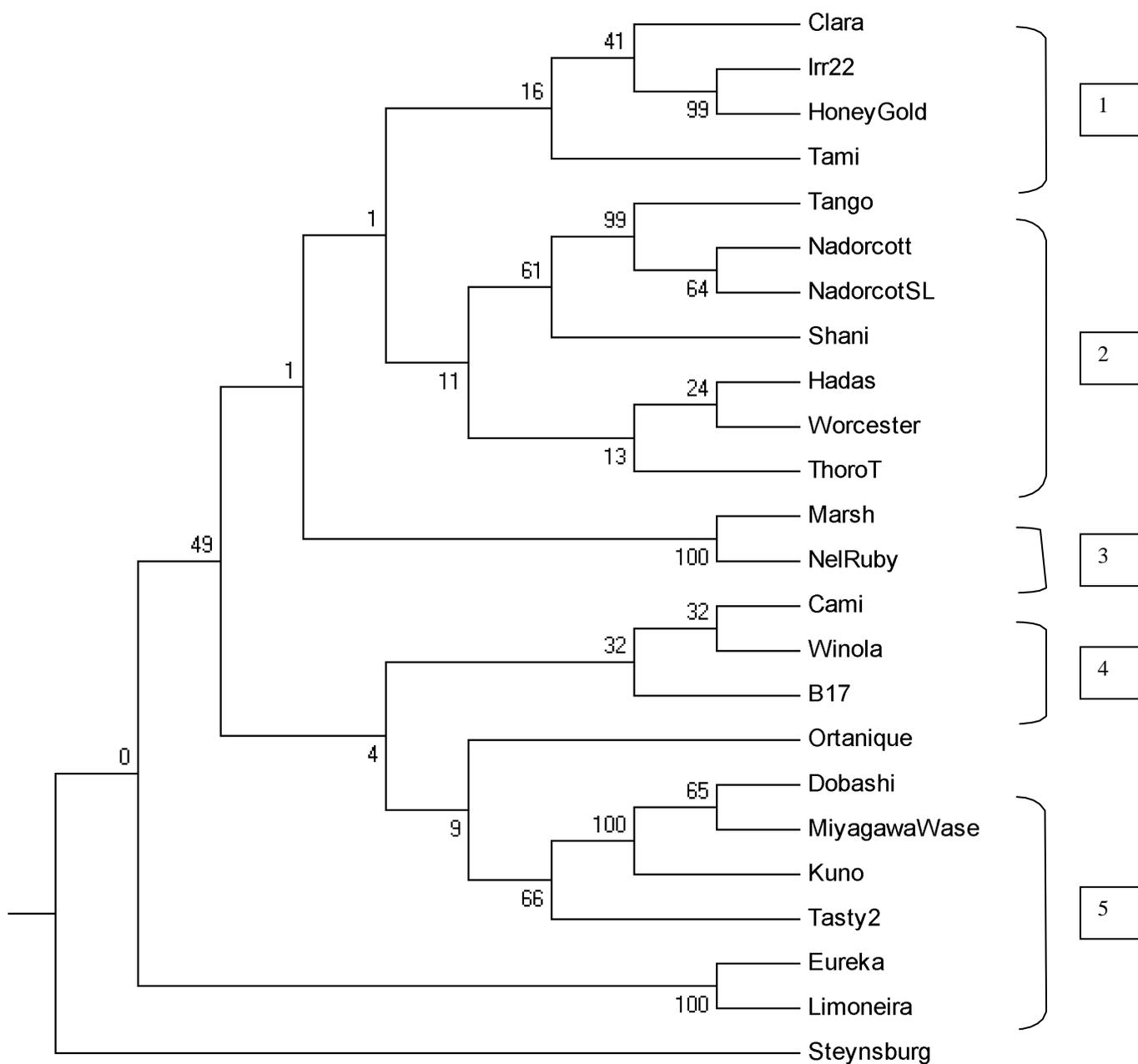


Figure 5.4.26.4. UPGMA dendrogram for mandarins group M2 (CCC = 0.994, MSD)

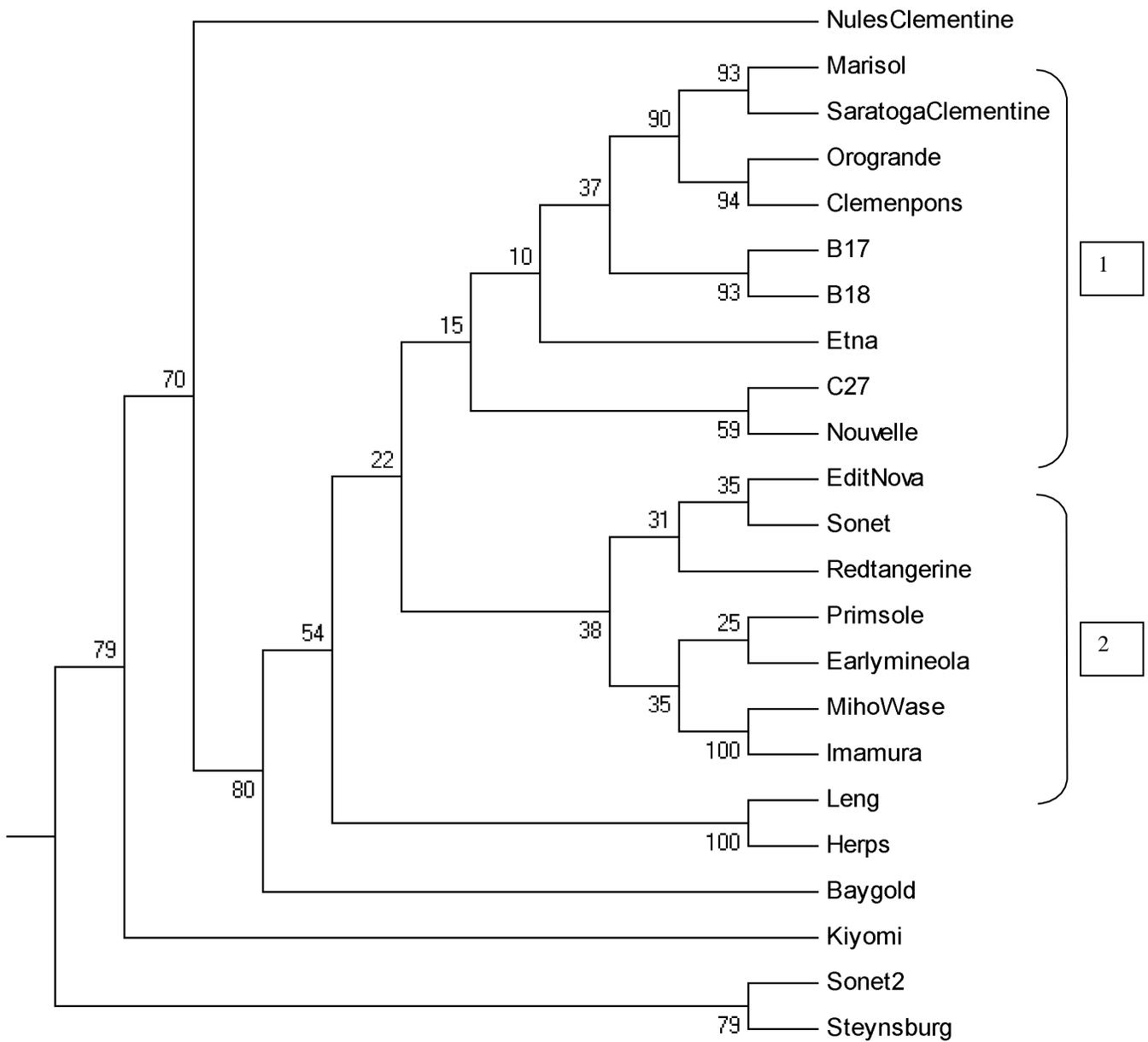


Figure 5.4.26.5. UPGMA dendrogram for mandarins group M3 (CCC = 0.880, MSD).

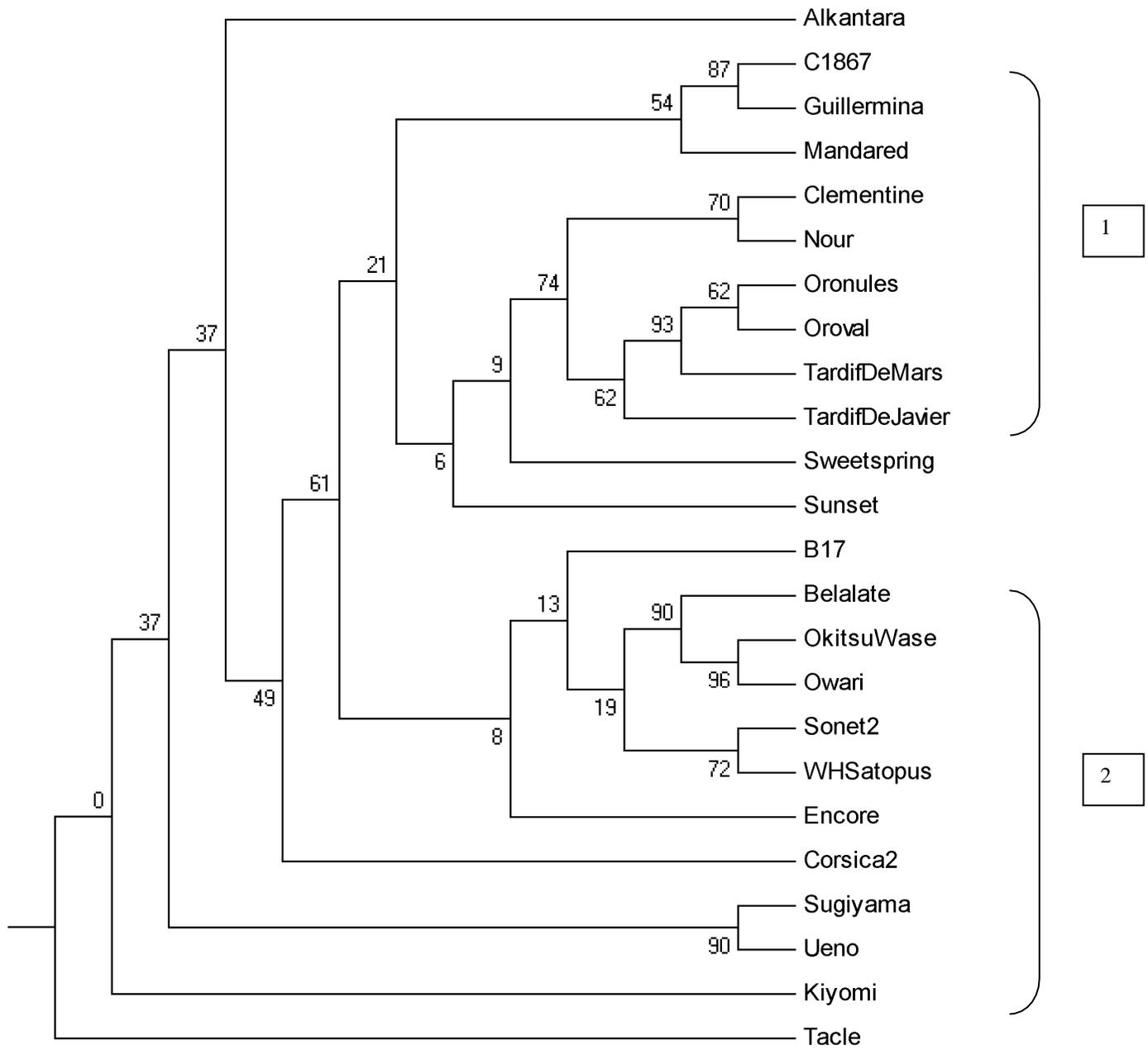


Figure 5.4.26.6. UPGMA dendrogram for mandarins group M4 (CCC = 0.979, RMSD).

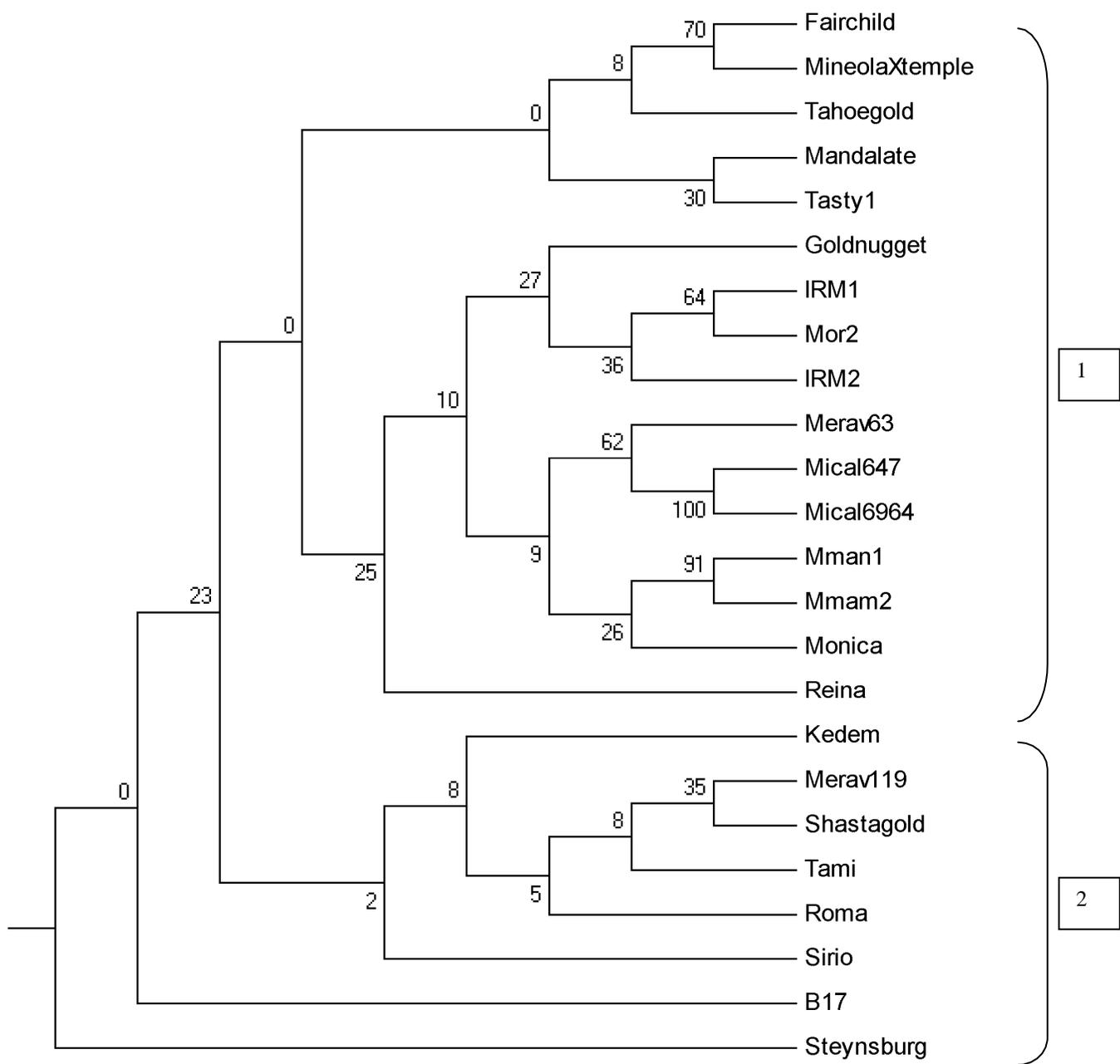


Figure 5.4.26.7. UPGMA dendrogram for mandarins group M5 (CCC = 0.997, RMSD).

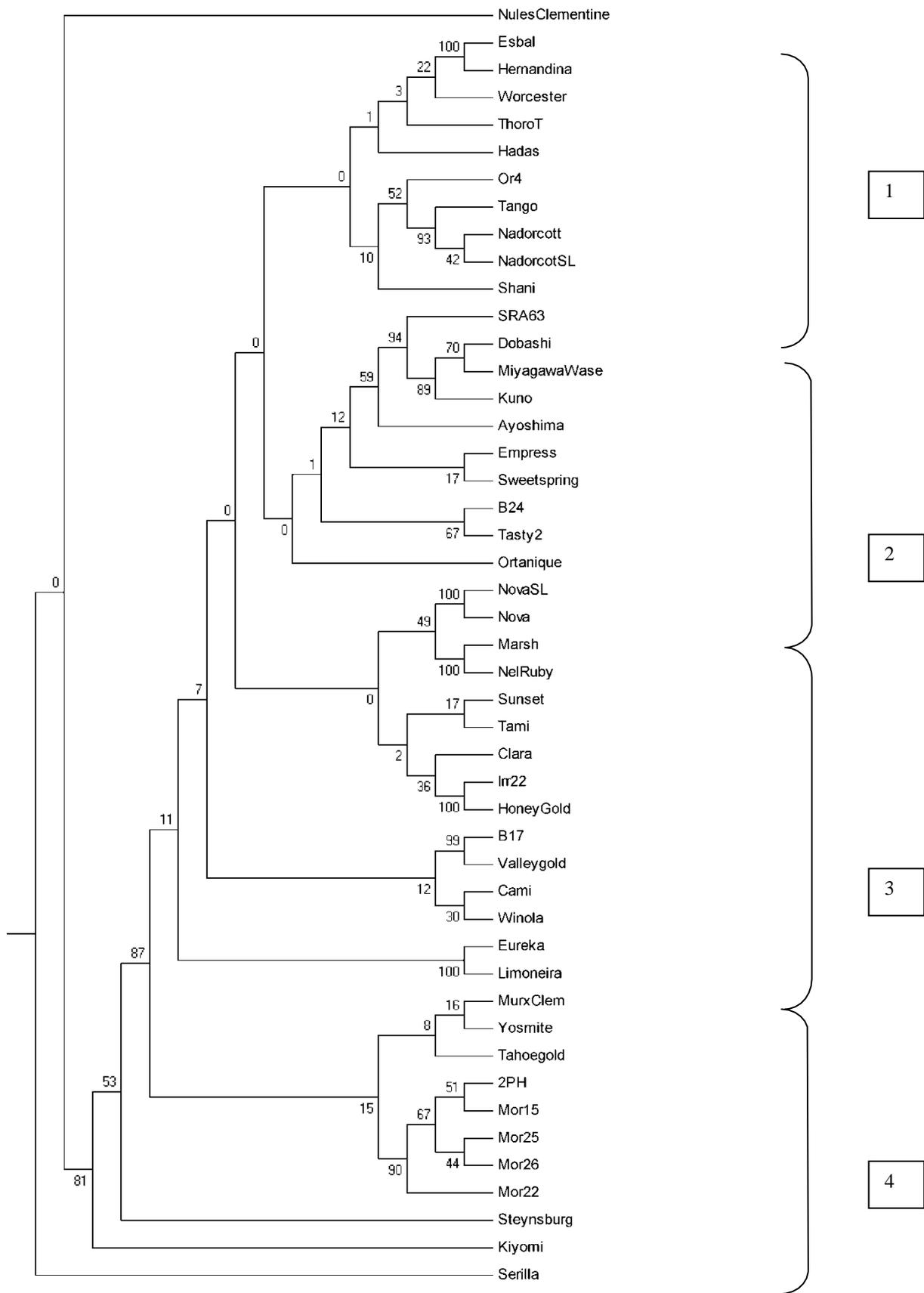


Figure 5.4.26.8. UPGMA dendrogram for mandarins M1 and M2 (CCC = 0.995, RMSD).

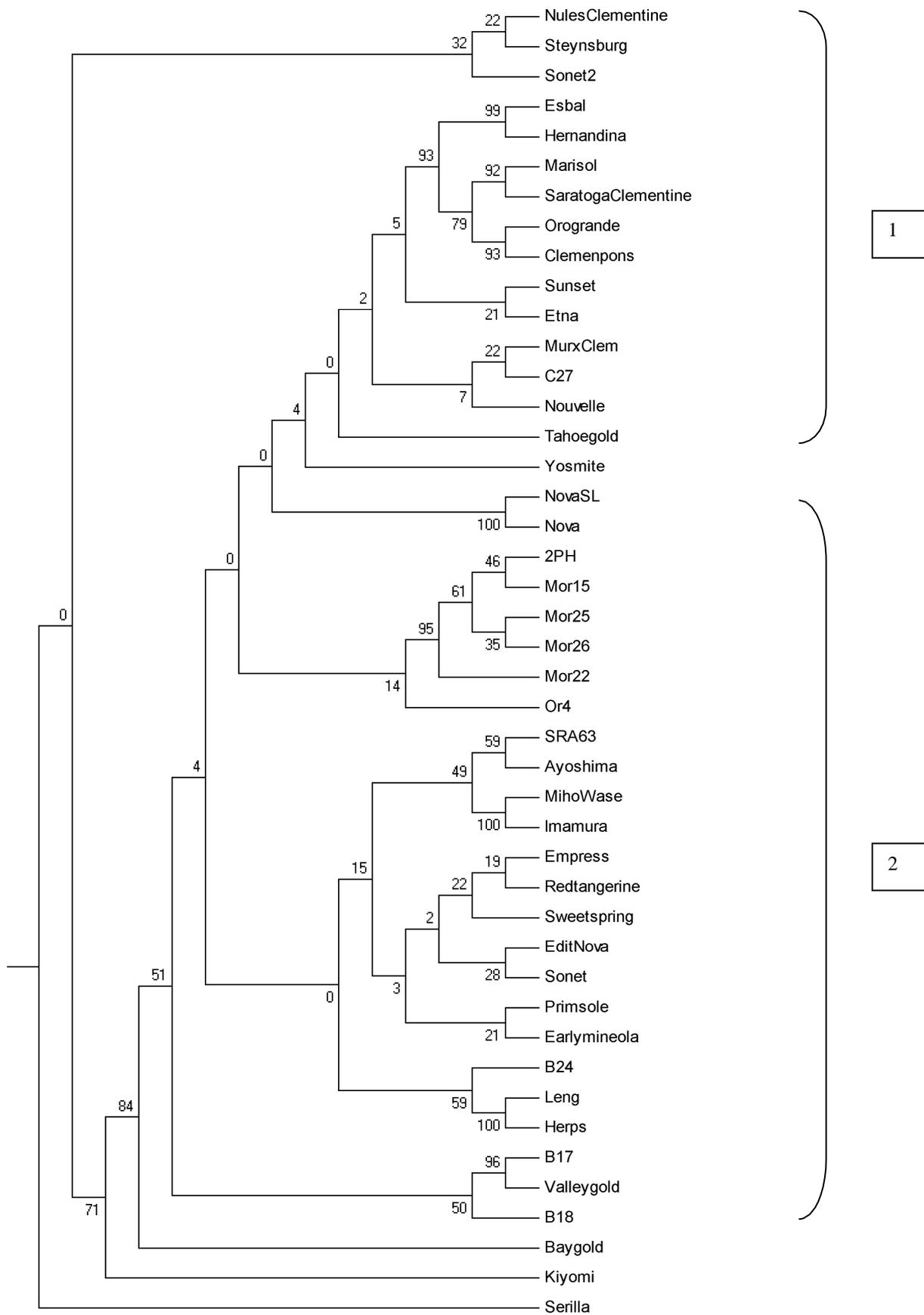


Figure 5.4.26.9. UPGMA dendrogram for mandarins group M1 and M3 (CCC = 0.951, MSD).

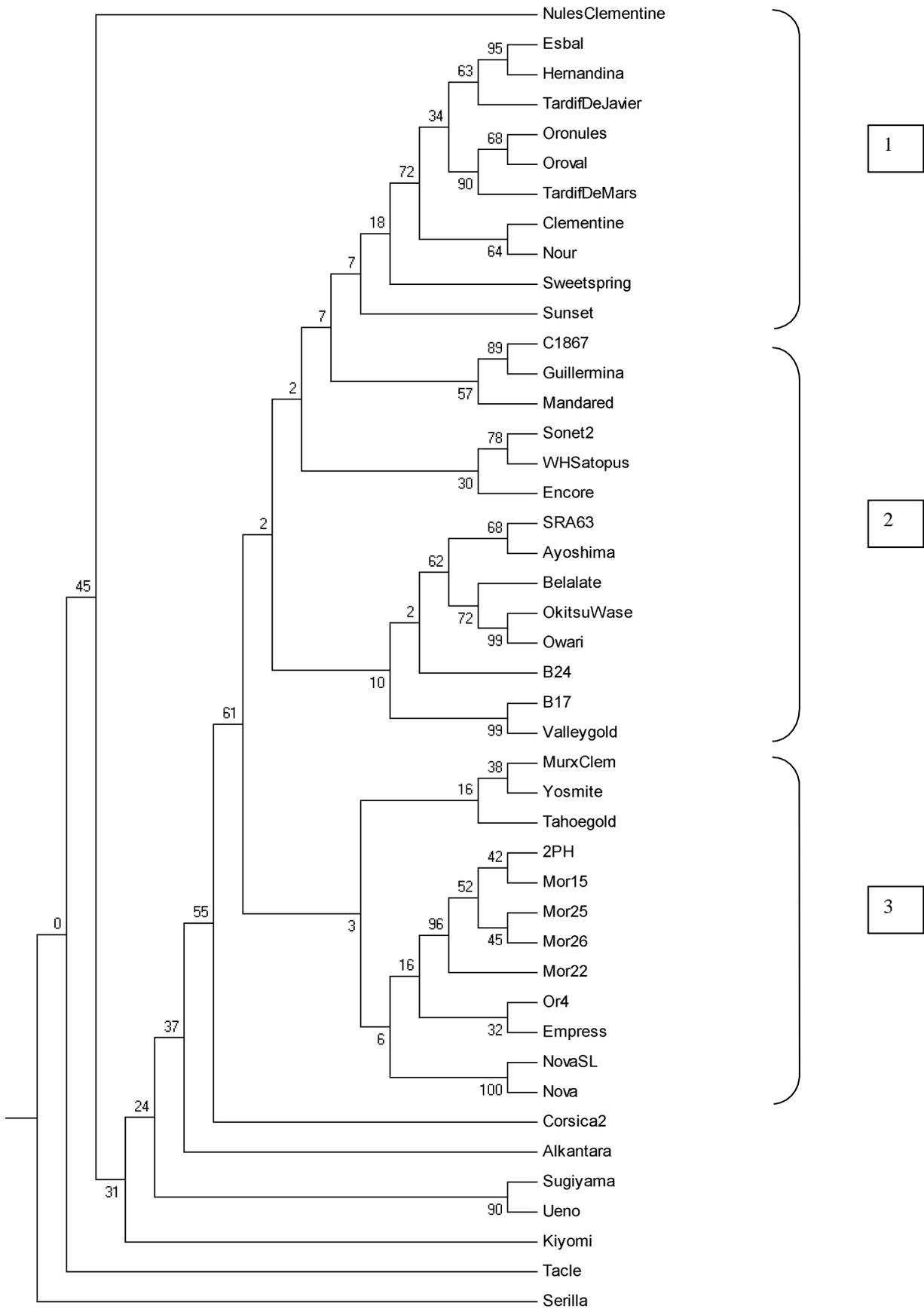


Figure 5.4.26.10. UPGMA dendrogram for mandarins group M1 and M4 (CCC = 0.946, RMSD).

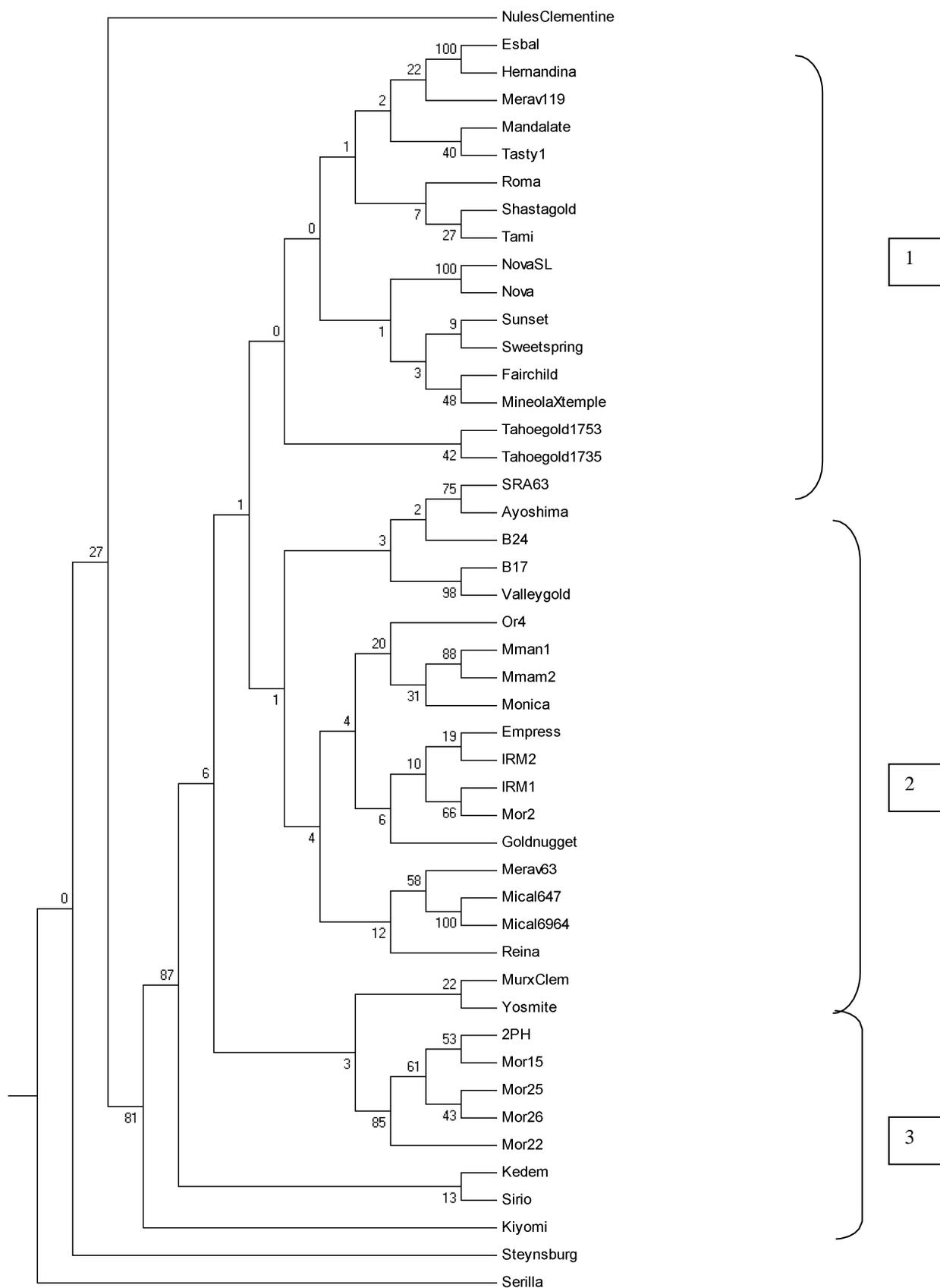


Figure 5.4.26.11. UPGMA dendrogram for mandarins group M1 and M5 (CCC = 0.994, RMSD).



Figure 5.4.26.12. UPGMA dendrogram for mandarins group M2 and M3 (CCC = 0.907, RMSD).

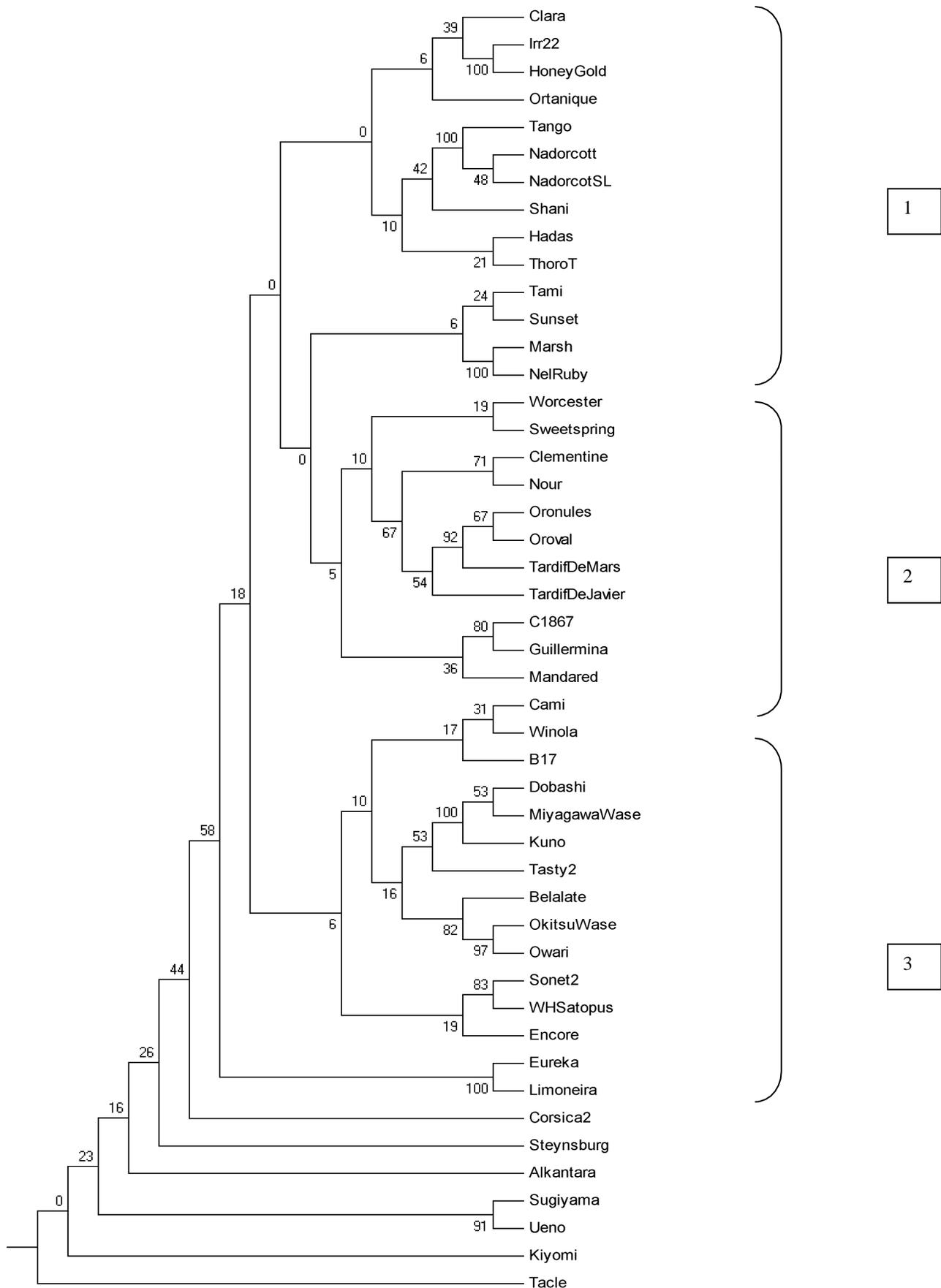


Figure 5.4.26.13. UPGMA dendrogram for mandarins group M2 and M4 (CCC = 0.983, RMSD).

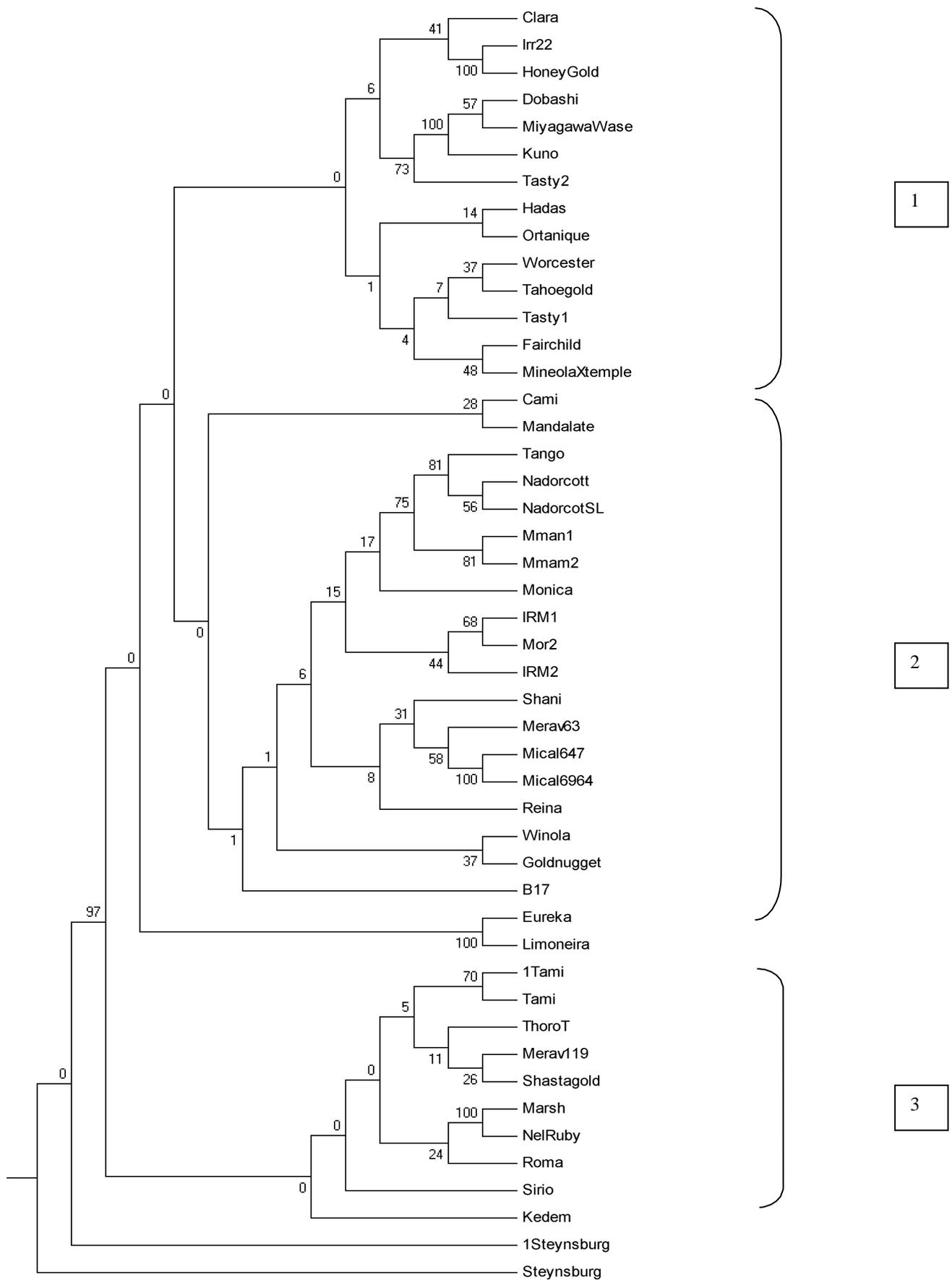


Figure 5.4.26.14. UPGMA dendrogram for mandarins group M2 and M5 (CCC = 0.998, MSD).

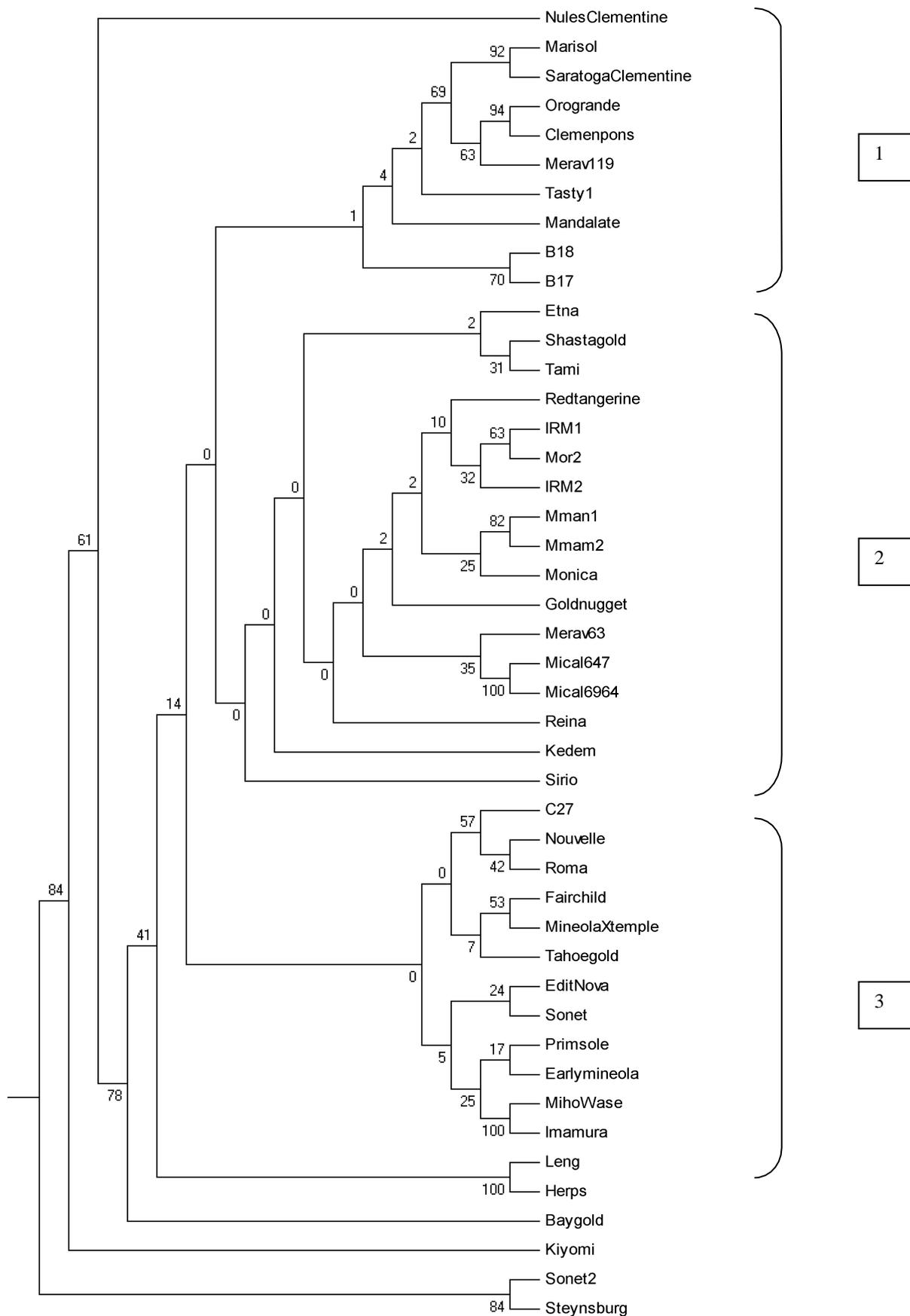


Figure 5.4.26.16. UPGMA dendrogram for mandarins group M3 and M5 (CCC = 0.888, RMSD).

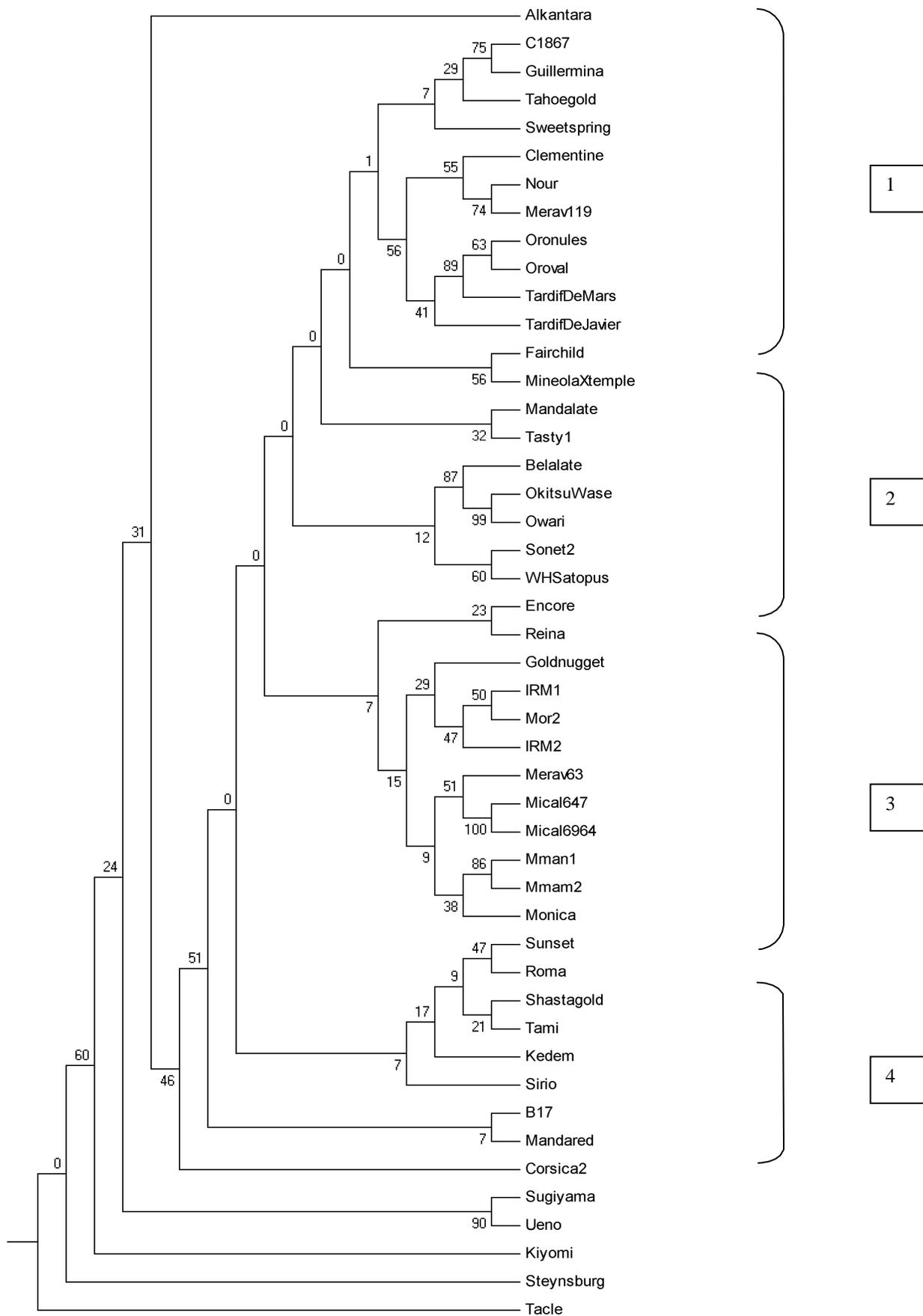


Figure 5.4.26.17. UPGMA dendrogram for mandarins group M4 and M5 (CCC = 0.992, RMSD).

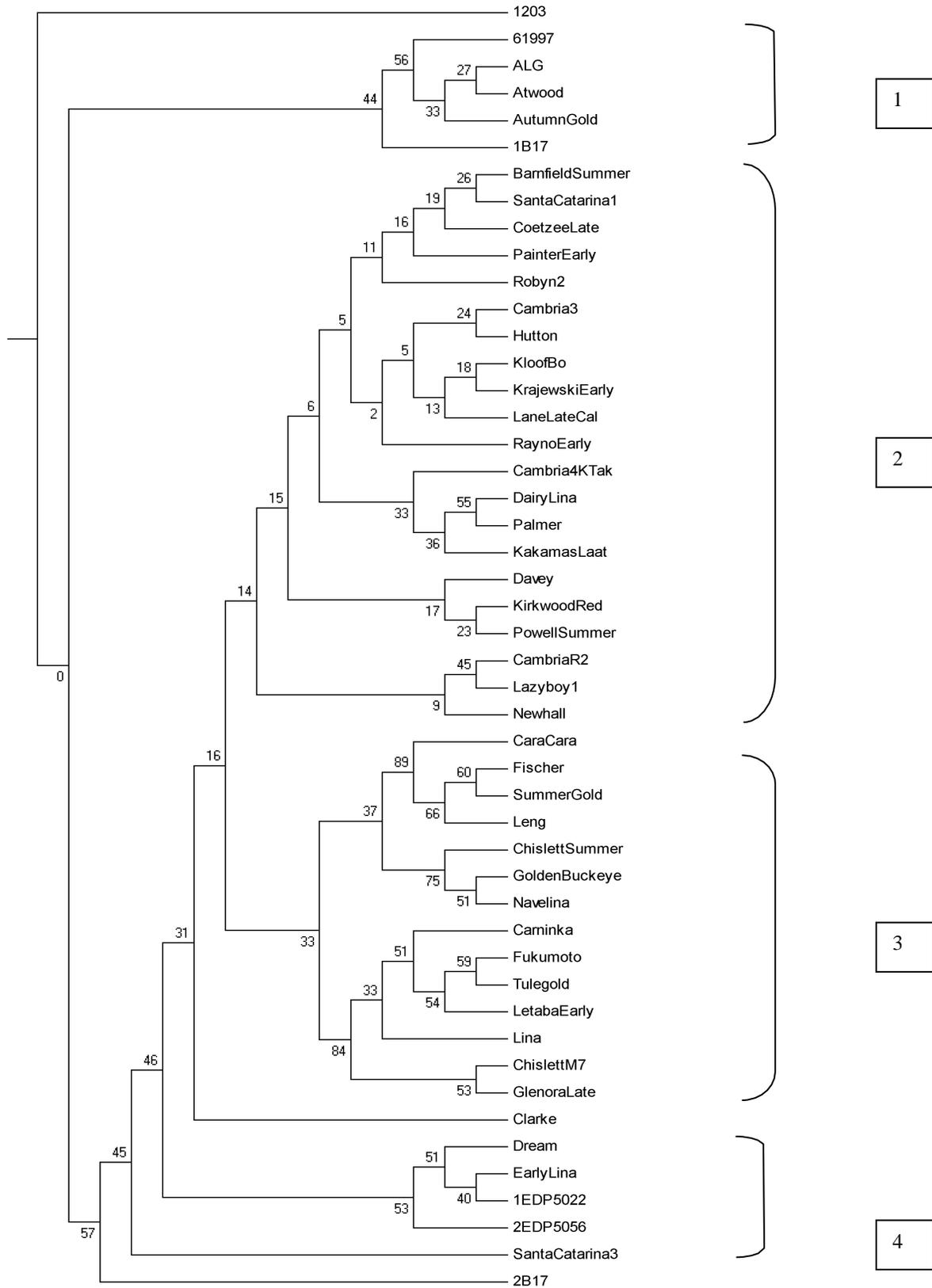


Figure 5.4.26.18. UPGMA dendrogram for oranges group O1 using SSR markers (CCC = 0.972, RMSD).

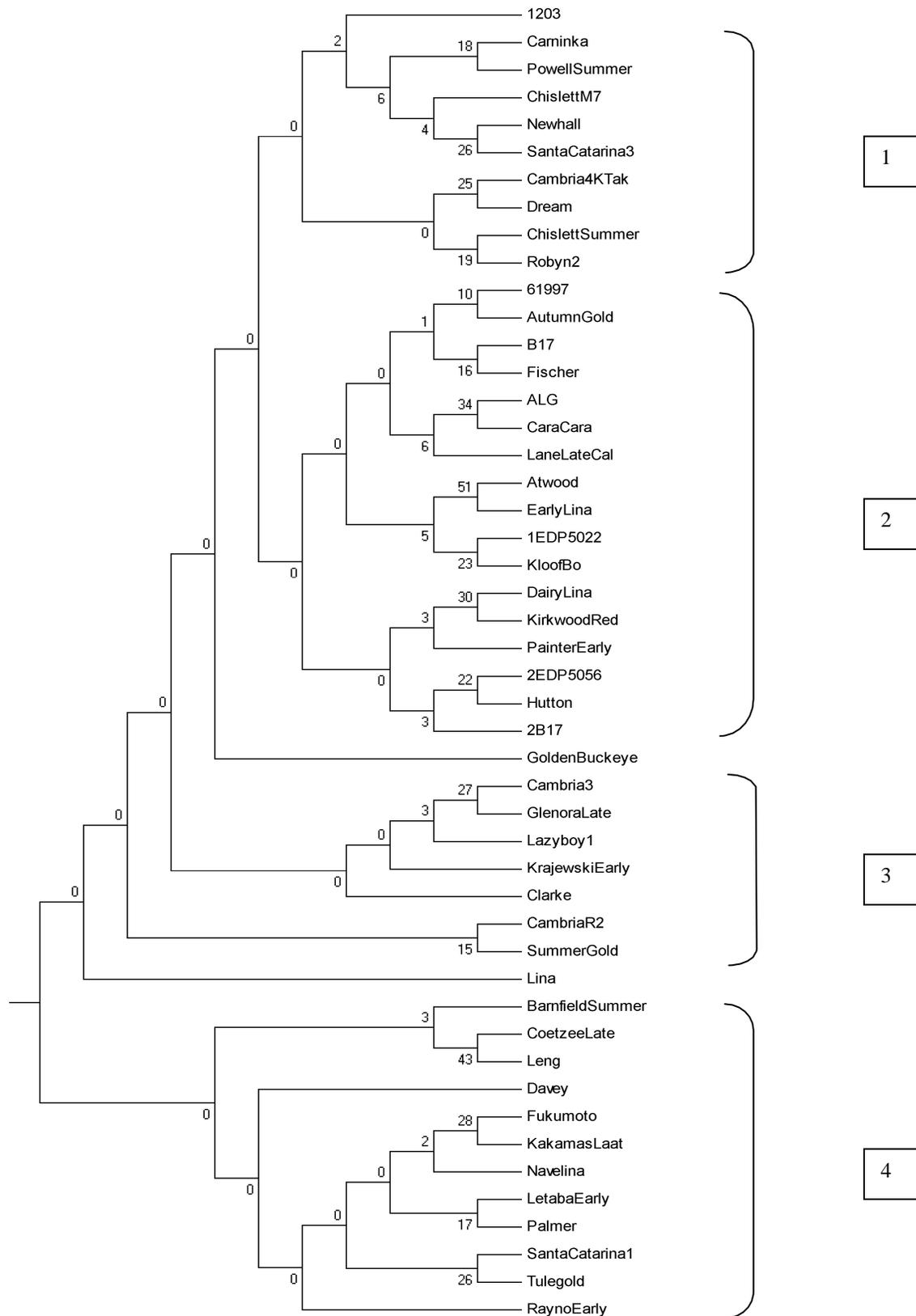


Figure 5.4.26.19. UPGMA dendrogram for oranges group O1 using SRAP markers (CCC = 0.220, Dice).

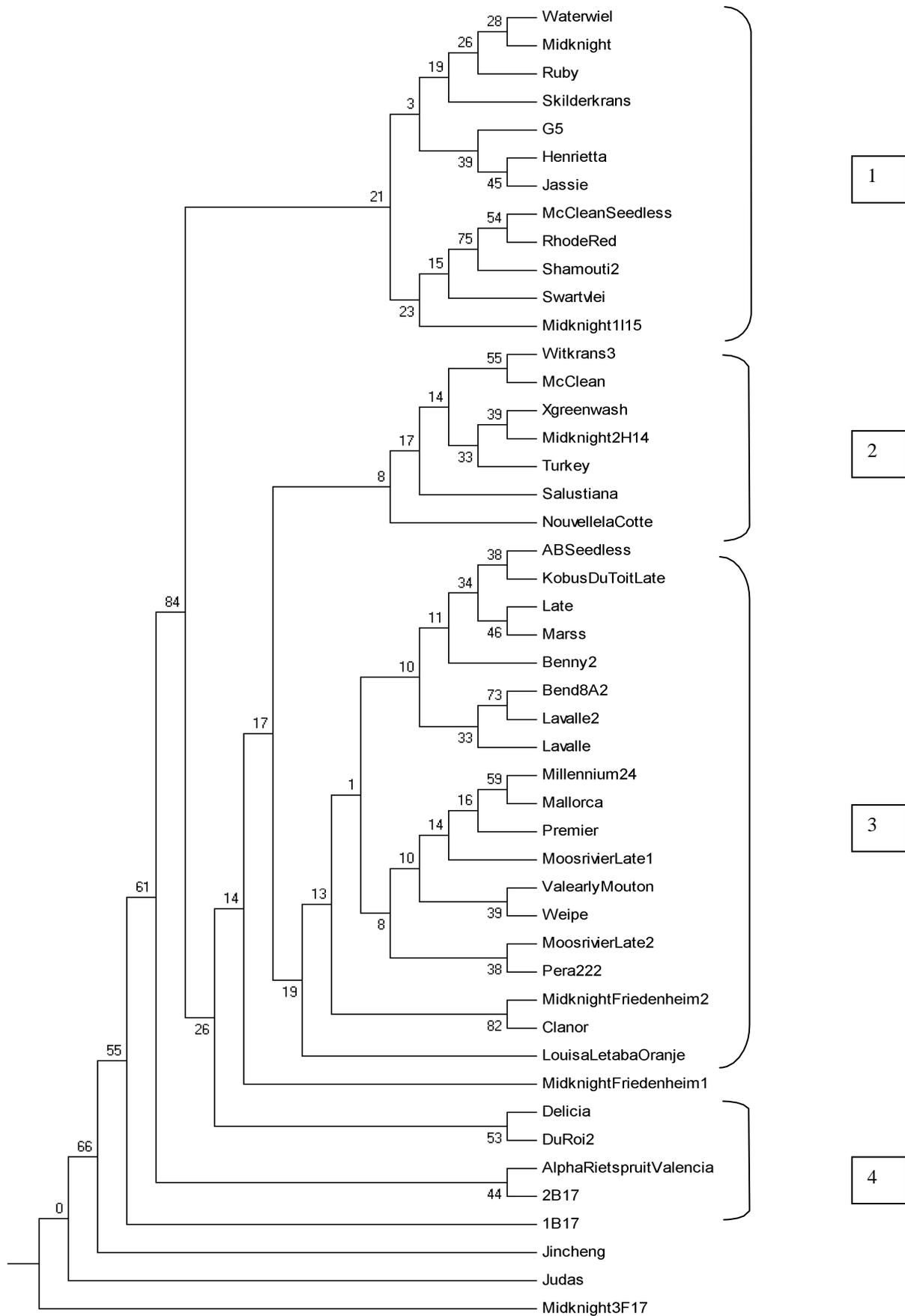


Figure 5.4.26.20. UPGMA dendrogram for oranges group O2 using SSR markers (CCC = 0.988, RMSD).

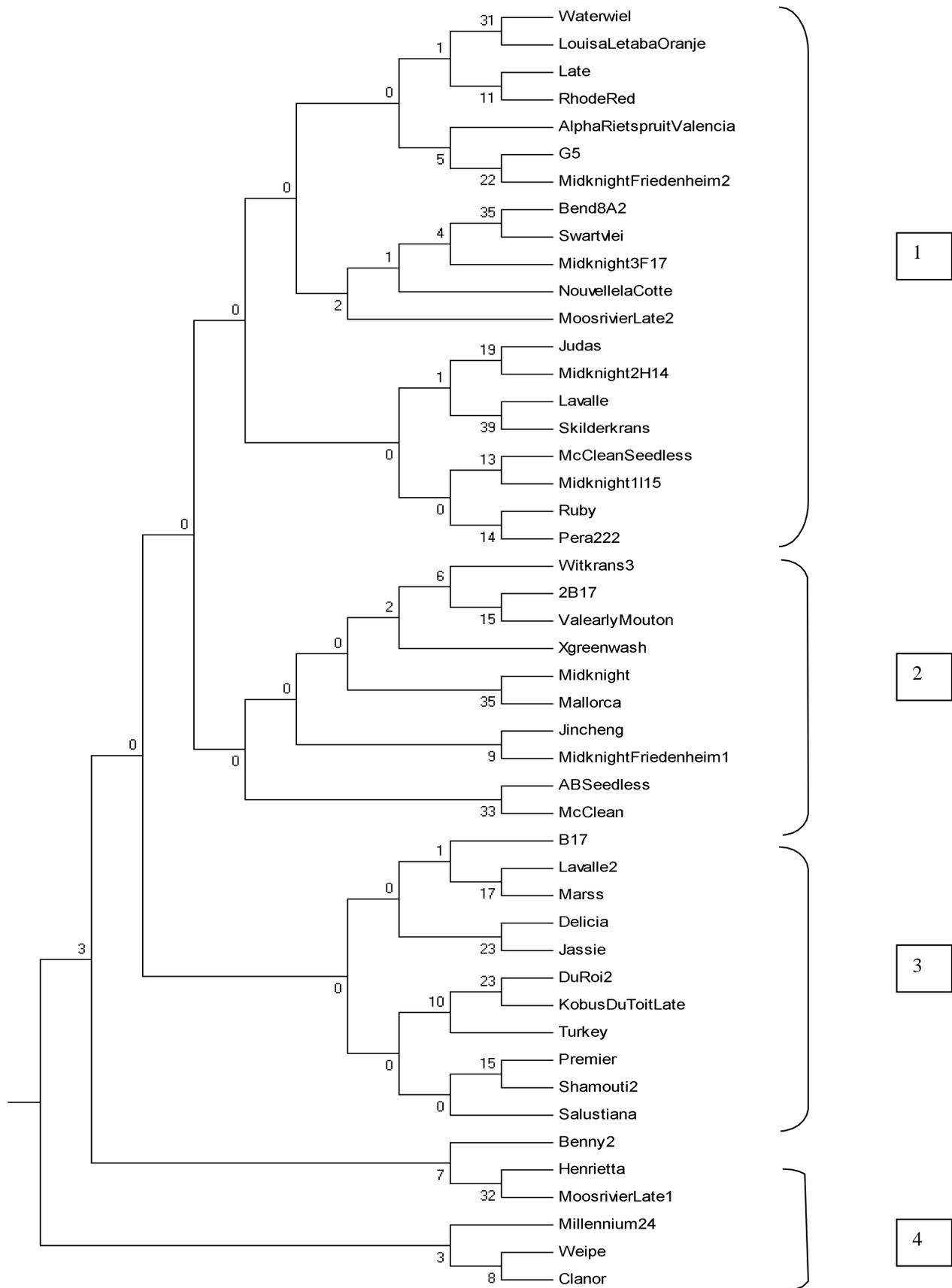


Figure 5.4.26.21. UPGMA dendrogram for oranges group O2 using SRAP markers (CCC = 0.022, Dice).

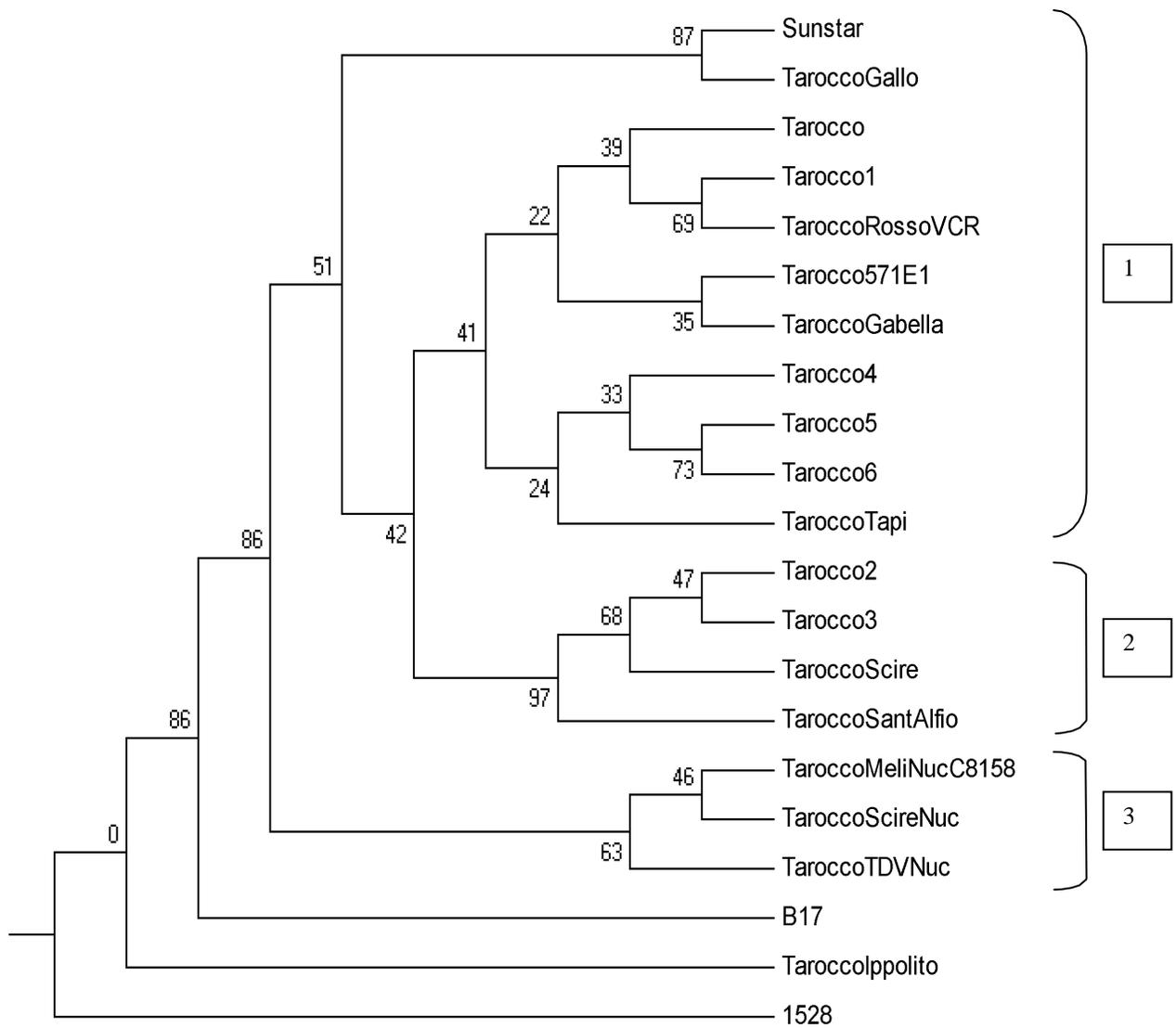


Figure 5.4.26.22. UPGMA dendrogram for oranges group O3 with SSR markers (CCC = 0.956, RMSD).

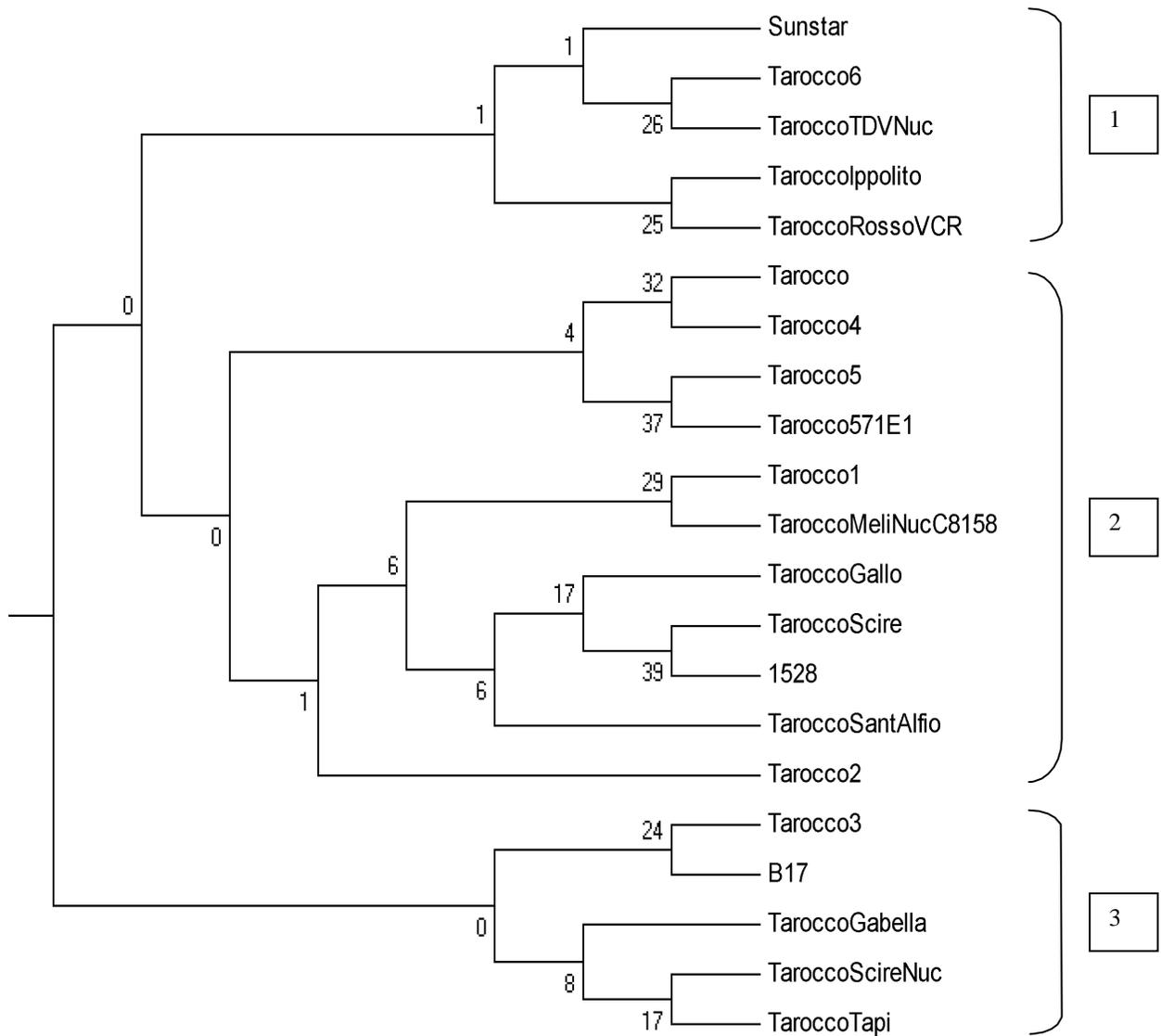


Figure 5.4.26.23. UPGMA dendrogram for oranges group O3 using SRAP markers (CCC = 0.100, Dice).

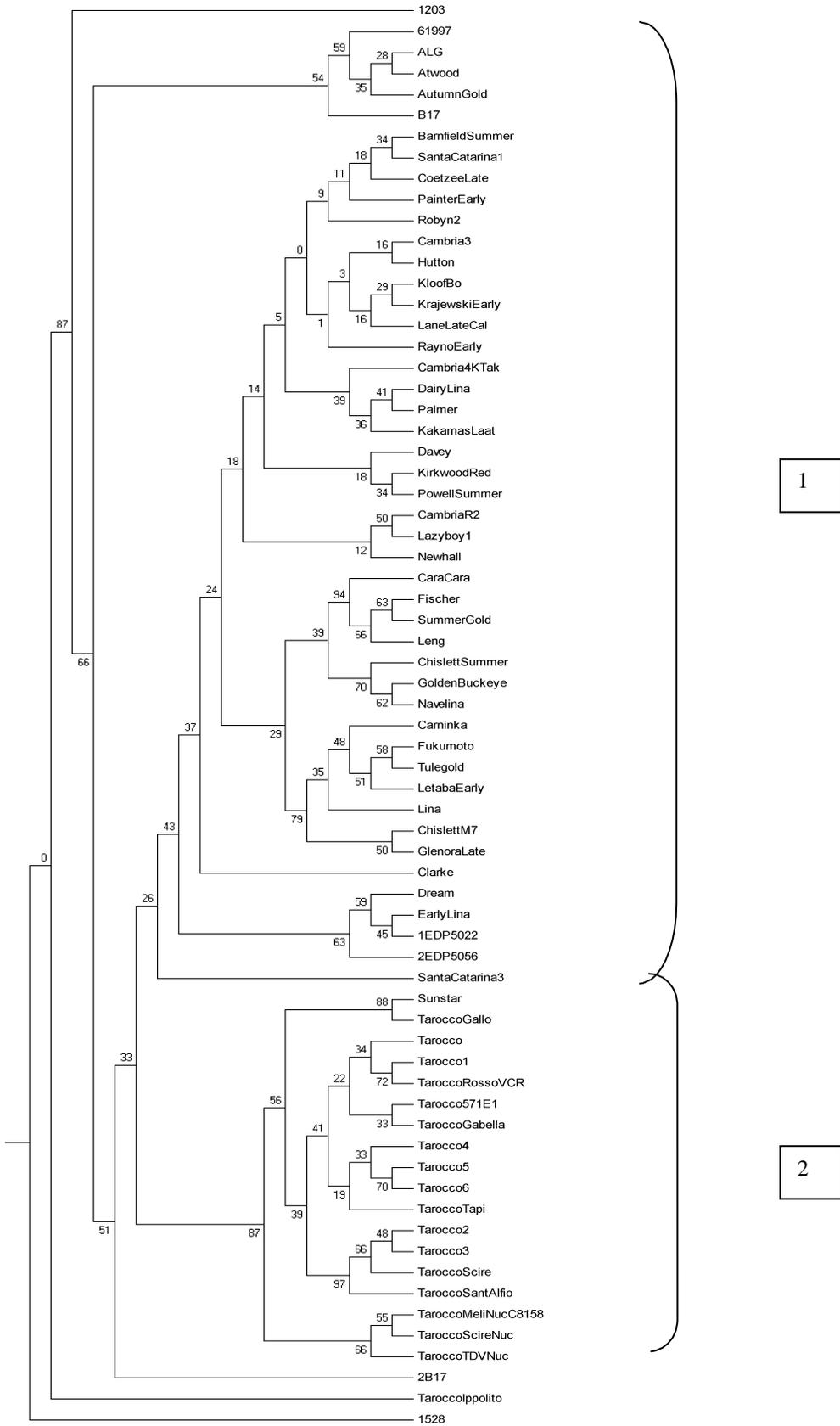


Figure 5.4.26.24. UPGMA dendrogram for oranges group O1 and O3 using SSR markers (CCC = 0.882, MSD).

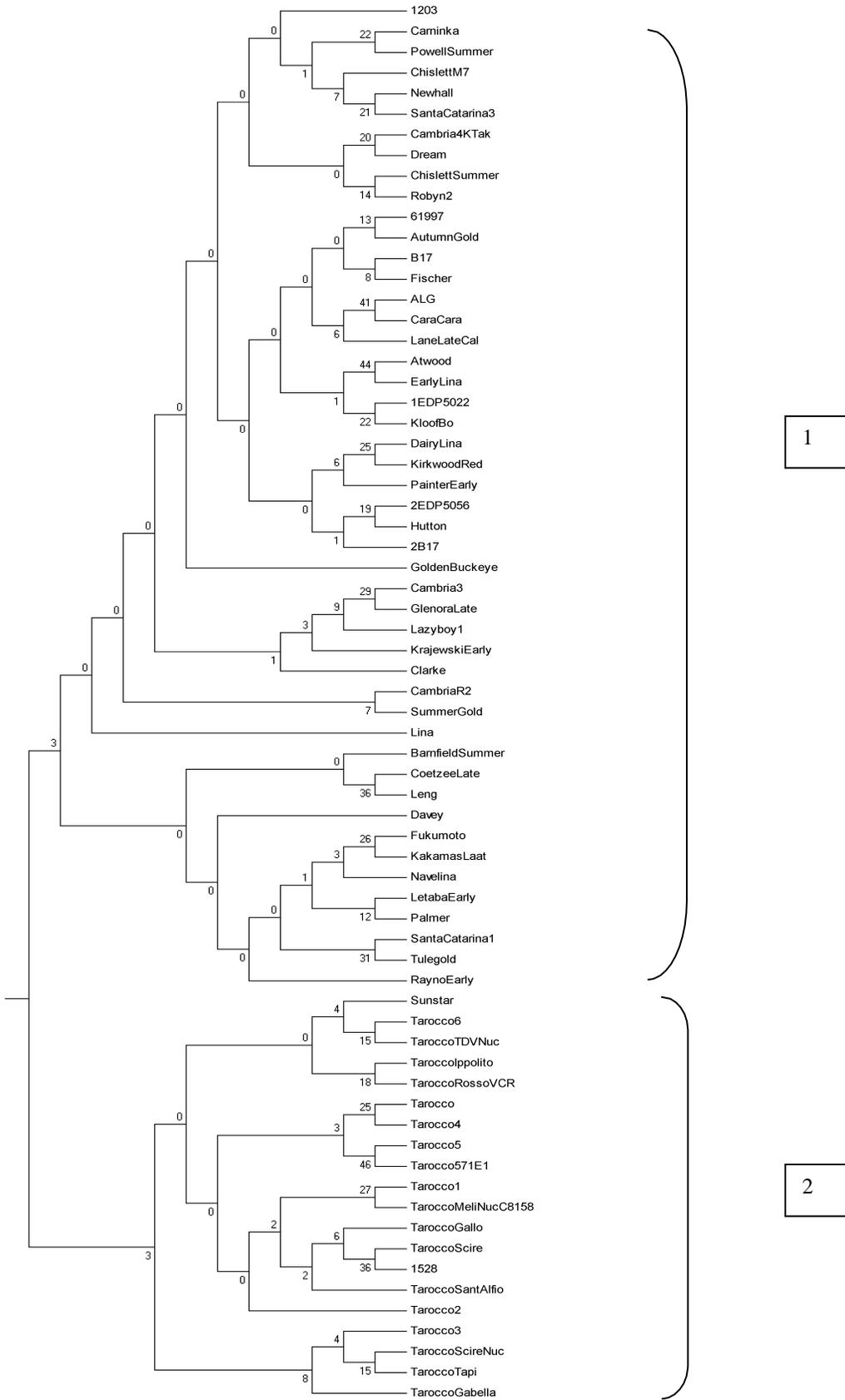


Figure 5.4.26.25. UPGMA dendrogram for oranges group O1 and O3 using SRAP markers (CCC = 0.143, Dice).

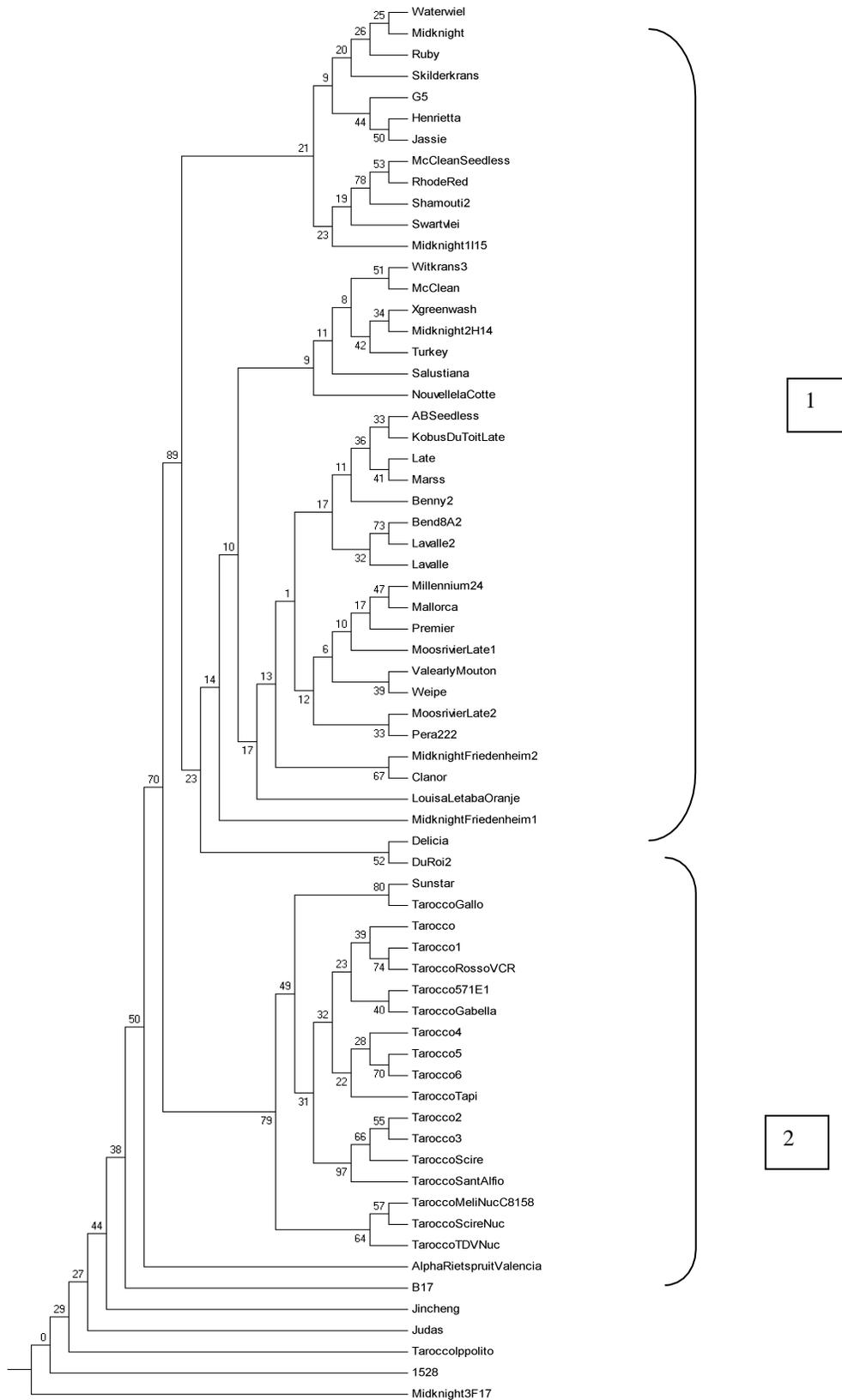


Figure 5.4.26.26. UPGMA dendrogram for oranges group O2 and O3 using SSR markers (CCC = 0.943, MSD).

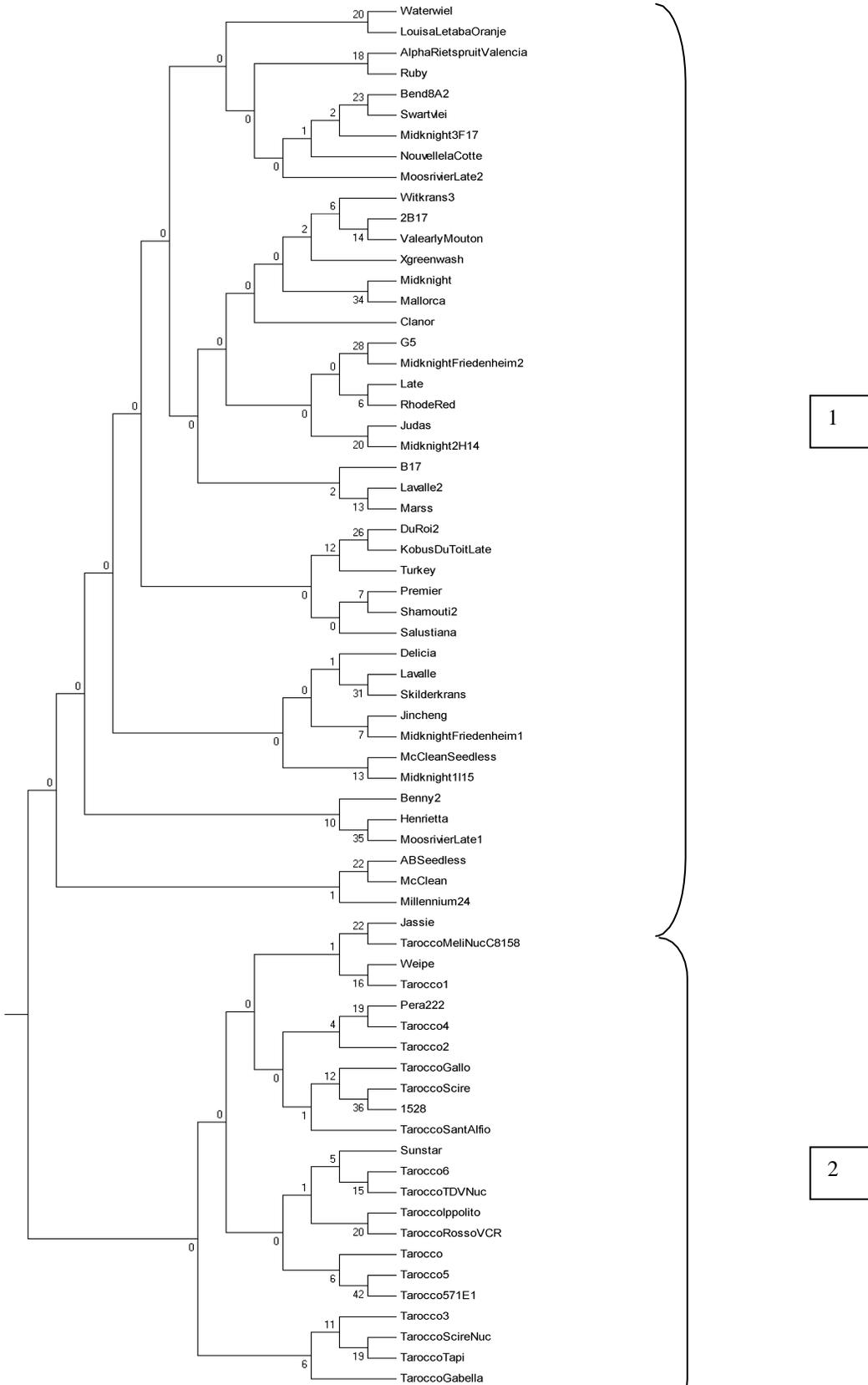


Figure 5.4.26.27. UPGMA dendrogram for oranges group O2 and O3 using SRAP markers (CCC = 0.087, Dice).

Technology transfer

Poster:

Verification and Genetic Diversity Analysis for Tropical and Subtropical Crops

Authors: Severn-Ellis A.A.¹, Froneman I. ¹, Penter M.¹, Nonyane D.¹, Sippel A.D¹, Cook G.² and Maqutu V.Z.Z.²

¹ ARC – Institute for Tropical and Subtropical Crops, Nelspruit, South Africa.

² Citrus Research International, Nelspruit, South Africa

Article published:

A.A. Severn-Ellis, A. Sippel, Z. Dlamini, and B. Manicom, 2012. Establishment of a molecular genotype reference database for mandarin accession verification. *Acta Hort.* 1007, ISHS 2013 p.753-756.

Proceedings of the 2nd All African Horticultural Conference, Skukuza, South Africa. Eds.: K. Hannweg and M. Penter

References cited

- Aleza, P., Juarez, J., Hernandez, M., Pina, J. A., Ollitrault, P. and Navarro, L., 2009. Recovery and characterization of a *Citrus clementina* Hort. Ex Tan. 'Clemenules' haploid plant selected to establish the reference whole *Citrus* genome sequence. *BMC Plant Biol* 110:1–17.
- Amar, M.H., Biswas, M.K., Zhang, Z. and Guo, W-W., 2011. Exploitation of SSR, SRAP and CAPS-SNP markers for genetic diversity of Citrus germplasm collection. *Sci Hort* 128:220-227.
- Amar, M.H. 2012. Comparative analysis of SSR and SRAP sequence divergence in *Citrus* germplasm. *Biotech* 11:20-28.
- Barkley, N.A., Roose, M.L., Krueger, R.R. and Federici, C.T., 2006. Assessing genetic diversity and population structure in a citrus germplasm collection utilizing simple sequence repeat markers (SSRs). *Theor. Appl. Genet.* 112:1519–1531.
- Barrett, H.C. and Rhodes, A.M., 1976. A numerical taxonomic study of affinity relationships in cultivated Citrus and its close relatives. *Syst Bot* 1: 105–136.
- Bretting, P.K. and Widrechner M.P., 1995. Genetic markers and plant genetic resource management. *Plant Breeding Rev* 13:11-86.
- Cristofani-Yaly, M., Novelli, V.M., Bastianel, M and Machado, M.A., 2011. Transferability and level of heterozygosity of microsatellite markers in Citrus species. *Plant Mol Biol Reps* 29:418-423.
- Corazza-Nunes, M.J., Machado, M.A., Nunes, W.M.C, Cristofani, M. and Targon, M.L.P.N., 2002. Assessment of genetic variability in grapefruits (*Citrus paradisi* Macf.) and pummelos (*C. maxima* (Burm.) Merr.) using RAPD and SSR markers. *Euphytica* 126:169–176.
- Das, A., Mandal, B., Sarkar, J. and Chaudhuri, S., 2007. Occurrence of zygotic twin seedlings in mandarin orange plants of the northeastern Himalayan region. *Curr Sci* 92:1488–1489.
- El-Mouei, R., Choumane, W. and Dway, F. 2011. Characterization and estimation of genetic diversity in *Citrus* rootstocks. *Int J Agric Biol* 13:571–575
- Federici, C.T., Fang, D.Q., Scora, R.W. and Roose, M.L., 1998. Phylogenetic relationships within the genus *Citrus* (Rutaceae) and related genera as revealed by RFLP and RAPD analysis. *Theor Appl Genet* 96:812-822.
- Froelicher, Y., Dambier, D., Costantino, G., Lotfy, S., Didout, C., Beaumont, V., Brottier, P., Risterucci, A.M., Luro, F., Ollitrault, P., 2008. Characterization of microsatellite markers in Citrus reticulate Blanco. *Mol Ecol Resour* 8:119–122.
- Gulsen, O. and Roose M.L., 2001. Lemons: diversity and relationships with selected *Citrus* genotypes as measured with nuclear genome markers. *J Amer Soc Hort Sci* 126:309–327.
- Gulsen, O., Uzun, A., Seday, U. and Kafa, G. 2011, QTL analysis and regression model for estimating fruit setting in young *Citrus* trees based on molecular markers. *Sci Hort* 130:418-424.
- Koehler-Santos, P., Dornelles, A.L.C. and Freitas, L.B., 2003. Characterization of mandarin citrus germplasm from Southern Brazil by morphological and molecular analyses. *Pesqui Agropecu Bras* 38:797–806.
- Krueger, R. R. and Roose, M.L., 2003. Use of molecular markers in the management of citrus germplasm resources. *J Amer Soc Hort Sci* 128:827-837.
- Li, G. and Quiros, C.F., 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. *Theor Appl Genet* 103: 455–461.

- Luro, F., Rist, D. and Ollitrault, P., 2001. Evaluation of genetic relationships in *Citrus* genus by means of sequence tagged microsatellites. In *Proceedings of the International Symposium on Molecular markers for characterizing genotypes and identifying cultivars in horticulture: 6–8 March 2000. Volume 546*. Edited by Doré C, Dosba F, Baril C. ISHS Acta Horticultarum; 2001: 237- 242. .
- Luro, F.L. Costantino, G., Terol, J., Argout, X., Allario, T., Wincker, P., Talon, M., Ollitrault, P. and Morillon, R., 2008. Transferability of the EST-SSRs developed on Nules clementine (*Citrus clementina* Hort ex Tan) to other *Citrus* species and their effectiveness for genetic mapping. *BMC Genomics* 2008, **9**:287 doi:10.1186/1471-2164-9-287
- Nicolosi, E., Deng, Z.N., Gentile, A., La Malfa, S., Continella, G. and Tribulato, E., 2000. Citrus phylogeny and genetic origin of important species as investigated by molecular markers. *Theor Appl Genet* 100:1155-1166.
- Novelli, V.M., Cristofani, M., Souza, A.A. and Machado, M.A., 2006. Development and characterization of polymorphic microsatellite markers for the sweet orange (*Citrus sinensis* L. Osbeck). *Genet Mol Biol* 29:90–96.
- Meyer, A.S., Garcia, A.A.F., de Souza, A.P. and de Souza, C.L. 2004. Comparison of similarity coefficients used for cluster analysis with dominant markers in maize (*Zea mays* L). *Genet Mol Biol* 27:83–91
- Morton, C.M., Grant, M. and Blackmore, S., 2003. Phylogenetic relationships of the Aurantioideae inferred from chloroplast DNA sequence data. *Am J Bot* 90:1463 -1469.
- Ollitrault, F., Terol, J., Pina, J.A., Navarro, L., Talon, M., Ollitrault, P., 2010. Development of SSR markers from *Citrus clementina* (Rutaceae) BAC end sequences and interspecific transferability in Citrus *Am J Bot* 97:124–129.
- Pang, X-M., Hu, C-G. and Deng, X-X,. 2007. Phylogenetic relationships within *Citrus* and its related genera as inferred from AFLP markers. *Genet Resour Crop Ev* 54:429-436.
- Rahman, M.M., Nito, N. and Isshiki, S., 1994. Genetic analysis of phosphoglucosomerase isozymes in 'true citrus fruit trees'. *Sci Hort.* 60:17–22.
- Ramadugu, C., Pfeil, B., Keremane, M.L., Lee, R.F., Maureira-Butler, I.J. and Roose, M.L., 2013. A six nuclear gene phylogeny of Citrus (Rutaceae) taking into account hybridization and lineage sorting. *PLoS ONE* 8:7 e68410
- Risterucci, A.M., Duval M.F., Rohde W. and Billotte N., 2005. Isolation and characterization of microsatellite loci from *Psidium guajava* L. *Mol Ecol* 5: 745–748.
- Snoussi, H., Duval, M-F., Garcia-Lor, A., Belfalah, Z., Froelicher, Y., Risterucci, A-M., Perrier, X., Jacquemoud-Collet, J-P., Navarro, L., Harrabi, M. and Ollitrault, P., 2012. Assessment of the genetic diversity of the Tunisian citrus rootstock germplasm. *BMC Genet* 13: 16.
- Tanaka, T., 1977. Fundamental discussion of citrus classification. *Stud Citrolog* 14:1–6.
- Uzun, A., Yesiloglu, T., Aka-Kacar, Y., Tuscu, O. and Gulsen, O., 2009. Genetic diversity and relationship within Citrus and related genera based on sequence related amplified polymorphism markers (SRAPs). *Sci Hort* 121: 306–312.
- Uzun, A., Yesiloglu, T., Polat, I., Aka-Kacar, Y., Gulsen, O., Yildirim, B., Tuzcu, O., Tepe, S., Canan, I. and Anil, S., 2011. Evaluation of genetic diversity in lemons and some of their relatives based on SRAP and SSR markers. *Plant Mol Biol Reps* 29:693-701.

ADDENDUM: ACTA HORT ARTICLE
ESTABLISHMENT OF A MOLECULAR GENOTYPE REFERENCE
DATABASE FOR MANDARIN ACCESSION VERIFICATION

A.A. Severn-Ellis, A. Sippel, Z. Dlamini, and B. Manicom
ARC - Institute for Tropical and Subtropical Crops, Nelspruit.

ABSTRACT

Citrus (*Citrus reticulata*) accessions entering the Virus Free Nucleus Block at the Institute for Tropical and Subtropical Crops have been characterised based on important morphological or agronomical features. These defining morphological or agricultural characteristics are not continuously expressed within the potted greenhouse environment. It is therefore not always possible to check or verify the trueness-to-type of an accession which may prevent the detection of misidentifications or duplicates. A large number of microsatellite (SSR) markers have been developed for Mandarin genotyping. In this study a set of 13 SSR markers was selected and assessed for their ability to distinguish between 32 Mandarin accessions and future use in the establishment of a molecular genotype database for mandarin accession verification.

INTRODUCTION

The last several decades have seen the evolution of molecular markers as tools with great potential application to the challenges of germplasm characterization. (Ford-Lloyd et al., 1997) These markers have a distinct advantage over morphologically based phenotypic characterization, as they are generally unaffected by the host of factors able to influence plant or organ characteristics. This allows comparisons between accessions within a collection or among collections at different locations at any time of year, while phenotypic characteristics can be masked by environmental or cultural affects. Molecular characterization has a number of applications in the management of germplasm collections. These include the identification of gaps and redundancies in the collection; correction of misidentified accessions, assessment of the actual genetic diversity present within the collection; development of core subsets and characterization of newly acquired germplasm (Bretting & Widrechner, 1995; Krueger and Roose, 2003).

Simple sequence repeat (SSR) markers have been used in various citrus genetic studies, (Luro et al. 2001, 2008; Gulsen and Roose 2001; Barkley et al. 2006; Ollitrault et al. 2010) as they are highly polymorphic, codominant, generally locus specific and randomly dispersed throughout the plant genome and have the potential to unravel the genetic diversity in citrus at the interspecific, intraspecific and intra-population level (Froelicher et al., 2008). The aim of this study was to assess the ability of selected SSR markers to distinguish between 32 Mandarin accessions as basis for future use in the establishment of a molecular genotype reference database.

MATERIALS AND METHODS

DNA Extraction

Plant leaf material was collected from 32 selected mandarin cultivars housed within the virus free CIP collection at the ARC in Nelspruit. DNA was extracted by grinding 0.4g of leaf tissue in 5ml extraction buffer in plastic envelopes. Homogenized samples were pipetted into micro-centrifuge tubes. The DNA was extracted according to a modified CTAB DNA extraction procedure (Risterucci et al., 2005). The DNA concentration was determined after precipitation using a spectrophotometer.

PCR Amplification

The PCR reaction consisted of 45ng of template DNA, 12.5ul EmeraldAmpMax HS (Takara) master mix, 0.025uM forward and reverse primer to a final volume of 25ul. PCR amplification of samples was performed using a G-Storm thermocycler. Cycling conditions consisted of an initial hot start at 95 °C for 4 min; 35 cycles of 94 °C for 30 sec, 50-60°C for 1 min, and 72 °C for 1 min, and a final extension step at 72 °C for 5 min. Amplified PCR products were resolved and scored on 4% agarose gels (Fig.1). GeneTools (Syngene) software was used to visualise gel images, analyse and determine length of PCR products.

Phylogenetic Analysis

GenAlEx 6.3 and Arlequin 3.11 were used to create a genetic distance matrix and summarize information on unique alleles, frequency and distribution of alleles. Neighbor was used to construct an UPGMA tree and Treeview to draw the tree.

RESULTS AND DISCUSSION

The 13 SSR primer pairs used in this study were selected from two sets of 28 and 43 SSR primers respectively described by Barkley et al., 2006 and Froelicher et al., 2008 based on polymorphism revealed. The level of genetic variation of these microsatellite loci was estimated by genotyping 32 (N) randomly selected mandarin cultivars. The number of alleles per locus (N_a), observed heterozygosity (H_o) and expected heterozygosity (H_e) of the SSRs were calculated using Arlequin 3.11 and are provided in Table 1. Significant deviation (P-value) from the Hardy-Weinberg equilibrium was recorded for all thirteen loci possibly due to the small population size and number of mutations amongst the selected mandarin cultivars (Table 1).

Probability of identity (PI) by locus and for increasing combinations of loci indicated that the number of microsatellite loci used were sufficient in differentiating between the 32 mandarin cultivars in this study (Table 1, Fig. 2). The polymorphism information content (PIC) values of the loci evaluated ranged from 0.089 to 0.909. Most informative markers were TAA15, GT03, Ci0A05b, mCrCIR07A12 and mCrCIR06B04 (Table 1). The UPGMA dendrogram generated clustered the mandarin cultivars into 4 groups consisting of a distinct Satsuma group and 3 mandarin hybrid groups (Fig.3).

CONCLUSION

The 13 selected primer pairs distinguished between most of the 32 mandarin cultivars. Furthermore, a high level of heterozygosity was obtained with 11 of the microsatellite loci. These microsatellite loci will be further used in the genotyping of the remainder of the mandarin cultivars and in the establishment of a molecular genotype database for mandarin accession verification.

Literature cited

- Barkley, N. A., M. L. Roose, R. R. Krueger, and C. T. Federici. 2006. Assessing genetic diversity and population structure in a citrus germplasm collection utilizing simple sequence repeat markers (SSRs). *Theor. Appl. Genet.* 112:1519-1531.
- Bretting, PK, and MP Widrechner. 1995. Genetic markers and plant genetic resource management. *Plant Breeding Rev.* 13:11-86.
- Froelicher, Y., Dambier, D., Costantino, G., Lotfy, S., Didout, C., Beaumont, V., Brottier, P., Risterucci, A.M., Luro, F., Ollitrault, P., 2008. Characterization of microsatellite markers in *Citrus reticulata* Blanco. *Mol. Ecol. Resour.* 8:119–122.
- Gulsen, O. and Roose M.L., 2001. Lemons: diversity and relationships with selected *Citrus* genotypes as measured with nuclear genome markers. *J Amer Soc Hort Sci.* 126:309–327.
- Hokanson, S.C., Szewc-McFadden, A.K., Lamboy, W.F., McFerson, J.R., 1998. Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a *Malus domestica* borkh. core subset collection. *Theor. Appl. Genet.* 97:671–683.
- Krueger, R. R. and Roose, M.L., 2003. Use of molecular markers in the management of citrus germplasm resources. *J. Amer. Soc. Hort. Sci.* 128:827-837.
- Luro, F., Laigret, F., Bove, J.M. and Ollitrault, P., 1995. DNA amplified fingerprinting, a useful tool for determination of genetic origin and diversity analysis in *Citrus*. *Hort. Sci.* 30:1063–1067.
- Ollitrault, F., Terol, J., Pina, J.A., Navarro, L., Talon, M., Ollitrault, P., 2010. Development of SSR markers from *Citrus clementina* (Rutaceae) BAC end sequences and interspecific transferability in *Citrus*. *Am.J. Bot.* 97:124–129.
- Risterucci, A.M., Duval M.F., Rohde W. and Billotte N., 2005. Isolation and characterization of microsatellite loci from *Psidium guajava* L. *Molecular Ecology Notes* 5: 745–748.

Tables

Table 1. Characterization of 13 microsatellite primer pairs.

Locus	N	Na	Ho	He	P-value	PI by locus	PIC
Ci01C07	32	5	0.625	0.743	0.0099	0.108	0.701
Ci01C09	32	6	0.188	0.546	0.0000	0.267	0.485
Ci02B07	32	4	0.375	0.674	0.0000	0.172	0.608
Ci06A05b	32	14	0.844	0.888	0.0000	0.023	0.878
Ci07E06	32	4	0.000	0.588	0.0000	0.233	0.525
Ci08C05	32	5	0.094	0.559	0.0000	0.235	0.519
mCrCIR01F04a	32	10	0.500	0.748	0.0000	0.084	0.727
mCrCIR01F08a	32	4	0.063	0.091	0.0493	0.828	0.089
mCrCIR06B04	32	17	0.969	0.916	0.0000	0.013	0.909
mCrCIR07A12	32	14	0.500	0.866	0.0000	0.031	0.853
mCrCIR07E12	32	4	0.000	0.682	0.0000	0.167	0.616
TAA15	32	9	0.750	0.763	0.0000	0.087	0.732
GT03	32	10	0.750	0.858	0.0006	0.036	0.842

Figures

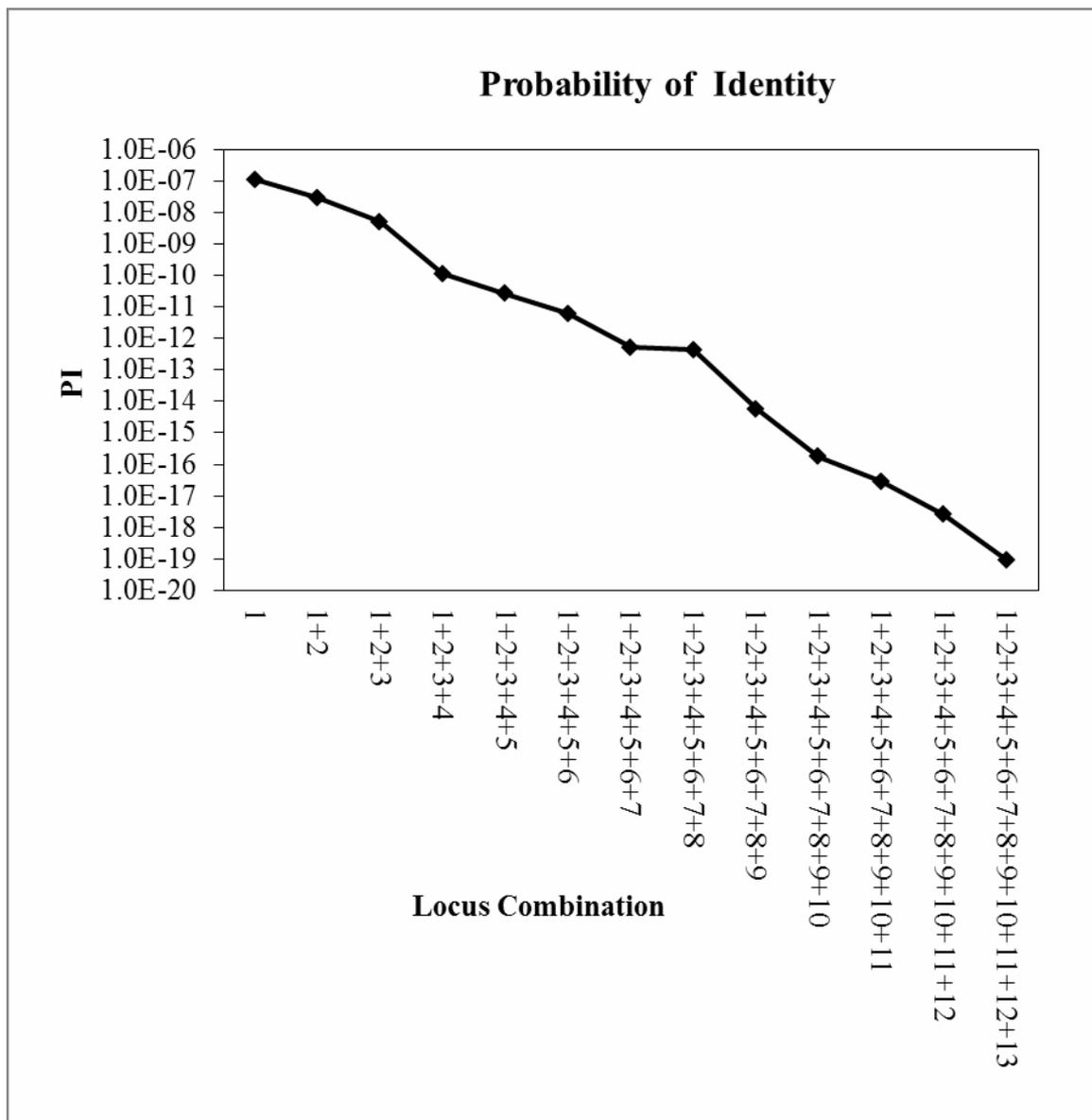


Figure 1. Probability of identity (PI) values for SSR loci. Determination of the number of markers required to reach a discriminant PI value for cultivar identification. Y axis is represented on a logarithmic scale

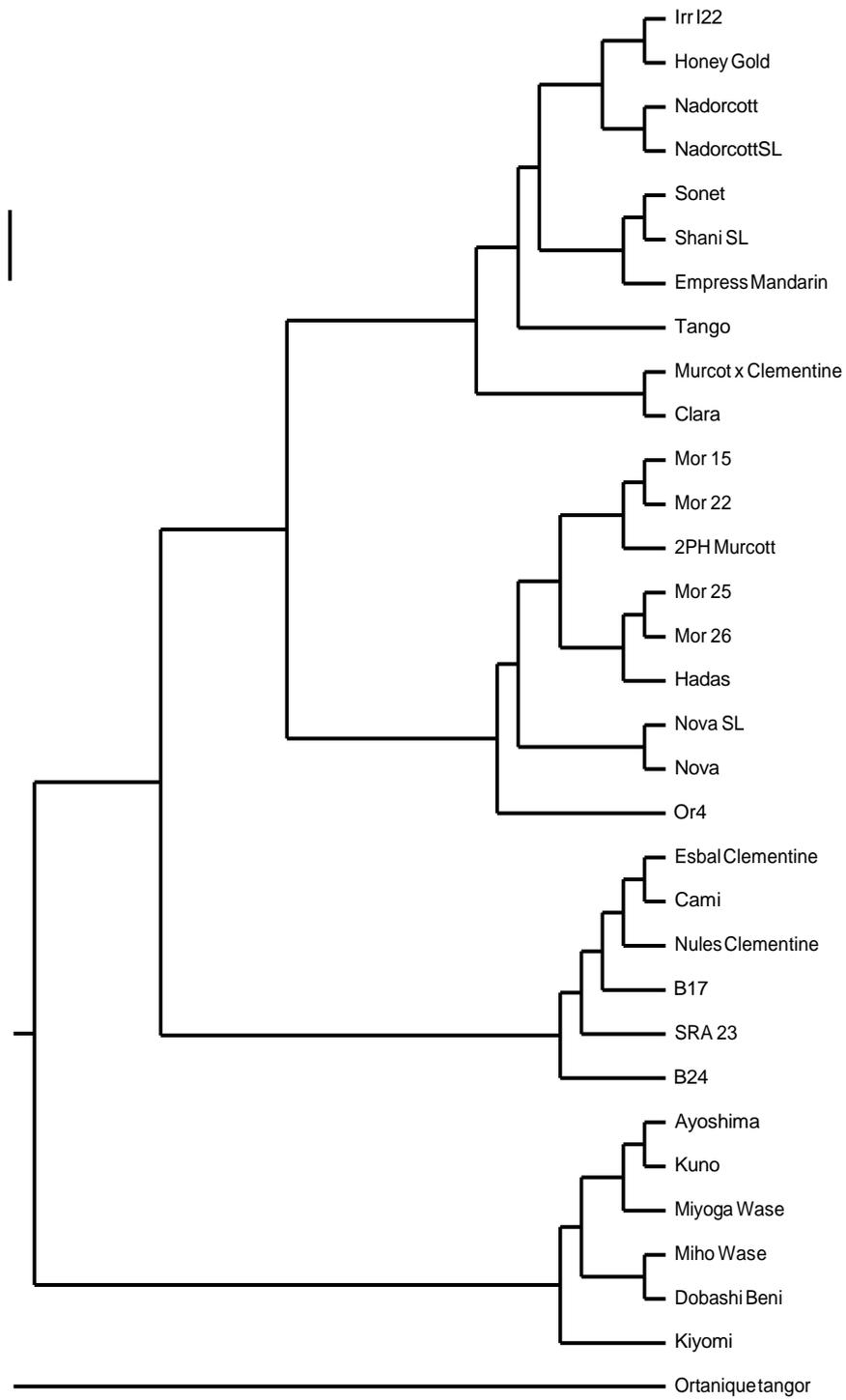


Figure 2. UPGMA generated tree showing relationships within and between accessions germplasm based on the allelic diversity generated.

5.5 Climatic Regions of Southern Africa and cultivars being evaluated

CLIMATIC REGION	AREA	PLACE	CULTIVARS
Hot-Dry	Limpopo	Tshipise	Grapefruit
			Valencias
			Mandarin hybrids (Late)
		Musina	Grapefruit
			Valencias
			Mandarin hybrids (Late)
		Letsitele	Grapefruit
			Valencias
			Mandarin hybrids (Late)
		Hoedspruit	Grapefruit
			Valencias
			Mandarin hybrids (Late)
Hot-Humid	Mpumalanga	Malelane	Grapefruit
			Valencias
			Mandarin hybrids (Late)
		Komatipoort	Grapefruit
			Valencias
			Mandarin hybrids (Late)
	KwaZulu-Natal	Pongola	Grapefruit
			Valencias
			Mandarin hybrids (Late)
		Nkwaleni	Grapefruit
			Valencias
			Mandarin hybrids (Late)
	Swaziland	Lowveld	Grapefruit
			Valencias
			Mandarin hybrids (Late)
Mozambique	Southern	Grapefruit	
		Valencias	
		Mandarin hybrids (Late)	
Intermediate	Limpopo	Tom Burke	Navels (Mid/Late)
			Valencias
			Mandarin hybrids (Mid/Late)
			Lemons
		Letaba	Navels (Mid/Late)
			Valencias
			Mandarin hybrids (Mid/Late)
			Lemons
		Levubu	Navels (Mid/Late)
			Valencias
			Mandarin hybrids (Mid/Late)
			Lemons
		Marble Hall	Navels (Mid/Late)

	Mpumalanga	Nelspruit	Valencias
			Mandarin hybrids (Mid/Late)
			Lemons
			Navels (Mid/Late)
		Karino	Valencias
			Mandarin hybrids (Mid/Late)
			Lemons
			Navels (Mid/Late)
		Hazyview	Valencias
			Mandarin hybrids (Mid/Late)
			Lemons
			Navels (Mid/Late)
	Schagen	Valencias	
		Mandarin hybrids (Mid/Late)	
		Lemons	
		Navels (Mid/Late)	
Swaziland	Ngonini	Navels (Mid/Late)	
		Valencias	
		Mandarin hybrids (Mid/Late)	
		Lemons	
Cold/Coastal	Eastern Cape	East Cape Midlands	Midseasons
			Navels/Valencias
			Mandarin hybrids/Satsumas
		Gamtoos River Valley	Lemons
			Mandarin hybrids
			Navels
	Satsumas/Clementines		
	Sundays River Valley	Lemons	
		Mandarin hybrids	
		Navels/Valencias	
	KwaZulu-Natal	Richmond	Lemons
			Navels
		Ixopo/Umzimkhulu	Lemons
			Navels
	Western Cape	Knysna	Lemons
			Mandarin hybrids
		Heidelberg	Navels
			Mandarin hybrids
			Lemons
		Paarl	Navels
Mandarin hybrids			
Satsumas/Clementines			
Wolseley	Navels		
	mandarin hybrids		

		Citrusdal	Satsumas/Clementines	
			Navels/Valencias	
			mandarin hybrids	
		Lemons	Clanwilliam	Navels/Valencias
				Mandarin hybrids
				Lemons
		Swellendam	Navels/Valencias	
			Mandarin hybrids	
			Lemons	
		Satsumas	Robertson	Navels/Valencias
				Mandarin hybrids/Satsumas
				Lemons
Cool-Inland	North-West	Rustenburg	Navels (Mid)	
			Navels (Late)	
			Mandarin hybrids	
	Limpopo	Zebediela	Navels (Mid)	
			Navels (Late)	
			Mandarin hybrids	
		Mokopane	Navels (Mid)	
			Navels (Late)	
			Mandarin hybrids	
		Burgersfort	Navels (Mid)	
			Navels (Late)	
			Mandarin hybrids	
		Ohrigstad	Navels (Mid)	
			Navels (Late)	
			Mandarin hybrids	
Mpumalanga	Ngodwana/Schoemanskloof	Navels (Mid)		
		Navels (Late)		
		Mandarin hybrids		
Semi-Desert	Northern Cape	Kakamas/Blouputs	Navels (Late)	
			Valencias	
			Grapefruit	
			Mandarin hybrids (Late)	
		Groblershoop/Upington	Navels (Late)	
			Valencias	
			Grapefruit	
			Mandarin hybrids (Late)	
		Vaalharts	Midseasons	
			Navels (Late)	
			Valencias	
			Mandarin hybrids (Late)	

5.6 Approximate maturity periods

Approximate Clementine Maturity Periods in the Cape region of South Africa

	March				April				May				June				July				Aug				Sept			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Early Clementine (Exp)			■	■	■																							
Oronules				■	■	■																						
Marisol					■	■	■																					
Early Oroval								■	■	■																		
SRA63									■	■	■																	
Oroval										■	■	■																
Nules										▨	▨	▨	▨	▨	▨													

Exp = Experimental Cultivar

■	Solid blocks indicate average maturity periods for the area overall
▨	Striped blocks indicate variation due to microclimates

Approximate Grapefruit Maturity Periods in the Northern region of South Africa

		March				April				May				June				July				Aug				Sept							
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	Star Ruby																																
	Marsh / Nartia																																
	Jackson																																
	Ray Ruby																																
	Henderson																																
	Rosé																																
	Flamingo																																
	Star Ruby late																																

Exp = Experimental Cultivar

Approximate Lemon Maturity Periods in the Cape region of South Africa

		March				April				May				June				July				Aug			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	Eureka																								
	Eureka SL (Exp)																								
	Genoa																								
	Lisbon																								
	Limoneira																								

Exp = Experimental Cultivar

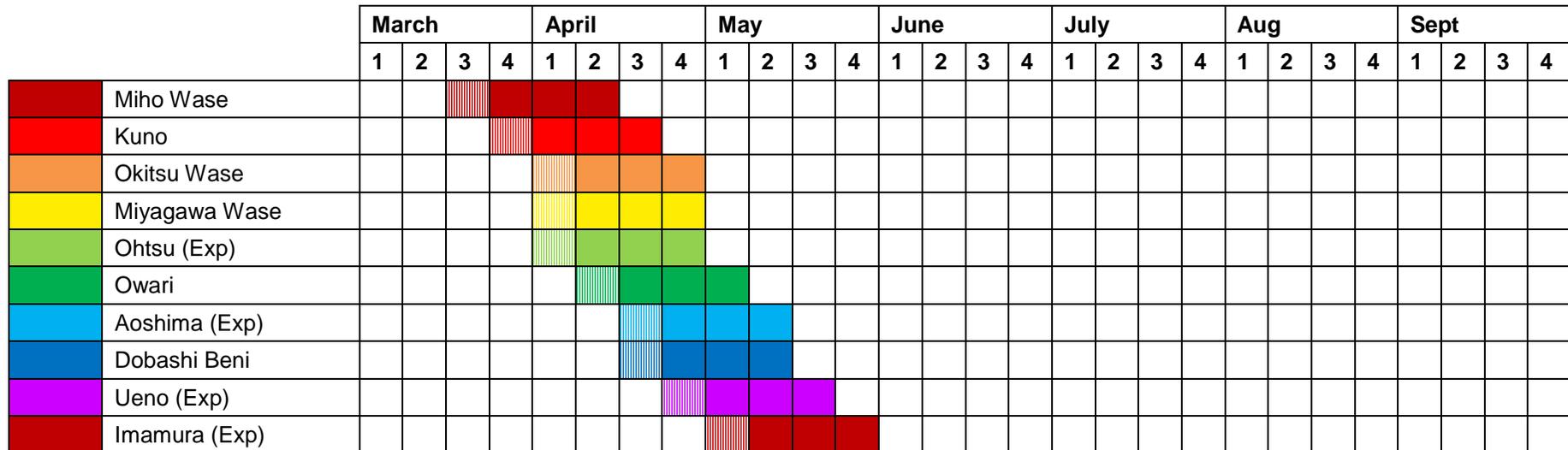
Approximate mandarin hybrid Maturity Periods in the Cape region of South Africa

	March				April				May				June				July				Aug				Sept			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Nova																												
HEM (Exp)																												
African Sunset (B24)																												
Or																												
Furr (Clemcott)																												
Valley Gold (B17)																												
Nectar (Exp)																												
Sweet Spring (Exp)																												
Nardorcott1																												
Tango (Exp)																												
Tasty 1 (Exp)																												
Tahoe Gold (Exp)																												
Mor																												
Yosemite Gold (Exp)																												
Shasta Gold (Exp)																												
Tasty 2 (Exp)																												
Gold Nugget (Exp)																												
Winola (Exp)																												

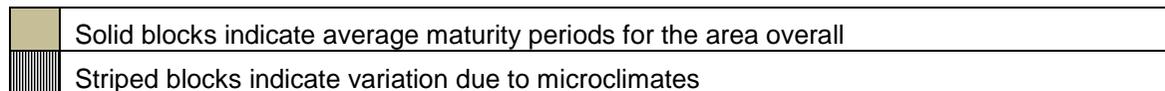
Exp = Experimental Cultivar

	Solid blocks indicate average maturity periods for the area overall
	Striped blocks indicate variation due to microclimates

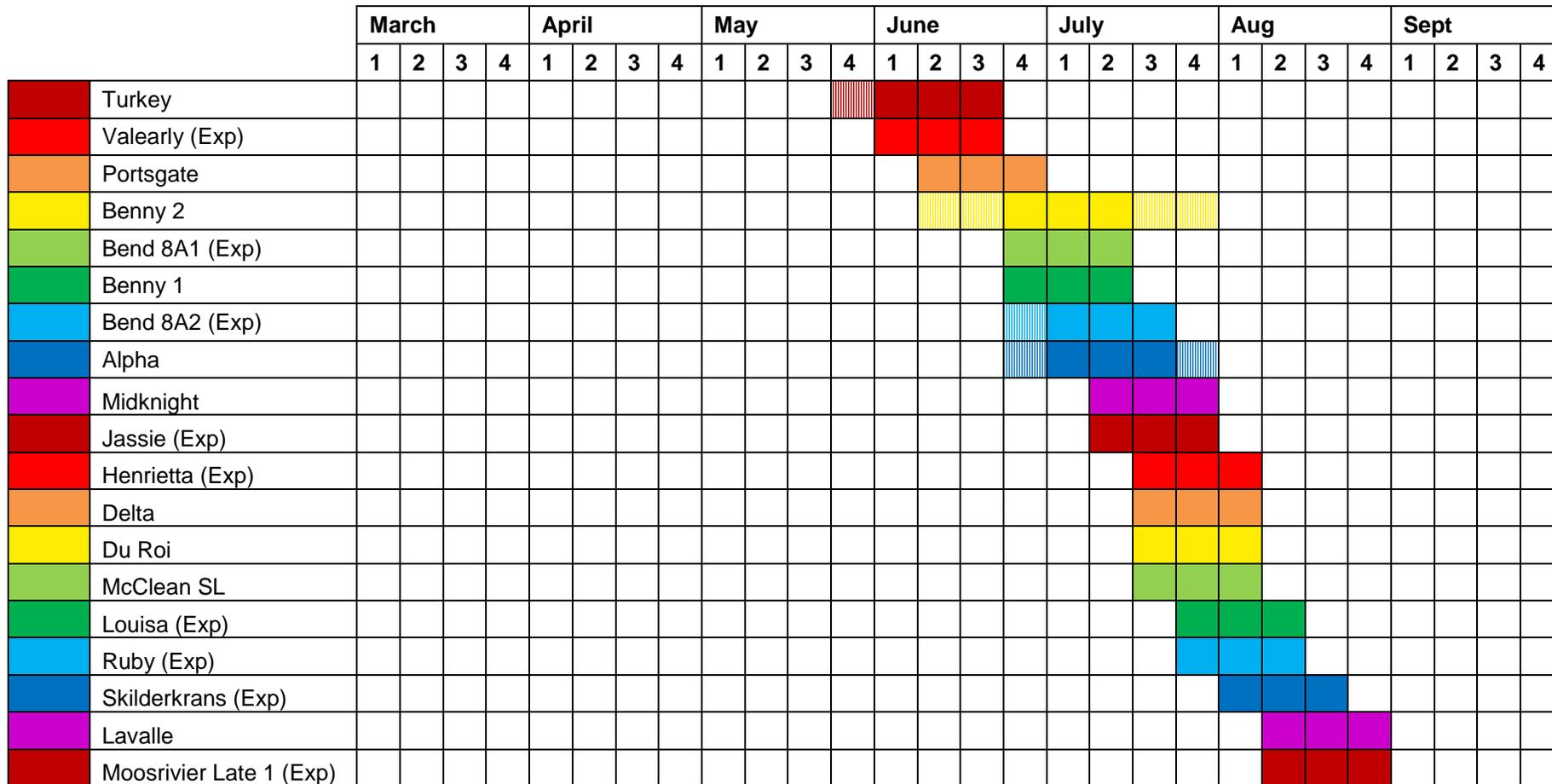
Approximate Satsuma Maturity Periods in the Cape regions of South Africa



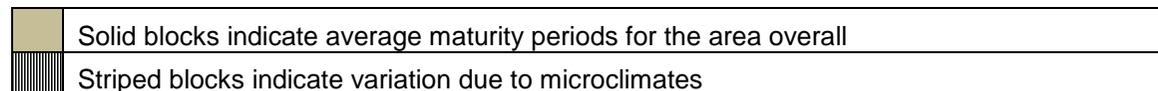
Exp = Experimental Cultivar



Approximate Valencia Maturity Periods in the Northern region of South Africa



Exp = Experimental Cultivar



Approximate Navel Maturity Periods in the Cape region of South Africa

	March				April				May				June				July				Aug				Sept			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Fukumoto																												
Newhall/Navelina																												
HAEN (Exp)																												
Fischer																												
DRN (Exp)																												
EHN (Exp)																												
Bahianinha																												
Palmer																												
Washington																												
Cara Cara																												
EDPN (Exp)																												
Autumn Gold																												
Barnfield Summer																												
Summer Gold																												
Powell Summer																												
Witkrans																												
Lane Late																												
KSEN (Exp)																												
Cambria																												
Glen Ora Late																												

6 CITRUS IMPROVEMENT SCHEME (CIS)

By P.H. Fourie, M.M. N. du Toit, M. le Roux, L. Olivier, S.P. van Vuuren, J.H.J. Breytenbach and G. Cook (CRI)

Summary

The purpose of the CIS is to enhance the standard of the South African citrus industry by ensuring that only horticulturally superior plants, which are free of viruses, diseases and pests, are supplied to producers and certified. The Citrus Growers Association of southern Africa (CGA) is responsible for the CIS and delegated its authority to CRI. In order to achieve this objective, close co-operation is required between CRI, the Agricultural Research Council's Institute for Tropical and Subtropical Crops (ARC-ITSC), DAFF's Directorate of Plant Health (DPH) and citrus nurseries represented by the South African Citrus Nurserymen's Association (SACNA). Additionally, Cultivar and Pathology sub-committees co-ordinate the respective CIS activities. The organisations and committees, as well as all participating role players in the CIS are represented on the CIS Advisory Committee (CISAC), which advises CRI on the CIS operations as specified in its Procedural Guide. This annual report summarises citrus seed and budwood production and supply from the Citrus Foundation Block (CFB) outside Uitenhage, nursery and tree certification, diagnostic services conducted by CRI in order to ensure the phytosanitary status of propagation material in the CIS, as well as CIS biosecurity services in southern Africa. Highlights from the 2014/15 season, include a record budwood supply year (23.6% more buds supplied than in 2013/14) with CFB continuing to improve toward its central supply objectives by reducing the percentage of certified buds cut in nurseries from 40% in 2012/13 to 28.5%. The phytosanitary status of the CIS is ensured by virus-elimination and diagnostic services prior to CIS introduction and was confirmed through re-indexing of mother trees. These activities are reported as well as further investigations aimed at improving diagnostics and cross-protection against *Citrus tristeza virus*. Citrus biosecurity in Africa initiatives involve continued engagements and propagation material supply to southern African countries, as well as surveys and awareness campaigns against African Citrus Greening, and the feared exotic threat Asiatic Citrus Greening and its Asian Citrus psyllid vector.

Opsomming

Die doel van die Sitrus Verbeteringskema (SVS) is om die standaard van die Suid-Afrikaanse sitrusbedryf te verbeter deur te verseker dat slegs plante van die beste hortologiese gehalte, vry van virusse, siektes en peste, aan produsente gelewer en gesertifiseer word. Die Sitrus Produsente Vereniging van suider-Afrika (CGA) is verantwoordelik vir die SVS en delegeer sy gesag aan CRI. Ten einde hierdie doelwit te bereik, word noue samewerking tussen CRI, die Landbou Navorsingsraad se Instituut vir Tropiese en Subtropiese Gewasse (LNR-ITSG), DAFF se Direkoraat van Plantgesondheid (DPH) en sitruskwekerye wat verteenwoordig word deur die Suid-Afrikaanse Sitrus Kwekers Vereniging (SACNA). Daarbenewens koördineer Kultivar en Patologie komitees die relevante SVS-aktiwiteite. Hierdie organisasies en komitees, asook al die deelnemende rolspelers in die SVS, word op die SVS Advieskomitee (CISAC) verteenwoordig en adviseer CRI oor die SVS-bedrywighede soos gespesifiseer in die operasionele riglyne. Hierdie jaarverslag som die volgende op: die sitrus saad en okuleerhout produksie en verskaffing vanaf die SVS Sitrus Grondvesblok (SGB) geleë buite Uitenhage; die kwekery en boom sertifisering, diagnostiese dienste gelewer deur CRI om die fitosanitêre status van voortplantingsmateriaal in die SVS te verseker, sowel as SVS biosekuriteit-aktiwiteite in suider Afrika. Hoogtepunte van die 2014/15 seisoen sluit 'n rekord okuleerhoutsverskaffings jaar in (23.6% meer ogies verskaf as in 2013/14) en die SGB wat steeds verbeter in hul doelwit van sentrale okuleerhout verskaffing met 'n afname in die persentasie van gesertifiseerde ogies wat in kwekerye gesny was van 40% in 2012/13 tot 28.5%. Die fitosanitêre status van die SVS word verseker deur virusreiniging en diagnostiese dienste voor SVS-insluiting en word deurlopend bevestig deur middel van die herindeksing van moederbome. Hierdie aktiwiteite word gerapporteer asook verdere ondersoek gemik op die verbetering van diagnostiese tegnieke en kruis-beskerming teen *Citrus tristeza virus*. Sitrus biosekuriteit inisiatiewe in Afrika behels voortgesette skakeling en verskaffing van voortplantingsmateriaal aan ander suider Afrikaanse lande, asook opnames en bewusmakingsveldtogte teen Afrika Sitrus Vergroening en die gevreesde eksotiese bedreiging: Asiatiese Sitrus Vergroening en sy Asiatiese Sitrus psylla vektor.

6.1 Budwood

This report summarises the seasonal supply of budwood from June 2014 to May 2015. A total of 4,437,048 buds were supplied by the Citrus Foundation Block (CFB) and authorised for cutting in certified nurseries. This is 26.8% more buds than in the same period of 2014 and 43.0% more buds than in the same period of 2013. During this period 78,614 buds were exported to neighbouring countries. Increased demand was mostly from the Western Cape (26.7%), Eastern Cape (23.0%) and Limpopo (10.8%) nurseries, whereas a decrease in demand was experienced in the Northern Cape (-24.4%) and Mpumalanga (-10.6%). North

West and KwaZulu-Natal had a below average demand during 2013/14 and this resulted in a significant increase in demand from these nurseries during 2014/15: North West (261.1%) and KwaZulu Natal (57.8%). Mandarin (40.3%) was the most popular citrus type, followed by lemon (28.2%), Valencia (12.8%) and navels (9.0%); in 2013 this proportion was 42.8%, 20.3%, 18.3% and 10.5%, respectively (Tables 6.1.1 and 6.1.2). The top 30 varieties comprised 90.5% of total number of buds supplied. Eureka Lemon (2nd most popular cultivar in 2013 and most popular cultivar in 2012) was the most popular cultivar in 2014, followed by Nadorcott 1 mandarin and Tango mandarin (Table 6.1.3). The need for authorised cutting in nurseries has decreased from 40.0% in 2012/13 to 31.0% in 2013/14 to 28.5% in 2014/15 (Figure 6.1.1).

Table 6.1.1. Buds supplied during the period June to May 2013-2015.

Area	2012/13	2013/14	2014/15	3-yr average
Local	3 151 265	3 549 064	4 437 048	3 712 459
Exported	7 650	13 315	78 614	33 193
Total	3 158 915	3 562 379	4 515 662	

Local	2012/13	2013/14	2014/15	Increased demand on 2013/14 (%)
Eastern Cape	627 553	768 210	944 832	23.0%
KwaZulu-Natal	41 800	24 400	38 500	57.8%
Limpopo	1 236 830	1 196 363	1 325 441	10.8%
Mpumalanga	229 125	265 757	237 560	-10.6%
North-West Province	121 000	46 500	167 900	261.1%
Northern Cape	149 669	188 600	142 530	-24.4%
Western Cape	745 288	1 059 234	1 580 285	49.2%
Total	3 151 265	3 549 064	4 437 048	

Exported	2012/13	2013/14	2014/15	3-yr average
Angola			43 014	43 014
Botswana	7 500	7 000		7 250
Congo		5 750	11 350	8 550
Swaziland			450	450
Zambia		565		565
Zimbabwe	150		23 800	11 975
Total	7 650	13 315	78 614	

Cultivar group	2012/13	%	2013/14	%	2014/15	%	3-yr average
Clementine	117 800	3.7%	81 910	2.3%	263 820	5.8%	154 510
Diverse	6780	0.2%	5885	0.2%	4495	0.1%	5 720
Grapefruit	88 670	2.8%	126 395	3.6%	45 800	1.0%	86 955
Kumquat	7 860	0.3%	11 950	0.3%	18 400	0.4%	12 737
Lemon	719 200	22.8%	721 665	20.3%	1 272 962	28.2%	904 609
Lime	37 170	1.2%	12 625	0.4%	28 570	0.6%	26 122
Mandarin Hybrid	924 237	29.3%	1 525 018	42.8%	1 819 503	40.3%	1 422 919
Midseason	9 440	0.3%	6 490	0.2%	4 978	0.1%	6 969
Navel	555 401	17.6%	375 426	10.5%	404 407	9.0%	445 078
Pummelo	850	0.0%	3 515	0.1%	2 420	0.1%	2 262
Rootstock		0.0%	80	0.0%	70	0.0%	75
Satsuma	37 270	1.2%	39 866	1.1%	74 004	1.6%	50 380
Valencia	654 237	20.7%	651 554	18.3%	576 233	12.8%	627 341
Total	3 158 915	100.0%	3 562 379	100.0%	4 515 662	100.0%	

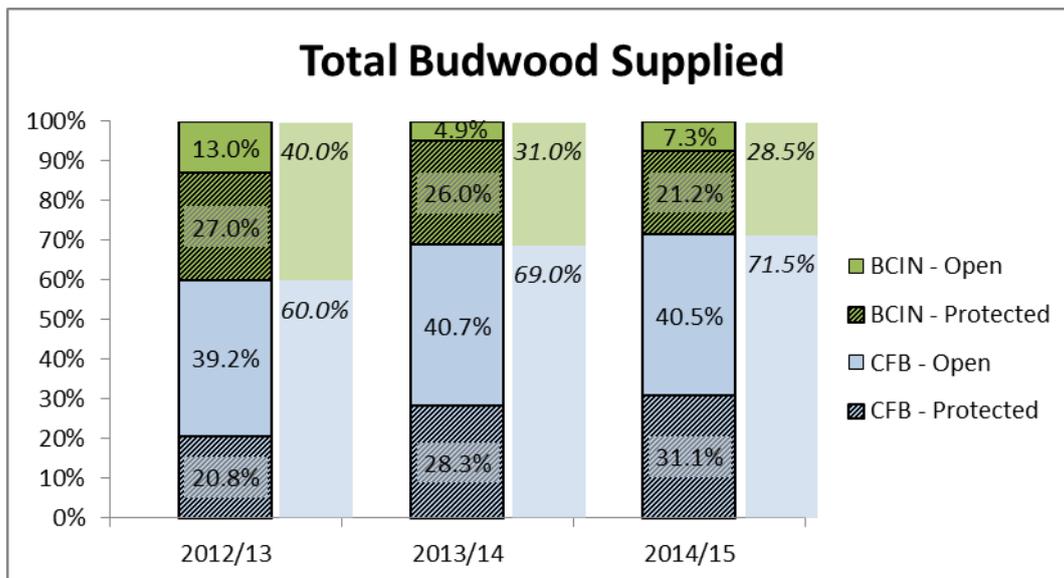


Figure 6.1.1. Budwood (% of total) supplied by the CFB and authorised for cutting in nurseries during the periods June to May from 2012-2015.

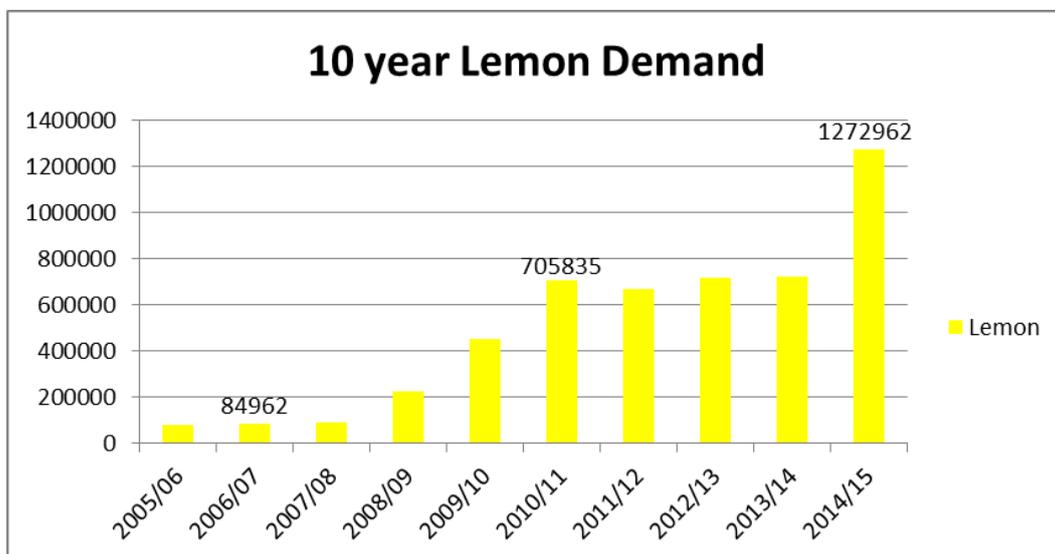


Figure 6.1.2. Lemon budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries during the periods June to May from 2005-2015.

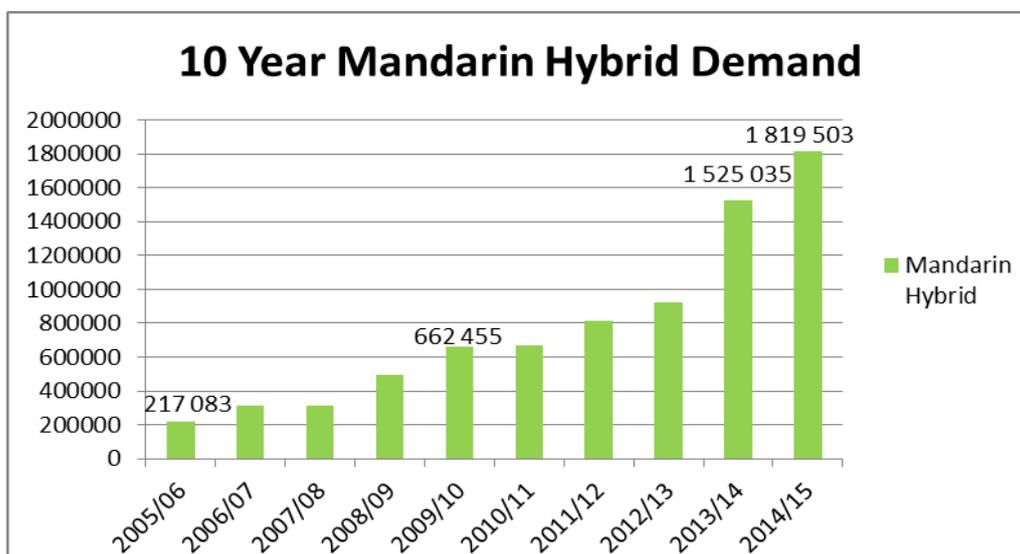


Figure 6.1.3. Mandarin hybrid budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries during the periods June to May from 2005-2015.

Table 6.1.2. Buds supplied per cultivar group per area (total number of buds per season) during the periods June to May from 2012-2015.

Variety Type	Year	Eastern Cape	KwaZulu-Natal	Limpopo	Mpumalanga	North-West Province	Northern Cape	Other African States	Western Cape	Total
Clementine	2012/13	2 600	500	10 000	600		7 000		97 100	117 800
	2013/14	19 952		7 500	85		5 500	900	47 973	81 910
	2014/15	37 759		29 350	330		7 000	5 400	183 981	263 820
Diverse	2012/13	180		3 100		400		150	1 150	4 980
	2013/14	275	1 750	1 900	40		200		170	4 335
	2014/15	10		1 100	450	800		100	2 025	4 485
Ellendale	2012/13						300		1 500	1 800
	2013/14					1 200			350	1 550
Grapefruit	2012/13	1 100	2 000	54 660	28 120			1 000	1 790	88 670
	2013/14		1 400	32 870	41 600		49 550	705	270	126 395
	2014/15		1 500	24 050	14 600	3 500		1 450	700	45 800
Kumquat	2012/13	650	3 000	200	200	500	10		3 300	7 860
	2013/14		2 000	3 400	1 400	3 100	1 000		1 050	11 950
	2014/15			8 300	3 100	4 000		500	2 500	18 400
Lemon	2012/13	194 460	7 000	323 280	18 305	47 700	41 500		86 955	719 200
	2013/14	264 768	3 750	271 300	31 545	5 200	28 300	655	116 147	721 665
	2014/15	431 061	17 500	358 345	54 490	63 800	56 260	880	290 626	1 272 962
Lime	2012/13	5 560	500	10 200	2 860	9 000	550		8 500	37 170
	2013/14	20		2 800	3 460	0		195	6 150	12 625
	2014/15	10		7 400	1 000			14 220	5 940	28 570
Mandarin Hybrid	2012/13	201 252	500	345 860	41 435	26 100	40 700	1 000	267 390	924 237
	2013/14	300 717	7 500	364 663	50 690	23 900	83 200	690	693 658	1 525 018
	2014/15	330 685	5 000	416 586	66 285	20 500	47 450	1 200	931 797	1 819 503
Midseason	2012/13	100							9 340	9 440
	2013/14	215			40	700			5 535	6 490
	2014/15	20							4 958	4 978
Navel	2012/13	154 928	11 500	84 240	55 660	18 200	28 540	2 500	199 833	555 401
	2013/14	143 764	1 000	72 200	15 475	7 100	13 450	4 560	117 877	375 426
	2014/15	113 999	6 000	123 150	22 050	19 950	14 500	22 736	82 022	404 407
Pummelo	2012/13			600			50		200	850
	2013/14			3 500				15		3 515

	2014/15			2 400					20	2 420
Rootstock	2013/14				80					80
	2014/15	70								70
Satsuma	2012/13	5 700	12 000	3 500	1 660				14 410	37 270
	2013/14	4 635	5 000	6 800	225	900		1 000	21 306	39 866
	2014/15	8 825	1 000	4 700	3 510	1 000	6 700		48 269	74 004
Seville	2014/15								10	10
Valencia	2012/13	61 023	4 800	401 190	80 285	19 100	31 019	3 000	53 820	654 237
	2013/14	33 864	2 000	429 430	121 117	4 400	7 400	4 595	48 748	651 554
	2014/15	22 393	7 500	350 060	71 745	54 350	10 620	32 128	27 437	576 233
Total		2 340 595	104 700	3 758 634	732 442	335 400	480 799	99 579	3 384 807	11 236 956

Table 6.1.3. Top 30 cultivars based on total number of buds supplied for seasons June to May from 2012-2015.

	2012/13	BCIN	CFB	Total	%	2013/14	BCIN	CFB	Total	%	2014/15	BCIN	CFB	Total	%
1	Eureka Lemon	260 185	285 205	545 390	17.3%	Nadorcott 1 Man.	433 088	191 909	624 997	17.5%	Eureka Lemon	192 173	554 347	746 520	16.5%
2	Or 4 Man.	194 950	50 800	245 750	7.8%	Eureka Lemon	60 750	380 410	441 160	12.4%	Nadorcott 1 Man.	276 805	294 737	571 542	12.7%
3	Midknight Val.	35 025	179 472	214 497	6.8%	Tango Man.	305 440	112 468	417 908	11.7%	Tango Man.	293 746	163 335	457 081	10.1%
4	Late Val.	71 000	123 590	194 590	6.2%	Late Val.	44 000	168 220	212 220	6.0%	Midknight Val.		243 515	243 515	5.4%
5	Chislett M7 Navel	111 404	45 530	156 934	5.0%	Midknight Val.		170 605	170 605	4.8%	Lisbon Lemon	27 100	188 422	215 522	4.8%
6	ARCCIT1614 (B17) (Valley Gold) Man.	118 750	37 000	155 750	4.9%	ARCCIT1614 (B17) (Valley Gold) Man.	71 456	92 637	164 093	4.6%	ARCCIT1614 (B17) (Valley Gold) Man.	35 660	130 421	166 081	3.7%
7	Nadorcott 1 Man.	55 932	99 001	154 933	4.9%	Limoneira 8A Lemon	53 800	75 466	129 266	3.6%	Witkrans 3 Navel	54 303	111 111	165 414	3.7%
8	Nova Man.	2 000	120 959	122 959	3.9%	Witkrans 3 Navel	39 798	69 740	109 538	3.1%	2PH Eureka SL Lemon	87 912	69 335	157 247	3.5%
9	Tango Man.	76 359	45 451	121 810	3.9%	Star Ruby Grapefruit		107 960	107 960	3.0%	Nova Man.		139 324	139 324	3.1%
10	Witkrans 3 Navel	53 610	41 290	94 900	3.0%	Lisbon Lemon	9 000	93 846	102 846	2.9%	Nules Clem.	64 221	67 625	131 846	2.9%
11	Lisbon Lemon	21 000	70 330	91 330	2.9%	Nova Man.		83 157	83 157	2.3%	Late Val.		116 178	116 178	2.6%
12	Carninka Late Navel	54 605	25 502	80 107	2.5%	Or 4 Man.	32 300	39 515	71 815	2.0%	Or 4 (2) Man.		101 805	101 805	2.3%
13	Star Ruby Grapefruit		79 760	79 760	2.5%	Cambria 3 Navel	13 300	41 574	54 874	1.5%	Royal Honey Man.	82 200	2 310	84 510	1.9%
14	Andes 1 Clemenluz Clem.	65 400	6 250	71 650	2.3%	Gusocora (G5) Val.	8 700	42 595	51 295	1.4%	Limoneira 8A Lemon		84 024	84 024	1.9%
15	Benny 2 Val.	8 000	57 571	65 571	2.1%	Benny 2 Val.		50 495	50 495	1.4%	Mor 26 Man.		77 985	77 985	1.7%
16	Cambria 3 Navel	32 058	32 871	64 929	2.1%	Mor 26 Man.		48 457	48 457	1.4%	Genoa Lemon		62 743	62 743	1.4%
17	Cambria 4 (K-tak) Navel	36 763	17 018	53 781	1.7%	Delta Val.		47 989	47 989	1.3%	Or 4 Man.		59 530	59 530	1.3%
18	Gusocora (G5) Val.	23 500	27 082	50 582	1.6%	Alpha Val.		47 006	47 006	1.3%	Nules (2) Clem.	26 740	32 785	59 525	1.3%
19	Limoneira 8A Lemon		44 540	44 540	1.4%	Nules Clem.		34 984	34 984	1.0%	Alpha Val.	7 200	48 065	55 265	1.2%
20	Delta Val.		43 042	43 042	1.4%	Chislett M7 Navel		33 911	33 911	1.0%	Andes 1 - Clemenluz Clem.	23 469	29 850	53 319	1.2%
21	Genoa Lemon		37 190	37 190	1.2%	Empress Man.		33 500	33 500	0.9%	Cambria 3 Navel		46 873	46 873	1.0%
22	Bearss Lime		36 770	36 770	1.2%	Lane Late (Cal.) Navel		30 403	30 403	0.9%	IR M2 (QDPI #283) Man.	38 800	850	39 650	0.9%
23	Mor 26 Man.		34 270	34 270	1.1%	Glenora Late Navel		26 303	26 303	0.7%	Bahianinha Navel		36 250	36 250	0.8%
24	Empress Man.	5 500	26 510	32 010	1.0%	Nules (2) Clem.		25 520	25 520	0.7%	Empress Man.	3 000	31 200	34 200	0.8%
25	Lavalle 2 Val.		26 000	26 000	0.8%	Carninka Late Navel		25 296	25 296	0.7%	Washington Navel		33 615	33 615	0.7%
26	Nules Clem.		21 500	21 500	0.7%	Bahianinha Navel		24 435	24 435	0.7%	Autumn Gold Navel	23 219	7 436	30 655	0.7%
27	Bahianinha Navel		20 940	20 940	0.7%	2PH Eureka SL Lemon		23 951	23 951	0.7%	Delta Val.		30 323	30 323	0.7%
28	Miho Wase Satsuma	1 000	17 870	18 870	0.6%	Or 4 (2) Man.		23 435	23 435	0.7%	Nadorcott SL Man.	20 800	8 360	29 160	0.6%
29	Washington Navel	3 000	15 407	18 407	0.6%	Genoa Lemon		20 211	20 211	0.6%	Gusocora (G5) Val.		28 385	28 385	0.6%
30	Palmer Navel		16 270	16 270	0.5%	Washington Navel		17 827	17 827	0.5%	Belalate Satsuma		28 330	28 330	0.6%
		1 230 041	1 684 991	2 915 032	92.3%		1 071 632	2 183 825	3 255 457	91.4%		1 257 348	2 829 069	4 086 417	90.5%
		1 263 571	1 895 344	3 158 915	100%		1 103 521	2 458 858	3 562 379	100%		1 285 648	3 230 014	4 515 662	100%

6.2 Seed

Uncharacteristically low and variable germination rates were experienced during 2013/14. Exports were stopped early to address local demand. During that period 1065 litres were replaced locally and 17 litres were replaced for SADC countries. Carrizo citrange seed was imported from Australia and Carrizo citrange, Swingle citrumelo and Rough lemon were imported from the United States of America to address the shortages in 2013/14. During May to April 2015, 4132 litres of seed were supplied locally by the CFB and 321 litres of seed were exported; 955 litres were produced by the CIS nurseries (Table 6.2.1). Unprecedented increase in demand for Eureka compatible rootstocks in 2014/15 has necessitated the need to import seed from the USA: 113 litres of Rough lemon and 118 litres of Volkameriana were imported. Carrizo citrange remains the most popular rootstock (42.4%), followed by Swingle citrumelo (14.3%) and C35 citrange (13.7%), Rough lemon (11.8%) and X639 (7.9%) (Table 6.2.2).

Table 6.2.1. Seed (litres) supplied by the CFB and Seed Produced by Nurseries (SPIN) during the periods May to April 2012-2015.

Area	2012/13*			2013/14*				2014/15			
	CFB	SPIN	Total	CFB	SPIN	Imported	Total	CFB	SPIN	Imported	Total
Local	3073		3073	2893		890	3783	4132	955	231	5318
Exported	230		230	340		1	341	321			321
Total	3303		3303	3233		891	4124	4453	955	231	5639

RSA supply	2012/13*			2013/14*				2014/15			
	CFB	SPIN	Total	CFB	SPIN	Imported	Total	CFB	SPIN	Imported	Total
Eastern Cape	337		337	610		224	834	649	620	68	1337
Gauteng			0				0	0			0
KwaZulu-Natal	14		14	16			16	35		2	37
Limpopo	1833		1833	1441		222	1663	2164		10	2174
Mpumalanga	11		11	43		34	77	74	335		409
North-West Province	98		98	48		1	49	98		0	98
Northern Cape	75		75	154		45	199	157		15	172
Western Cape	706		706	581		364	945	954		136	1090
Total	3073		3073	2893		890	3783	4132	955	231	5318

Seed exports	2012/13*			2013/14*				2014/15			
	CFB	SPIN	Total	CFB	SPIN	Imported	Total	CFB	SPIN	Imported	Total
Australia	72		72	33			33	15			15
Egypt	7		7				0				0
Indian Ocean Islands			0				0	10			10
Morocco	15		15	151			151				0
Other African States	15		15	35		1	36	47			47
Portugal	100		100	80			80	140			140
South America	21		21	41			41	103			103
United Arab Emirates			0				0	6			6
Total	230		230	340		1	341	321	0	0	321

*SPIN not determined

Table 6.2.2. Rootstock cultivar supply (litres seed) from 2012/13 to 2014/2015.

Rootstock Cultivar	2012/13			2013/14				2014/15			
	CFB	SPIN *	Total	CFB	SPIN *	Imported	Total	CFB	SPI N	Imported	Total
79 AC			0.0%				0.0%	1			0.0%
79AB-6/14			0.0%				0.0%	3			0.1%
AT			0.0%				0.0%		5		0.1%
BC			0.0%				0.0%		15		0.3%
C35	255		7.7%	115 5			28.0%	670	103		13.7 %
CC	168 4		51.0%	100 1		588	38.5%	1975	419		42.4 %
CM	5		0.2%	12			0.3%	14			0.2%
FD	227		6.9%	120			2.9%	140			2.5%
MXT	74		2.2%	98			2.4%	146	5		2.7%
RL	378		11.4%	274		252	12.8%	461	92	113	11.8 %
SC	435		13.2%	332		51	9.3%	551	256		14.3 %
SXB			0.0%				0.0%		25		0.4%
TC	75		2.3%	44			1.1%	10	7		0.3%
VA	57		1.7%	75			1.8%	58		118	3.1%
X639	78		2.4%	82			2.0%	414	29		7.9%
YC	36		1.1%	36			0.9%	10			0.2%
Total	330 3		100.0 %	323 3		891	100.0%	4453	955	231	100.0%

*SPIN not determined

6.3 Production

With multiplication trees in production, the CFB presently carries a potential budwood stock of >7 million buds of approximately 330 varieties per year. As the top 30 varieties comprise 90.5% of demand, multiplication tree stocks are being managed in order for CFB to be able to timeously supply demand of the sought-after varieties. During April to March 2015, 6151 increase trees were budded in greenhouses 2 and 4. These consisted of 17 new releases from the ARC-ITSC, 18 from CRI and re-multiplication of 13 existing cultivars. A further 2688 seedlings in 2-L bags will be budded during April 2015 in the heated rapid multiplication tunnels. These tunnels allowed for faster multiplication of a much higher concentration per surface area of increase trees. Thus far this initiative seems to be successful as active growth was observed into winter and budwood was already harvested in the summer on the small increase trees. The 6705 trees in 2-L and 230-ml pots that were budded last season in the rapid multiplication tunnel were replanted in 10-L bags in Greenhouse 4 C. A further 7248 seedlings in 230-ml polytubes and 7776 seedlings in 2-L bags are available for budding in autumn and spring of the next reporting period. Fifteen different rootstock varieties (total of 1205 trees) were planted in two new seed source orchards with a high density planting distance of 6 x 1.5 m to increase early yields; alternate trees will be removed at a later stage to reduce the planting density.

Table 6.3.1. New cultivar introductions from 2012-2015.

Area	2012/13	2013/14	2014/15
New introductions from ARC's STG laboratory	14	17	17
New introductions from CRI's STG laboratory	9	11	18
Re-multiplication of existing cultivars	99*	64	13

*This includes new mother trees for greenhouse 5

6.4 Tree Certification

There were 2,115,243 trees certified during April 2014 to March 2015. This is 1,229,359 more trees than in the same period of 2013/14 and 1,136,790 more than in 2012/13 (Table 6.4.1). The implementation of an extensive database to verify the pathogen status of nursery trees as a certification criterion has resulted in a

number of pending tree certification requests. Michelle le Roux has also been on maternity leave in the first quarter, which necessitated Louise Olivier to focus much of her time on training and performance of other duties. The backlogged certification requests were concluded in winter and spring of 2014. During 2013/14 and 2014/15, 143,453 and 44,656 trees, respectively, were not eligible for certification. This was mostly because of the Phytophthora status or tree age that exceeded 30 months after budding.

Table 6.4.1. Trees certified during the period April to March from 2012-2015.

Citrus type	Year	Other African States								South Africa								Total	
		Angola	Botswana	Congo	Mozambique	Namibia	Other African States	Zambia	Zimbabwe	Eastern Cape	Gauteng	KwaZulu-Natal	Limpopo	Mpumalanga	North-West Province	Northern Cape	Swaziland		Western Cape
Clementine	2012/13					560			7 931			4 500	10 212				20 140	43 343	
	2013/14								1 200	5 560		20 699	4 620				34 043	66 122	
	2014/15			2 000	1 332	200	115		3 780	310	2 200	670	20	2 334			24 711	37 672	
Diverse	2012/13					270			340			230	100				380	1 320	
	2013/14													1 441			425	1 866	
	2014/15									300		19	430					749	
Grapefruit	2012/13					20			2 582		14 700	27 752	1 400	10	3 012	1 700	5 925	57 101	
	2013/14											3 340	6 050	970				10 360	
	2014/15							100	626		1 112	4 874	9 014				500	16 226	
Lemon	2012/13					1 150			106 080		4 894	24 103	12 120	2 000	4 277		56 321	210 945	
	2013/14	1 500			2 500				94 689		3 250	10 523	26 300	6 150			15 924	160 836	
	2014/15			100	9 090	150	700		151 698		11 520	128 822	49 319	12 407	600	5 000	63 664	433 070	
Lime	2012/13											5 276	2 000				2 470	9 746	
	2013/14											1 500					435	1 935	
	2014/15			50			300				1 500	504	2 103				500	4 957	
Mandarin Hybrid	2012/13		500			40			34 087			13 953	17 898	1 290	4 634		78 597	150 999	
	2013/14		500						40 145		6 000	35 414	23 385	33 435			167 823	306 702	
	2014/15			500	16 164	100	4 800		207 534		5 150	160 783	158 703	59 460	22 247		128 686	764 127	
Navel	2012/13		1 000			2 565			51 342		7 600	27 769	38 462	6 261		7 500	26 229	168 728	
	2013/14	1 100				2 000			78 773	50	1 500	2 492	62 632	13 860	5 847		14 650	182 904	
	2014/15			840	5 575	380	7 770		105 957			59 290	45 556	6 593	3 600	6 100	99 455	341 116	
Satsuma	2012/13								10 635			6 563	7 175				2 965	27 338	
	2013/14	1 930							9 900	230		1 153	1 720	940	400		14 274	30 547	
	2014/15			300	717				20 240	4 010		5 703	1 458				18 957	51 385	
Valencia	2012/13		1 000			110			28 939		14 950	198 533	20 286	3 481	2 620	3 520	35 494	308 933	
	2013/14		1 500			200			12 770	4 560	1 600	64 795	22 867	8 300			8 020	124 612	
	2014/15			1 010	1 665		29 480		6 000	18 213	330	520	259 595	87 853	21 365	6 410	33 500	465 941	
Total		4 530	4 500	4 800	37 043	7 745	43 165	100	6 000	987 461	15 350	76 496	1 068 855	611 683	180 297	53 647	23 820	854 088	3 979 580

Table 6.4.2. Trees not meeting the certification criteria during the period April to March from 2012-2015 (*Recorded from June 2013, only)

Certified vs Not Certified	Year	Angola	Botswana	Congo	Mozambique	Namibia	Other African States	Zambia	Zimbabwe	Eastern Cape	Gauteng	KwaZulu-Natal	Limpopo	Mpumalanga	North-West Province	Northern Cape	Swaziland	Western Cape	Total
Certified	2012/13		2 500			4 715				241 936		42 144	308 679	109 653	13 042	14 543	12 720	228 521	978 453
Certified	2013/14	4 530	2 000		2 500	200				237 477	10 400	12 350	139 916	147 574	65 096	6 247		255 594	885 884
Certified	2014/15			4 800	34 543	830	43 165	10 000	6 000	508 048	4 950	22 002	620 260	354 456	102 159	32 857	11 100	369 973	2 115 243
Not Certified *	2012/13																		
Not Certified *	2013/14									55 143			600		7 310			80 400	143 453
Not Certified	2014/15									9 879			560					34 217	44 656

6.5 Nursery Certification

Twenty-six nurseries were visited during the May 2014 audits. Twenty-one nurseries retained their certification status, while 5 nurseries were provisionally certified. Upon completion of the outstanding requirements, these 5 nurseries could be certified.

Twenty-five nurseries were visited during the November 2014 audits. Twenty-three nurseries retained their certification status, while one previously certified nursery was provisionally certified and one lost its certification. The increase in Phytophthora infestation in certain nurseries is of concern and additional support is given to assist these nurseries to correct the problem. The following table lists the certified nurseries.

Table 6.5.1: CIS Certified Nurseries in March 2015

Nursery	Address	Town	Province	Code	Contact Person	Tel	Fax	Cell	Email
Apapanzi Kwekery	PO Box 147	Kirkwood	Eastern Cape	6120	Nellis Meiring	042 230 1483	042 230 0923	082 550 6210	nellis@srvalley.co.za
Augsburg Kwekery	PO Box 195	Clanwilliam	Western Cape	8135	Alta Laing	082 952 8127	086 661 4372	079 527 0316	admin@augsburnursery.co.za
BF Joubert Kwekery	PO Box 193	Kirkwood	Eastern Cape	6120	Francois Joubert	042 230 0309	042 230 0280	084 951 1922	bfjkweek@srvalley.co.za
Casmar Kwekery	PO Box 3	Mooi-nooi	North West	0325	Neville Wenhold	014 574 3152	014 574 3798	082 881 4189	casmarnursery@absamail.co.za
Cederberg Tree Nursery	PO Box 273	Citrusdal	Western Cape	7340	Patricia Willemsse	022 921 3526	022 921 3957	076 622 7007	info@cederbergtreenursery.co.za
Du Roi Kwekery	PO Box 66	Letsitele	Limpopo	0885	Felix Hacker	015 345 1650	015 345 1414	082 879 5923	felix@duroibugs.co.za
Esselen Kwekery	PO Box 100	Malelane	Mpumalanga	1320	Leon Esselen	013 790 0160	013 790 0492	083 325 0565	esselenk@mweb.co.za
Gamtoos Kwekery	PO Box 140	Patensie	Eastern Cape	6335	Keuler Engela	042 283 0506	042 283 0978	072 260 9813	keuler@rikusld.co.za
H J Joubert Kwekery	PO Box 207	Montagu	Western Cape	6720	Herman Joubert	023 614 2237	023 614 2237	082 578 5747	hopewell@breede.co.za
Henley Citrus	PO Box 1686	Letsitele	Limpopo	0885	Charles Boyes	015 386 0211	015 386 0248	082 264 9916	charles@bigday.co.za
Letsitele Kwekery	PO Box 114	Letsitele	Limpopo	0885	Barend Vorster	015 345 1600	015 345 1601	083 259 5590	barend@mahela.co.za
Loskop Kwekery	PO Box 1101	Marble Hall	Limpopo	0450	Jan Odendaal	082 413 9707	086 623 0912	082 413 9707	kynomel@vodamail.co.za
Mistkraal Nursery	PO Box 106	Kirkwood	Eastern Cape	6120	Tyna Ferreira	042 230 0614	042 230 1461	082 789 5150	beans@srvalley.co.za
Namakwaland Sitrus	PO Box 44	Clanwilliam	Western Cape	8135	Tobias Basson	027 482 2503	027 482 1562	082 784 4123	tobias@namakwalandsitrus.com
Ngwenya Kwekery	PO Box 36	Malelane	Mpumalanga	1320	Milanie v/d Merwe	013 790 3004	013 790 3480	082 418 7693	milanie@riversidefarm.co.za
Oase Sitrus Kwekery	PO Box 2606	Hartswater	Northern Cape	8570	Gerrit Schlebusch	053 474 2080	053 474 2080	082 907 1562	oasekwekery@lantic.net
Oranjerivier Sitrus Kwekery	PO Box 875	Kakamas	Northern Cape	8870	Blom Rossouw	054 441 0183	086 544 9691	083 306 0622	osk@vodamail.co.za
Rietvlei Kwekery	PO Box 2436	Tzaneen	Limpopo	0850	Lucas McLean	083 630 3236	086 672 8450	083 630 3236	rietvlei@global.co.za
Sondagsrivier Kwekery	PO Box 304	Kirkwood	Eastern Cape	6120	Willem Truter	042 230 0349	042 230 0510	083 227 6655	willem@srvalley.co.za
Stargrow Kwekery	PO Box 189	Citrusdal	Western Cape	7340	Marco du Toit	022 921 2232	022 921 2747	082 563 0795	stargrowcitrus@alazon.co.za
Tulbagh Kwekery	PO Box 99	Tulbagh	Western Cape	6820	Bredell Roux	023 230 0694	023 230 1353	082 214 2520	admin@tulbaghnursery.co.za
Tweeling Kwekery	PO Box 190	Kirkwood	Eastern Cape	6120	Jan Potgieter	042 230 1408	042 230 1408	082 560 2179	tweeling@srvalley.co.za
Waterfall Nursery	PO Box 339	Adelaide	Eastern Cape	5760	Rudi van der Meulen	046 684 0738	046 684 1451	082 695 3433	waterfall@intekom.co.za
Witkrans Kwekery	PO Box 17	Boshhoek	North West	0301	Linda Grobler	014 573 3036	014 573 3036	082 414 4739	witkrans1@mweb.co.za

6.6 Statutory Improvement Scheme

The statutory CIS proposal was extensively discussed and debated in meetings with all participating citrus nurseries, a retail nursery, cultivar management companies and growers. A status document stating the benefits and detriments of a voluntary or compulsory statutory improvement scheme, including summarised feedback and inputs from all stakeholders, was discussed at a public workshop facilitated by the NAMC on 9 April 2014. The workshop was attended by 38 persons representing stakeholders, including growers, SACNA, nurserymen, cultivar managers, CGA, CRI and DAFF representatives. The workshop debated matters arising from the consultation process on which more clarity or consensus was required. The NAMC meeting concluded, as was reported in 2013/14, that a compulsory scheme offered the most advantages as well as protection from biosecurity risks for the citrus industry in South Africa, but that the needs of all role players including those not supportive of a compulsory scheme should be considered. Subsequently, meetings were also held with SACNA, of whom certain members opposed a compulsory scheme, as well as the ARC who did not attend the workshop. This issue will receive ongoing attention.

6.7 Protective zone surrounding the Citrus Foundation Block

The legislation, declaring a radius of 5 km around the CFB as a citrus free area, was published in the Government Gazette on 21 January 2011. Orders to remove all citrus trees were issued by DAFF. Most residents have removed their citrus trees. DAFF has made several follow-up visits to owners refusing to remove trees, and is addressing the matter with the remaining two owners.

6.8 Shoot tip grafting (STG), pre-immunisation and nucleus block management

Project 790 by J.H.J. Breytenbach S.P. van Vuuren and G. Cook (CRI)

Summary

Shoot tip grafting (STG) is used to eliminate graft transmissible pathogens from citrus material before introduction into the Citrus Improvement Scheme. During the current year two new selections were received for STG and a further 34 submissions, from previous years, are at various stages in the process before release. A virus-free gene source is maintained in an insect-free tunnel at CRI. Virus-free material is pre-immunised with a suitable *Citrus tristeza virus* source before it is supplied to the Citrus Foundation Block (CFB) at Uitenhage. Seventeen new selections were supplied to the CFB and added to the CRI gene source, which now comprises 294 cultivars and selections. Virus-free material is also supplied to various cultivar owners on request for either export or trial purposes.

Opsomming

Groeipuntenting (GPE) word gebruik om sitrus materiaal skoon te maak van ent-oordraagbare patogene voor toevoeging tot die Sitrusverbeteringskema se genebron. Gedurende die jaar is twee nuwe seleksies ingedien vir GPE en 'n verdere 34 seleksies van vorige introduksies is in verskeie fases voor vrystelling. Virusvrye boompies van verskillende cultivars en seleksies word as 'n genebron in 'n insekvrige tunnel by CRI bewaar. Virusvrye materiaal word met 'n toepaslike *Citrus tristeza virus* bron gepeïmuniseer voordat dit aan die Sitrus Grondvesblok (GVB) by Uitenhage vrygestel word. Sewentien nuwe seleksies is aan die GVB voorsien en die is by die CRI genebron gevoeg, wat tans uit 294 kultivars en seleksies bestaan. Virusvrye materiaal word aan verskeie cultivar eienaars op versoek verskaf wat deur hulle gebruik word vir uitvoer of eksperimentele doeleindes.

Introduction

The overall objective of the southern African Citrus Improvement Scheme (CIS) is to enhance the productivity of the industry by ensuring supply of the highest quality propagation material. Graft transmissible diseases (GTD) have detrimental effects on the growth and production of citrus trees and are responsible for stunting, decline, small fruit and a range of other harmful effects. Shoot tip grafting (STG) is the standard method for the elimination of pathogens (Navarro *et al.*, 1975). Some pathogens are more difficult to eliminate and heat therapy should be incorporated with the STG process (Roistacher, 1977). The STG technique was developed by Murashige *et al.* (1972) and improved by Navarro *et al.* (1975) and de Lange (1978). Some cultivars and selections of the virus-free gene source maintained at the ARC-ITSC have been duplicated in part at CRI Nelspruit as a back-up source. STG facilities at CRI are used to introduce new virus-free cultivars and selections which are added to the gene source after STG and indexing. Cross-protection for severe CTV infection is a function of the CIS and specific pre-immunising CTV sources are applied to all citrus varieties before supply to the CFB.

Objectives

Receive and introduce new cultivar selections.

Do STG of new editions and index for GTD to ensure that they are virus-free.

Maintain the virus-free gene source in an insect-free tunnel.

Pre-immunise selections with a suitable cross-protecting *Citrus tristeza virus* (CTV) source before budwood supply to the CFB at Uitenhage.

Materials and methods

In vitro cultured rootstocks: The standard method used for *in vitro* cultured rootstocks is to expose the cotyledons by removing the seed coat of Troyer citrange seed and surface sterilise in 1% sodium hypochlorite (NaOCl) for 10 minutes followed by three rinses in sterile distilled water. Three to four seeds are planted in growth tubes containing sterile Murashige and Skoog (MS) agar medium (Murashige & Skoog, 1962). Germination takes place at a constant temperature of 28°C in continuous darkness. When the seedlings have reached a height of 30 to 40 mm, they are stored at 4°C in darkness.

Scion preparation: Method 1; buds of the source plant are budded on a standard rootstock in the glasshouse. After bud growth and maturation (approximately 3–4 months), the source plant is defoliated by hand to induce flushing. Ten to 14 days later, the new shoots are harvested and surface sterilised on a flow bench for 5 minutes in 0.52% NaOCl and then rinsed three times in sterile distilled water. Method 2; bud sticks from the source plant are cut in 50 mm lengths and surface sterilised by immersion for 10 minutes in 1% NaOCl containing a wetting agent. After 3 rinses in sterile distilled water the bud sticks are cultured in 250 ml glass bottles containing sterile wet sand. The cultures are incubated at 32°C and exposed to 16 h light/day. Ten to 14 days later new shoots are harvested and treated as in method 1.

STG: The seedling rootstock is aseptically decapitated about 50 mm above the cotyledons. The cotyledons and their auxiliary buds are removed and an inverted T incision is made, 1 mm vertically and 1 – 2 mm horizontally approximately 10 mm from the top. The cuts are made through the cortex to reach the cambium. A shoot tip consisting of the apical meristem and 2 to 3 leaf primordia is excised from the growth point of the collected shoots under a stereo microscope. The growth tip, containing the leaf primordia, is placed on the horizontal cut of the incision on the rootstock. The grafted plant is transferred to sterile MS liquid medium and cultured at constant 28°C exposed to 16 h light/day.

STG plant increase. The shoot tip starts growing 3 to 4 weeks after STG. The growing shoot tip is micro-grafted with the seedling rootstock onto a vigorous-growing virus-free rootstock in the glasshouse. After micro-grafting, the graft is closed by a plastic bag for 8 days. Once the graft has sufficiently grown, buds for indexing are taken from this material.

Virus indexing. Elimination of graft transmissible pathogens is established by indexing the STG material on sensitive biological indicators as described by van Vuuren and Collins (1990). Biological indexing results are thereafter confirmed with molecular diagnostic techniques. Reverse-Transcription Polymerase Chain Reaction (RT-PCR) is used to detect viroids, CPsV and ASGV. PCR is used to detect the bacterial pathogen causing citrus greening. Virus-free plants are maintained in an insect-free tunnel containing the gene sources from where material is taken, multiplied and pre-immunised with suitable CTV cross-protection sources (van Vuuren and Collins, 1990), prior to release to the CFB at Uitenhage.

Results and discussion

Objective / Milestone	Achievement
<ul style="list-style-type: none">• Receive and maintain new selections/cultivars.	Ongoing: 34 brought forward from previous year; 2 new selections received in current year.
<ul style="list-style-type: none">• Do shoot tip grafting (STG) of new selections/cultivars and index for graft transmissible diseases, to ensure they are virus-free.	Ongoing: 66 STGs, 7 successful micro-grafts.
<ul style="list-style-type: none">• Maintain the virus-free nucleus block in an insect-free tunnel.	Ongoing: currently 294 cultivars and selections.
<ul style="list-style-type: none">• Establish a pre-immunised source of the new selection/cultivar with a suitable CTV cross-protection source and supply budwood to the CFB.	Ongoing: 17 additions supplied to CFB.
<ul style="list-style-type: none">• Re-index the virus-free selection every three years.	Ongoing: Partially indexed for CVd, CPsV and Greening

STG:

The STG procedure was initiated at CRI in 2004 and the existing facilities completed in 2005. The introductions for STG and subsequent releases to the CFB from 2008 to date are summarised in Table 6.8.1. Two new selections of two cultivar owner groups were submitted for STG in the current year and 34 brought forward from the previous year. During this report period a total of 66 STGs were done on these introductions, including failed grafts. Of these, 7 were successfully micro-grafted.

Fifteen successful STGs have been indexed. Fourteen of the fifteen successful STGs indexed negative for CTV, ASGV and CVd by biological indexing, and one tested positive for CVd (Table 6.8.2). Thirteen STG's were biologically indexed for CPsV and CID and were all negative (Table 6.8.3). On average it takes 24 to 30 months to obtain a virus-free STG followed by the scheduled indexing to confirm the virus-free status of the cultivar. However, delays can occur with elimination of some pathogens. The reason for these "difficult to remove" cases is unknown.

To facilitate a faster turn-around with the STG process, new introductions are tested directly with PCR prior to STG to determine the viroid and CTV status as well as post STG, once enough material is available for testing. These additional steps help to identify the pathogens to be eliminated and allow quicker detection of pathogens not eliminated by an STG step. This shortens the time before the STG can be repeated if positive. This is quicker than biological indexing used previously. This process does not replace the biological indexing and PCR is done as a confirmation of pathogen free status prior to final release of the accession. These additional tests are routinely done and the number of tests conducted is not reported here.

Confirmation of biological indexing by PCR on a number of STG submissions prior to final release is reflected in Table 6.8.4. Seventeen STG submissions free of CTV, CVd, and ASGV were pre-immunised successfully and budwood was supplied to the CFB.

Table 6.8.1. STG submissions in the pipeline for graft transmissible disease elimination and indexing.

Cultivar Group ²	STG introductions and releases 2010 to 2014 ¹															
	2010			2011			2012			2013			2014			Balance
	Bf	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	
C	0	1	0	1	0	0	1	5	1	5	1	0	6	0	3	3
G	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mi	1	0	2	1	0	0	1	0	0	1	0	0	1	0	0	1
Ma	2	3	2	3	0	2	1	0	1	0	4	0	4	0	0	4
N	19	6	3	22	11	4	29	10	5	34	2	4	32	0	13	19
R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
V	6	0	0	6	2	2	6	2	2	6	0	2	4	1	0	5
Or	2	0	1	1	0	0	1	0	0	1	0	0	1	1	0	2
Rs	1	0	1	0	1	0	1	1	0	2	0	1	1	0	1	0
Total	32	10	10	34	14	8	40	18	9	49	7	7	49	2	17	34

¹ Bf = Brought forward from previous year; Balance = Balance for the current reporting year.

² Cultivar/variety group: C = Clementine; G = Grapefruit; L = Lemon; Mi = Midseason; Ma = Mandarin; N = Navel; R = Reticulata; V = Valencia; Or = Ornamental; Rs = Rootstock.

Table 6.8.2. STG submissions indexed biologically for CTV, ASGV and CVd.

Variety Group	Number of plants	Negative	Positive
Navel	11	11	-
Midseason	-	-	-
Mandarin	-	-	-
Valencia	1	-	1
Clementine	3	3	-
Grapefruit	-	-	-
Lemon	-	-	-
Ornamental citrus	-	-	-

Rootstock	-	-	-
Total	15	14	1

Table 6.8.3. STG submissions indexed biologically for CPsV and CID.

Variety Group	Number of plants	Negative	Positive
Navel	9	9	-
Midseason	-	-	-
Valencia	1	1	-
Reticulata	-	-	-
Mandarin	-	-	-
Grapefruit	-	-	-
Clementine	-	-	-
Lemon	-	-	-
Ornamental	1	1	-
Rootstock	2	2	-
Total	13	13	-

Table 6.8.4. STG plants indexed by PCR for CVd, ASGV and CPsV and Greening.

Cultivars	CVd	ASGV	CPsV	Greening
Navel	11	11	9	7
Midseason	-	-	-	-
Valencia	-	-	1	1
Reticulata	1	1	-	-
Mandarin	-	-	-	-
Grapefruit	-	-	-	-
Clementine	3	3	-	3
Lemon	-	-	-	-
Ornamental	-	-	1	-
Rootstock	-	-	2	-
Total	15	15	13	11

Maintaining the virus-free gene source:

The number of selections maintained at CRI is listed per cultivar/variety group in **Table 6.8.5**. Seven new additions were made to the gene source this reporting period (**Table 6.8.1**). Two trees of each selection are maintained in the gene source and trees have to be re-budded to new rootstocks every five years as part of the routine maintenance.

Table 6.8.5. The number of accessions per cultivar group maintained at the CRI nucleus block.

Variety Group	No. of selections maintained at CRI
Clementine	27
Diverse (Citron, Sour orange, etc.)	2
Ellendale	4
Grapefruit	18
Kumquat	1
Lemon	20
Lime	4
Mandarin	5
Midseason	27
Navel	72
Ornamental	4
Pummelo	7
Reticulata	33
Rootstock	23
Satsuma	8
Valencia	47
Total	294

Conclusion

- Successful elimination of GTDs from new selections was achieved. On average it takes 30 months for the entire process from STG to final release (the quickest being 21 months) although some selections proved to be problematic and still remain infected despite repeated STG.

- Seventeen new selections were added to the gene source and also released to the CFB.
- Two new selections were received this year for elimination of GTD and are in the STG process.

Technology transfer

Annexure 9 to CIS Procedural guide added: “Citrus Graft Transmissible Diseases in SA”
Proposed amendment to CIS Procedural Guide – “Certification of interim source material”

Further objectives and work plan

Quarterly milestones for Apr-Jun, Jul-Sep, Oct-Dec 2014 and Jan-Mar 2015

- Receive material
- Bud to virus-free rootstocks and maintain at high temperature
- Prepare liquid and solid Murashige & Skoog culture mediums
- Prepare and plant seed in culture tubes with solid medium
- Germinate seed in darkness
- Store rootstocks at 4°C
- Prepare rootstocks under stereo microscope under aseptic conditions
- Collect new shoots from source maintained at high temperature
- Prepare etiolated rootstock from culture tube
- Under the stereo microscope, cut and place shoot tip on rootstock
- Put the rootstock with shoot tip into a culture tube with liquid medium
- Keep tubes in growth room (do weekly trimmings of rootstock suckers)
- Graft shoot tip with rootstock on virus-free rootstocks in the glasshouse
- Let shoot tip grow for indexing
- Index for graft transmissible agents
- Pre-immunise rootstock with suitable cross protector
- Bud virus-free shoot tip grafted material to pre-immunised rootstock
- Do ELISA to confirm pre-immunisation
- Multiply pre-immunised budwood on virus-free rootstocks
- Supply budwood to Citrus Foundation block
- Maintain virus-free material in nucleus block

References cited

- Bar-Joseph, M., S.M. Garnsey, D. Consalves, M. Mocouitz, D.E. Pecifull, M.F. Clark & G. Loebenstein. 1979. The use of enzyme-linked immunosorbent assay for the detection of *Citrus tristeza virus*. *Phytopathology* 69: 190 – 194.
- De Lange, J.H. 1978. Shoot tip grafting – a modified procedure. *Citrus and Subtrop. Fruit J.* 539: 13 – 15.
- Murashige, T. & F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15: 473 – 497.
- Murashige, T., W.S. Bitters, T.S. Rangan, E.M. Nauer, C.N. Roistacher & B.P. Holliday. 1972. A technique of shoot apex grafting and its utilisation towards recovering virus-free *Citrus* clones. *HortScience* 7: 118 – 119.
- Navarro, L., C.N. Roistacher & T. Murashige. 1975. Improvement of shoot tip grafting *in vitro* for virus-free citrus. *J. Amer. Soc. Hort. Sci.* 100: 471 – 479.
- Roistacher, C.N. 1977. Elimination of citrus pathogens in propagative bud-wood. Budwood selection, indexing and thermotherapy. *Proc. Int. Soc. Citriculture* 3: 965 – 972.
- Roistacher, C.N. 1991. Graft-transmissible diseases of citrus – Handbook for detection and diagnosis. IOCV, FAO, Rome.
- Van Vuuren, S.P. & R.P. Collins. 1990. Indexing of transmissible pathogens and pre-immunisation with *Citrus tristeza virus* for the South African Citrus Improvement Programme. *Subtropica* 11(11): 17 – 19.

6.9 Diagnostic services for graft transmissible diseases

Project 796 by J.H.J. Breytenbach, S.P. van Vuuren and G. Cook (CRI)

Summary

The success of the Citrus Improvement Scheme (CIS) relies on the diagnostic detection of pathogens, the elimination thereof, and the maintenance and distribution of healthy propagation material. Biological and molecular indexing is done on new introductions prior to release to the CFB as well as on accessions

maintained at the CFB to establish whether graft transmissible disease agents are present or have been inadvertently introduced. The ongoing activities of these CIS functions are reported. The mother trees maintained at the CFB are indexed every two years on a rotating basis for the presence of severe CTV strains and for the presence of citrus viroids (CVd). The biological evaluation of CTV severity in 161 mother trees was completed and concluded the indexing for the current cycle. No indications of severe strains were detected, but a number of cultivars lacked the presence of CTV indicating a loss of the pre-immunising sources, notably in soft citrus and to a lesser extent some navel and Valencia types. Additionally the strain composition in certain cultivars differed from that of the original CTV sources. The effect of temperature on strain composition in sources were investigated in a glasshouse trial within this project and more widely in Project 1100 to understand the strains required for cross-protection. The results of the trial in this project indicate that temperature is not responsible for the lack of certain strains and that this problem may be ascribed to an interaction between the host (cultivar tolerance/resistance) and the CTV strains. The viroid indexing was initiated earlier than scheduled due to the availability of indicator plants and 194 mother trees were biologically indexed and results confirmed by PCR. One accession was found positive and clean material was resupplied to the CFB. General diagnostics and investigations into *ad hoc* industry problems and concerns relating to graft transmissible diseases are also reported within this project.

Opsomming

Die sukses van die Sitrusverbeteringskema (SVS) berus op 'n fitosanitêre program wat op 'n diagnostiese opsporing van die teenwoordigheid van skadelike patogene gebaseer is. Die SVS behels die eliminerings van die patogene en die onderhou en verpreiding van gesonde voortplantingsmateriaal. Biologiese en molekulêre indeksering word gedoen op nuwe toevoegings tot die SVS voordat die materiaal aan die Grondvesblok verskaf word asook her-indeksering van moederbome wat by die Grondvesblok gehuisves word. Daar word hier verslag gelewer op hierdie voortdurende aktiwiteite van die SVS. Die moederbome by die Grondvesblok word op 'n rotasie basis elke tweede jaar ge-herindekseer om te bepaal of enige strawwe CTV rasse, of enige sitrus viroïede, in die moederbome voorkom. Die biologiese evaluasie van die CTV virulensie in 161 moederbome is gedurende die jaar voltooi. Geen tekens van strawwe rasse is waargeneem nie, maar CTV was afwesig in sekere kultivars, hoofsaaklik in sagte sitrus maar ook in 'n mate by navel en Valencia seleksies. 'n Ondersoek het ook getoon dat die CTV ras samestelling in sekere kultivars verskil van die oorspronklike pre-immuniseringsbronne. Die invloed van temperatuur op die ras samestelling van CTV bronne is in hierdie projek asook in projek 1100 ondersoek om 'n beter begrip te kry van CTV rasse vir kruisbeskerming. Die resultate van die proef in hierdie projek toon aan dat temperatuur nie verantwoordelik is vir die afwesigheid van CTV nie en dat die probleem moontlik toegeskryf kan word aan die interaksie tussen gasheer (cultivar verdraagsaamheid/bestandheid) en die CTV rasse. Viroïed indeksering van 194 moederbome is ook voltooi en biologiese resultate is bevestig d.m.v. PCR. Een cultivar is positief gevind en skoon materiaal is aan die grondvesblok verskaf. Algemene diagnostiese dienste en ondersoeke na probleme t.o.v. ent-oordraagbare siektes in die industrie word op 'n *ad hoc* basis gedoen en verslag word ook in hierdie projek gelewer.

Introduction

As with any commercial tree crop, citrus species are susceptible to various graft transmissible diseases (GTD) caused by viruses, viroids, bacteria and, in some cases, unidentified pathogens. The GTD affect the vigour, longevity of the trees, as well as the yield and quality of fruit. The framework of disease-free planting material is a phytosanitary programme based on diagnosis, detection and elimination of causal agents and maintenance and distribution of healthy propagation material. Shoot tip grafting (STG) is used to eliminate these diseases (Navarro, 1976) and is used in South Africa since 1977 (de Lange *et al.*, 1981).

Indexing, or establishing whether GTD disease agents are present in plant material, is done mostly by means of biological indicator plants. A range of virus-free plants are propagated in the glasshouse, each of which is used for detection of a specific graft transmissible pathogen. Previously only biological indexing was used for the detection of GTD following STG, but this is now supplemented with molecular based techniques, which target regions of the pathogen's nucleic acid are used to specifically identify the pathogen. These techniques such as Reverse-Transcription Polymerase Chain Reaction (RT-PCR), PCR and dot-blot have an enhanced sensitivity compared to symptom expression on indicators.

Since *Citrus tristeza virus* (CTV) and its vector, *Toxoptera citricida*, are endemic in South Africa, virus-free material should be protected by pre-immunisation with a suitable cross-protection source (Müller & Costa, 1987). Currently three CTV sources are used for cross-protection in the southern African Citrus Improvement Scheme (CIS) depending on the scion material to be protected (von Broembsen & Lee, 1988; van Vuuren *et al.*, 1993a; van Vuuren *et al.*, 1993b; van Vuuren *et al.*, 2000). ELISA is used to confirm pre-immunisation with CTV (Roistacher, 1991). The STG and pre-immunisation procedures have been improved to suite South

African conditions (Fourie & van Vuuren, 1993). Re-indexing of the mother trees, maintained at the Citrus Foundation Block (CFB), is done to ensure these trees remain free of graft transmissible pathogens and that the pre-immunising CTV remains mild within these cultivars. CTV severity indexing is done on an annual basis, indexing for Citrus viroids (CVd) is done biennially and other GTD are indexed every 10 years.

Indexing for GTD is also done to support growers where field problems are experienced and is necessary to ensure appropriate recommendations. Budwood sent in by growers or collected during field visits, are budded to indicator plants and kept in the glasshouse at optimum temperatures according to the requirements for disease detection.

Objectives

1. Biological and molecular indexing of material that went through STG.
2. Biological and molecular re-indexing of mother trees at the CFB.
3. Requests from growers and institutions to index suspected material for graft GTD.
4. *Ad-hoc* indexing as required

Materials and methods

Specific virus-free indicator plants are propagated from seed or clonally from virus-free material for the detection of the various graft transmissible diseases (GTD). These are maintained in an insect-free glasshouse and kept in stock until needed. When budwood for indexing is received, two buds are budded on each of three indicator seedlings for each disease. For CPsV indexing 4 buds are used to inoculate each of 3 indicator plants. Hereafter the plants are cut back to force new growth and kept in the glasshouse at a temperature required for symptom expression of the specific disease. Known positive and negative control samples are included. A minimum indexing time of 6 months is required for CTV, CVd, *Apple stem grooving virus* (tatter leaf) (ASGV) and greening, while 12 months are required for *Citrus psorosis virus* (CPsV) and Citrus Impietratura Disease (CID) indexing.

Field material is usually not suitable for the serological or molecular techniques (PCR, s-PAGE, DOT blots etc.), since the organisms are usually present in low concentrations or are poorly distributed, and therefore false negative results may be obtained. Field material is inoculated on suitable indicator plants, maintained at optimal temperatures in the glasshouse and then tested at least 3 months after inoculation for specific pathogens. Molecular results are regarded as a confirmation of the biological result.

Results and discussion

Objective / Milestone	Achievement
<ul style="list-style-type: none"> • Biological and molecular indexing of STG plants for CTV, CVd, ASGV, CPsV and CID. 	Achieved and ongoing.
<ul style="list-style-type: none"> • Annual biological and molecular indexing of the CFB mother trees (every year for CTV severity; every third year for the presence of CVd; every 10 years for the presence of CPsV and ASGV). 	Achieved and ongoing.
<ul style="list-style-type: none"> • Indexing samples sent in by growers and institutions using ELISA, PCR and biological indicators. 	Samples were received for indexing for possible ASGV, CPsV, CVd and CTV infection. Results communicated to clients.

1. STG material

After the STG process, citrus cultivars undergo initial biological indexing for CTV, ASGV and CVd (Table 6.9.1). Once indexed negative for CTV, ASGV and CVd, a process which takes 6 months, the source is pre-immunised with a suitable CTV cross-protection source. After confirmation of positive pre-immunisation, budwood is supplied to the CFB to establish mother trees. The cultivar is then also introduced into the nucleus block (Table 6.9.4). Following interim releasing to the CFB, plants are further biologically indexed for CPsV and CID (Table 6.9.3). The presence of greening is continuously monitored since all the cultivars and selections, except the trifoliate types, are self-indexing with symptoms clearly visible if the pathogen is present, but PCR confirmation is done prior to final release to the CFB. Fifteen cultivars, further in the release process, were tested by PCR for the presence of CVd, thirteen for the presence of ASGV and eleven for Greening. All indexed negative for these pathogens (Table 6.9.2).

Table 6.9.1. Status of STG plants indexed biologically for CTV, ASGV and CVd¹.

Cultivar	Number of plants	CTV			ASGV			CVDs		
		+	-	±	+	-	±	+	-	±
Navel	11	-	11	-	-	11	-	-	11	-
Midseason	-	-	-	-	-	-	-	-	-	-
Mandarin	-	-	-	-	-	-	-	-	-	-
Valencia	1	-	1	-	-	1	-	1	-	-
Clementine	3	-	3	-	-	3	-	-	3	-
Lemon	-	-	-	-	-	-	-	-	-	-
Grapefruit	-	-	-	-	-	-	-	-	-	-
Ornamental citrus	-	-	-	-	-	-	-	-	-	-
Rootstock	-	-	-	-	-	-	-	-	-	-
	15	0	15	0	0	15	0	1	14	0

¹ + = positive; ± = awaiting final results; - = negative.

Table 6.9.2. STG plants indexed by PCR for CVd, ASGV, CPsV and Greening.

Cultivars	CVd	ASGV	CPsV	Greening
Navel	11	11	9	7
Midseason	-	-	-	-
Valencia	-	-	1	1
Reticulata	1	1	-	-
Mandarin	-	-	-	-
Grapefruit	-	-	-	-
Clementine	3	3	-	3
Lemon	-	-	-	-
Ornamental	-	-	1	-
Rootstock	-	-	2	-
Total	15	15	13	11

Table 6.9.3. STG plants indexed biologically for CPsV and CID.

Variety Group	Number of plants	Negative	Positive
Navel	9	9	-
Midseason	-	-	-
Valencia	1	1	-
Reticulata	-	-	-
Mandarin	-	-	-
Grapefruit	-	-	-
Clementine	-	-	-
Lemon	-	-	-
Ornamental	1	1	-
Rootstock	2	2	-
Total	13	13	

Table 6.9.4. Pre-immunisation status and new cultivar additions to the gene source and Foundation Block.

Cultivars	Number of plants pre-immunised	Pre-immunisation to be confirmed	Pre-immunisation confirmed by ELISA	Supplied to the CFB and additions to Nucleus Block
Navel	13	-	13	13
Midseason	-	-	-	-
Valencia	-	-	-	-
Reticulata	-	-	-	-
Mandarin	-	-	-	-
Grapefruit	-	-	-	-
Clementine	3	-	3	3
Lemon	-	-	-	-
Ornamental	-	-	-	-
Rootstock	1	-	1	1
Total	17	0	17	17

2. Re-indexing of mother trees at the CFB

Citrus tristeza virus:

The CTV severity status of 161 mother trees, were indexed as part of the re-indexing program. Mother trees indexed are presented in Table 6.9.5. The Mexican lime indicators were inspected for growth, the presence of mild to severe stem pitting and vein clearing 6 months after inoculation. Seventy-six mother trees tested negative for CTV after inspection and CTV absence was confirmed by ELISA. There were no mother trees with severe CTV strains.

Table 6.9.5. Mother trees at the CFB indexed for CTV severity.

Cultivar	Number of mother trees	Number of trees with severe SP*	Number of trees with mild CTV	Number of trees negative for CTV
Total Mandarins [10 cultivars]	31	0	8 [1 cv all positive; 3 cvs some positive; 6 cvs all negative]	23
Total Midseasons	0	0	0	0
Total Navels [8 cultivars]	37	0	25 [4 cvs all positive; 3 cvs some positive; 1 cv all negative]	12
Total Valencias [9 cultivars]	37	0	25 [5 cvs all positive; 2 cvs some positive; 2 cvs all negative]	12
Total Satsumas [1 cultivar]	6	0	3 [1 cv some positive]	3
Total Clementines [2 cultivars]	9	0	0 [2 cvs all negative]	9
Total Grapefruit [1 cultivar]	6	0	6 [1 cv all positive]	0
Total Lemons [7 cultivars]	30	0	13 [1 cv all positive; 2 cvs some positive; 4 cvs all negative]	17
Total Limes [1 cultivar]	5	0	5 [1 cv all positive]	0
Total Kumquats	0	0	0	0
Grand Total	161	0	85	76

No indications of severe strains were detected, but a number of cultivars lacked the presence of CTV indicating a loss of the pre-immunising sources, notably in soft citrus and to a lesser extent some navel and Valencia types. Accessions tested within the 2-year cycle showed that 60% of both mandarin and Clementine mother trees no longer contain the CTV pre-immunising source. This was also noted in Satsuma, Valencia and Navels in 31%, 24% and 20% of the mother trees respectively.

The incidence of the absence and variation of CTV in the mother trees is unknown and may be due to one or a combination of different factors. Two attempts were made to try and elucidate the problem; firstly to establish which strains of a cross-protecting source is present in the mother tree and what is the effect of the strains on growth, vein clearing and stem pitting of the Mexican lime indicator and secondly, what is the effect of temperature on the survival of single strains in Mexican lime indicator plants and its effect on growth and stem pitting and does temperature interfere with the composition of composited CTV sources.

A. The effect of the cultivar on the composition of the cross-protecting CTV source with multiple strains:

Materials and methods:

During re-indexing, two mother trees of three cultivars from each of Valencia, navel and soft citrus which differed slightly from each other in symptom expression on the Mexican lime host, were selected (Table

6.9.6). The cross-protecting source for all the mother trees was LMS6. The biological response of the Mexican lime indicators (growth, vein clearing, stem pitting) was evaluated normally. In addition, bark samples were taken and RNA extracted. RT-PCR was employed using strain specific primers for strain identification. Band intensity was used as an indication of strain titre; however, this was not a quantitative analysis.

Results and discussion:

The biological indexing results of CFB mother trees and the CTV strains that were present in each tree in comparison with the original pre-immunising source are given in Table 6.9.6.

Table 6.9.6. Symptom expression of Mexican lime after inoculation with material from different CFB mother trees and the presence of CTV strains in each tree in comparison with the LMS6 pre-immunising source

Cultivar	Mother tree	Growth cm	VC rating ¹	SP rating ²	CTV strains ³			
					T68	HA16-5	RB1	RB2
Valencia 1	A	77	+	+	+++	+++	-	-
Valencia 1	B	68	+	+	+++	+++	-	-
Valencia 2	A	66	+	+	+++	+++	-	-
Valencia 2	B	62	+	+	+++	+++	-	-
Valencia 3	A	80	+	++	+++	++	-	-
Valencia 3	B	62	+	++	+++	++	-	-
Navel 1	A	88	-	-	+++	++	-	-
Navel 1	B	84	-	-	+	+	-	-
Navel 2	A	74	+	++	+	+++	-	-
Navel 2	B	47	+	++	+++	++	-	-
Navel 3	A	60	+	+	+++	++	-	-
Navel 3	B	71	+	+	+++	+++	-	-
Satsuma 1	A	69	+	+	+++	+++	-	-
Satsuma 1	B	72	-	+	++	++	-	-
Mandarin 1	A	82	-	-	++	++	-	-
Mandarin 1	B	78	-	+	++	++	-	-
Clementine 1	A	88	-	-	+	+	-	-
Clementine 1	B	69	-	-	-	+	-	-
LMS6 Control		69	+	+	+++	+++	+++	+++

* Differ significantly from the other mother tree of the same cultivar at the 5% level (Fisher's LSD).

¹ Vein clearing rating: - = none; + = mild; ++ = moderate; +++ = severe.

² Stem pitting rating: - = none; + = mild; ++ = moderate; +++ = severe.

³ Band intensity: - = none; + = faint; ++ = moderate; +++ = intense.

Significant growth differences of the sensitive Mexican lime seedlings occurred when inoculated with CTV from the two mother trees of navel 2 and the two mother trees of Clementine 1; this might be indicative of better CTV colonization in mother trees, or effects of the CTV strains in those mother trees. The differences in growth between the other mother trees of the same cultivar were not significant.

Vein clearing varied from absent to mild but there was no correlation with growth. The mild expression was similar to the LMS6 control.

Stem pitting was absent in the navel 1 mother trees, the mandarin 1 A mother tree and both the Clementine 1 mother trees. Vein clearing was also absent in these trees and biologically these mother trees would have been declared virus-free.

The RB strains that are present in the LMS6 source were absent in all the mother trees tested. The T68 and HA16-5 strains that were detected varied in occurrence and titre from tree to tree. These two strains were the most consistent in the Valencia mother trees.

The variations might be due to interactions among strains in specific hosts or the tolerance mechanism within the hosts.

B. The effect of temperature on the survival of single CTV strains and the composition of strains within composite samples.

Materials and Methods:

Virus-free Mexican lime seedlings were bud-inoculated separately with six single CTV strains in six replicates (2 pots). Similarly CTV sources with multiple strains were inoculated. The inoculated plants were left at 28°C for seven days and then transferred to two temperature ranges. One pot of each CTV source was transferred to 24-28°C and the other to 28-32°C. The plants were cut back to above the inoculation point and the re-growth trained to one shoot.

After 6 months growth of plants of each treatment was measured, cut and the bark removed to assess stem pitting. Two centimeters of the top from each plant were taken for PCR analysis. Bark from each sample was peeled separately and RNA extracted. RT-PCR was employed using strain specific primers for strain identification. The CTV strain identification was unsuccessful and the plants were left for another 6 months at the same temperatures. The plants were evaluated again 6 months later and samples taken for RT-PCR analysis.

Results and Discussion:

The effect of the CTV sources on the growth of the Mexican lime plants during the first 6-month cycle is shown in Table 6.9.7. There were significant differences among the CTV sources but no difference between low and high temperature for each source.

Table 6.9.7. Growth (cm) of Mexican lime plants at two temperature ranges during the first 6-month cycle after inoculation with different CTV sources and strains

CTV strain or source	Temperature	
	24-28°C	28-32°C
Virus-free	44.3 a	41.3 abc
HA16-5	40.3 abcd	36.3 abcdefg
RB1	48.3 a	40.0 abcde
RB2	42.0 abc	33.3 abcdefgh
T3	9.2 i	18.0 hi
T68	28.7 bcdefgh	40.3 abcd
VT	20.7 ghi	28.0 cdefgh
CTVSC 1	24.0 efghi	38.0 abcdef
GFMS12	23.3 fghi	32.7 abcdefgh
GFMS35	29.0 bcdefgh	37.0 abcdef
LMS6	24.3 defghi	34.3 abcdefg
Nartia A	23.0 fghi	30.3 bcdefgh

Values in the body of the table followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Growth of the Mexican lime plants during the second 6-month period is presented in Table 6.9.8. Overall growth was significantly better at the high temperature. However, there was no significant difference in growth between the two temperatures where RB1, RB2, HA16-5 and T68 CTV strains were inoculated. Significant differences occurred where T3, VT, LMS6, GFMS35, Nartia A, CTVSC 1 and GFMS12 were inoculated. The un-inoculated control plants at the low temperature were significantly smaller than those at the high temperature indicating that the low temperature was below the optimum temperature for growth.

Table 6.9.8. Growth (cm) of Mexican lime plants at two temperature ranges during the second 6-month cycle after inoculation with different CTV sources and strains

CTV strain or source	Temperature	
	24-28°C	28-32°C
Virus-free	35.3 cdefgh	63.0 a
HA16-5	38.0 bcdefgh	51.3 abc
RB1	40.0 bcdefg	53.3 ab
RB2	39.0 bcdefgh	47.3 abcd
T3	9.7 i	41.0 bcdefg
T68	32.7 defgh	47.3 abcd
VT	31.0 efgh	49.0 abc
CTVSC 1	25.3 ghi	44.3 bcdef
GFMS12	23.0 hi	48.3 abcd
GFMS35	29.7 efgh	54.0 ab
LMS6	29.7 efgh	47.3 abcd

Nartia A	29.3 fgh	45.7 bcde
Mean**	30.2 x	49.3 z

* Values in the body of the table followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

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

The stem pitting ratings are presented in Table 6.9.9. Overall the stem pitting was significantly more severe at the low temperature. This can be more attributed to the effect of CTV sources T68, CTVSC 1, GFMS12 and LMS6. Plants with CTV strain T3 had the most severe stem pitting at both temperatures.

Table 6.9.9. The occurrence of stem pitting in Mexican lime plants that were inoculated with different CTV sources and kept at two temperature ranges for six months

CTV strain or source	Stem pitting rating**	
	Temperature	
	24-28°C	28-32°C
Virus-free	1.0 a	1.0 a
HA16-5	1.3 ab	1.0 a
RB1	1.0 a	1.0 a
RB2	1.3 ab	1.0 a
T3	4.0 f	4.0 f
T68	2.0 bcd	1.0 a
VT	2.7 de	2.0 bcd
CTVSC 1	2.7 de	1.3 ab
GFMS12	4.0 f	2.0 bcd
GFMS35	1.7 abc	1.3 ab
LMS6	3.3 ef	1.3 ab
Nartia A	2.3 cd	2.0 bcd
Mean**	2.3 x	1.6 z

* Values in the body of the table followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

** Stem pitting rating: 1 = no pitting; 2 = mild pitting; 3 = moderate pitting; 4 = severe pitting.

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

The effect of temperature on CTV strain survival and composition in sources are presented in Table 6.9.10. All the single strains were present in the host at low and high temperature. The pre-immunising sources were also stable at both temperatures. The only variation occurred where CTVSC 1, a source that is currently being evaluated in soft citrus, at both temperatures. The original source contains six CTV strains and it is possible that so many strains interact among each other or that the distribution in the host is not equal. At the low temperature strains T3 and VT, and at the high temperature only T3, were not detected.

Table 6.9.10. CTV strains detected by RT-PCR in Mexican lime plants a year after inoculation with single strains and sources containing multiple strains and maintained at two temperature ranges

CTV strain or source	Temperature range	CTV strain							
		HA16-5	RB1	RB2	T3	T30	T36	T68	VT
Virus-free	24-28°C	-	-	-	-	-	-	-	-
Virus-free	28-32°C	-	-	-	-	-	-	-	-
HA16-5	24-28°C	+	-	-	-	-	-	-	-
HA16-5	28-32°C	+	-	-	-	-	-	-	-
RB1	24-28°C	-	+	-	-	-	-	-	-
RB1	28-32°C	-	+	-	-	-	-	-	-
RB2	24-28°C	-	-	+	-	-	-	-	-
RB2	28-32°C	-	-	+	-	-	-	-	-
T3	24-28°C	-	-	-	+	-	-	-	-
T3	28-32°C	-	-	-	+	-	-	-	-
T68	24-28°C	-	-	-	-	-	-	+	-
T68	28-32°C	-	-	-	-	-	-	+	-
VT	24-28°C	-	-	-	-	-	-	-	+
VT	28-32°C	-	-	-	-	-	-	-	+
CTVSC 1	Original	-	+	+	+	+	-	+	+
CTVSC 1	24-28°C	-	+	+	-	+	-	+	-

CTVSC 1	28-32°C	-	+	+	-	+	-	+	+
GFMS12	Original	-	-	-	-	-	-	+	-
GFMS12	24-28°C	-	-	-	-	-	-	+	-
GFMS12	28-32°C	-	+	+	-	-	-	+	-
GFMS35	Original	-	+	+	-	-	-	-	-
GFMS35	24-28°C	-	+	+	-	-	-	-	-
GFMS35	28-32°C	-	+	+	-	-	-	-	-
LMS6	Original	+	+	+	-	-	-	+	-
LMS6	24-28°C	+	+	+	-	-	-	+	-
LMS6	28-32°C	+	+	+	-	-	-	+	-
Nartia A	Original	-	+	+	-	-	-	+	-
Nartia A	24-28°C	-	+	+	-	-	-	+	-
Nartia A	28-32°C	-	+	+	-	-	-	+	-

2. Re-indexing of mother trees at the CFB (continued)

Citrus viroids:

Additionally, 194 mother trees were indexed for the presence of CVd (Table 6.9.11). Three mother trees, of one cultivar were positive for CVd.

Table 6.9.11. Mother trees at the CFB indexed for CVd.

Cultivar	Number of mother trees	Number negative	Number positive
Total Mandarins [10 cultivars]	31	31	0
Total Midseasons	0	0	0
Total Navels [11 cultivars]	49	46	3 [1 cv all positive]
Total Valencias [13 cultivars]	47	47	0
Total Satsumas [3 cultivars]	14		0
Total Clementines [3 cultivars]	12	12	0
Total Grapefruit [1 cultivars]	6	6	0
Total Lemons [7 cultivars]	30	30	0
Total Limes [1 cultivars]	5	5	0
Total Kumquats	0	0	0
Grand Total	194	191	3

3. ITSC-ARC and CRI collaborative work

Shoot tip grafting for the CIS is done at both the CRI and ARC-ITSC laboratories. To confirm the pathogen free status of new accessions prior to release to the CFB, duplicate molecular testing is done on these accessions and sample numbers are summarized in Table 6.9.12.

Table 6.9.12. Numbers of new accessions subjected to duplicate testing for the various pathogens.

Pathogen	ARC-ITSC accessions	CRI accessions
CVd	29	17
CTV	40	17
ASGV	29	17
CPsV	31	17
Greening Disease	0	17

4. Test optimization for direct testing of CFB multiplication blocks for viroids.

For re-indexing of multiplication blocks older than 3 years and cultivars without CFB mother trees, a sampling and testing protocol was required which is sensitive, but also enables pooling of samples from the trees in multiplication blocks to make the screening manageable. This optimization was done by the ARC-ITSC to

determine the maximum number of samples that can be pooled and to determine which extraction protocols are best suited for a large screening. It was also necessary to develop more rapid test assays. Primers and protocols for real-time PCR detection have been developed and are in the final testing phase. One-step RT-PCR assays are being optimized to enable higher through-put.

5. Implementation of SSR Markers for cultivar accession verification.

The molecular SSR markers optimized for cultivar verification by the ARC-ITSC has been very useful to confirm identity for the mandarin types. However, in an investigation of a Clementine variety, where there was uncertainty regarding the supply material, it was not possible to differentiate four different Clementine varieties using the appropriate markers based on the compiled database. This suggests that a further refining of this database is required.

6. Ad-hoc indexing

6.1. General indexing for growers

Citrus material submitted by growers or collected during visits, are indexed for specific diseases (Table 6.9.13). Once the results are available, they are communicated to the submitting parties.

Table 6.9.13. Indexing of material sent in by growers or collected during visits.

Disease	No of samples	Results
CTV	11	Communicated to clients
CVd	17	Communicated to clients
CPsV	14	Communicated to clients
ASGV	1	Communicated to clients

6.2. Viroid field trial

In the Citrus Improvement Scheme (CIS) all graft transmissible agents, including CVd, are removed from all cultivars by shoot tip grafting (STG) but then re-inoculated with a selected *Citrus tristeza virus* (CTV) source for cross protection since this virus is endemic due to the abundance of aphids. Recently claims were made by cultivar owners and agents that the removal of CVd from some cultivars by STG, changes the performance of the cultivar under field conditions. This may include productivity, fruit colour and size and it was requested that CVd infected material be maintained and supplied by the CIS. No empirical evidence supports these claims and it is necessary to evaluate these cultivars with and without CVd to substantiate the approach of the CIS. Project 1074 was initiated to compare STG and old clone material. Indexing to determine the CTV and CVd status of various sources of three cultivars to be used in the project was done. The procedures and results are given in the Annual Report of Project 1074.

6.3. Delta Valencia

Biological and molecular indexing was conducted on a field sample submitted from the Limpopo province displaying psorosis-like fruit symptoms in the previous report period. The sample indexed negative for CPsV with PCR, but oak-leaf symptoms developed on the biological indicator indicating a probable viral-like disease. Similar symptoms were reported this season at a different location, also in the Limpopo province and samples again tested negative for CPsV. Sources were established on indicator hosts. Samples will be investigated for the presence of potential pathogens using Next Generation Sequencing (NGS) at the University of Stellenbosch.

Conclusion

Efficient pathogen detection and elimination enables supply of healthy budwood to the industry and is the primary objective of this project. Additionally, diagnostic services were provided and analysis of industry problems and concerns relating to graft transmissible diseases were addressed.

Technology transfer

None

Further objectives and work plan

Quarterly milestones for Apr-Jun, Jul-Sep, Oct-Dec 2014 and Jan-Mar 2015

- Propagate virus-free indicator plants, for the different graft transmissible diseases.
- Bud any bud wood send in, to these indicator plants.

- Keep plants in the glasshouse according to the disease diagnostic requirements.
- Use ELISA and PCR for detection of the presence of a pathogen.
- Discussion of results with the party involved.
- Improvement and validation of diagnostic techniques and development of new techniques for indexing for the various graft transmissible diseases (on-going)
- Source virus-free seed of various herbaceous plants for a host range study. Determine optimum growing conditions and maintain seedlings, mechanically inoculate various “psorosis”-sources. Submit certain samples for electron microscopic examination.
- Perform double-stranded RNA isolations and poly-acrylamide gel electrophoresis (PAGE).

References cited

- de Lange, J.H., van Vuuren, S.P. & Bredell, G.S. 1981. Groeipunt-enting suiwer sitrusklone vir die superplantskema van virusse. *Subtropica* 2(5): 11-16.
- Fourie, C.J. & van Vuuren, S.P. 1993. Improved procedures for virus elimination and pre-immunisation for the South African Citrus Improvement Programme. Proc. IV World Congress of the International Society of Citrus Nurserymen: 61-66.
- Müller, G.W. & Costa, A.S. 1987. Search for outstanding plants in tristeza infected orchard: The best approach to control the disease by pre-immunisation. *Phytophylactica* 19: 197-198.
- Navarro, L. 1976. The citrus variety improvement program in Spain. Proc. 7th Conf. IOCV: 198-203.
- Roistacher, C.N. 1991. Graft transmissible diseases of citrus: Handbook for detection and diagnosis. FAO, Rome, Italy.
- Van Vuuren, S.P., Collins, R.P. & da Graça, J.V. 1993a. Evaluation of *Citrus tristeza virus* isolates for cross protection of grapefruit in South Africa. *Plant Disease* 77: 24-28.
- Van Vuuren, S.P., Collins, R.P. & da Graça, J.V. 1993b. Growth and production of lime trees pre-immunised with different mild citrus tristeza virus isolates in the presence of natural disease conditions. *Phytophylactica* 25: 49-52.
- Van Vuuren, S.P., van der Vyver, J.B. & Luttig, M. 2000. Diversity among sub-isolates of cross-protecting citrus tristeza virus isolates in South Africa. Proc. 14th Conf. IOCV: 103-109.
- Von Broembsen, L.J. & Lee, A.T.C. 1988. South Africa's Citrus Improvement Program. Proc. 10th Conf. IOCV: 407-4

6.10 Citrus Biosecurity activities

Given the capacity constraints within DAFF, a lot of support is provided by CRI. More resources are urgently required for the HLB/ACP surveys and African Biosecurity actions. Feedback from those biosecurity activities that are linked to the CIS are briefly discussed in this report.

6.10.1 African Citrus Greening surveys

In the Western Cape, ACG delimiting surveys were conducted around the ACG positive trees detected in Piketberg. These trees were removed and to date no new positive find were detected. The source of the Piketberg trees is being investigated. One ACG positive lemon tree outside Plettenberg Bay, which is within the Western Cape Greening Buffer zone, was removed; the source of this tree is still unknown.

In the Eastern Cape, the EC Citrus Greening working group followed up on existing destruction orders for ACG positive trees detected in Transkei area and conducted further surveys. Trees were removed and no more positive trees were detected. However, it was evident that the illegal “bakkie-trade” of citrus trees from Kwazulu-Natal was still active, and from nurseries that were previously officially ordered to refrain from this practice. The matter is being followed up with the respective nursery. The awareness campaign in EC targets the Eastern Cape Directorate for Rural Development and Agrarian Reform (EC-DRDAR) extension officers, but training has unfortunately not been done due to communication issues. Training is, however, planned for winter 2015, and will be combined with oriental fruit fly training.

6.10.2 Asiatic Citrus Greening (HLB) and Asian Citrus Psyllid (ACP) surveys

A national HLB/ACP action plan was developed by CRI and approved by DAFF. Important actions are contingency surveys and awareness programmes. These are coordinated between DAFF, CGA and CRI at bi-annual Greening Stakeholder meetings. Given the seriousness of this risk to the southern African citrus growers, it has to be acknowledged that insufficient resources are being allocated to these actions. DAFF, with inputs from CRI, developed and submitted a project proposal in order to secure additional funding for the surveys. Unfortunately, this project did not receive funding and surveys will have to be conducted under

the normal operational budget. In 2014/15 no surveys for HLB / ACP were conducted due to the capacity constraints on DAFF, particularly brought about by urgently required CBS interventions.

6.10.3 Citrus biosecurity in Africa

a) Angola

In the recent past, CRI became aware that citrus nursery trees were being imported from Brazil. These imports are extremely risky as many of the feared citrus diseases that occur in Brazil, that are exotic to Africa, could have been exported with these consignments. On invitation from CRI, a delegation of the Angolan ministry of Agriculture visited the CFB, CIS nurseries and DAFF in 2013. They acknowledged the risk of importing propagation material and indicated that they will immediately cease to authorize imports from Brazil. Follow-up engagements involved Dr Hennie le Roux presenting a talk on Citrus Biosecurity at an agricultural trade show in Angola (December 2013). Future engagements, which have been postponed due to capacity reasons, will involve a survey of citrus orchards planted with trees from Brazil to determine whether serious diseases were inadvertently introduced. This survey is planned for August 2015.

b) Mozambique

Evidence of citrus development in Mozambique with Brazilian links raised concern that they might use Brazilian-made trees. MC Pretorius and Hennie le Roux arranged a meeting with Mozambique officials to discuss the citrus biosecurity risks and to promote the use of CIS nurseries. The meeting was postponed twice, the second time due to the elections in Mozambique. A meeting will be scheduled as soon as possible with the newly elected officials. The ARC also referred proponents of a very large tobacco-industry funded citrus-for-backyards project to the CIS, who supplied rootstock seed and will also supply budwood. The ARC will train the project participants in tree production. The ARC must be commended for its valuable role in supporting the Citrus biosecurity in Africa initiative.

c) Ethiopia

Dr Hennie le Roux from CRI visited Ethiopia and found HLB 700 km further south than initial detection in that country. Dr Tim Grout from CRI is involved in project that will survey for ACP and HLB in Kenya, and through CRI collaboration in this project they will possibly expand surveys to Ethiopia.

7 INTERNATIONAL VISITS

7.1 G.C. SCHUTTE

7.1.1 Report on a visit to Holland, Germany and Spain from 10-16 August 2014

Purpose: Inspection of European ports and plant health inspection services to gather information regarding their inspection protocols and techniques.

a) Holland

Visit to the Netherlands Food and Consumer Product Safety Authority in Wageningen (11 August 2014)

A meeting was held with Marjan Folkers (senior officer plant health), Bart van de Vossenbergh (molecular biologist) both of the Netherlands Food and Consumer Product Safety Authority, Peter Rozenboom (specialist phytosanitaire) of Kwaliteits-Controle-Bureau and Marcel van Raak (researcher in mycology) of the Ministry of Agriculture, Nature and Food Quality. They are all situated in Wageningen except for Peter Rozenboom who is situated in Den Haag.

After introducing ourselves we had an open and informative meeting regarding the inspections, sampling and testing laboratories. Questions were asked regarding sampling, inspections and protocols.

Sampling strategies and inspections:

The Dutch are meticulous regarding their sampling and labelling of interceptions, each receiving a bar code that can be traced at any time during the testing process. Five boxes per 1000 boxes are inspected (see below).

Nederlandse Voedsel- en Warenautoriteit

Register: Steekproefgrootte bij import Tab 06 13

Steekproefgrootte * Inspecteren per kleinste mogelijke fytosanitaire eenheid	Intensiteit	Grootte van de partij	Aantal te inspecteren eenheden per partij	Te inspecteren eenheden
		61 - 100 colli	5	colli
		> 100 colli	5 + 1 per 100 colli	colli
Gladiol	Iedere zending	< 25000 knollen	1 colli 50 knollen kaal maken	
		> 25000 knollen	2 colli 100 knollen kaal maken	
Overige bloembollen	Iedere zending	1 - 30 colli	1	colli
		31 - 100 colli	2	colli
		> 100 colli	2 + 1 per 200 colli	colli
Zakken leegstorten, kisten en dozen tenminste voor de helft				
Zaaiaden (zakken)	Iedere zending		1 colli	
Hout	Iedere zending monitoren			
Verpakkingshout				
GROENTEN & FRUIT				
	iedere zending	< 100 colli	2	colli
		100 - 300	3	colli
		301 - 500	3	colli
		501 - 1000	4	colli
		1001 - 2000	5	colli
		2001 - 3000	7	colli
		3001 - 4000	9	colli
		4001 - 5000	11	colli
		5001 - 6000	13	colli

A document was presented on request regarding their visual inspections at the harbour by inspectors (see below). What is interesting under the heading "Biologie" the following is stated "in de aangetaste plekken worden pycnidien, ongeslachtelijke sporen, gevormd. De rol van deze sporen in dhet infectieproces wordt over het algemeen klein geacht." Asked if the lesions on the photographs as published in "Datashets Fytobewaking" were verified with PCR, the answer was no.

Guignardia citricarpa

Engelse naam: citrus black spot

EU-status

IIAI

Systematiek

Het geslacht *Guignardia* behoort tot de Ascomyceten.

Biologie

Infectie vindt voornamelijk plaats door middel van ascosporen, die zich ontwikkelen op dood blad in de boomgaard. Deze sporen komen vooral vrij tijdens regen, en soms ook tijdens beregening. Ascosporen worden door de lucht verspreid, en aantasting van de vruchten is mogelijk vanaf vruchtzetting tot 4 a 5 maanden later. De eerste symptomen verschijnen niet eerder dan 6 maanden na vruchtzetting. Symptoomontwikkeling op rijpe vruchten wordt bevorderd door hogere temperatuur, hogere lichtintensiteit, droogte en slechte conditie van het gewas.

In de aangetaste plekken worden pycniden, ongeschlachtelijke sporen, gevormd. De rol van deze sporen in het infectieproces wordt over het algemeen klein geacht.

Ascosporen, de belangrijkste bron van infecties, worden alleen gevormd op afgevallen, aangetast blad, niet op vruchten.

Symptomen/schade

Guignardia citricarpa is de veroorzaker van "black spot" bij *Citrus*. Deze schimmel is op de EU quarantainelijst geplaatst, omdat het niet in de Europese Unie voorkomt, en als een bedreiging wordt gezien voor de *Citrus* teelt in het Mediterrane gebied.

Waardplanten

Guignardia citricarpa komt alleen voor bij *Citrus* soorten.

Een zeer nauw verwante soort is onlangs beschreven als *Guignardia mangiferae*. Deze schimmel heeft een zeer brede waardplantenreeks, waaronder *Citrus* spp., maar veroorzaakt op *Citrus* geen symptomen.

Referenties

1.) Nonpathogenic Isolates of the Citrus Black Spot Fungus, *Guignardia citricarpa*, Identified as a Cosmopolitan Endophyte of Woody Plants, *G. mangiferae* (*Phyllosticta capitalensis*), R.P. Baayen, P.J.M. Bonants, G. Verkley, G.C. Carroll, H.A. van der Aa, M. de Weerd, I.R. van Brouwershaven, G.C. Schutte, W. Maccheroni, C. Glienki de Blanco, J.L., Azevedo, *Phytopathology* **92(5)**: (2002) 464-477.

2.)

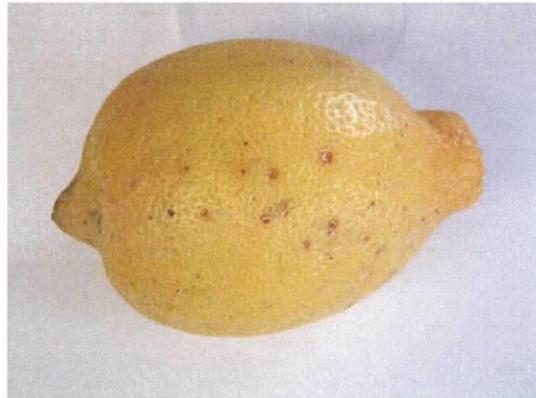
http://www.eppo.org/QUARANTINE/fungi/Guignardia_citricarpa/GUIGCI_protocol.pdf

Organisme	Bayer-code	Waardplant	Herkenning/symptomen
<i>Guignardia citricarpa</i>	GUIGCI	Citrus-soorten	Black spot symptomen op vruchten

Beginnende symptomen op sinaasappel



Beginnende
symptomen op
citroen



Symptomen op citroen

©Wageningen 2007. Het auteursrecht van deze publicatie berust bij de Plantenziektenkundige Dienst te Wageningen.
Niets uit deze uitgave mag zonder voorafgaande toestemming van genoemde dienst op enigerlei wijze worden vermeerderd, openbaar gemaakt
of voor commerciële doeleinden worden gebruikt.

Versie 1

Ingangsdatum: 24-7-2007

Pagina 2 van 2

Laboratory testing:

Asked if the testing is done according to a protocol, they gave me a copy of the European and Mediterranean Plant Protection Organization (PM 7/17) diagnostic protocol for *Guignardia citricarpa* (see Addendum 1). Everything is stipulated there. Of interest was Fig. 2 on page 321. In the flow diagram under "Fruits with black spot symptoms" and "Isolation" is where the wheels came off. Asked if they ever test for viability, the answer was no. Their excuse is that it takes too long for the fungus to grow which cause a bottleneck at the harbours. They claim that they only have to proof that the pathogen is present irrespective if it is alive or dead.

Asked if they keep photographic records of the lesions, they said they do and would show me later. They asked me why this is necessary I told them that it was for management purposes. If there were red lesions on the fruit means that it went through the packhouse and develop during transport while black lesions showed CBS went undetected through the packhouses. We can then go back to these packhouses to ensure that they must jack up their inspection procedures. Asked what type of lesions they commonly see, KCB said it is only the common hard spot lesions, which was good news.

Addendum 1: European and Mediterranean Plant Protection Organization (PM 7/17) diagnostic protocol for *Guignardia citricarpa*.

European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes

PM 7/17 (2)

Diagnostics

Diagnostic

Guignardia citricarpa

Specific scope

This standard describes a diagnostic protocol for *Guignardia citricarpa*.¹

Specific approval and amendment

First approved in 2002–09.
Revised in 2009–09.

Introduction

Guignardia citricarpa is a damaging pathogen on *Citrus* spp., occurring in many areas where *Citrus* is cultivated including Asia, Australia, South America, Southern Africa, Central America and the Caribbean region (CABI/EPPO, 1998; EPPO/CABI, 1997; CABI, 2006). The disease has not been reported from Europe or North America. It is mainly a foliage and fruit disease. The pathogen has significant economic impact mainly due to the external blemishes that make citrus fruit unsuitable for the fresh market. Severe infections may cause premature fruit drop (Kotzé, 2000). Some losses due to fruit drop occur in years favourable for disease development and when fruit is held on the trees past peak maturity (CABI, 2006). In addition, latently infected (asymptomatic) fruit at harvest may still develop symptoms during transport or storage (Kotzé, 1996).

In areas where only one disease cycle occurs annually, perithecia with ascospores, produced exclusively on leaf litter, are the main source of inoculum. However, in areas, where rain is not confined to a single season or citrus flowering occurs more than twice per year, conidia of the anamorph *Phyllosticta citricarpa* are as important as ascospores as inoculum sources (Spósito *et al.*, 2001). Ascospores released from the perithecia during rainfall or irrigation are carried by wind throughout the canopy and long distances beyond. The critical period for infection starts at fruit set and lasts for 4–5 months after which fruit becomes resistant (Kotzé, 2000). Leaves are susceptible for up to 10 months after development (Trute *et al.*, 2004). Following infection, the fungus remains in a quiescent state until the fruit becomes fully grown or mature, with disease symptoms being produced many months after infection has taken place (Kotzé,

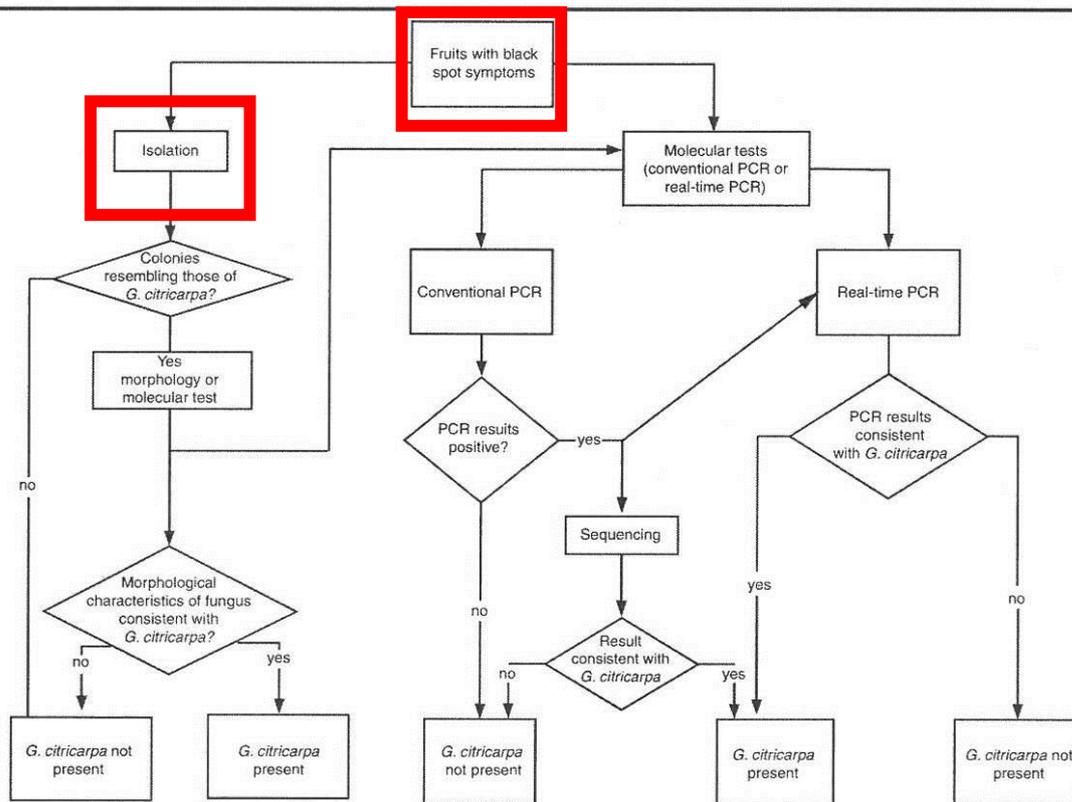
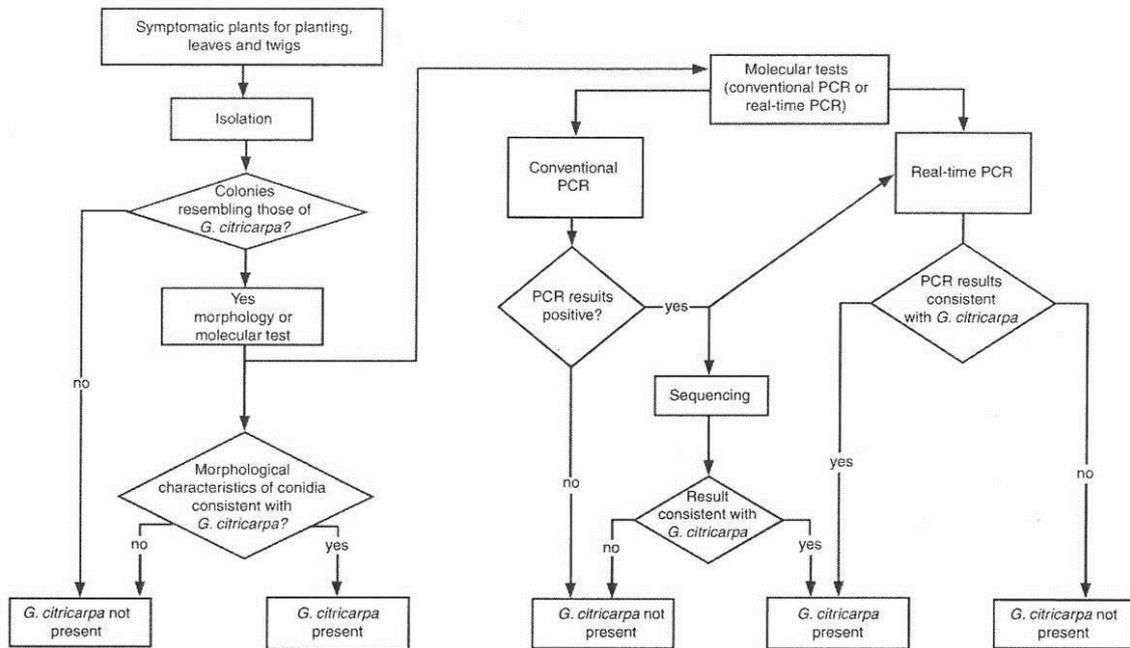
2000). Pycnidia with conidia are produced on symptomatic citrus fruit and leaf litter (Kotzé, 2000). They may be splash-dispersed onto the canopy or washed off from infected late-hanging fruit onto young fruit and leaves that are still at the susceptible stage (Spósito *et al.*, 2001). Perithecia develop within 40–180 days after leaf drop (Kotzé, 2000).

Spread of *G. citricarpa* to continents previously free from the disease is assumed to have taken place mainly through infected plants for planting (nursery stock and other planting material, e.g. for botanical gardens), rather than through infected fruits.

Phytosanitary regulations cover fruits of *Citrus*, *Fortunella*, *Poncirus* and their hybrids², other than fruits of resistant late-hanging fruit. They should originate from countries or areas recognized to be free from 'pathogenic strains' of *G. citricarpa* or at least the orchard and the fruits should be free from symptoms caused by 'pathogenic strains' of *G. citricarpa*. The regulations refer to strains of *G. citricarpa* pathogenic to citrus because non-pathogenic, endophytic *G. citricarpa*-like strains have been reported from symptomless *Citrus* plants as well as from host plants other than *Citrus* (Chiu, 1955; McOnie, 1964). These non-pathogenic strains were considered in the past to be saprophytic forms (Sutton & Waterston, 1966) or avirulent strains of *G. citricarpa* (Kotzé, 2000), although McOnie (1964) reported that these strains belong to a distinct *Guignardia* species (or, perhaps, variety or form). Baayen *et al.* (2002) have recently confirmed the latter and have shown that such strains belong to a distinct species, *Guignardia mangiferae* (anamorph *Phyllosticta capitalensis*), a common endophyte in many plant families. *Guignardia mangiferae* can be distinguished from *G. citricarpa* by cultural, morphological and molecular characters, and has never been found associated with typical black spot symptoms (Baayen *et al.*, 2002). *Guignardia mangiferae* is sometimes isolated from

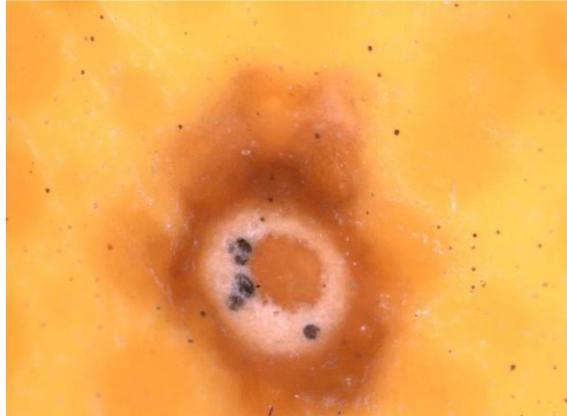
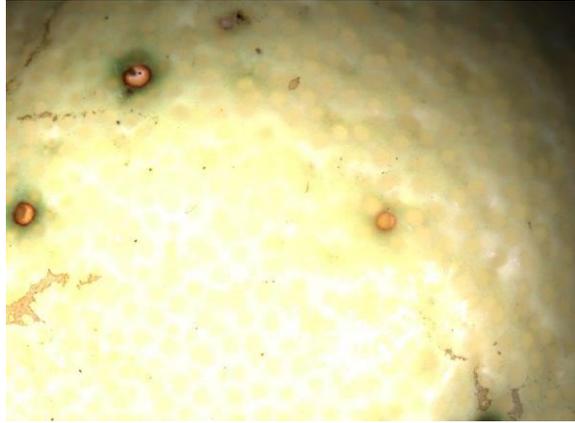
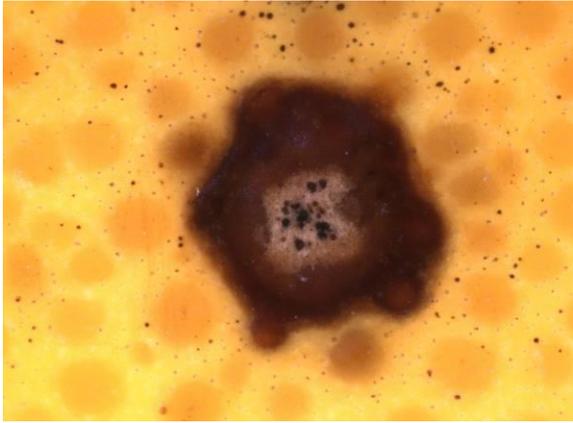
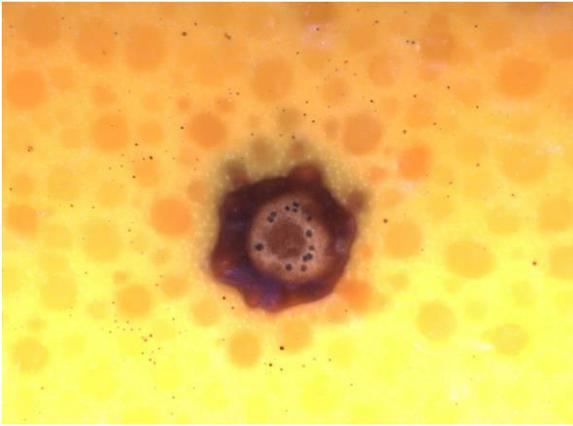
¹Use of brand names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable.

²*Poncirus* and its hybrids are citrus rootstocks, so that their fruits are not in practice traded.



to
ISE

Fig. 2 Flow diagram for the identification of *Guignardia citricarpa*.



Looking at the above photographs, only the bottom two lesions seem to be viable.

b) **Germany**

Visit to the Julius Kuhn Instituut for National and International Plant Health in Braunschweig (12 August)



Left to right: Katrin Kaminski, Dr. Magdalene Pietsch, Dr Clovis Douanla-Meli (senior scientist in Mycology), and Axel Moehrke from Dole.

Sampling strategies and inspections:

As in Holland, there is nothing wrong with their traceability of samples which is handled by their Federal inspectors of the -ministry of Food and Agriculture. As no inspectors were present, it could not be determined as to how they do sampling and inspection and where it is done. A brochure regarding CBS symptoms and other disease symptoms was handed over that can be confused with CBS. Some of the lesions as shown on the photographs are not CBS (see Addendum 2 below) as none of them have been verified using PCR and no pycnidia are present. This confusion about how CBS actually looks was evident when a box full of intercepted fruit came to the laboratory during the visit. There similar samples that look like boll-worm / insect damage was also inspected, but it was left to them sort it out.

Laboratory testing:

Dr Clovis Douanla-Meli, the scientists responsible for the identification and qPCR, is originally from Cameroon. He mentioned that nothing was in place last year to do any inspections of fruit that landed in Germany. Therefore he went to Wageningen to get training from the Dutch how to perform qPCR tests according to the protocol. Although the same protocol (EPPO Standard PM 7/98) was used, he made a couple adjustments.

The German's qPCR results and protocol was sent to Dr. Hano Maree at the University of Stellenbosch for his comments. He highlighted several critical shortcomings as follows:

- a) Lack of replicates
- b) High Ct Values
- c) Low efficiency
- d) Calculation of threshold
- e) Input DNA not standardised
- f) PCR is not a relevant test as DNA detection does not determine viability of the pathogen. It should be a grow-out test.

A copy of the EU document was handed over: "Commission implementing decision of 2 July 2014 – setting out measures in respect of certain citrus fruits originating in South Africa to prevent the introduction into and the spread within the Union of *Phyllosticta citricarpa* (McAlphine) Van der Aa (notified under document C92014) 4191". From the content of this document it is clear that South Africa is singled out as the only

culprit excluding Argentina, Brazil and Uruguay (Addendum 3). Moreover, the prescribed procedure specifies the need for identification of the pest only, and does not address viability at all.

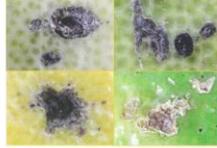
In general they were open and had no problem in sharing information and protocols with us. Clovis, was so kind as to copy photographs of the lesions on fruit from Weipe that were intercepted in Hamburg during July 2014 (see Addendum 4 below).

Addendum 2: Brochure for German inspectors

Verwechslungsmöglichkeiten

Zitruskrebs

(Bakterium) *Xanthomonas axonopodis* pv. citri (Hans) Vauterin



Krebs-Läsionen (vereinzelt oder zusammenhängend) reichen von 1 - 10 mm Größe. Anfängliche Anzeichen sind gehobene korkartige Partien mit aufgebogenen silbrigen bis weissgrünen Rändern, wobei auf grünen Früchten ein gelbes Halo deutlich sichtbar ist. Später entsteht nach Zermahlen die Oberhaut eine kotzerbliche Vertiefung mit Rissen sowie braunes zuckerartiges knuspriges Material.

Verdacht und Nachweis

Früchte, die beim visuellen CBS-Inspektionsverfahren auffällig sind, müssen prinzipiell einer Diagnose bzw. einem Laborverfahren unterworfen werden. Dies gilt für klare CBS-Symptome als auch für Zweifelsfälle.

Eine Bestätigung des Verdachts mit der klassischen Isolierung des Pathogens auf Agarmedium hat nur eine Erfolgsquote von 10%. Daher beruht das EU-weit einheitliche CBS-Nachweisverfahren auf einem qPCR-basierten Protokoll, an dessen Entwicklung derzeit im JKI Institut Pflanzengesundheit gearbeitet wird.

Bei Verdacht auf CBS können vorläufig über die amtliche Pflanzenschutzbehörde des jeweiligen Bundeslandes Proben in das JKI (Dr. Christa Gausel-Maly) gesendet werden.

Verwechslungsmöglichkeiten

Septoria Flecken

(Pilz) z.B. *Septoria citri* Pass.



Erst kleine bräunliche bis rotbraune Narben von 1 - 2 mm Durchmesser. Die Läsionen werden später dunkler und stärker ein. Sie können zu großen Schädlingen zusammenfließen. Wie bei CBS-Läsionen können Septoria-Flecken auch Pyknidien (winzige schwarze Gebilde) entwickeln.

Informationsblatt des JKI: Zitrus-Schwarzfleckenkrankheit

Als Download finden Sie das Informationsblatt unter: <http://www.jki.bund.de/forschung.html>

Text: Christa Gausel-Maly und Anne Georg-Engler, Institut für nationale und internationale Antragsverfahren zum Pflanzenschutz des JKI

Redaktion und Layout: Dr. Gertraud Hochgraf (Pressestelle) und Anja Wack (Informationszentrum)

Abbildungen: Christa Gausel-Maly

Kennzeichen: Julius Kühn-Institut, Bundesforschungsanstalt für Kulturpflanzen, Marsweg 11/12, 80504 Braunschweig

Telefon: 0531 396-1200, e-mail: kontakt@jki.bund.de

Die JKI ist eine Einrichtung im Geschäftsbereich des Bundesministeriums für Ernährung und Landwirtschaft (BMEL).

DOI: 10.5073/JKI.2014.002



Zitrus-Schwarzfleckenkrankheit (CBS)

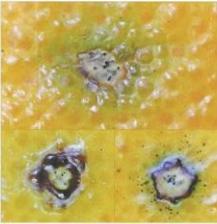
Phyllosticta citricarpa (= *Gaigardaria citricarpa*)
Symptome und Verwechslungsmöglichkeiten



Albtraum von Zitrusanbauern

Zitruspflanzen und -früchte werden von verschiedenen Schadorganismen befallen. Einige Krankheiten führen einem Produktionsausfall herbei, während andere eher eine Wertminderung von Früchten verursachen. Die Schwarzfleckenkrankheit (CBS) verursacht den Rückgang von beiden, Fruchtqualität und -quantität, und ist von allen Zitrusanbauern gefürchtet. Sie wird durch den pilzlichen Erreger *Phyllosticta citricarpa* (= *Gaigardaria citricarpa*) ausgelöst. CBS ist politisch wie wirtschaftlich aktuell von größerer Bedeutung für den Zitrushandel zwischen Arabisländern und der EU. Bisher konnte der Pilz in der EU nicht vor und unterliegt daher strengen Quarantäneerregulungen. Um eine Einschleppung des Pilzes in die EU zu verhindern, müssen alle EU-Mitgliedsstaaten eine Kontrolle von Zitrusimporten auf CBS durchführen. Dabei erfolgt im ersten Schritt die Identifizierung der Symptome auf den Früchten. Diese sind jedoch nicht nur sehr variabel in ihrer Erscheinung, sondern je nach Entwicklungsphase sehr leicht mit Symptomen anderer Zitruskrankheiten zu verwechseln.

Typische CBS-Symptome



Harte Flecken mit Pyknidien stellen typische diagenetische CBS-Symptome dar. Läsionen sind unregelmäßig kreisförmig mit einem Ø von 3 - 10 mm, oft ziegelrot mit brauner bis grauer Mitte und ausgeprägter brauner bis schwarzer Umrandung. Ein grüner Halo um die Läsionen ist Anzeichen des Entwicklungsstadiums. Bereits an frühzeitig geernteten unreifen Früchten können sich nach dem Pflücken auch die harten Läsionen entwickeln. Pyknidien sind meistens an der grauen Mitte als kleine schwarze Punkte vorhanden und mit Hilfe der Handlupe gut erkennbar.



Harte Flecken ohne Pyknidien können auch als einheitlich rötliche, eingesunkene Läsionen auftreten. Die Anregung der Pyknidienbildung auf solchen Läsionen kann mit einer Inkubation bei 27°C unter konstanten Beleuchtungsbedingungen vorgenommen werden.

Typische CBS-Symptome



Sommergerostete Früchte können sich an einer schweren Infektion. Sie treten meist kurz vor der Ernte auf. Das Erscheinungsbild ist eine Anhäufung dunkelbrauner kleiner Läsionen (1 - 3 mm Ø). Zum Teil sind sie eingesunken, möglicherweise mit Pyknidien. Sie sind unterschiedlich gefärbt, von rotlich mit dunkelbrauner Umrandung bis braunlich, grau oder farblos. Die Läsionen können sich später oder während der Lagerung zu vitulierten oder harten Flecken weiterentwickeln.



Winter CBS-Läsionsarten, die hier nicht vorgestellt wurden (z.B. falsche Meliose), treten überwiegend an unreifen Früchten auf. Dabei ist ihr Vorkommen auf importierten Früchten unwahrscheinlich.

Verwechslungsmöglichkeiten

CBS-Symptome können leicht übersehen oder mit denen anderer Zitruskrankheiten verwechselt werden, da sie sehr unterschiedlich ausfallen. Das erweitert die symptomatische Erkennung von CBS für Umlageübte wesentlich.

Braunfleckung der Mandarine

(Pilz) *Alternaria alternata* (Fr.) Keiseler



Verschiedenartige Symptome auf Zitrusfrüchten werden *Alternaria* Arten zugeschrieben. So die Braunfleckung, die oft auf Mandarinen vorkommt. Hier ersetzen die Läsionen erst als Braunung, dann folgt die Bildung eines korkigen Gewebes, das sich später abblät. Oft entsteht ein Krater oder eine Pockennarbe, aber niemals Pyknidien.

Schwarznarbigkeit (Black Pit) der Zitrusfrüchte

(Bakterium) *Pseudomonas syringae* pv. *syringae* var. *Hall*



Typische Black Pit Symptome sind deutlich eingesunkene hellbraune Flecken, die später dunkelbraun bis schwarz werden. Oft treten konzentrische braune Ringe auf einem hellen Grund auf (Abb. oben li.). Unterscheidung zu CBS: kein deutlicher Halo und keine Pyknidienbildung.

Addendum 3: EU decision on South African fruit dated 2 July 2014.

17.2014

EN

Official Journal of the European Union

L 196/2

COMMISSION IMPLEMENTING DECISION

of 2 July 2014

setting out measures in respect of certain citrus fruits originating in South Africa to prevent the introduction into and the spread within the Union of *Phyllosticta citricarpa* (McAlpine) Van der Aa

(notified under document C(2014) 4191)

(2014/422/EU)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community ⁽¹⁾, and in particular the third sentence of Article 16(3) thereof,

Whereas:

- (1) *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*) is listed in point (c) 11 of Section I of Part A of Annex II to Directive 2000/29/EC as a harmful organism not known to occur in the Union. Since 2011, following the approval of a new code for fungal nomenclature by the International Botanical Congress, that organism has been referred to as *Phyllosticta citricarpa* (McAlpine) Van der Aa, hereinafter 'the specified organism'.
- (2) The European Food Safety Authority (hereinafter 'the Authority') published a pest risk assessment on the specified organism on 21 February 2014 ⁽²⁾. In light of that pest risk assessment it is concluded that the requirements regarding the specified organism set out in Directive 2000/29/EC for the introduction into the Union of citrus fruits originating in fields outside an area recognised as being free from the specified organism are not sufficient to protect the Union against the introduction of that organism. Given the recurring high number of interceptions in the previous years of citrus fruits originating in South Africa infested with the specified organism, it is necessary to take stricter measures without delay in order to improve protection of the Union against the introduction of that organism. Given that many of those interceptions have been on fruits of *Citrus sinensis* (L.) Osbeck 'Valencia', those fruits should be subject to testing for latent infection in addition to the measures applying to all citrus fruits.
- (3) However, the introduction into the Union of the specified organism through the import of fruits of *Citrus latifolia* Tanaka is rated by the Authority as very unlikely. It is therefore appropriate to exclude *Citrus latifolia* Tanaka from the measures provided for in this Decision.
- (4) In case of interceptions of citrus fruits originating in South Africa which are infected with the specified organism, the Commission will assess whether the arrival of infected fruits is the result of failures in the procedures for official monitoring and certification in South Africa. In case of recurring interceptions due to failing monitoring and certification procedures within the same year, the Commission will review this Decision before the sixth interception has been notified.
- (5) For reasons of clarity, Commission Implementing Decision 2013/754/EU ⁽³⁾ should be repealed.
- (6) The measures set out in this Decision should apply from 24 July 2014 in order to allow operators sufficient time to adapt to the new requirements.
- (7) The measures provided for in this Decision are in accordance with the opinion of the Standing Committee on Plant Health,

⁽¹⁾ OJ L 169, 10.7.2000, p. 1.

⁽²⁾ EFSA PLH Panel (EFSA Panel on Plant Health), 2014. Scientific Opinion on the risk of *Phyllosticta citricarpa* (*Guignardia citricarpa*) for the EU territory with identification and evaluation of risk reduction options. EFSA Journal 2014;12(2):3557, 243 pp. doi:10.2903/j.efsa.2014.3557.

⁽³⁾ Commission Implementing Decision 2013/754/EU of 11 December 2013 on measures to prevent the introduction into and the spread within the Union of *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*), as regards South Africa (OJ L 334, 13.12.2013, p. 44).

ANNEX

REQUIREMENTS FOR INTRODUCTION OF THE SPECIFIED FRUITS REFERRED TO IN ARTICLE 1

1. Requirements concerning the specified fruits

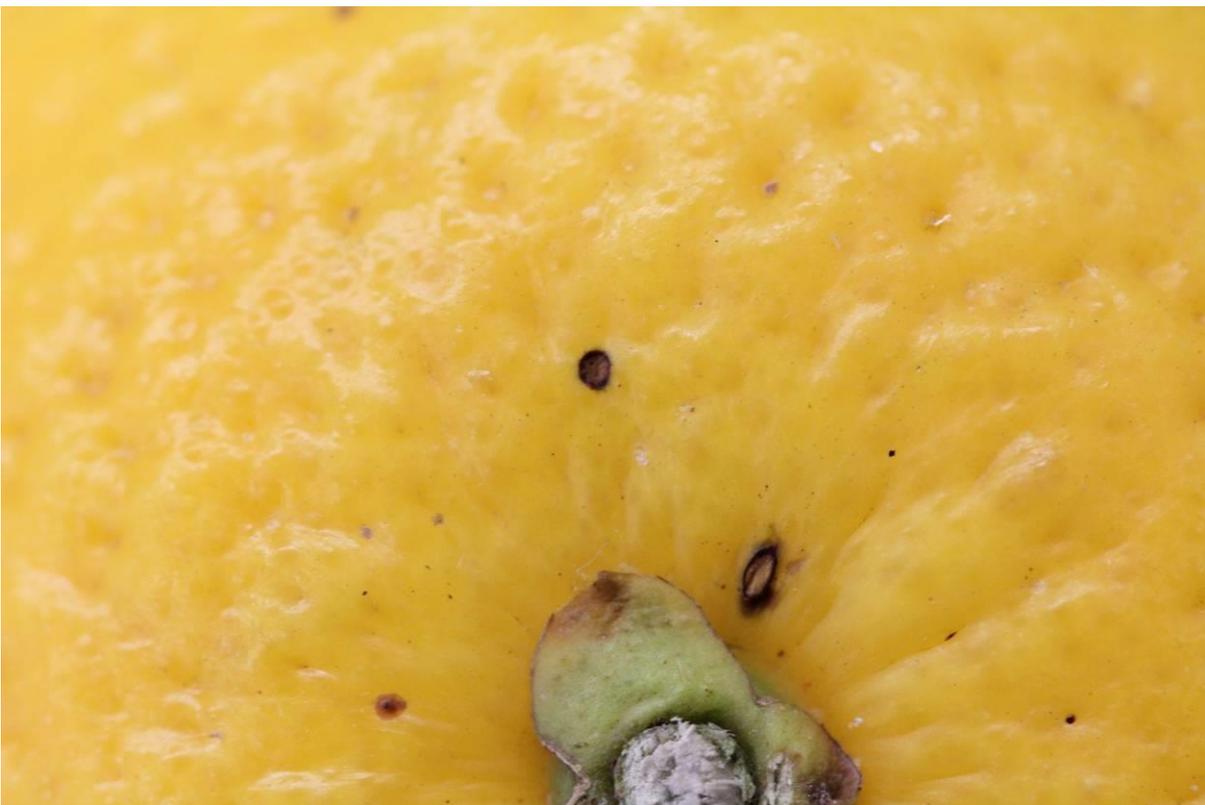
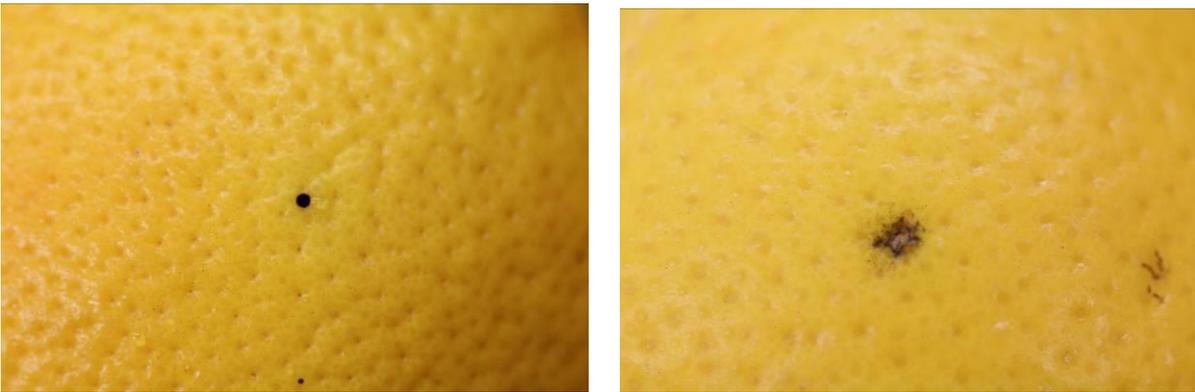
- 1.1. The specified fruits shall be accompanied by a phytosanitary certificate, as referred to in the first subparagraph of point (ii) of Article 13(1) of Directive 2000/29/EC, which includes under the heading 'Additional declaration' the following statements:
- the specified fruits originate in a field of production which has been subjected to treatments against the specified organism carried out at the appropriate time since the beginning of the last cycle of vegetation;
 - an official inspection has been carried out in the field of production during the growing season and no symptoms of the specified organism have been detected in the specified fruit since the beginning of the last cycle of vegetation;
 - a sample has been taken along the line between arrival and packaging in the packing facilities of at least 600 fruits of each species per 30 tonnes, or part thereof, selected as much as possible on the basis of any possible symptom of the specified organism; all sampled fruits showing symptoms have been tested and found free of the specified organism;
- 1.2. In the case of *Citrus sinensis* (L.) Osbeck 'Valencia', the phytosanitary certificate shall, under the heading 'Additional declaration', also include the statement that a sample per 30 tonnes, or part thereof, has been tested for latent infection and found free of the specified organism.
- 1.3. Complete traceability of the specified fruits shall be ensured as follows:
- the field of production, the packing facilities, exporters and any other operator involved in the handling of the specified fruits shall be officially registered for that purpose;
 - detailed information on the pre- and post-harvest treatments shall be kept;
 - throughout their movement, from the field of production to the point of export to the Union, the specified fruits shall be accompanied by documents issued under the supervision of the National Plant Protection Organisation of South Africa, as part of a documentary system on which information is made available to the Commission by South Africa.

2. Requirements concerning inspections within the Union

- 2.1. The specified fruits shall be visually inspected at the point of entry or at the place of destination established in accordance with Commission Directive 2004/103/EC⁽¹⁾. Those inspections shall be carried out on samples of at least 200 fruits of each species of the specified fruits by batch of 30 tonnes, or part thereof, selected on the basis of any possible symptom of the specified organism.
- 2.2. If symptoms of the specified organism are detected during the inspections referred to in point 2.1, the presence of the specified organism shall be confirmed or refuted by testing of the fruits showing symptoms. If the presence of the specified organism is confirmed, the batch from which the sample has been taken shall be subjected to one of the following measures:
- refusal of entry into the Union;
 - destruction, other than by processing.

⁽¹⁾ Commission Directive 2004/103/EC of 7 October 2004 on identity and plant health checks of plants, plant products or other objects, listed in Part B of Annex V to Council Directive 2000/29/EC, which may be carried out at a place other than the point of entry into the Community or at a place close by and specifying the conditions related to these checks (OJ L 313, 12.10.2004, p. 16).

Addendum 4: Fruit from Weipe that were intercepted in Hamburg. Note the absence of pycnidia in the lesions



I argued that, after looking at the photographs, these lesions are not viable. They did not realize it, and as in the case with the Dutch, do not test for viability, but test just for the presence of the pathogen.

c) Spain

Visit to the harbour at Cartagena (13 August)

Sampling strategies and inspections:

Salvador Palanca escorted me to the harbour at Cartagena where the cold room facilities were visited. They were very secretive regarding what or what not to show. After a while, while some “inspectors” were changing “guard”, they quickly opened a container for and showed “chem free” fruit of South Africa. Thereafter the cold rooms filled to capacity with Brazilian, Argentinian, Uruguayan and South African fruit were visited. What was obvious is that more South African pallets were loosened to remove the top layer of boxes for inspection than any of the South American countries.



Salvador Palanca (SA San Miguel South Africa) in a cold store with South African fruit that was rejected due to CBS.

Salvador Palanca noted that although the inspectors cleared a consignment of fruit from Addo in SA, personnel of the University of Valencia (prof. Jose Garcia-Jimenez) came to draw a second sample which later tested “positive” for CBS (see addendum 4 below). It was recommended to him to demand photographs and PCR results. A South African example of such a tested was shown to him.

Asked if other inspection facilities could be visited, they refused. That was it. In some other office an Argentinian inspector, Luis Bautista Fassi, was introduced from the “Citrus Exporters Association from North Eastern Argentina” (CECNEA), Entre Rios. He could not speak English, but from what could be gathered, he assists the inspectors in the harbour.

During lunch in Cartagena the “Director Del area de Agricultura Y Pesca” of the Murcia province, Joachim Rodriguez Navarro, was introduced. He could not speak English but Salvador could translate the conversations. Then he came up with the ridiculous story that black spot can be introduced like *Pseudomonas syringae* for instance on pears has. Asked how he can compare a bacterial disease with a fungal disease which has a certain life cycle. At that stage it was 40°C outside and asked how can pycnidiospores survive such extreme heat? He could not answer any of the questions properly.

They were informed about the high ranking visit that the Spanish ambassador and other EU representatives had at Rosle Boerdery at Groblersdal of which they had no knowledge. This should at least be made public in our Fruit Journal with photographs of the delegates to be sent to these people to show what we are doing to curb the disease.

Laboratory testing:
No information was gathered in this regard.

Addendum 4: Diagnostic report of prof Jose Garcia-Jimenez (University of Valencia) regarding the presence of CBS in a second sample from asymptomatic fruit from Addo in the Eastern Cape.



GRUPO DE INVESTIGACIÓN EN HONGOS FITOPATÓGENOS
INSTITUTO AGROFORESTAL MEDITERRÁNEO
UNIVERSIDAD POLITÉCNICA DE VALENCIA
Camino de Vera, s.n. 46022- Valencia- ESPAÑA

Tel.: 96 387 9251 Fax: 96 387 9269 / 96 387 7331
E-mail: gihf@upv.es

Juan José Martínez Mora
PIF Cartagena
Muelle de San Pedro
30202 Cartagena, Murcia

INFORME DE DIAGNÓSTICO

Muestra n^o: 2421-2424

Material: Dos muestras de naranjas (3011400848 / MSCU7324348, PUC: L0681 y L7479) y (3011400848 / MSCU7324348, PUC: L7299, L7125 y L7331), y dos muestras de limones (3011400851 / MSCU8562619, PUC: L1875, L1719 y L7114) y (3011400851 / MSCU8562619, PUC: L7481 y L7306), procedentes de Sudáfrica. Se solicita la determinación de la posible presencia de hongos de cuarentena.

Pruebas realizadas: Observación directa bajo lupa y microscopio. Aplicación de los protocolos moleculares de detección de *Guignardia citricarpa* Kiely (Diagnostic Protocols for Regulated Pests PM7/17. EPPO Bulletin 2003, 33: 271-280) y de *Elsinoë* spp., mediante la técnica PCR descrita por Hyun *et al.* (Plant Dis. 91: 865-870).

Diagnóstico: Se ha detectado *Phyllosticta citricarpa* (McAlpine) Aa (= *Guignardia citricarpa*) en la muestra de naranjas (3011400848 / MSCU7324348, PUC: L0681 y L7479), correspondiente a la ficha con número 2421 del registro del LNR.

En el resto de muestras no se han detectado hongos de cuarentena.

P.O.

José García Jiménez

EUROPEAN UNION: NOTIFICATION OF INTERCEPTION OF A CONSIGNMENT OR HARMFUL ORGANISM		EU Interception Nr. 89437
1. Consignor a. Name: SAN MIGUEL FRUITS (PTY) LTD b. Address: 9 JUTLAND CRESCENT, ST GEORGES PARK, PORT ELIZABETH, SUDAFRICA c. Country: ZA [SOUTH AFRICA]		2. Interception file a. National Reference Number: ES/MAGRAMA/2014/1261 g. Revision Nr. & Date: 00/2014-08-21 Consignment from: b. Third Country: [+] c. EU: [-] Request for message to be sent: d. to Member States: [+] e. to EPP0: [+] f. to Country of Export: [+] 4. Envelope a. Plant protection organization of: ES[SPAIN] b. To: FVO 5. Export a. Exporting Country: ZA [SOUTH AFRICA] b. Place of export: SUDÁFRICA 6. Origin a. Country of origin: ZA [SOUTH AFRICA] b. Place of origin: SUDÁFRICA
3. Consignee a. Name: AGRICOMMERCE SA. b. Address: SAINT CHARLES INTERNATIONAL -BP, BD PERIPHERIQUE MAGASIN 128 (66030), PERPIGNAN CEDEX, FRANCIA c. Country: FR [FRANCE] d. Destination Country: FR [FRANCE] e. Destination Place: PERPIGNAN (PEC)		7. Transport a. Mode(s) of transport: SEA b. Mean(s) of transport: BARCO c. Identification(s): HELENA SCHEPERS 8. Point Of Entry a. POE's Country: FR [FRANCE] b. POE's Place: PERPIGNAN (PEC)
9. Identification of Consignment a. Type of document: PHYTOSANITARY CERTIFICATE b. Document number: PGT 1125479 c. Country of issue: ZA [SOUTH AFRICA] d. Place of issue: PORT ELIZABETH e. Date of issue: 2014-07-16		10. Description of the intercepted part of the consignment a. Type of package(s)/container(s): CONTAINER b. Distinguishing mark(s) of package(s)/container(s): TEMU 9006010 c. Number(s) on package(s)/container(s): d. Plant, plant product or other object: CIDS1 [CITRUS SINENSIS] e. Class of commodity: OTHER LIVING PLANTS : FRUIT & VEGETABLES
11. Net Mass/Volume/Number of units in consignment a. MVN: 20498 b. Unit of measure: KGM [KILOGRAM]		12. Net Mass/Volume/Number of units in intercepted part a. MVN: 20498 b. Unit of measure: KGM [KILOGRAM]
13. Net Mass/Volume/Number of units in contaminated part a. MVN: 20498 b. Unit of measure: KGM [KILOGRAM]		14. Reasons for interception a. Reason: OTHER REASONS : PRESENCE OF HARMFUL ORGANISM b. Scientific name of the harmful organism: GUIGCI [PHYLLOSTICTA CITRICARPA] c. Extent of contamination: PLANT, PLANT PRODUCT OR OTHER OBJECT
15. Measure(s) taken on consignment a. Measure RELEASE OF THE CONSIGNMENT TEST IN LABORATORY OF THE CONTAMINATED PRODUCTS QUARANTINE IMPOSED c. Quarantine Begin date: d. Quarantine Anticipated end date: e. Quarantine End date: f. Country of Quarantine: g. Place of Quarantine:		16. Free Text LA MUESTRA DE 20 FRUTOS DE ESTE ENVIO FUE ENVIADA AL LABORATORIO DE REFERENCIA COMO ASINTOMÁTICA, LA MERCANCÍA FUE LIBERADA, POSTERIORMENTE, EN EL ANÁLISIS DE LA MUESTRA, ESTA DIO POSITIVO A PHYLLASTICTA CITRICARPA. PUC: L 6111
b. Extent of measure THE INTERCEPTED PART OF THE CONSIGNMENT THE INTERCEPTED PART OF THE CONSIGNMENT LABORATORY TEST h. Sample submission date: 2014-08-06 i. Anticipated result date: j. Result date: 2014-08-07 k. Country of Test: ES [SPAIN] l. Place of Test: VALENCIA		17. Information on the interception a. Place/Check point: CARTAGENA PUERTO b. Official service: MAGRAMA c. Date: 2014-08-06
18. Sender of the message a. Official service: MAGRAMA b. Signed and authorised by: LORCA ALEJANDRO c. Contact: inspfito@magrama.es d. Sender Website URL: e. Date: 2014-08-21		

7.2 A. MANRAKHAN

7.2.1 Report on a visit to Bangkok, Thailand, on the 9th International Fruit Fly Symposium of Economic Importance, 12-16 May 2014

INTRODUCTION

The International Symposium on Fruit Flies of Economic Importance (ISFFEI) is a quadrennial event which brings together scientists working on fruit fly pests in different parts of the world. The 9th International Fruit Fly Symposium of Economic Importance took place from the 12 to 16 May 2014 in Bangkok, Thailand.

The objectives of my participation in the 9th ISFFEI were to:

1. Obtain new information on fruit fly biology and management.
2. Meet and connect with other fruit fly workers.
3. Conduct the first kick off meeting for the ERAfrica fruit fly project which is co-ordinated by CRI.
4. Present a key note paper on "Use of male annihilation technique for control of pest species in the *Bactrocera* group on mainland Africa" (Session 6 on Thursday 15 May 2014)

The scientific programme of the symposium covered various aspects of fruit fly biology, ecology and management. The programme consisted of 10 sessions (See Annex1) under the following topics (1) Area-wide and Action Programs, (2) Biology, Ecology, Physiology and Behaviour, (3) Morphology and Taxonomy, (4) Genetics and Evolution, (5) Chemical Ecology and Attractants, (6) Control methods and supporting technology, (7) Natural enemies and biological control, (8) SIT principles and applications and (9) Risk assessment and quarantine. Unfortunately, there was no coverage of post-harvest treatments during the course of this symposium.

KEY POINTS FROM KEY ORAL PRESENTATIONS

Area-wide and Action Programs

Applications of geo-informatics in area-wide pest management by Anon Snidvongs & Sujinda Thanaphum, Geo-informatics and Space Technology Development Agency (GISTDA), Bangkok, Thailand.

Geo-informatics is being used for area wide fruit fly management including Sterile Insect Technique in some fruit production areas in Thailand. Fruit fly monitoring data are overlaid on high resolution images of fruit orchards and data on environmental variables which are accessed from specific satellites such as Sentinel and ALOS-2. Some satellite data are available for free.

Can polyphagous Tephritid pest populations remain undetectable over years under favourable climatic and host conditions by Donald Mc Innis et al. ARS-USDA, US.

Five scenarios were presented following incursions of a polyphagous fruit fly in an area with favourable climate and host availability: (1) Undetected incursion does not survive, (2) incursion detected early and eradicated, (3) incursion not suppressed and allowed to grow exponentially, (4) new incursion of the same pest occurring after extinction either naturally or artificially (aided by control products), (5) existing population remaining undetected for years. The last scenario was debated in the presentation. Polyphagous fruit flies such as Medfly would need to have balanced stable populations in order to be able to exist even at low population levels. If they do not have the balanced state, populations would naturally die out. In areas containing high density of traps with especially powerful attractants, the probability of detection of polyphagous fruit flies would be high. Therefore claiming establishment of polyphagous flies without proof of population existence (trapping data) is damaging to international trade.

Population dynamics of the Mediterranean fruit fly in coffee areas located in the Guatemala Chiapas, Mexico, Region and its implications in IPM strategies by Walther Enkerlin, Programa Regional Moscamed, Guatemala

The wind patterns of Medfly influenced dispersal of Medfly. Aerial sprays of GF-120 were used to control Medfly. Aerial GF-120 Sprays were applied at **150 ft (45 m)** above tree canopy every 10 days at ~ 4 L of GF-120 mixture per ha. Droplet sizes of GF-120 varied between 3 and 6 mm in diameter. Sterile Insect Technique was also used for Medfly control and applied at a 100:1 Sterile: Fertile ratio.

Morphology and Taxonomy

Led Zeppelin and the DNA Barcoding of fruit flies: “Stairway to heaven” or “Babe, I’m gonna leave you”?- A pragmatic approach towards workable solutions by Massimiliano Virgilio, Royal Museum for Central Africa, Belgium

DNA barcodes exist for 10-15% of described species www.boldsystems.org. For different insect orders, the availability of DNA barcodes are as follows: Diptera: 28%; Lepidoptera: 36%; Hymenoptera: 17% and Coleoptera: 8%. The question was how can we work with an incomplete DNA barcode library? Two criteria are used for identification: Best close match criterion (BCM) & Best Match (BM), whereby identification is accepted based on distance query-match. The use of Adhoc thresholds – a R package is being proposed for insect identification and identification of Tephritids.

Resolution of key pest members of *B. dorsalis* species complex by Anthony Clarke et al., Queensland University of Technology, Australia

For some members of the *B. dorsalis* complex such as *B. papayae*, *B. carambolae*, *B. philippinensis*, *B. dorsalis* s.s & *B. invadens*, there are no robust diagnostics to separate them. The species were split too far. Morphological variations were found between the species mainly in terms of colour of thorax, with darker thoracic colours for species in *B. dorsalis* complex occurring in Asia compared to those in Africa. The morphological traits considered discriminatory (aedeagus length, postsutural lateral vittae etc) are continuous between sibling species. When looking at molecular data, *B. invadens* had a separate cluster but that could be due to population structure. Haplotypes are shared between these four members of the *B. dorsalis* complex. The pheromone composition was different for *B. carambolae* and hybrid viability was reduced following cross mating with the other members in the complex. The current position is that there is a lack of any distinct differences between *B. philippinensis*, *B. papayae*, *B. invadens* and *B. dorsalis* except for those commensurate with population level variation. This has led the authors to conclude that the different taxa form one species- *B. dorsalis*. *B. carambolae* though remain a distinct but close species in the complex. Drew & Romig (2013) have synonymised *B. philippinensis* and *B. papayae*

A paper was submitted to Systematic Entomology proposing the synonymy of *B. papayae* and *B. invadens* with *B. dorsalis*. The paper is being reviewed. Acceptance of the name change remains however a political one.

An integrative approach to unravel the *Ceratitis* FAR cryptic species complex by Marc De Meyer et al., Royal Museum for Central Africa, Belgium

The females in the *Ceratitis* FAR complex (*C. rosa*, *C. fasciventris* and *C. anonae*) cannot be reliably separated on morphological grounds. Although some pheromone compounds were similar for the species, a number of species had a few unique compounds. Different cuticular hydrocarbons- were found for the different species. Landmarks on the wings and wing banding separated *C. rosa*, *C. fasciventris* and *C. anonae*. Oral ridges and accessory plates of the larvae of the three species were also different and clearly separated the species. Microsatellite markers used to determine the population genetic structure of the three species further revealed 2 separate entities of *C. rosa*, 2 entities of *C. fasciventris* and 1 entity of *C. anonae*. Current results on developmental physiology, mating compatibility and geographical distribution are confirming the two separate entities of *C. rosa*. Preliminary results have shown very low mating compatibility between the two *C. rosa* types.

Chemical Ecology and Attractants

Bait manufactured from beer yeast waste and its use for fruit fly management by Shanmugam Vijaysegaran, Queensland University of Technology, Australia

Beer waste was used successfully in Malaysia as protein bait (in spot sprays) for control of *Bactrocera* pests. Beer waste plants have been set up in Vietnam. The beer waste is heated to remove gas, alcohol and excess water. Enzyme is added to digest the waste. A food grade preservative is added to provide a 2 year shelf life and the processing time of the protein bait is 48 hours. In previous bait production plants, bait was produced from beer waste using a two tank system whereby one tank would heat the waste to remove alcohol and excess water and after heating the waste would be pumped to 2nd tank for enzyme treatment. Currently bait plants have an automated modern single-tank system. The commercial beer waste baits contain 18-25% sugars and 12-18% proteins. The recommended insecticides for use with the beer waste baits are malathion (0.2% a.i), fipronil (0.01% a.i) and spinosad.

Control methods and supporting technology

Detection of *B. dorsalis* in Mauritius and rapid response by Preaduth Sookar, Ministry of Agro Industry and Food Security, Mauritius

In March 2013, *B. dorsalis* was detected in routine surveillance network in Mauritius. The action plan modelled from South Africa was used to implement delimiting surveys and control actions. The last detection in traps was in November 2013 and last detection in fruit was in June 2014. Infestation was recorded on carambola, guava, citrus, Indian almond, golden apple and acerola. Eradication actions have stopped in April 2014. The monitoring will be carried out for another 12 weeks in order to confirm eradication.

Area-wide management of *C. capitata* in fruit trees in Israel- successful implementation and new challenges by Miriam Silberstein, MIGAL, Israel

The situation in Israel is that of many small production plots by many farmers. Mass trapping seems to be more effective than aerial sprays and area-wide control seems better than control of individual plots. The control is effected over a whole year.

Integrated pest management of fruit flies on rose apples in Thailand by Sunyakee Srikachar, Department of Agriculture, Thailand

B. dorsalis completes its life cycle in 17-21 days in rose apple. Fruit infestation starts at 21 days after stamens fall off (mature green). Bagging helped reduce infestation. Spunbong bag and white plastic bags reduced infestation.

Natural enemies and biological control

Technical competition and the fate of augmentative biological control by John Sivinski, USDA ARS, Florida, US

Augmentative biological control is in direct competition with SIT. Early season augmentation and at the source of the pest rather than in the crop can keep fly populations low to protect grapefruit export zones. The number of parasitoids required for successful control of Caribbean fruit fly was 60 000 per km². However cost of biological control is expensive. In order to make biological control more cost effective, costs of rearing and handling must be lowered, release rates must be lowered (0.2-0.4 lower than that of sterile male releases) and more efficient parasitoids are required. The costs of rearing can be reduced by using mass reared females. Efficient parasitoids such as *Fopius arisanus* that can attack the egg stage should be used. The use of limonene and volatile from tephritid larvae (PEA) can be used as oviposition cues for parasitoids. Are there niches for augmentative biological control with improvement in SIT techniques (sexing strains, cytoplasmic incompatibility, Trojan females, improved performance of irradiated males, anoxia treated flies for improved mating)? Augmentative parasitoids could replace bait sprays in the future and be incorporated in the programme.

Autodissemination and pathogen dynamics in *Bactrocera invadens*: Screening, Horizontal transmission and suppression in a mango agroecosystem by Samira Mohamed, ICIPE, Kenya

The holistic IPM approach for *B. invadens* is through the use of different techniques: Bait spray, male annihilation, biopesticide, monitoring, parasitoid, orchard sanitation and the use of augmentorium. The focus of the talk was on biopesticide and on *Metarhizium anisoplae* isolates developed by ICIPE and tested on *B. invadens*. One of the *M. anisoplae* isolate- ICIPE 69 was found to be effective in the lab and has now been commercialized by Real IPM and being traded as Campaign. When *M. anisoplae* were tested in the laboratory, conidial uptake could take place in 10 seconds when flies were drawn to an autoinoculator (Methyl eugenol as an attractant inside a trap and *M. anisoplae* placed over a velvet material glued to the inside wall of the trap). Horizontal transmission of the conidia occurred during mating and transmission could even take place with a 3rd female recipient. Methyl eugenol baited traps loaded with Campaign (0.8 g of conidia per trap) were set at 50/ha and were found to provide effective control of *B. invadens*. The conidia were recharged every 4 weeks. Infection of females in the field was determined.

Area-wide suppression of *Bactrocera* fruit flies in Dragon fruit orchards in Binh Thuan, in Vietnam by Thi Thanh Hien Nguyen, Plant Protection Research Institute, Vietnam.

In dragon fruit orchards, they are using an interesting method with a 100 ha core area of MAT (ME MAT blocks at 50 m intervals), BAT (Beer waste and fipronil mixture) and orchard sanitation for *B. dorsalis* control and a buffer area of 300 ha containing MAT and orchard sanitation only.

Risk assessment and quarantine

Pest risk analysis for economically important Tephritidae: the crossroads between science, plant protection and safe trade by Alison Neeley, Centre for Plant Health Science and Technology

The issue of *Bactrocera dorsalis* taxonomy was given as an example as a case where science could impact on the risk analysis and trade. The resolution of sibling species into one species or multiple species could impact management policies. There would be separate management policies required in the case of separate species.

International standards for phytosanitary measures on fruit flies by Rui Pereira, IAEA, Austria

The phytosanitary treatments for *B. dorsalis* will be soon discussed on a technical panel. An annex to ISPM 26 (Establishment of fruit fly free areas) on control measures for an outbreak within a fruit fly free area will be soon finalised and adopted. The determination of host status for fruit flies was blocked.

Eradication of Tephritid fruit flies: Lessons from Gerda by David M. Suckling, Plant and Food Research, New Zealand

In total there have been 173 eradication campaigns against 94 species worldwide. The global eradication database is located on a website B3.net.nz/gerda. Eradication actions were carried out worldwide on 17 species of Tephritidae. The trend observed on the database is that as the area of infestation increases, success of eradication decreases.

Socio-Economic analyses of area-wide management of mango fruit fly in India by Abraham Verghese, National Bureau of Agriculturally Important Insects, India

In India the loss of mangoes due to *B. dorsalis* infestation ranges between 18% and 80%. *B. dorsalis* is the main pest in South India while *B. zonata* is the main pest in the northern parts of India. The bait sprays used for the control of *B. dorsalis* are a mixture of 10% jaggery (similar to molasses) and toxicant. The sprays are applied on trunks of trees.

KEY POINTS FROM KEY POSTER PRESENTATIONS

Area-wide management of fruit flies –Dream or reality by Andrew Jessup et al. Department of Primary Industries, Australia

Currently there are a number of different area-wide programmes across Australia. There is a proposal to now set up a national area wide fruit fly management programme across Australia. The programme will bring together different industries, federal, state, researchers and regional communities

***Ceratitis capitata*-Wolbachia symbiosis: the impact of the symbiont on host development and male competitiveness by Kyritsis G. A. et al., IAEA, Austria**

Wolbachia pipientis is an endosymbiont alphaproteobacterium that commonly induces cytoplasmic incompatibility. *Wolbachia* infected lines of Medfly have been established. The survival, fecundity, fertility, flight ability and mating performance of the lines were determined. Incompatibility Insect Technique can be established for fruit fly species where Genetic Sexing Strains.

Effect of citrus peel in the viability of *Anastrepha fraterculus* and *Ceratitis capitata* immature stages by Ruiz Josephina et al., Universidad Nacional de Tucuman, Argentina

Compounds from peel of lemon and grapefruit such as limonene and citral were found to affect viability of eggs and larvae. Limonene was less toxic than citral at the egg stage but not at the larval stage.

Search for new fruit fly attractants from plants by Nishida R. & Tan K-H., Kyoto University

β -Caryophyllene was not attractive to *B. dorsalis*. The latter compound however was more attractive to *B. correcta* compared to methyl eugenol

Electronantennogram of *Ceratitis capitata* and field responses on *Bactrocera dorsalis* with Cera trap by Sierras N. et al, Bioberica, Spain

Cera trap at 50 traps per hectare was found to successfully control *B. dorsalis* in Thailand.

Simplified identification key for larvae of tephritid species the most regularly intercepted on imports in Europe by Valerie Blame, Unite entomologie et plantes invasives, France

A simplified key was presented to distinguish between frequently intercepted fruit fly larvae in Europe. The flies identified included *Ceratitis capitata*, *Ceratitis cosyra* and *Bactrocera invadens/B. dorsalis*.

FORMAL CONTRIBUTION BY ARUNA MANRAKHAN TO ISFFEI:

Use of Male Annihilation Technique For *Bactrocera* Control On Mainland Africa

Aruna Manrakhan¹, Timothy G. Grout¹, Jan-Hendrik Venter², Tertia Grove³ & Christopher W. Weldon⁴

¹Citrus Research International, PO Box 28, Nelspruit 1200, South Africa. E-mail:aruna@cri.co.za; ²Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa; ³Agricultural Research Council-Institute for Tropical and Subtropical Crops, Nelspruit, South Africa ; ⁴University of Pretoria, Department of Zoology and Entomology, Pretoria.

Fruit fly pest species in the *Bactrocera* group, particularly those responding to the male attractant methyl eugenol, can be effectively suppressed by the male annihilation technique (MAT). In MAT, the male fruit flies are targeted through deployment of stations or substrates containing a mixture of male attractant and an insecticide. The aim of MAT is to realise high levels of male kill thereby reducing the number of matings and fruit fly population level. The use of MAT on mainland Africa for suppression of recently introduced methyl-eugenol responding *Bactrocera* pest species is increasingly being considered and used.

In South Africa, the use of MAT in combination with protein bait application and orchard sanitation was effectively used to control *Bactrocera invadens* in the northern regions. The male annihilation method most commonly used and trialled so far for *B. invadens* control was the use of wooden fibre board blocks impregnated with methyl eugenol and malathion. Other male annihilation methods such as SPLAT technology containing methyl eugenol and spinosad and gels containing methyl eugenol and permethrin have recently been registered on an emergency basis for *B. invadens* control in South Africa. The efficacy of these different methods for *B. invadens* control is being investigated in South Africa.

The use of MAT for control of *Bactrocera* pest species in Africa is expensive given that the parapheromones have to be imported. As such, only effective and affordable male annihilation methods for control of *Bactrocera* pest species would eventually become more widely used on the continent.

Key words: Bactrocera, parapheromones, male annihilation technique

Meetings with international collaborators

During ISFFEI, two meetings were scheduled with international collaborators for two CRI projects which have and will receive international funding. One was the ERAfrica fruit fly kick off meeting and the other was Horizon 2020 meeting.

ERAfrica kick off meeting (Monday 12 May 2014, 18:00 -20:00)

The ERAfrica kick off meeting was held in conjunction with meetings of two other regional fruit fly projects: The FRUITFLYNET project of Royal Museum of Central Africa and the Indian Ocean Regional TC project of the IAEA, which have some overlapping activities. The objective of the joint meeting was to introduce the three projects to all participants of the three regional projects and to discuss common grounds in order to streamline activities and avoid duplication.

The three projects were presented by the co-ordinators of the three projects: ERAfrica (A Manrakhan, CRI), FRUITFLYNET (M De Meyer, RMCA) and Indian Ocean Regional TC project (R Pereira, IAEA).

ERAFRICA Fruit fly Project

ERAFRICA is a European Union project aimed at promoting a unified European approach to collaborating with Africa in the field of science and technology research for innovation and sustainable development. The ERAFRICA project is funded by the European Commission, with different institutions in participating European and African countries providing the funding for different project themes. The funding for the ERAFRICA fruit fly project was approved in January 2014 and the three year project is due to start not later than 1 June 2014. The partners in ERAFRICA fruit fly project are Citrus Research International- CRI (South Africa), Royal Museum for Central Africa- RMCA (Belgium), CIRAD (Reunion, France) and Centre National de Recherche Agronomique- CNRA (Ivory Coast). The project is co-ordinated by Aruna Manrakhan, CRI. The aims of the project are to develop effective and accurate detection methods for fruit fly pests in Africa and the Indian Ocean region. The specific objectives of the project are to (1) determine the efficacy and sensitivity of different trapping systems for monitoring Afrotropical fruit fly pests, (2) analyse the population genetic structure of key indigenous and exotic fruit fly pests in the Afrotropical region for a better understanding of their geographic ranges and dispersal patterns, (3) develop identification tools for Afrotropical fruit flies and (4) set up a standardised fruit fly detection system in Africa and the Indian Ocean region.

Trapping surveys with new and standard attractants will be conducted over 2 years in selected areas on the African continent (principally in South Africa and Ivory Coast). The CIRAD team in Reunion will focus on development of fruit volatiles for monitoring of female Afrotropical fruit flies which have a poor response towards currently available food-based attractants. The population genetic structure of key fruit fly pests will be studied through molecular analysis of sample populations collected in different areas across Africa and the Indian Ocean region. The RMCA team in Belgium will use specimens and information obtained from the trapping studies to test, modify and improve currently existing identification tools (multi-entry keys and barcode based identification methods). Findings from this project will be used to set up a standardised fruit fly detection system through the development of a fruit fly detection protocol for Africa and the Indian Ocean region. The protocol will be in the form of an electronic manual which will contain recommendations of trapping systems for major fruit fly pests in Africa and the Indian Ocean region. The protocol will also include identification methods for fruit flies and will have links to the DNA barcode library and the multi entry key.

FRUITFLYNET

FRUITFLYNET is a networking initiative funded by the Belgian Science Policy (BELSPO) of the Belgian Federal Government. The general objective is to facilitate the creation of a network between a Belgian Federal Research Institution (like the Royal Museum for Central Africa in Tervuren, RMCA) and non-European partners in order to initiate a long-term consolidated network. In the particular case of the FRUITFLY network, RMCA intends to develop a network with three other African partners: the Stellenbosch University (Stellenbosch, South Africa; SU), the Sokoine University of Agriculture (Morogoro, Tanzania; SUA) and the Eduardo Mondlane University (Maputo, Mozambique; EMU). The network activity is focused on and provides funds for organizing meetings to discuss on how monitoring and surveying activities with regard to fruit flies and conducted by these institutions, can be standardized and harmonized.

The selection of the partner institutions and countries is based on the following:

All three countries are more or less in the same geographical region (southern to eastern Africa) and have extended experience in fruit fly research. They also all have a thriving horticultural industry, focusing on (sub)tropical and temperate climate fruits like mango, papaya, avocado, citrus and grapes. There is a large overlap both in fruit fly diversity and in pest species management. All three face similar problems regarding native fruit flies like the mango fruit fly (*Ceratitidis cosyra*), the Natal fruit fly (*C. rosa*) and the Mediterranean fruit fly (*C. capitata*), but also the exotic *Bactrocera invadens*, and to a lesser extent *B. cucurbitae*.

The African partners have been involved in fruit fly research for some time, and RMCA has already a longstanding collaboration with these institutions on an individual basis. These collaborations are directed towards, among other activities, the detection and long term monitoring of fruit flies, and surveying campaigns for native and exotic fruit fly pests. SUA and RMCA have embarked on surveys for *Bactrocera latifrons*, and have been monitoring the populations of *B. invadens* in Central Tanzania for the last 10 years. Between 2008 and 2011, RMCA assisted EMU in surveys throughout Mozambique to establish the spread of *B. invadens* in the country and currently has a monitoring project in Central Mozambique. Finally, RMCA has been providing identification services lately for surveys in the Western Cape and Namibia conducted by SU. Each of these countries have national or regional programs for fruit fly monitoring. Each of these programs is developed on an individual basis but there is little to no regional approach despite the overlap in pest species, and targeted crops. It was, therefore, deemed important to try and bring actors of the different institutions together to compare and discuss their approaches, and to see if common grounds can be

defined. The RMCA plays a pivotal role in this as it is the institution that is providing identification and other technical support to the individual activities.

The networking initiative will run for two years, starting in May 2014. During these two years, four meetings are planned:

- A kick-off meeting in conjunction with the International Fruit Fly Symposium in Bangkok;
- A final meeting in conjunction with the regional TEAM (tephritid workers of Europe, Africa and the Middle East) which is planned for 2016 to be held in South Africa and organized by SU;
- Two field meetings in between, that will take place in two different African partner countries.

During these meetings, the different approaches will be discussed and demonstrations will be given of the methodologies. The ultimate objective is to write up a proposal for a regional surveying program that would enable to gather data in a standardized way so that results obtained from the different countries, can be compared.

Indian Ocean Regional TC project

The IAEA Technical Cooperation regional project RAF5062 on “Preventing the Introduction of Exotic Fruit Fly Species and Implementing the Control of Existing Species with the Sterile Insect Technique and Other Suppression Methods” is financially supported by the IAEA and has the aiming of sharing among the countries of the Indian Ocean (IO) region the knowledge on the status of tephritid fruit fly pests in each of the participating countries and the control techniques in use, including the possibility of applying the Sterile Insect Technique (SIT), and coordinate the joint efforts required to avoid the introduction of the exotic fruit fly pests. This can be achieved through the strengthening of the quarantine and pest risk analysis for each of the participating countries. Also through the installation of accurate monitoring system to detect early exotic fruit fly pest introductions, with the objective to eradicate at incipient stages of the invasion to maintain the region free of these pests.

This networking initiative includes the participation of the Member States of the Indian Ocean Region, namely France (La Réunion), Madagascar, Mauritius, Mozambique, Seychelles and the United Republic of Tanzania. The project was approved for 2012-2015 and has as main outcomes the National Plant Protection Organizations of Member States in the region networked in terms of increased awareness and technical capacity to prevent or detect and address invasive exotic tephritid fruit fly pest outbreaks and Increased technical capacity of some Member States in the region to integrate, as part of a phased conditional approach, the Sterile Insect Technique (SIT).

Several networking activities took place under the ongoing RAF5062 project during the last two years:

- Coordination Meeting of the Indian Ocean TC regional project RAF5062, February 2012 in Mauritius.
- Regional Training Course on Fruit Fly Detection, Taxonomy and Identification for Indian Ocean, November 2012 in La Réunion, France.
- Regional Meeting on Common Emergency Action Plan for Exotic Fruit Flies, June 2013 in Mauritius.
- Signature of a memorandum of understanding by the participating Member States, June 2013 in Mauritius
- Regional Training Course on Quarantine and International Standards for Phytosanitary Measures for the Indian Ocean, July 2013 in Mozambique.

There are already other activities planned for 2014 like the present meeting (this time in collaboration with other fruit fly initiatives in Africa) and the:

- Regional Training Course on Use of GIS for Area-Wide Fruit Fly Programmes in Indian Ocean, 16-20 June 2014. Zanzibar, United Republic of Tanzania

Additionally, due the involvement of the contacts of the other two fruit fly initiatives, in some training/meetings conducted under this project and the common problems shared by the participating countries (sometimes overlapping) open the opportunity to broadening the vies by the inclusion of participants of the different initiatives. Counterparts will work closely and under the same protocol. Through interaction with other stakeholders linkages will be made to transfer the knowledge and material to end users. Hopefully this can contribute for the sharing of experiences and for the harmonization of techniques in the Region.

The common grounds of the meeting were discussed under three sections: (1) Identification, (2) trapping methodologies and (3) survey protocols. For adult specimen confirmation, materials can be sent to M De Meyer, RMCA. A multi entry key will be provided to participants of all regions in order to enable easy identification. For the trapping methodologies, a list of trapping activities will be compiled by the co-ordinators of the three projects and wherever possible the trapping methods will be standardized. For the survey protocols, protocols available as annex to ISPM No. 26 will be followed. Soon, a fruit sampling protocol will also be uploaded by IAEA.

Further to the introductory meeting of ERAfrica fruit fly project, individual meetings with principal investigators took place in order to discuss conduct and timeline of activities. Principal investigators of the ERAfrica fruit fly project who were present were: M De Meyer (RMCA), Francois N'klo Hala (Ivory Coast) and Helene Delatte & Pierre-Francois Duyck (CIRAD) who represented Serge Quilici.

Horizon 2020 meeting (Friday 16 May 2014, 13:00- 14:00)

Horizon 2020 is an EU research and innovation programme providing funding to projects for the period 2014-2020. In February 2014, CRI was invited to participate with other European and non- European institutions on a project encompassing the theme of Precision Area Wide Management of native and alien plant pests. The main co-ordinator of the project is Nikolaos Papadopoulos, University of Thessaly, Greece.

A meeting was held with Nikolaos Papadopoulos and Sonya Broughton (Australia) on the last day of ISFFEI where we were informed that the proposed project made it to the second round and that we would be required to urgently submit a number of documents for preparation of the detailed project proposal. The role of CRI would be to test precision detection methods developed during the first year of the project mainly on *B. invadens*/*B. dorsalis*.

Meeting for the next TEAM conference in Stellenbosch in 2016 (Thursday 15 May 12:00 -13:30)

The next meeting of Tephritid workers of Europe Asia and the Middle East will be held in Stellenbosch in March/April 2016. The University of Stellenbosch will be hosting the meeting. The local organising committee (P. Addison, University of Stellenbosch; C Weldon. University of Pretoria; J-H Venter, Department of Agriculture, Forestry and Fisheries; A. Manrakhan, CRI) met with M. De Meyer (Chair of TEAM) and N. Papadopoulos (Previous Chair of TEAM and previous organiser of the TEAM meeting in 2012). The different items discussed were: time of meeting (proposed to be held in March/April 2016), venue of the meeting (either at conference centre at US or at Spier- will depend on costs), local organising committee (already assembled and included also T. Grove, ARC and M. Karsten, US) and sponsorship (IAEA sponsored 10, 000 Euros for the last meeting and could be approached to sponsor the coming conference). Sponsorship will also be sought from DAFF, ARC, Industries and chemical companies. The conference will be held over 3 days with a field trip organised on the 4th day. It was suggested that African Entomology be approached to publish the proceedings of the conference. P. Addison will arrange to set up a website providing the details of the meeting and subsequently for registration.

Other interactions during ISFFEI

Following my presentation, I was asked if SIT against *B. invadens* could be considered in Africa and the reply was that it was possible but the numbers would have to be reduced to low levels to ensure effective overflooding ratios and the numbers should be reduced by the use of baits, MAT and orchard sanitation prior to start of SIT programme.

T. Vera, Argentina, suggested we consider the use of Lufenuron (Chitin synthesis Inhibitor) mixed with Methyl Eugenol for *B. invadens* control and compare it to Male Annihilation Technique. The lufenuron will act as a chemo sterilant, reducing fecundity and fertility.

P. Cook, U. S. would like CRI to test some new dispensers containing specific attractants for Medfly and *B. invadens*.

M. Mwatawala, Tanzania confirmed the ability of his institute to host Louisa Makumbe (PhD student AT University of Pretoria) who is working on dispersal capacity of *B. invadens*, project funded by CRI. Louisa will be possibly initiating the mark –release –recapture studies in August 2014.

S. Mohamed, ICIPE, Kenya agreed to provide us with *B. invadens* pupae required for the mark-release-recapture studies conducted in Tanzania under the *B. invadens* dispersal capacity project.

Field visit to area-wide IPM of fruit fly in mangosteen, rambutan and durian orchards in Rhayong Province. (Wednesday 14 May 07:00 – 19:00)

We visited the experimental orchard of the Agricultural Extension of Rayong Provincial Office. There was presentation on the different crops being planted in the Province and the fruit fly damage recorded on these crops. The main crops grown in the province: mangosteen, long kong, rambutan, mangoes and jackfruit. *B. correcta* and *B. umbrosa* were the main pests of the fruit grown in that province and they were controlled by the use of male annihilation technique during season together with bait sprays and orchard sanitation. The

fruit collected during orchard sanitation were processed to produce organic fertilizer (See Plate 1). The male annihilation technique used there consists of plastic bottles with four holes (2-3 mm in diameter) punched on two sides of the bottle (See Plates 2 A & B). A wick impregnated with methyl eugenol is suspended inside the bottle. The bottles are placed at a density of 60 per ha and contain no insecticide. Methyl Eugenol is added every week onto the wick

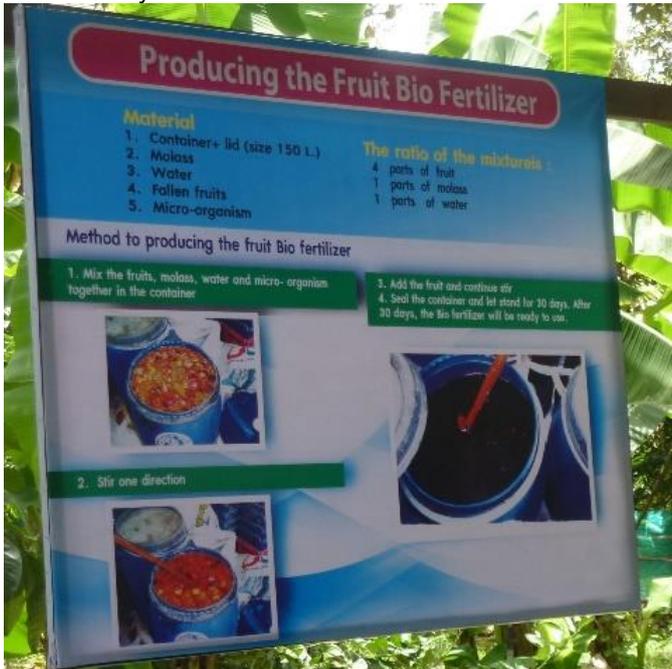


Plate 7.2.1. Processing of fruit following orchard sanitation in Rhayong Province, Thailand. Fruit are chopped and mixed together with molasses, water and microorganisms in plastic containers. The containers are sealed for 30 days after which the biofertilizer is ready to use.

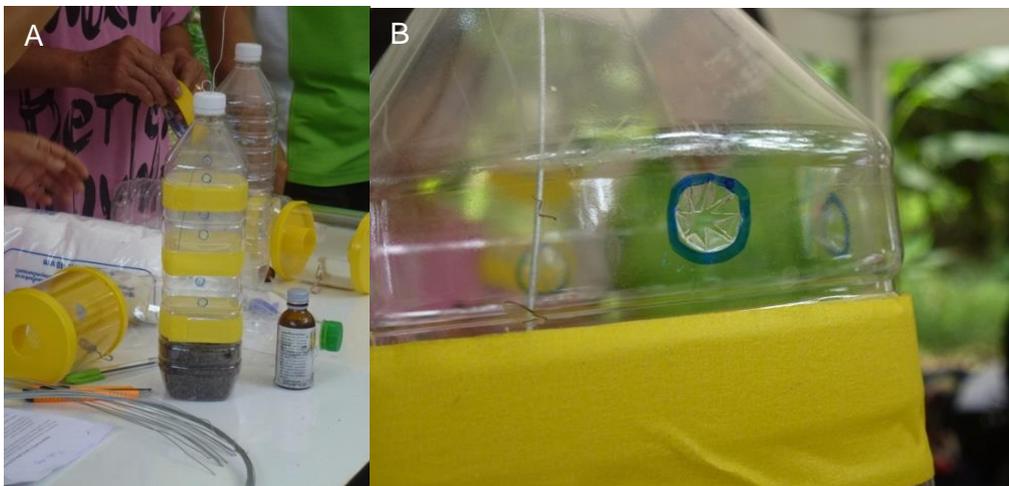


Plate 7.2.2. (A) Methyl eugenol traps for Male Annihilation Technique in fruit orchards in Rhayong Province, Thailand. **(B)** Entry holes (2-3 mm in diameter) in traps for *B. dorsalis* males.

CONCLUSION

During my participation in the 9th ISFFEI, I was able to obtain new information on biology and control of various fruit fly pests. The symposium also offered a platform to interact and connect with other fruit fly workers. This was also an opportunity to meet with international fruit fly workers currently collaborating on CRI projects.

ACKNOWLEDGEMENTS

Citrus Research International for funding my participation in the 9th International Symposium of Fruit Flies of Economic Significance.

Annex 1

The 9th International Symposium on Fruit Flies of Economic Importance

12-16 May 2014, Bangkok, THAILAND



SCIENTIFIC PROGRAMME

Monday, 12 May 2014

07.30-08.30 Registration & Poster Mounting

08.30-09:45 Opening Ceremony

09:45-10:30 **Opening Speaker** : *Dr. Anond Snidvongs*

*Executive Director, Geo-Informatics and SpaceTechnology
Development Agency, Ministry of Science and Technology*

Applications of Geo-Informatics in Area-Wide Integrated Pest Management

10:30-11:00 BREAK

Session 1: Area-wide and Action Programs

Coordinators: *Pablo Liedo and Sunyanee Srikachar*

11:00-11:30 **Keynote Speaker:** *Donald McInnis*

Can Polyphagous Tephritid Pest Populations Remain Undetectable over Years under Favorable Climatic and Host Conditions?

11:30-11:50 **OP 1.1** : Population Dynamics of the Mediterranean Fruit Fly (*Ceratitidis capitata*, Wied.) in Coffee Areas Located in the Guatemala - Chiapas, Mexico, Region, and Its Implications in IPM Strategies

By Walther Enkerlin

11:50-12:10 **OP 1.2** : Fruit Flies Area-wide Integrated Control Program in Thailand Success or Failed?

By Suksom Chinvinijkul

12:10-12:30 **OP 1.3** : Suppression of Mediterranean Fruit Fly Using Sterile Insect Technique in Neretva River Valley of Croatia

By Mario Bjeliš

12:30-13:30 LUNCH

Session 2: Biology, Ecology, Physiology and Behaviour

Coordinators: *Serge Quilici and Suksom Chinvinijkul*

13:30-14:00 **Keynote Speaker** : *Phillip Taylor*

The Tephritid Tardis: How Queensland Fruit Flies Escape in Time

14:00-14:20 **OP 2.1** : Pupal Diapause Development and Termination is Driven by Low Temperature Chilling in *Bactrocera minax*

By Changying Niu

14:20-14:40 **OP 2.2** : Bacterial Symbionts as an Essential Component of the Olive Fly, *Bactrocera oleae* (Rossi), Nutritional Ecology

By Michael Ben-Yosef

14:40-15:00 **OP 2.3** : Remating Inhibition in *Anastrepha ludens* (Loew)

By Diana Pérez-Staples

15:00-15:30 BREAK

16:00-17:00 Poster Discussion : Session 1 and Session 2

Discussion Leaders: *Beatriz Sabater and A. Vergese*

17:00-17:45 General Discussion

The 9th International Symposium on Fruit Flies of Economic Importance
12-16 May 2014, Bangkok, THAILAND



Tuesday, 13 May 2014

Session 3: Morphology and Taxonomy

Coordinators: Teresa Vera and Weerawan Amornsak

- 08:30-09:00 **Keynote Speaker : Massimiliano Virgilio**
Led Zeppelin and the DNA Barcoding of Fruit Flies: “Stairway to heaven” or
“Babe, I’m gonna leave you”? – A Pragmatic Approach Towards Workable Solutions.
- 09:00-09:20 **OP 3.1 :** Resolution of Key Pest Members of the *Bactrocera dorsalis* Species Complex
By Anthony Clarke
- 09:20-09:40 **OP 3.2 :** An Integrative Approach to Unravel the *Ceratitis* FAR Cryptic Species Complex
By Marc De Meyer
- 09:40-10:00 **OP 3.3 :** New Insights in the Definition of the *Anastrepha fraterculus* Cryptic
Species Complex
By Teresa Vera and Janisete Silva
- 10:00-10:30 Group Photo and BREAK

Session 4: Genetics and Evolution

Coordinators: Anna Malacrida and Sujinda Thanaphum

- 10:30-11:00 **Keynote Speaker : Marc F. Schetelig**
Past, Present and Future of Strain Development
- 11:00-11:20 **OP 4.1:** From Transgenesis to Functional Genomics: Novel Tools for the Study of
Reproduction in a Severe Pest, the Mediterranean Fruit Fly, *Ceratitis capitata*
By Francesca Scolari
- 11:20-11:40 **OP 4.2:** Population Structure of *Ceratitis rosa* Karsch in South Africa: Using Molecular
and Morphological Markers to Estimate Gene Flow and Dispersal Ability
By Minette Karsten
- 11:40-12:00 **OP 4.3 :** Genetic Structure Analysis of Three Kinds of Fruit Fly of Economic
Importance in China
By Qinge Ji
- 12:00-13:30 LUNCH

Session 5: Chemical Ecology and Attractants

Coordinators: Nancy Epsky and Sirada Thimprasert

- 13:30-14:00 **Keynote Speaker :Shanmugam Vijaysegaran**
Bait Manufactured from Beer Yeast Waste and Its Use for Fruit Fly Management
- 14:00-14:20 **OP 5.1 :** Recent Developments on Chemical Ecology
By Eric Jang
- 14:20-14:40 **OP 5.2 :** Responses of Dacini Fruit Flies to Novel Phenylpropanoids in Australia and
Papua New Guinea
By Jane E. Royer

The 9th International Symposium on Fruit Flies of Economic Importance

12-16 May 2014, Bangkok, THAILAND



14:40-15:00 **OP 5.3** : Effect of Methyl Eugenol Consumption by Male *Bactrocera umbrosa* Fabricius (Diptera: Tephritidae): Implications on Sexual Attraction, Maturation, and Mate Selection
By Suk-Ling Wee

15:00-15:30 BREAK

16:00-17:00 Poster Discussion: Session 3, Session 4 and Session 5
Discussion Leaders: Yoav Gazit, Serge Quilici and Marc De Meyer

17:00-17:45 General Discussion

18:00-21:00 Meeting of International Fruit Fly Steering Committee (open to SC members)

Wednesday, 14 May 2014

7:00-19:30 Technical tour

Thursday, 15 May 2014

Session 6: Control Methods and Supporting Technology

(e.g. Geographic Information System-GIS and Surveillance)

Coordinator: Nikos Koulousis and Sirilux Noikeaing

08:30-09:00 **Keynote Speaker : Aruna Manrakhan**
Use of Male Annihilation Technique for Control of Pest Species in the *Bactrocera* group on Mainland Africa

09:00-09:20 **OP 6.1:** Detection of *Bactrocera dorsalis* (Hendel) in Mauritius and Rapid Response
By Preeaduth Sookar

09:20-09:40 **OP 6.2:** Area Wide Management of *Ceratitidis capitata* in Fruit Trees in Israel - Successful Implementation and New Challenges
By Miriam Silberstein

09:40-10:00 **OP 6.3:** Integrated Pest Management of Fruit Flies on Rose Apple in Thailand
By Sunyane Srikachar

10:00-10:30 BREAK

Session 7: Natural Enemies and Biological Control

Coordinators: Pablo Montoya and Narit Thuochan

10:30-11:00 **Keynote Speaker : John Sivinski**
Technical Competition and the Fate of Augmentative Biological Control

11:00-11:20 **OP 7.1:** Autodissemination and Pathogen Dynamics in *Bactrocera invadens* Drew, Tsuruta & White: Screening, Horizontal Transmission and Suppression in a Mango Agroecosystem
By Sunday Ekesi

11:20-11:40 **OP 7.2:** Towards a Rapid Identification Technique for Immature Parasitoids
By Olivia Reynolds

11:40-12:00 **OP 7.3:** Bane or Boon? The Effect of Weaver Ants on Mango Fruit Flies and their Parasitoids
By Valentina Migani

12:00-13:30 LUNCH

The 9th International Symposium on Fruit Flies of Economic Importance

12-16 May 2014, Bangkok, THAILAND



Session 8: SIT Principles and Applications

Coordinators: Rui Pereira and Wanitch Limohpasmanee

- 13:30-14:00 **Keynote Speaker: Daniel Hahn**
Management of Dormancy: A Review and Discussion of Importance for Biological Control Programs Including SIT
- 14:00-14:20 OP 8.1: New Mediterranean Fruit Fly Emergence and Release Facility at Tapachula, Chiapas, Mexico
By José Luis Zavala
- 14:20-14:40 OP 8.2: Micro-SIT : a Novel and Sustainable Control for *Ceratitidis capitata* in Israel
By Gal Yaacobi
- 14:40-15:00 OP 8.3: Area-Wide Suppression of *Bactrocera* Fruit Flies in Dragon Fruit Orchards in Binh Thuan, Vietnam
By Thi Thanh Hien Nguyen
- 15:00-15:30 BREAK
- 16:00-17:00 Poster Discussion : Session 6, Session 7 and Session 8
Discussion Leaders: Sunday Ekes, Olivia Reynolds and Abdeljelil Bakri
- 17:00-17:45 General Discussion
- 19:00 FAREWELL DINNER

Friday, 16 May 2014

Session 9: Risk Assessment, Quarantine and Post-harvest

Coordinators: Kenneth Bloem and Tasanee Pradyabumrung

- 08:30-09:00 **Keynote Speaker: Stephanie Bloem**
Pest Risk Analysis for Economically Important Tephritidae: The Crossroads between Science, Plant Protection and Safe Trade
- 09:00-09:20 OP 9.1: Revised Quarantine Distances for Domestic and International Trading
By Bernie Dominiak
- 09:20-09:40 OP 9.2: International Standards for Phytosanitary Measures on Fruit Flies
By Rui Pereira
- 09:40-10:00 OP 9.3: Eradication of Tephritid Fruit Flies: Lessons from Gerda
By David M. Suckling
- 10:00-10:30 BREAK

Session 10: Additional Topics

- 10:30-10:50 OP 10.1: Socio-Economic Analyses of Area-Wide Management of Mango Fruit Fly in South India
By Abraham Verghese
- 10:50-11:10 OP 10.2 : Economic Evaluation of the Moscamed Program in Guatemala and Its Impacts in Such Country, Mexico, The United States and Belize
By Diznarda Salcedo

The 9th International Symposium on Fruit Flies of Economic Importance
12-16 May 2014, Bangkok, THAILAND



11:10-11:30 **OP 10.3:** Beyond Compliance: Integrated Systems Approach for Pest Risk Management in South East Asia

By John D. Mumford

11:30-12:00 Poster Discussion : Session 9

Discussion Leader: Kenneth Bloem

12:00-12:30 General Discussion

12:30-13:00 CLOSING SESSION

13:00-15:00 LUNCH

15:00-15:30 Poster Removing

8 VOORLIGTING 2014-15

Deur Hennie le Roux, Hannes Bester, M.C. Pretorius, Keith Lesar, Dawid Groenewald, Andrew Mbedzi en Melton Mulaudzi (CRI)

Die 2014 Seisoen

Die afgelope seisoen is oorheers deur die swartvlek-situasie tov uitvoere na Europa. Die toepassing en bestuur van die maatreëls tov swartvlek het groot uitdagings gebied, en die geweldige hoeveelheid tyd en energie wat aan sitruswartvlek (CBS) spandeer is, het die gewenste resultate gelewer. Ten spyte van frustrasies wat van tyd tot tyd ervaar is, het samewerking tussen al die verskillende rolspelers bygedra tot suksesvolle uitvoere na die EU.

Suurlemoene het 'n ongekende goeie seisoen beleef. Die uitsonderlike hoë pryse vir suurlemoene het reeds groot bestellings bome by kwekerye tot gevolg en daar is tekens dat beduidende hoeveelhede meer suurlemoene in die nabye toekoms aangeplant gaan word.

Die pryse vir sagtesitrus en lemoene was oor die algemeen ook goed, met die uitsondering van die laat variëteite. Verder was dit 'n baie goeie seisoen vir laat mandaryne ook, maar pomelo's het weer 'n moeilike seisoen beleef en produsente sal sterk op snoei en vruguitdunning moet fokus om te verseker dat vrugte van die gesogte grootte geproduseer word om kostes van oes en verpakking te beperk, druk op die mark te verlig en inkomste per karton te verhoog.

Die 2015 Seisoen

Op skatting is die totale uitvoervolumes effens laer as verlede seisoen op 113.1m kartonne, met lemoene en pomelo's wat effens laer is, suurlemoene wat effens op is en sagtesitrus dieselfde as verlede seisoen. Produsente sal sinvolle besluite moet neem om nie ongewenste tellings en spesifikasies uit te voer nie. Dit is veral van toepassing op pomelos. Die pomeloprodusente het besluit om uitvoere hierdie seisoen beter te struktureer om 'n ooraanbod op enige gegewe stadium in 'n spesifieke marksektor te voorkom.

'n Afvaardiging van die EU FVO het Suid Afrika besoek. CRI Voorligters het hulle en DAFF na die onderskeie sitrusstreke vergesel sodat hulle kon bevestig dat die maatreëls wat deur Suid-Afrika ingestel is om die voorkoms van sitruswartvlek te beheer, voldoende is. Hulle was tevrede met die maatreëls, maar dieselfde streng vereistes geld as verlede jaar, nl dat daar slegs vyf onderskeppings van swartvlek toegelaat sal word.

CRI Produksiewerkswinkels

Die reëlings tov die CRI Produksiewerkswinkels is op versoek van die meerderheid produsente in die verskillende produksiestreke aangepas om gedurende September saam te val met die CRI Plaagbeheer & Siektebestuur Werkswinkels. Die addisionele druk wat produsente ervaar tov marktoegangsvereistes, veral na die EU, maak dit moeilik om enige werkswinkels gedurende die piekseisoen by te woon.

Die gekombineerde CRI Produksie, Plaagbeheer & Siektebestuur werkswinkels is gedurende die laaste twee weke van September tot eerste week Oktober in die onderskeie streke aangebied, waartydens daar ook terugvoer oor die CRI Navorsingssimposium, wat gedurende 17–22 Augustus 2014 plaasgevind het, gegee is. Tydens hierdie werkswinkels is CBS-spuitprogramme vir elke produksiestreek uitgewerk, wat na finalisering aan DAFF deurgegee is vir implementering in die RMS (Sien Snykant 189).

Hoewel die terugvoer vanaf die produsente wat dit bygewoon het baie positief was, is die onbetrokkenheid van die CGA Direkteure, as leiersfigure in hul onderskeie streke, eintlik kommerwekkend. Dit is juis tydens hierdie werkswinkels wat produsente en ander rolspelers in die sitrusbedryf eerstehands by navorsing en voorligting betrokke kan raak, waardevolle insette kan lewer en op hoogte kan kom met die werksaamhede op voetsoolvlak binne CRI. As die leiersstruktuur, wat essensieel wesenlike besluite rakende CRI moet neem, onbetrokke is en nie ten volle verbind is tot die werksaamhede binne CRI nie, kan daar nie verwag word dat die sitrusbedryf as eenheid volhoubaar gesond sal funksioneer en alle leierfigure instaat sal wees om deurentyd in belang van die hele bedryf te kan optree nie.

Gedurende Januarie en Februarie 2015 is ses CRI Na-oes Werkswinkels in die grootste produksiestreke aangebied. In Limpopo en Mpumalanga is drie aangebied, met een elk in KZN, die Oos-Kaap en die Wes-Kaap. Hierdie werkswinkels is gesamentlik deur CRI en DST-PHI befonds. 'n Wye reeks onderwerpe is gedek met die fokus op fitosanitêre & uitvoer regulasies, die sitruswaardeketting, uitvoerstandaarde, patologiese & fisiologiese gehalte-aspekte, verpakking & verpakkingsmateriaal, verkoeling, logistiek en voedselveiligheid.

Die CRI Na-oes werkswinkels het 'n baie belangrike rol begin speel tov die oordraging van kennis aan al die na-oes rolspelers in die sitrusbedryf, soos die pakhuispersoneel, tegniese adviseurs, verpakkingsmateriaal vervaardigers, chemiese bedryf, waksvervaardigers, studente, uitvoerders, DAFF en PPECB. Die waarde wat deur die bedryf op die Na-oeswerkswinkels geplaas word, is enorm.

CRI-PTF

Toets van kartonne as deel van die akkreditasie-proses is uiters belangrik. Navorsings- en ontwikkelingswerk op meer koste-effektiewe kartonne is hoog op die CRI-PTF se prioriteitslys. Papier-kombinasies speel 'n groot rol as dit kom by die prys van kartonne. Die CRI-PTF het ook begin om die prys van die papierkomponent per kartontipe te bepaal. Dit is baie belangrik dat hierdie pryse vergelyk word en daar moes op een of ander wyse vasgestel word of laer pryse a.g.v ligter papier-kombinasies aan die produsente deurgegee word.

As deel van 'n rasionalisasieprogram by Sappi het hulle besluit om die vervaardiging van Stackkraft (SK) en Superflute (SF) te staak. Tydens verskeie dringende en ernstige gesprekke met Sappi se topbestuur, waartydens CRI-PTF ernstige kommer hieroor uitgespreek het, het hulle die boodskap baie duidelik gekry dat hulle eenvoudig sal moet kom met nuwe verbeterde en meer koste-effektiewe produkte in die plek van die SK en SF. Indien dit nie gebeur nie sal Sappi baie marktaandeel in die sitrusbedryf verloor. As gevolg hiervan het Sappi die nuwe 165g/m² Ultraflute, wat die 175 SF vervang, ontwikkel. In proewe op A15C- en E15D kartonne is die 175g/m² Superflute uiters suksesvol met die nuut ontwikkelde 165g/m² Ultraflute vervang. Sappi het ook begin met die vervaardiging van 'n nuwe produk om SK te vervang.

Ekperimentele E15D en A15C kartonne is met 'n nuut ontwikkelde 165g/m² Sappi Ultraflute vervaardig. Dit is opgevolg met proewe met A15C kartonne wat van 'n nuwe hoë Kappa 175g/m² Kraftpride en 165UF vervaardig is. Gebaseer op die lysprys van papier, kan dit 'n besparing van R0.70 per karton meebring.

Om swamgroeï op veral dennehout palette te voorkom, moet palette met 'n 6% SOPP oplossing behandel word. As 'n alternatief vir SOPP is 'n proef met RT14 by Schoeman Boerdery gedoen. Gedurende die afseisoen sal verdere proewe met SOPP en RT14 by CRI se fasiliteite in Nelspruit met reeds besmette planke gedoen word.

Om verskeie redes gebruik die Wes-Kaap produsente nie die 1210x1010mm sitrus palet vir uitvoere na die VSA nie, maar die sogenaamde wit- en rooiblok palette. Dit het skielik in 'n krisis ontwikkel nadat houtboorders in hierdie palette gevind is. Net nadat die lewendige hootboorders in die VSA gevind is, was die Wes-Kaap produsente verplig om die vrugte wat nog vir die res van die 2014 seisoen na die VSA verskep moes word, op plastiese palette te pak. Die plastiese palette wat toe vir die res van die seisoen gebruik is, kon nie in rakke geplaas word nie en bo en behalwe dit, was die prys van dié palette ook nog meer as twee maal duurder as hout palette. Gegewe al bogenoemde probleme verkies die VSA mark houtpalette.

Gedurende die 2014 seisoen is ernstige probleme met swak kwaliteit bulk bins (BB) ondervind. Die BB wat die probleme veroorsaak het, was hoofsaaklik afkomstig vanaf twee pakhuse en is deur een spesifieke verskaffer vervaardig. Dis kommerwekkend dat sekere pakhuse/produsente steeds nie ag slaan op die CRI-PTF se aanbevelings om slegs pakmateriaal aan te skaf wat aan die minimum spesifikasie voldoen nie.

Kartonne is weer deur Sappi getoets vir die akkreditasie-stelsel en alle kostes is weer deur hulle gedra. Die 2015 Pakmateriaal Spesifikasies en Palettiserings Protokolle Dokument is opgestel en aan alle rolspelers en belanghebbendes in die bedryf versprei.

Na-oes voorligting

Pakhuse is weer op 'n een tot een basis besoek en die houding en terugvoering is weereens baie positief, met goeie interaksie en samewerking met die pakhuse. Pakhuisbestuur is meer tegemoetkomend en gewillig om hulle idees en vertroulike informasie tov terugvoering oor bederf, residu-resultate ens. te bespreek, en is ook bereid om die nodige aanbevole veranderinge aan te bring.

Die latentepatogeen, Diplodia, wat stingelentverrotting veroorsaak, het voorgekom, veral op die suurlemoene. Daar is vanaf die begin van die seisoen 'n algehele tekort aan TBZ (thiabendazole) agv 'n probleem met vervaardiging van die produk in China, en dit bemoeilik die behandeling en beheer van die latentepatogene.

Die 2014 seisoen is afgesluit met 'n suksesvolle rondte van besoeke/konsultasies aan 75 pakhuse. Dit is positief om te sien hoeveel pakhuse poog om hulle pakhuis-kritiesebeheerstelsels reg te bestuur, veral die sanitasie van die pakhuse. Baie pakhuse het meer "klinies" geword, wat pakhuissanitasie betref, maar daar

is steeds pakhuis wat nie genoeg aandag aan pakhuis-sanitasie gee nie. Die bestuur van pakhuis-sanitasie gaan ook hand aan hand met die bestuur van die pakhuis-behandelings en die toediening van die regte konsentrasies en residuladings van die belangrike swamdoders.

Die grootste na-oes probleme tydens die 2014 seisoen was die skilprobleme, meestal op die satsumas, in die Wes-Kaap, en tot 'n mindere mate in die ander gebiede. Uitvoerders het ook genoem dat "pitting" heelwat op die Valencias voorgekom het, veral op die Turkeys en Bennies, as gevolg van verskeping teen lae temperature (4.5°C). Vrugkleur was ook in die markte 'n probleem. Laat nawels was nie ontgroen nie, en die vrugte het wel te groen in die markte aangekom. Vruggrootte was ook 'n probleem met klein nawels in sekere markte. Baie koueskade is ook op sensitiewe vrugte, soos op die mandaryne en pomelo's, in sekere van die kouesteri markte gesien.

Biosekuriteit en Marktoegang

Biosekuriteit: *Bactrocera invadens* het besonder vining oor 'n wye gebied in die land versprei. Dit is reeds so ver suid as Suid-KZN, Vaalharts en die Benede-Oranjerivier. Ten spyte van al die pogings tussen die verskillende rolspelers om dit uit te wis en die verspreiding hok te slaan, is dit 'n duidelike voorbeeld van die mate waartoe die Sitrusbedryf in Suid-Afrika blootgestel is aan die potensiële vernietigende effek van indringer-spesies van buite. Biosekuriteit is die gesamentlike verantwoordelikheid van alle rolspelers in die bedryf, maar dit op sigself is 'n risiko, aangesien dit nie altyd deur almal op 'n verantwoordelike wyse gedryf word nie.

Tydens die 6de Internasionale Nematologiekongress wat in die Kaap gehou is, is heelwat tyd saam met Proff Larry Duncan en Joe Noling van Florida gespandeer. Volgens hulle het Asiatische vergroening (HLB) reeds 50% van Florida se sitrusproduksie vernietig en nog is dit die einde niet. Die suider-Afrikaanse sitrusbedryf moet deeglik hiervan kennis neem wanneer daar besin word oor biosekuriteitsaangeleenthede, soos die monitoring vir *Liberibacter asiaticus* en *Diaphorina citri*.

Marktoegang: Die CGA het gedurende 2014 'n uiters waardevolle oefening gedoen deur die EU en Spaanse ambassadeurs, die Italiaanse landbou attache en ambassade personeel te nooi na 'n sitruslandgoed, Rosslee Boerdery, om aan hulle te demonstreer hoeveel voorsorg daar getref word om te verseker dat swartvlek-besmette vrugte nie in die EU sal beland nie.

Tydens 'n privaat besoek aan Ethiopië het Hennie le Roux monsters teruggebring na Suid Afrika vir ontleding vir *Liberibacter*. Al vier die monsters het positief getoets vir *Candidatus Liberibacter asiaticus*, wat die organisme is wat Huanglongbing veroorsaak, en wat tans chaos veroorsaak in talle sitrusproduserende lande soos Florida, Texas, Mexico en Brazilië. Hierdie resultate bewys dat HLB minstens 700 km suid beweeg het vanaf North Wollo waar dit vir die eerste keer in Afrika in 2010 ontdek is, tot 35 km noord-wes van Adaama.

Die 8^{ste} CRI Sitrusnavorsings simposium

CRI het die 8ste Sitrus-navorsings simposium aangebied vanaf 17-20 Augustus 2014 by Champagne Sports Resort in die Drakensberge. Hierdie simposium was, soos in die verlede, 'n reuse sukses en het aan die suider-Afrikaanse sitrusbedryf weereens 'n geleentheid gegee om te netwerk soos nog nooit tevore nie. Daar was nie minder nie as 55 mondelinge aanbiedinge en 38 plakkate wat onderwerpe ingesluit het soos Marktoegang, Biosekuriteit, Kultivars en Onderstamme, Plantpatologie, Hortologie en Entomologie.

Daar was 61 borge wat R1.4 miljoen bygedrae het. Die hoofborg was Bayer CropScience, Yield het 'n uiters suksesvolle Golfdag aangebied, Distell die Verwelkomingsdrankies, Arysta LifeScience die Verwelkomingsete, XPS die Maandag-aand se buffet, River BioScience die "Happy Hour", SAPPI die gala-ete, BASF die Radio Kalahari orkes en ADAMA die wegneemetes na afloop van die simposium. Die goue en silver borge het almal uitstallings gehad wat in twee sale en op die grasperk buite die simposiumlokaal aangebied is.

Goedbeplande vraelyste is wyd onder produsente en belanghebbende rolspelers versprei om insette te kry wat bepalend vir die beplanning van die volgende simposium in 2016 sal wees. Hierdie vraelyste is ook na alle sitrusprodusente toe uitgestuur en ook deur die kursesgangers tydens die CRI werksinkels in September ingevul. Meer as 90% van die candidate voel steeds dat Champagne Sports Resort die mees geskikte plek is om die simposium in 2016 aan te bied.

6de Internasionale Nematologie Kongres

Gedurende 4 – 9 Mei 2014 is die 6de Internasionale nematologie kongres (6ICN) in Kaapstad aangebied deur die plaaslike Nematologie Vereeniging van Suidelike Afrika (NSSA). Meer as 450 afgevaardigdes van

meer as 32 lande het die geleentheid, wat algemeen beskryf is as die beste nematologiekonferensie nog, bygewoon. MC Pretorius was deel van die reëlingskomitee.

SA Citrus Improvement Scheme

Twee sitruskwekerie is laat in die jaar besoek, en hoewel dit geensins verteenwoordigend van al die kwekerie is nie, is die gehalte van die bome beslis aan die verswak. Dit word ook in die probleme weerspieël wat in die algemeen in boorde op jong aanplantings, afkomstig uit 'n verskeidenheid kwekerie, gesien word. Dit het ook aan die lig gekom dat die uitslae van die monsterneming tydens self-oudits nie werklik die status tov skadelike patogene reflekteer nie. Die risiko tov verliese vir kwekerie is bloot te groot.

Daar word geweldig baie klem gelê op die teenwoordigheid of afwesigheid van skadelike patogene, wat oorwegend die bepalende faktor vir sertifisering van 'n bepaalde boombestelling is. Te min klem word op worteltoestand en tuinboukundige faktore gelê. Sommige kwekerie sal eerder verseker dat hul monsters negatief vir patogene toets, aangesien hul voorbestaan van die suksesvolle kweek en bemerking van bome afhang, as om verliese te ly, onderhewig aan die knyptang van stygende kostes.

Die enorme kostes om sitrus te vestig, wat in die orde van bykans R100 000 per hektaar beloop, vereis dat meer aandag aan die suksesvolle kweek van gesertifiseerde sitrusbome gegee behoort te word om patogeenvrye bome van uitstaande tuinboukundige gehalte te verseker. Die omvang van die Suid-Afrikaanse Sitrusbedryf regverdig die aanstelling van 'n voltydse kwekery-adviseur om bogenoemde rol toegewyd te vervul.

Navorsings-Portofilio vergaderings

Voorligting was teenwoordig op al vier die vergaderings waarop daar bepaal word watter van die navorsingsvoorstelle gefinansier sal word. Omdat daar onvoldoende befondsing is, is slegs voorstelle met 'n 3 gradering, wat die hoogste gradering is, befonds. Die befondsing spreek sowat 90% van die bedryf se navorsingsbehoeftes aan.

CGA Summit

'n Uitsers suksesvolle "Citrus Summit" is deur die CGA by Phalaborwa aangebied, en dis deur CRI Voorligting bygewoon, waar lesings aangebied is deur CRI personeel oor oa Marktoegang, Navorsing, Voorligting, die SVS en Biosekuriteit. Tydens die daaropvolgende CGA Direksievergadering is besluit dat 'n CRI Bestuurder: Biosekuriteit aangestel moet word en die pos is goedgekeur.

8.1 TRANSFORMATION MANAGERS' ANNUAL REPORT

The Citrus Study Groups

The Technology Transfer Groups (TTGs) play a major role in technically positioning citrus farmers in nowadays farming environment. The study group environment offers citrus farmers the opportunity to engage in a more in-depth discussion with peers, sharing information and knowledge about a subject or an issue they are collectively involved in. Being a member of a study group where everyone actively strives to learn and remain on task can be very advantageous toward one's success in citrus farming. The conducting of the citrus study group sessions went well during the 2014/2015 season in Eastern Cape, Limpopo and Kwazulu Natal provinces.

Information Days

The Limpopo and the Eastern Cape provinces started information days called the Citrus Field Day and the Transformation Grower Day respectively. These have become traditional events in these two provinces. The aim of these events is to encourage the developing citrus growers, agricultural officers and other stakeholders in the citrus industry to come together and share citrus production and marketing information. These events are held annually and the host district in these provinces chooses the theme of the information day.

The Citrus Field Day

During 2014 the Citrus Field Day event was held on the 23rd of July at the Sunningdale farm, Mookgopong in the Waterberg district and the theme was "Back to Basics: Doing Things the Right Way in the Citrus Value Chain". A total of 105 people attended representing 3.5 million cartons. The Citrus Academy and CRI staff attended, supported by the Limpopo Department of Agriculture, DAFF officials, as well as citrus farmers from as far as Weipe and Mooinooi near Marikana, in the North West.

The Transformation Grower Day

During 2014 the Transformation Grower Day event was hosted at Cape College, KAT River Valley in Fort Beaufort. The event was organized by Citrus Research International (CRI), Citrus Growers Association of

Southern Africa (CGA) and Eastern Cape Department of Rural Development and Agrarian Reform (ECDRDAR). A total of 86 delegates attended the grower day and there is an improvement from last year's attendance although a total of 100 people were anticipated.

CRI Postharvest Workshop

The 2015 CRI Citrus Packhouse workshops were held in January, February and March at the Fairview Hotel in Tzaneen, Loskop Dam in Groblersdal, CRI Boardroom in Nelspruit, Mentors Kraal in Jeffreys Bay and Blue Waters hotel in Durban respectively. The workshops went on very well and the small and developing farmers, Department of Agriculture Forestry and Fisheries (DAFF), officials from Provincial Department of Agriculture (PDA) and Citrus Academy students also attended these workshops. These workshops were preparing the citrus farmers for the upcoming packing season.

CRI Production, Pests and Diseases Management Workshops

The CRI Citrus Regional Extension Workshops in the northern region were held in August at Swadini Resort in Hoedspruit, Loskop Dam in Groblersdal, CRI Boardroom and in Nelspruit and in September at the Mentors Kraal in Jeffreys Bay respectively.

Citrus Emergent Excellence Export Training Workshop

The Citrus Academy (CA) has secured funding for the Citrus Emergent Export Excellence Training Workshop from Department of Agriculture Forestry and Fisheries (DAFF) Directorate Marketing. The training workshops were facilitated by Louis von Broembsen and Sam Louw from the Citrus Academy. The CRI Transformation Extension coordinators Melton Mulaudzi and Andrew Mbedzi provided support to the facilitators during the execution of these workshops.

Female Farmer of the Year Competition

The Department of Agriculture, Forestry and Fisheries (DAFF) in partnership with Total South Africa hosted the Annual Female Entrepreneur of the Year Awards on the 22nd of August at a Gala Dinner at Mmabathu Convention Centre, Mafikeng in the North West Province. Ms. Ivy Nokwanale Mzamo from Luthando Citrus Farming Trust at Kirkwood in the Sundays River Valley Municipality was the overall national winner of the competition. She participated in this competition from the Cadadu District Level and through to the provincial level. She won both on the district and the province levels.

Empangisweni Community Trust Farm

Empangisweni Community Trust farm is located near Gluckstadt, about 30km from Vryheid on the R34 road to Melmoth. The farm is in the process of establishing 100ha of citrus (60ha of lemons and 40ha of oranges). A total of 60ha of lemons has been established in the farm and they are going to start with the planting of oranges during September 2015.

Citrus Growers Development Chamber (CGDC)

The Chamber met on the 10th of March 2015 at the Cajori Hotel just before the first historic Citrus Summit Indaba in Phalaborwa. The purpose of this meeting was to report back to the Chamber about the progress of the formation of the CGA Developing Company. The entire Chamber members are in great support of the formation of this company. They were informed that Dr. Richard Bates of Bates Consulting has already drawn the CGA Developing Company prospectus and the Chamber Executive has accepted it and it is also going to be presented to the CGA Board of Directors during the Citrus Summit Indaba on the 11th and the 12th of March 2015 at the Hans Merensky Hotel in Phalaborwa. The Chamber members were informed by the transformation manager that if the CGA Board approves the CGA Developing Company prospectus at the Citrus Summit Indaba the company will be registered during April 2015.

The Chamber members took a resolution that during 2015/2016 financial year they should meet at least twice per year. The first meeting should look at the plans and the second meeting will look at the implementation of those plans. The Chamber Executive committee will hold four meetings per year and also when the need arises.

The Signing of MoU

The CGA Transformation office is in the process of working to re-sign a new MoU between the Citrus Growers Association of Southern Africa (CGA) and the Limpopo Department of Agriculture (LDA). The negotiations between the CGA and the LDA have already been initiated and the MoU is now on the hands of the legal representatives for the LDA.

CGA Grower Development Company

The Citrus Growers' Association (CGA), as a commodity organisation, established a Transformation Initiative in 2000 to contribute to the development of citrus growers. As such it has participated in a number of

Government initiatives focusing on transformation of the agricultural sector viz. Mentorship, Extension Services, Research and Development, and Grower Skills Development.

The industry has also supported the establishment of the Citrus Grower Development Chamber (CGDC) to facilitate transformation and out of this has arisen a proposal to establish the CGA Grower Development Company to further promote and support the development of black citrus growers.

A situational survey of 118 emerging growers was undertaken by the Citrus Academy in 2013/14 to establish a baseline of information about these growers. The findings, together with prevailing knowledge, give direction for the CGA Grower Development Company. The vision of the company is:

“To support the establishment and growth of sustainable and profitable black citrus growers with market linkages to ensure food security, jobs and wealth creation.”

CGA has agreed to supply grant funding on an annual basis for 4 years commencing in 2015. An amount of R6 million is earmarked for 2015. This will escalate at 5% per annum for the following three years to R26 million. The company's functions will focus on the following to support the development of black growers:

Function 1 - Production infrastructure and technical support

Function 2 - Production business management support

Function 3 - Facilitation of access to funding (grants/loans) by providing support

Function 4 - Facilitation of access to markets

Function 5 - Social Facilitation in respect of project governance

8th CRI Citrus Research Symposium

A total of 8 members from the Citrus Growers Development Chamber (CGDC) attended the CRI Citrus Research Symposium at the Champagne Sports Ground Resort. Five (5) of the members are representing the Chamber at the Variety Focus Group (VFG) and 3 members are representing the Chamber at the Citrus Marketing Forum (CMF) and were sponsored by Citrus Growers Association of Southern Africa (CGA) to attend the 8th CRI Citrus Research Symposium.

A total of 56 officials from Department of Agriculture Forestry and Fisheries (DAFF), Provincial Departments of Agriculture (PDAs) and Agricultural Colleges attended the 8th CRI Citrus Research Symposium.

CGA Citrus Summit Indaba

The Citrus Summit was proposed to the CGA Board of Directors by the commercial citrus growers to replace the CGA Roadshows which were held every year, in February/March to inform the growers about the previous season and plans for the coming season. It was also the opportunity for all growers to meet with CGA and associated company personnel and ask questions and clear up any misconceptions that may have occurred. Holding a Summit will be a way to bring all the growers of the different regions together so discussions can take place on a broader base than the more localised roadshows. There will be one Citrus Summit every second year (biannual).

The first Citrus Summit Indaba was held on the 11th and the 12th of March 2015 at the Hans Merensky Hotel in Phalaborwa. Most of the Citrus Growers Development Chamber (CGDC) members attended. The Chamber started the Citrus Summit by holding their first meeting of the year on the evening of the 10th of March 2015 at the Cajori Hotel in Phalaborwa.

Each of the CGA staff presented a presentation and was assisted by one of the CGA Directors and a special guest and enough time was made available for questions and answers after each session. Guest speakers and representatives from key organisations as well as the CGA overseas representatives attended the first Citrus Summit Indaba at the Hans Merensky Hotel in Phalaborwa.

CGA Mentorship Programme

There is still no funding for the CGA mentorship programme from the provincial departments of agriculture (PDAs). The CGA Transformation desk will continue to engage with the provincial departments of agriculture for the funding of the mentorship programme.

During November 2014 the Department of Agriculture, Forestry and Fisheries (DAFF) held a Commodity Based National Mentorship Implementation Framework workshop at the Sefala Building in Pretoria. The National Mentorship Implementation Framework aims to recommend and provide standards and criteria to be used in facilitating mentorship programme to smallholder and developing producers in the agriculture, forestry and fisheries sectors. The framework is intended to apply to cooperatives, financial institutions,

associations or unions and other departments as well as partners who are active or have interest in any leg of the agriculture, forestry and fisheries value chain.

Government Grant and Recap Funding

The Kat River Valley citrus farmers received R2.2 million from the Provincial Department of Agriculture, for buying fertilizers and pesticides. A total of 18 farmers benefitted from this grant. The MEC promised to assist the Ripplemead with an amount of R9,2 million for the rebuilding of the packhouse, while KAT River farmers will again receive another R4 million for fertilizers and pesticides. The Western District or Cacadu will receive R4 million to support selected farms at Patensie and Sundays River valley with inputs and infrastructure.

The recapitalization funding has been approved for the following farms:

- **Lovers Retreat Farm:** The second tranches of recap received, the farmer bought new tower boom spray, herbicides boom spray, harvesting trailer, a Toyota LDV and is also erecting a pack shed.
- **Torties Farm:** The second trenches of recap have been approved and the farmer is erecting a pack shed for parking tractors and other movable assets.
- **Orange Grange farm:** The store room and pack shed have been erected with second trench of recap. The store room will be used to store chemicals and fertilizer while the pack shed include the provision of an office.
- **Rallyvale Farm at Peddie:** The pack shed is also erected together with an office and store room for fertilizer and pesticides.

Challenges

- Some farmers in the KAT River Valley Experienced hail damage and most affected crops were Navel and Novas.
- Land transfer issue and tax clearance certificate, is causing a delay for some KAT River farmers to access their phase 2 recap funding.
- Poor communication between the responsible government officials and citrus growers with regard to recapitalization funding.
- Lack of budget for the training of the Limpopo Department of Agriculture extension officers who are coordinating citrus in the province.
- Most of the upcoming citrus growers are faced with financial challenges.
- Lack of funding for the CGA Mentorship Programme
- Shortage of credible mentors to assist in the CGA Mentorship Programme.

8.2 RESEARCH PRIORITIES / NAVORSINGSPRIORITEITE

The Citrus Research Priorities for 2014 were determined from July–October 2013 taking into account the inputs from the producers, the Exporters Technical Panel and the CRI Workshops. A communication was sent to all the citrus producers who are on CRI's Technology Transfer Group (TTG) list, listing all the research approved for 2013. This provided a means for growers to assess which of their previous requests were being addressed. Growers were requested to study these research projects and indicate any additional research required as a priority. They were advised to weight requirements from 1-3, with 3 the higher priority. Once the TTG's Technical Committee had received all the research requests from the area they compiled a summary and a meeting was held with the Area Extension Manager and the Technical committees. In a few cases the TTGs indicated that there were no changes to their research needs and cancelled the proposed meeting. The Area Extension Managers from the North and the South compiled the resultant research priorities and forwarded these to the Manager Research and Technical. Unfortunately many producers, including CRI directors, do not read their e-mails and are therefore not aware of the way in which the research priorities are determined and are therefore criticising the process. The respective TTGs are responsible themselves who they choose both as chairman and as technical committee for each of the areas.

The research priorities were also determined at the five CRI Post Harvest Workshops held in Limpopo, Mpumalanga, KwaZulu Natal, the Eastern Cape and the Western Cape in January/February 2013 as well as the CRI Production Workshops and the CRI Spring Pest Complex and Disease Management Workshops held in May/ June and September 2013 respectively. It was also determined for the Exporters Technical Panel and the growers involved in the Transformation process. The majority of the research priorities are the same as the previous years. The Research Priorities can be summarised as follows with the project numbers addressing each of these problems or the reason why a specific problem is not addressed.

1. DISEASE MANAGEMENT

1.1 CITRUS BLACK SPOT	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
The fact that South Africa would only be allowed 5 strikes in the EU, the fact that the EFSA report indicated that they do not accept South Africa's argument that the fruit do not pose a pathway to spread CBS and the fact that the disease cannot establish in a Mediterranean climate had a huge impact on the growers perception of what should be the citrus industry's highest priority. To the growers in the summer rainfall areas it was definitely CBS and the phytosanitary status of CBS. As in the latter part of 2012 the growers experienced enormous challenges to adhere to the latest DAFF regulations to be able to export into the EU.. It is thus of the utmost importance that the status of CBS should be changed from a phytosanitary to a cosmetic disease in order to get rid of these disruptions. This requires that all the research that was completed on CBS should be published in refereed journals. Something that were done over the last couple of years.	919 970 977 1012 1026 1088 Market Access			
A request by growers in CBS affected areas is still that the USA should be opened up for all citrus producing areas in South Africa, with the hope that the EU would then accept the fact that fruit do not pose a threat as a pathway to spread the disease.	1026,1 088 Market Access			
Develop alternative spray programmes and application methods which is more effective than the current programmes. It would be even better if these programmes could control <i>Alternaria</i> as well.	970,75 0			
Complete the study to determine the critical period for CBS infection in the Eastern Cape.	919,10 26			
Develop alternative strategies to interrupt the diseases life cycle. Protection of the leaves prior to falling should be re-investigated as well as the destruction of inoculum (dead leaves) during the rainy season. Genetic manipulation to build in resistance genes should also receive urgent attention..			X	
Convince the chemical companies selling strobilurens to change the labels in order to be able to spray these products without mancozeb as there is no threat of resistance of CBS against the strobilurines.	970			
Investigate the possibility to spray a third strobilurine late in February on old clone valencias and lemons to prevent a late infestation of CBS. Get the USDA to acknowledge the Weipe & Tshipise areas as areas of low pest prevalence.	970 Market Access			
Determine where does the third <i>Phylosticta</i> sp found on citrus fits in	1088			
Register the combination of the strobilurines with benomil without mancozeb.	970			
The MRL for citrus to Canada is 0,1ppm. However, in Canada itself the MRL for use on their produce is 0,5ppm. Paul Hardman to follow up.	Market Access			
CBS spray programmes for the EU cannot be statutory for everybody. There is groves in the areas of low pest prevalence that has never sprayed and has been CBS free to date because of other measures taken to prevent the introduction and spread of inoculum.	Market Access			

	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
Lower Orange River must be declared CBS free in the EU	Market Access			
CBS pest free areas must be maintained according to international law.	Market Access			
Find postharvest options to prevent development of CBS symptoms.	UKZN project in PHD		X	
1.2 ALTERNARIA				
Alternative spray programmes to control <i>Alternaria</i> more effectively with fewer sprays and more effective spraying techniques to reduce the volume of water needed to apply the chemicals effectively	750,891,1089,1096			
Establish an on-going project to screen all new cultivars for <i>Alternaria</i> tolerance.			X	
Determine the effect of shade netting on <i>Alternaria</i> on susceptible cultivars.	New ARC			
Confirm the possibility of <i>Alternaria</i> resistance against the strobilurins				X
1.3 BOTRYTIS				
Spray programmes to control <i>Botrytis</i> on lemons during flowering.	1015			
Determine the effect of <i>Botrytis</i> on lemons in the Sundays River Valley.	1015			
Identify fungal growth on blossoms of Satsumas and other varieties				DC
1.4 PHYTOPHTHORA CITROPHTHORA				
More effective control programmes.			X	X
Establish an ongoing project to screen all new cultivars against <i>P. citrophthora</i>			X	
Need effective control option for snails in W-Cape			X	
Current program only delays problem. Need effective control option			X	
1.5 POST-HARVEST DISEASES				
Optimisation of the flooder to allow commercial use in packhouses	1050, 1x new CRI@U SPP project			
Optimisation of fungicide treatments in the packhouse to be the most effective to protect the fruit and to prevent resistance developing.	936,1050, 3x new CRI@U SPP projects			
Optimising the GRAS chemicals such as sodium bicarbonate, especially in an imazilil protection programme. (pH correlations, concentration, temperature, exposure times etc.)	936, 1x new CRI@U SPP project			
Develop techniques to use the quaternary ammonium products safely for exports to Japan. Assist with the re-introduction of Sporekill	Market Access		X	
Development of wax standards			X	

Alternatives for Guazatine	123			
----------------------------	-----	--	--	--

	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
Determine the rate of residue breakdown of all products used in post-harvest treatments			X	X
Determine the viability to pack citrus under certain conditions as chem.-free without causing decay problems	UKZN			
Control options for <i>Rhizopus</i>			X	
Control options for sour rot and brown rot	123			
Need options to do away with warm water bath	1050, 1x new CRI@U SPP project			
Supermarkets requests third of EU MRL – need alternative control products that will be effective at low concentrations	936, 1050, 2x new CRI@U SPP project			
Need tool for early detection of decay in packhouses	1073		X	
Investigate guazatine burn	123			
Evaluate the use of Orosorb in the warm bath to get better run-off and drying in the tunnel		X		
Control and prevention of fungal growth on wooden pallets			X	
Investigate “stripping” of mancozeb to prevent residue issues to Canada			X	
1.6 PHYTOPHTHORA ROOT AND COLLAR ROT				
Alternative control options	1030			
Screening of new rootstocks against <i>Phytophthora</i>	UP/CRI 01/09			
More effective and safer phosphonate treatments			X	X
Determine the effect of compost teas and commercially applied microbial applications against <i>Phytophthora</i> root rot			X	
1.7 CITRUS NEMATODE				
Evaluation of pre-plant fumigation products on replant soils.	762			
Alternative control options (E.g. Imidacloprid?)	1030			
Control options for sheeth nematode			X	
1.8 ARMILLARIA ROOT AND COLLAR ROT				
Develop control options for <i>Armillaria</i> .	1068			
Test/screen all commercial and experimental citrus rootstocks against <i>Armillaria</i> .			X	
1.9 ROOT HEALTH				
Develop a more holistic approach to root health in general.	910		X	
1.10 CITRUS TRISTEZA VIRUS				
Optimising cross protection. There are TSR orchards in Hoedspruit with heavy stem pitting but the fruit is large and the tonnage 100 tons/ha.	738,739, 742,789, 968,885 885B, 1056, new			

	CRI@US project			
--	----------------	--	--	--

	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
Evaluation of different cultivars in either the selection or suppression of different CTV strains.	1056, new CRI@US project			
1.11 CITRUS GREENING (HUANGLONGBING)				
Development of greening resistant cultivars. Challenging the greening tolerant cultivars developed by Fanie van Vuuren must be accelerated. The genes must be identified and built into other varieties as well	815			
Monitoring the spread of African greening towards the Eastern Cape citrus producing areas.	CIS-Biosecurity			
Monitoring KwaZulu-Natal for a possible introduction of Asian HLB.	CIS-Biosecurity			
Develop methods to cure greening-infested trees			X	
Study the role of alternative hosts in the epidemiology and spread of greening				
Search for greening resistance through embryo rescuing	815			
Search for greening protection using mild strain CTV.			X	
Investigate the transmission and infection of <i>Candidatus Liberibacter africanus</i> at different times of the season.	988			
Confirm if there are any alternative hosts for Asian HLB in South Africa	886B			X
Confidor NB for control of psylla. Find alternative systemic products to control psylla	IPM priority			
1.12 VIROIDS				
Ensure that the CFB is free of all graft transmissible pathogens including the viroids.	CIS790, 796			
Study the effect of viroids on the horticultural characteristics of different cultivars eg. Fruit set, maturity, colour.	1074			
1.13 BIO-SECURITY				
Make industry aware of the Act on the Distribution of Plant Material	CIS-Biosecurity			X
2 INTEGRATED PEST MANAGEMENT				
2.1 FALSE CODLING MOTH				
Fine tuning the systems approach to ensure that the industry will be able to send citrus into the EU without cold steri.	1039 & 1085			
Develop more effective control methods. This includes the optimization of the SIT programme, more effective use of the granuloviruses, commercializing the entomopathogenic nematodes and the entomopathogenic fungi.	1024, 1042, 1049, 1065, 1079 & 1083			
Develop alternative mating disruption systems	955, 1063 & 1080			
Develop amelioration techniques to control FCM in fewer days during cold sterilization.	965 & IBB02/12			

	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
Develop techniques to detect FCM on the pack line.	976, 1022, 1066, 1071 & 1090			
Develop techniques to enhance the release of parasites late in the season.	1021			
Find alternative pheromones to attract FCM		X		
Need mating disruption to be effective on areas smaller than 10ha		X		
Need information to establish bats in the orchards		X		
Need information to establish bats in the orchards				
Evaluate EPN's later (Apr/May) to control FCM	1042			
Start to assess cold treatment at 2 degrees on semi commercial level.	1039			
Categorize lemons as low risk variety at commercial level.	1087			
Determine mating potential of FCM survivors after 2 degree treatment.	1039			
Carob moth increase in citrus. Need ID to distinguish between carob moth and FCM	US/ENT-08-02, US/ENT-11-A3 & 1051			
Quantify the effect of carob moth on citrus	US/ENT-11-A3 & 1051			
2.2 FRUIT FLY				
2.2.1 <i>Bactrocera invadens</i>				
Monitoring of the spread of <i>Bactrocera invadens</i> in South Africa, Zimbabwe, Botswana and Mozambique.	966, 1075 & RU(Timm)			
Investigating a M3 and MAT block barrier in the valleys between Vhembe and Tshipise to protect Tshipise from re-infestations with <i>Bactrocera</i> .	(1075 test feasibility)			X
Develop a risk mitigation strategy to be able to export citrus from areas where <i>Bactrocera</i> was detected. (on an orchard to orchard basis)				X(DAFF)
Get all SADEC countries together to deal with <i>Bactrocera</i> together.				X (DAFF)
Registration of Hym lure/Prolure + Spinosad both as aerial and ground sprays.	Contract trial			
RB is requested to sell methyl eugenol directly to the growers to recharge their MAT blocks themselves. Especially for Zimbabwe.	RB			
Put pressure on DAFF so that more MAT blocks and M3s are distributed amongst the rural villages				X (BiSC)
GF-120 not as effective as Malathion – need a shorter PHI for Malathion				X (BiSC)
Develop yeast to attract females (technique from Australia)	1093			
2.2.2 Mediterranean and Natal fruit fly				
Develop a more attractive attract and kill option than the M3 that will last as long but will reduce the number of traps /ha.	915			
Develop a M3 with both a male and a female attractant.		X		

	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
Study the role of entomopathogenic nematodes and – fungi on fruit fly larvae in the soil.	930, 980 & 1042			
Investigate EGO (enriched ginger oil) as attractant for males	Contract trial			
2.3 MEALYBUG				
Develop alternative control methods against mealybug to replace products such as Dursban, Applaud, Ultracide and Tokuthion.	985, 1017, 1029 & 1048			
Find biocontrol agents that can be used against mealybug.	As above			
Determine the possible role of entomopathogenic nematodes against mealybugs.	985			
Investigate the importation of parasites from Australia.		X		
Determine effect of Anagyrus as alternative predator	1017			
2.4 CAROB MOTH				
Study the morphology and control of carob moth in citrus.	US/ENT-08-02			
Study the effect of EPNs on carob moth.		X		
Monitoring of carob moth.	US/ENT-11-A3,1051			
Investigate the presence of carob moth in Tshipise				X
Carob moth increase in citrus. Need ID to distinguish between carob moth and FCM	US/ENT-08-02			
2.5 FRUIT PIERCING MOTHS				
Monitoring and control of fruit piercing moths.	1058			
2.6 LEAFHOPPERS				
Develop methods to control leafhopper.	942 & 1061			
Also need biological control option		X		
2.7 LEPIDOPTERAN PESTS				
Determine the effect of imidacloprid on lepidopteran pests in citrus.	954			
2.8 ANTS				
Develop and commercialize ant baits for both pugnacious and brown house ants.	857			
Find methods to keep ants out of trees	857			
2.9 THRIPS				
Develop alternative control options for thrips (Abamectin is overused but a registration @ 30ml/100l is needed) especially for November – January control.	1029 & 1061			
Investigate the importation of a predatory thrip from Koppert		X		
Investigate the effect of Cryptonem on the soilborne phase of thrips.	1042			
Thrips trials must be conducted in Letsitele.	1029 (elsewhere in Limpopo)			

	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
Find alternative to Abamectin	1029 & 1061			
Find attractant for use during Dec/Jan that is acceptable to all markets	(done years ago)			
2.10 MITES				
Registration of a generic for Mitigate to reduce its price.	NA			
Alternative control options to replace Acarol which is affordable and effective. Especially budmite is becoming an increasing problem even on Star Rubies.		X		
Revise registration of Mitigate in Canada (Paul Hardman)				CGA
2.11 RED SCALE				
Alternative control options for imidacloprid, especially on heavier soils.	1076			
2.12 SNAILS				
Control options more affordable than Moloxide.	Contract trial			
Moloxide not registered – need registered options	Na			
Need effective control for whole spectrum snails in W-Cape			X	
2.13 SLUGMOTH				
Epidemiology and control		X		
2.14 WOOLLY WHITEFLY				
Alternative control options	1061 & 1082			
Movento not registered – needs registered products				
2.15 PSYLLA				
Find alternative systemic insecticides to control psylla	1061			
Investigate the possible accelerated degradation of imidacloprid		X		
2.16 BOLLWORM				
Bolldex registered on stone fruit – Helicovir to be resistered asap	RB			
2.17 LEMON BORER MOTH				
Increasingly problematic – find control options	715 & 933			

3. CROP AND FRUIT QUALITY MANAGEMENT

3.1 Rind condition	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
3.1.1 Peteca				
Effective control measures.	833			
3.1.2 Creasing and splitting				
Effective control measures.			X	
Better understanding of the physiology of creasing.			X	
Test the product Oenosan.		X		
Investigate the effect of silica and boron on the uptake of calcium		X		
Splitting on navels, Midnights, soft citrus	1027			
3.1.3 Chilling injury				
Develop post-harvest treatments to control chilling injury.	832			
Determine step-down temperature for all varieties to prevent CI	832			
3.1.4 Rind breakdown				
Develop better methods to predict and control rind breakdown especially on Bennys and Turkeys	958,1031			
Need alternatives for TBZ. And find out why does TBZ help.	958			
3.1.5 Blossom end clearing				
Develop a better understanding of the problem and ways to prevent it. The problem is on the increase.			X	
3.1.6 Cold damage				
Develop techniques to protect fruit and trees against frost damage			X	
3.1.7 Shelf life				
Develop techniques to extend the shelf life of citrus fruit.			X	
Method to quantify over ripeness and puffiness.		X		
Monitor the trial which is conducted at Letaba with ozone.		X		
3.1.8 Tear staining				
Determine the cause of tear staining on Nadorcotts and how to prevent it.			X	
3.1.9 Silica				
Determine the role silica can play to reduce all of the above damages	974, 1057			
Determine the effect of silica on fruit set, size, sunburn, etc.	974, 1057			

3.2 FRUIT PRODUCTION & QUALITY	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
3.2.1 Flowering				
Alternating flower on Mors, Orrs and Nadorcotts a problem.	981			
Effect of netting on flowering			X	
3.2.2 Fruit set				
Fruit set a huge problem on Eureka Seedless! But also on Navels in the Eastern Cape and Deltas and Midnights in many citrus areas. Also with TSR in Nkwaleni.			X	
Effect of products such as Reflecto, Silica, Kaolin and Shade cloth on fruit set.			X	
Effect of gibb applications on the western side of the tree only.			X	
Sort out alternate bearing on Mor and Nadorcott	981			
Increase production on oranges and soft citrus in the south, without compromising on fruit size			X	
Investigate effect of higher concentrations Gibb on fruit set of mandarins			X	
Investigate effect of netting, and different colour netting, on set			X	
Evaluate girdling of framework branches vs trunk and timing for better set on mandarins			X	
Fine tune irrigation scheduling to minimise stress and improve set	986			
3.2.3 Regrowth				
A major problem especially on lemons and late mandarins especially when fruit set was poor. Need to test and register products such as Sunny, Cultar or Regalis			X	
Determine the MRLs for plant growth stimulants.			X	
3.2.4 Pruning				
Pruning techniques on late mandarins and lemons need to be developed.				X
Compare winter pruning with late pruning and summer pruning.				X
Do trials to manipulate fruit set, alternate bearing, fruit size and regrowth on soft citrus	981			
3.2.5 Fertilisation				
Recommendations needed to increase carbohydrate levels quicker after harvesting a large crop.	1028			
Manipulation of fertilization to decrease rind problems.	958			
Role of humic and fulvic acids. Does carbon fertilizers act as a carrier for the absorption of fertilisers or is it merely slowing the rate of N leaching from the soil?	1028			

	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
Role of silica.	974,1057			
Timing of first N applications on different cultivars.			X	
The influence of different formulations on the foliar uptake of elements	1037			
Prove that liquid carbon fertilizers does have an effect on the microbial life in the soil. (Not talking of normal organic material).			X	
Evaluate different foliar applications available in industry – too many snake oils		X		
Evaluate effect of silica on rind defects, fruit size, etc-		X		
3.2.6 Internal quality				
Ways to drop the acid levels		X		
Ways to increase the acid levels		X		
Optimization of internal quality under OHS systems			X	
Effect of shade nets on internal quality			X	
3.2.7 Sheepnose				
Climate effect on sheep nose of grapefruit			X	
Effect of shade netting on sheep nose			X	
3.2.8 Cold damage				
How to prevent frost damage (frost bite, Copper Silica, etc.)		X		
Influence of rootstocks on frost damage		X		
Effect of netting on cold damage			X	
3.2.9 Sunburn				
Methods to reduce sun burn				X
Alternatives for oil to reduce sun burn on grapefruit			X	
Effect of netting on sunburn				X
3.2.10 Fruit colour				
Methods to improve fruit colour, especially in the north			X	
Methods to initiate earlier colour			X	
Methods to intensify fruit colour to prevent cold damage during cold steri			X	
Effect of netting on fruit colour			X	
3.2.11 Fruit size				
Methods to increase fruit size on Deltas, Clementines and Rustenburg navels.			X	
3.2.12 Blossom end clearing on grapefruit				
Causes and control			X	
3.2.13 Evaluation of biostimulants				
Biostimulants such as Alexin, Citrox, CropBiolife, Cilic, Messenger, Mannitol, Sorbitol and GA14 should be evaluated.		X		

3.2.14 Water usage	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
The Water Research Council is looking at the water usage of the different fruit crops. They gave CRI the opportunity to be involved. This is important as this could affect water quotas in the future.	986			
3.2.15 Fish lips				
Producers are harassed in Letsitele by PPECB for what they call fish lips at the button end side of fruit. No mealy bug is involved.		X		
3.2.16 Weed control				
Investigate resistance to chemicals		X		
Find softer products for weed control		X		
Find solutions for control of 'motvanger' and 'cat's claw creeper'		X		
3.3 COLD CHAIN & PACKAGING				
3.3.1 Cold Chain Management				
Investigate optimum shipping temperature and RH to control waste.				X
Updated manual annually for decay control (Production Guidelines & Booklet).				X
Set time and temperature protocols for new varieties		X		
Investigate the correlation between variation in temperature on vessels and decay		X		
Determine effect of forced air cooling on rind disorders	832			
Determine influence of loading at room temperature on decay and shelf life	932			
Determine optimum pre-cooling temperature to prevent excessive condensation during handling in port and loading of vessels		X		
Determine optimum rate of cooling to restrict rind disorders		X		
Investigate the variation and influence of temperature and humidity during transport with Tautliners vs flat bed trucks.			X	
Do trials on ambient loading of soft citrus	832			
3.3.2 Packaging and Palletizing				
Evaluate new pallets and set minimum specifications for pallets, including fungal en pest treatments. Ongoing project.				X
Find alternative material to wood for manufacturing of pallets. Ongoing.				X
Investigate stronger board combinations to replace end pieces. Ongoing.				X
Set handling guidelines for all aspects of the cold chain. Ongoing.				X
Set guidelines and specifications with photos for palletizing of all cartons (strapping, securing sheets, corner pieces, etc.)				X

	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
Evaluate different sizes and types of corner pieces. Ongoing for new corner pieces				X
Accreditation process for packaging manufacturers and service providers in the cold chain should be implemented				X
Use of short corner pieces instead of end pieces in open tops should be investigated.				X
Set handling guidelines for all aspects of the cold chain. Cooling Working Group to finalize.				X
Evaluation of fruit in Supervent cartons under cold sterilization. Ongoing	832			X
Develop control options for wood rotting fungi on pallets.			X	

4. CULTIVAR DEVELOPMENT

Cultivar Development	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
Expand and update the Cultivar Fact Sheets	Yes, CFS			
4.1 Rootstocks				
More suitable rootstocks for high pH soils in general	922, 923			
More suitable replant rootstocks for high pH soils	922, 923			
More suitable dwarfing rootstocks on high pH soils	922, 923			
Lemon cultivar/rootstock trial for the Sundays River Valley	1010A			
Reintroduction of Flying Dragon from San Miguel to be tested on heavier soils	Yes, New			
Evaluation of Argentinean rootstocks	Yes, New			
Get clarity on incompatibility of Fukumoto and Mor with trifoliolate rootstocks	Fukumoto, 1007	Mor, No		
4.2 Cultivars				
Earlier and later Satsumas with a better internal quality	57A, 57 B, 57C, 57D			
Earlier and later Clementines	1000A 1000B 1000C 1000D			
Late mandarins of which the plantings are not restricted	964D			
Late mandarins for the hotter areas	812E, 75C,899B			
Navels that yield better with acceptable fruit size	812A, 899C, 963B,964C			
Early navel with round fruit with good yields	899C, 941A, 963B			
Earlier and later Star Ruby selections	Yes, 812G			
Early grapefruit which is not prone to sheeppose		No		
A better tasting red grapefruit	Yes, 812G			

	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
Earlier and later Valencia selections	812D 75A&B 740A 899A 963A 964B			
Olinda Valencia to be reintroduced to the Foundation Block	Yes			
Need cross pollination chart / table for soft citrus		No		

8.3 STUDY GROUP CHAIRMEN FOR 2014-15

TTG	Name	Tel. no	Email
Baviaans	Phillip Dempsey	082 498 2778	phillipdempsey@southernfruit.co.za
Beitbridge	Paul Bristow	072 701 9227	pbristow@iwayafrica.com
Benede-Oranjerivier (Kakamas)	Jacques de Wet Jannie Spangenberg	082 495 0632 082 556 8610	augpad@lantic.net santas@mweb.co.za
Breederivier	Sakkie Bruwer	083 226 2540	subtrop@netactive.co.za
Burgersfort	Albert Winterbach	079 508 3960	waterval.albert@gmail.com
Citrusdal	Sakkie Bruwer	083 226 2540	subtrop@netactive.co.za
Groblersdal/M. Hall	Pieter Engelbrecht	082 524 8925	pieter@dpet.co.za
Hoedspruit	Hannes Meintjies	082 460 5220	hannes@eden-fruit.com
Katrivier	Isabel Sparks	071 415 0288	technical@katco.co.za
Knysna	John Stanwix	082 789 5051	knycit@mweb.co.za
Komatipoort	Dirk Horn	013-7937536 083 259 3359	sommerreg@soft.co.za
Letsitele	Eddie Vorster	083 629 4949	evmv@mweb.co.za
Malelane	Leon Esselen	013-790 0160	esselenk@mweb.co.za
Midnight Study Group	Theuns Nieuwoudt	082 559 2992	sneht@ctecg.co.za
Nelspruit	Willem Kieviet	082 490 2991	wkieviet@vodamail.co.za
Nkwalini	Mike Wafer	083 278 6150	michaelwafer@yahoo.com
Ohrigstad (Kaspersnek)	Kobus Beetge	082 388 0011	mogaba@yebo.co.za
Paarl/Stellenbosch/Swartland	Stephan Venter	083 670 8030	Stephan@insectscience.co.za
Patensie	Gerhard van Vuuren	071 684 8102	gerhardj@patensiecitrus.co.za
Pongola	André Barnard	083 229 8539	mhlathi@idhweb.com
Rustenburg	Willem van Schalkwyk	082 773 8095	willem@svsboerdery.co.za
Southern Natal	Peter Button	082 488 8537	pbuttonuturenet.co.za
Sundays River	Dave Gerber	079 495 3162	technical@srcc.co.za

Swaziland	Gerd Höppner	09268-3232311	gerdh@rssc.co.sz
Swellendam	Sarel Neethling	082 551 2357	sarel@thornlands.net
Tshipise	Barend Vorster	082 651 2642	xmasbdy@lantic.net
Vaalharts (Hartswater)	Michael van Niekerk Danie Mathewson	082 948 2551 082 550 0293	orange@lantic.net saamfarm@lantic.net
Waterberg	Danie Janse van Rensburg		sitrus1@bufland.co.za
Weipe	Bertus Dillman	083 488 5522	noordgrens@lantic.net
Zimbabwe	John Perrott	09263 91223841 0726111478	johnwperrott@gmail.com

8.4 THE RELATIVE FUNDING SUPPORT FOR RESEARCH PORTFOLIOS AND PROGRAMMES FOR 2014-15

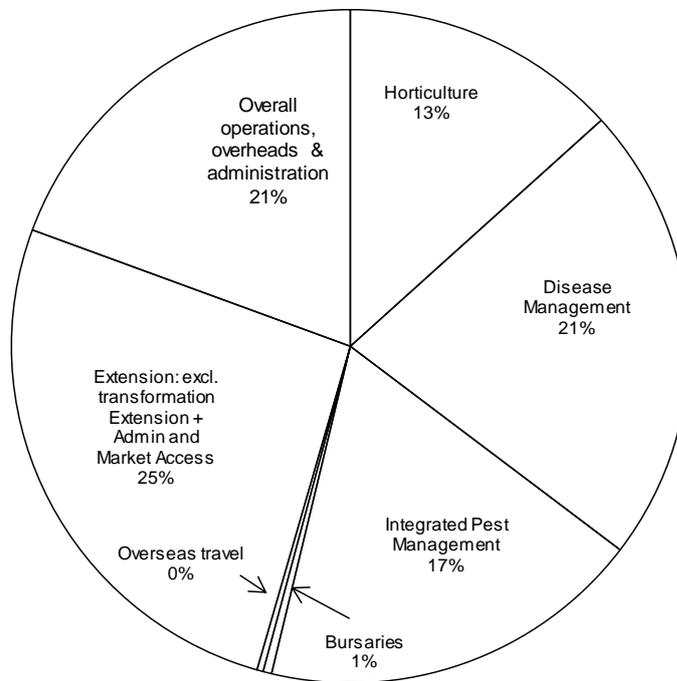


Fig. 8.4.1. Percentage funding in each CRI Portfolio and the rest of the budget for 2014-15.

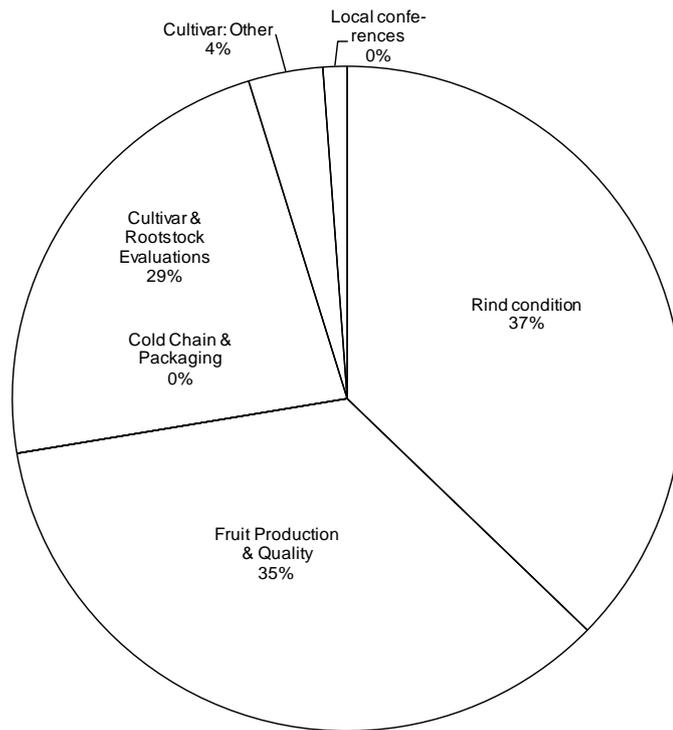


Fig. 8.4.2. Percentage funding to programmes in the CRI research Portfolio: Horticulture for 2014-15.

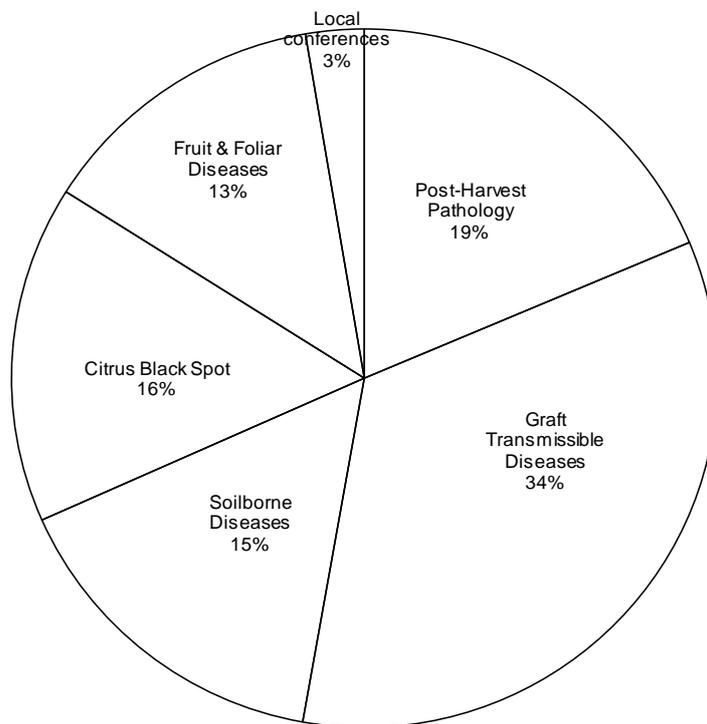


Fig. 8.4.3. Percentage funding to programmes in the CRI Portfolio: Disease Management for 2014-15.

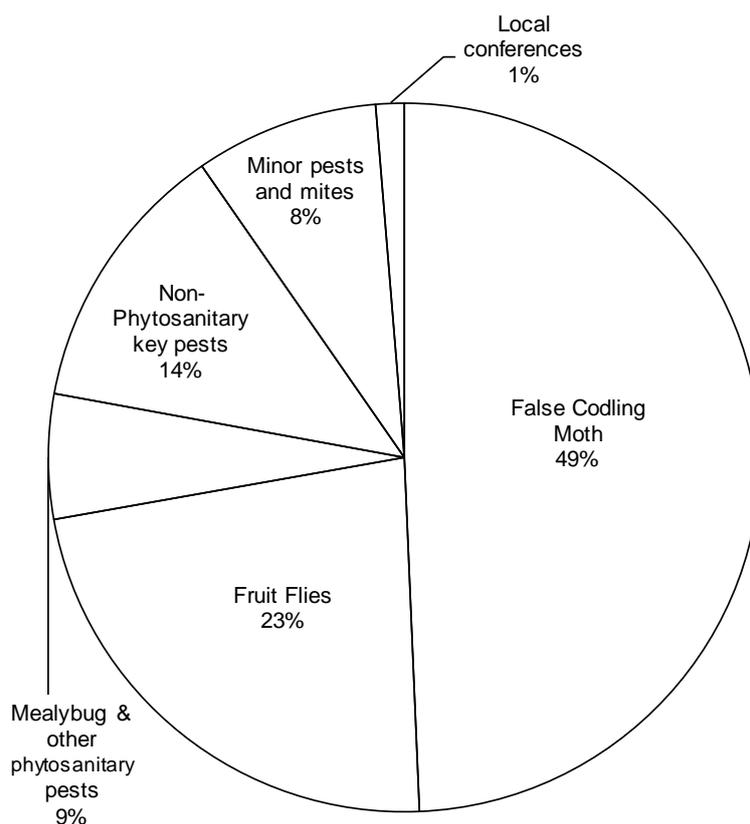


Fig. 8.4.4. Percentage funding to programmes in the CRI Research Portfolio: Integrated Pest Management for 2014-15.

8.5 EXTENSION PRESENTATIONS BY CRI RESEARCHERS IN 2014-15

Date	Place	Topic	Type of meeting
BASSON, E.			
17-20 August 2014	Drakensberg, SA.	The role of the CRI Diagnostic Centre in the SA Citrus Improvement Scheme	8 th Citrus Research Symposium
		Poster: The CRI Diagnostic Centre: a diagnostic service to the citrus industry	
		Poster: Variations of citrus black spot lesions	
BREYTENBACH, J.H.J.			
17-20 August 2014	Drakensberg, SA.	The effect of climate and host on <i>Citrus Tristeza Virus</i> cross-protection of grapefruit	8 th Citrus Research Symposium
CARSTENS, E.			
17-20 August 2014	Drakensberg, SA	Use of simple sequence repeat (SSR) markers to evaluate genetic diversity among SA isolates of <i>Phyllosticta citricarpa</i> .	8 th Citrus Research Symposium
		Market Access and Biosecurity as Critical Components of a sustainable southern African Citrus	
COOK, G.			
17-20 August 2014	Drakensberg, SA.	<i>Citrus Tristeza Virus</i> strain characterization of cross-	8 th Citrus Research Symposium

		protecting sources and CTV strain analysis in grapefruit field trial trees	
		Poster: Population dynamics and seasonal fluctuation in the percentage infection of <i>Trioza erytreae</i> with <i>Candidatus Liberibacter africanus</i> , the African citrus greening pathogen	
CRONJE, P.J.R.			
17-20 August 2014	Drakensberg, SA.	Colour development of 'Star Ruby' grapefruit and the impact on chilling injury susceptibility	8 th Citrus Research Symposium
		Ambient loading of citrus for cold sterilization markets: research feedback	
28-31 Jan 2015	Polokwane Loskopdam	CRI Packhouse Workshop	Workshop
12-14 Feb 2015	Port Elizabeth Duban	CRI Packhouse Workshop	Workshop
18-19 Feb 2015	Stellenbosch	CRI Packhouse Workshop	Workshop
DANEEL, J-H.			
17-20 August 2014	Drakensberg, SA.	Poster: A comparison between aerial and ground-based applications of bait sprays for fruit fly control	8 th Citrus Research Symposium
DU TOIT, M.			
17-20 August 2014	Drakensberg, SA.	Poster: The Citrus Improvement Scheme	8 th Citrus Research Symposium
		Poster: Citrus Tree Certification	
ERASMUS, A.			
17-20 August 2014	Drakensberg, SA.	At last! An effective and practical alternative to the fungicide dip tank	8 th Citrus Research Symposium
FOURIE, P.H.			
17-20 August 2014	Drakensberg, SA.	Is fresh citrus fruit a pathway for CBS into Europe? EFSA's pest risk assessment and technical response by an international expert panel	8 th Citrus Research Symposium
		The Southern African Citrus Improvement Scheme: a dynamic industry's foundation and security	
GILBERT, M.			
17-20 August 2014	Drakensberg, SA.	Monitoring of fruit flies and false codling moth on multi-crop farms	8 th Citrus Research Symposium
GROUT, T.G.			
17-20 August 2014	Drakensberg, SA.	Can GRAS fumigants offer a more sustainable control option for phytosanitary pests than pesticides?	8 th Citrus Research Symposium
16 Sept – 1 Oct	Hoedspruit Groblersdal Nelspruit Durban Jeffrey's Bay	Managing citrus pests in spring	
HATTINGH, V.			
17-20 August 2014	Drakensberg, SA.	Market Access and Biosecurity as Critical Components of a	8 th Citrus Research Symposium

		sustainable southern African Citrus	
JOUBERT, J.			
17-20 August 2014	Drakensberg, SA.	Performance of Mandarin hybrid selections in the different production areas (climatic regions)	8 th Citrus Research Symposium
		Citrus rootstock characteristics and new possibilities for the future	
		Influence of rind water content on Mandarin citrus fruit quality	
KIRKMAN, W.			
17-20 August 2014	Drakensberg, SA.	Seeing the invisible: X-ray for post-harvest detection of FCM	8 th Citrus Research Symposium
KOTZE, C.			
17-20 August 2014	Drakensberg, SA.	Rapid decline and trunk rot of citrus observed in some Eastern Cape orchards	8 th Citrus Research Symposium
LE ROUX, H.F.			
17-20 August 2014	Drakensberg, SA.	Citrus rootstock research: A case study of the negative effect of short term decisions on long term research priorities	8 th Citrus Research Symposium
MANRAKHAN, A.			
10 Feb 2015	Letsitele	Fruit Fly (Oriental)	Constantia Study Group
19 March 2015	Hoedspruit	Fruit Fly (Oriental)	Hoedspruit Study Group
17-20 August 2014	Drakensberg, SA.	Fruit Fly - A new fruit fly pest in South Africa: research on a new control technique and improvement on existing control methods	8 th Citrus Research Symposium
16 Sept-03 Oct 2014	Limpopo Mpumalanga KwaZulu-Natal E. Cape W. CApe	Fruit Fly pests of citrus	CRI Pest & Disease Workshop
MEEDING, S.			
17-20 August 2014	Drakensberg, SA.	Poster: Kultivarevaluasie proses en metodes	8 th Citrus Research Symposium
MOORE, S.D.			
17-20 August 2014	Drakensberg, SA.	Conservation and augmentation of entomopathogenic nematodes on citrus for control of false codling moth	8 th Citrus Research Symposium
		Cold treatments for post-harvest risk mitigation of FCM (and carob moth)	
		Poster: A survey for false codling moth in export lemons	
		Poster: The mirid bug, <i>Eurystylus capensis</i> , a new 'pest' on citrus	
16-17 Sep 2014	Hoedspruit	FCM	Grower meeting
18-19 Sep 2014	Groblersdal	FCM	Grower meeting
22-23 Sep 2014	Nelspruit	FCM	Grower meeting
25 Sep 2014	Durban	FCM	Grower meeting
30 Sep – 1 Oct 2014	Jeffrey's Bay	FCM	Grower meeting
2-3 Oct 2014	Citrusdal	FCM	Grower meeting

2-3 Oct 2014	Citrusdal	Spring complex	Grower meeting
Nov 2014	Letsitele	FCM	Grower study group meeting
27-28 Jan 2015	Tzaneen	FCM	Packhouse workshop
29-30 Jan 2015	Groblersdal	FCM	Packhouse workshop
10-11 Feb 2015	Nelspruit	FCM	Packhouse workshop
12-13 Feb 2015	Durban	FCM	Packhouse workshop
17-18 Feb 2015	Jeffrey's Bay	FCM	Packhouse workshop
19-20 Feb 2015	Stellenbosch	FCM	Packhouse workshop
MURRAY, C.			
17-20 August 2014	Drakensberg, SA.	Poster: <i>Citrus Tristeza Virus</i> strain characterisation of potential CTV cross-protecting sources for soft citrus	8 th Citrus Research Symposium
PRETORIUS, M.C.			
17-20 August 2014	Drakensberg, SA.	Diachronic study of abiotic and biotic factors associated with citrus decline	8 th Citrus Research Symposium
SCHUTTE, G.C.			
17-20 August 2014	Drakensberg, SA.	Spray programmes for the control of fruit and foliar diseases in SA	8 th Citrus Research Symposium
		Are low volume spray applications effective for the control of <i>Phytophthora</i> brown rot?	
STANDER, O.P.J.			
17-20 August 2014	Drakensberg, SA.	Practical applications of the synthetic auxin 2,4-dichlorophenoxy acetic acid (2,4-D) in the SA citrus industry	8 th Citrus Research Symposium
		Poster: The benefits of hand thinning of Nadorcott mandarin	
STEPHEN, P.R.			
17-20 August 2014	Drakensberg, SA.	Poster: Can vapour-heat treatments be a viable alternative to cold disinfestation for false codling moth?	8 th Citrus Research Symposium
VAHRMEIJER, J.T.			
17-20 August 2014	Drakensberg, SA.	Are citrus trees thirsty?	8 th Citrus Research Symposium
		The use of humates and fulvates to reduce nitrogen leaching in citrus	
VAN NIEKERK, J.M.			
17-20 August 2014	Drakensberg, SA.	Preventative and curative management of soilborne pathogens in citrus nurseries	8 th Citrus Research Symposium
VAN VUUREN, S.P.			
17-20 August 2014	Drakensberg, SA.	Re-indexing of <i>Citrus Tristeza Virus</i> cross-protecting sources in mother trees of the Citrus Foundation Block	8 th Citrus Research Symposium
		Poster: Evaluating sweet orange clones for greening resistance	
VAN ZYL, J.G.			
17-20 August 2014	Drakensberg, SA.	Understanding the science of spray application in citrus: getting industry back on track	8 th Citrus Research Symposium
		Poster: Evaluation of two novel spray applicators for fungicide	

		spray application in SA citrus orchards	
14 January 2015	Letaba Junction, Letsitele	Understanding and optimising fungicide spray application in citrus orchards through plant pathology research	Spray Application Workshop
23 January 2015	Bayer Cropscience, Paarl	Understanding and optimising fungicide spray application in citrus orchards through plant pathology research	Spray Application Workshop

8.6 OTHER MEANS OF TECHNOLOGY TRANSFER

8.6.1 SA Fruit Journal by Tim G Grout (CRI)

The SA Fruit Journal is received by every PUC holder and provides the best opportunity for detailed knowledge transfer because it allows for colour photographs and illustrations. During the report period there was a wide range of articles on plant pathology, entomology and horticulture as well as some shorter news articles (Table 8.6.1.1). Low resolution downloads of the latest version can be obtained from <http://www.safj.co.za/>.

Table 8.6.1.1. S A Fruit Journal articles in 2014-15.

Issue	Article	Author/s
April/May	Influence of sunlight exposure on fertility of <i>Phyllosticta citricarpa</i> pycnidia in citrus black spot lesions on grapefruit and Valencia orange rinds	G.C. Schutte, C. Kotze & H.J.G. Korf
June/July	Jaarlikse CRI Na-oes Werkswinkels groei ongekend	J.J. Bester, H.F. le Roux, A. Erasmus, D. Groenewald, K. Lesar & M.C. Pretorius
	New appointments at Citrus Research International	T.G. Grout
Aug/Sept	The mirid bug, <i>Eurystylus capensis</i> , a new "pest" on citrus	S.D. Moore, A. Ratnadass, M. Ferreira & W. Kirkman
	Naturally occurring entomopathogenic nematodes (EPNs) reduce infestation of citrus by false codling moth	A. Manrakhan, J-H Daneel & S.D. Moore
Oct/Nov	Novel pre-harvest usage of the synthetic auxin 2,4-dichlorophenoxy acetic acid (2,4-D) in the SA citrus industry	O.P.J. Stander
Dec/Jan	Can silicon be used to prevent <i>Alternaria alternata</i> in citrus trees?	N.M. Asanzi, N.J. Taylor & J.T. Vahrmeijer
	Agste CRI Sitrus-navorsingsimposium	H.F. le Roux & T.G. Grout
	The benefits of hand thinning 'Nardorcott' mandarin (<i>Citrus reticulata</i> Blanco)	O.P.J. Stander
Feb/Mar	40 Years in the citrus bug workshop	P.R. Stephen

8.6.2 CRI website by Tim G Grout (CRI)

Bandwidth usage increased significantly from 7.25 GB to 20.11GB during the report period while unique visitors to the site increased from 25 768 in 2013/4 to 39 338 in 2014/5 (Table 8.6.2.1). Most visits were once again from South African IP addresses, followed by unknown domains, dot-net domains and dot-com. Of countries that could be identified, the most bandwidth was used in decreasing order by Germany, Brazil, Turkey and Ukraine.

Table 8.6.2.1. Visits and page requests on www.cri.co.za since April 2014.

Month	Unique visitors	Number of visits	Pages	Hits	Bandwidth
Total 2013/4	25768	46448	211837	514378	7.25 GB
Apr 2014	5930	10290	30199	54450	884.30 MB

May 2014	3076	4765	16320	41249	880.06 MB
Jun 2014	1695	2864	15534	42919	793.01 MB
Jul 2014	1779	2947	11295	41962	906.83 MB
Aug 2014	1614	2711	9747	40621	711.01 MB
Sep 2014	1888	3041	15369	47440	829.26 MB
Oct 2014	1698	2797	10022	39020	757.49 MB
Nov 2014	1561	2556	9105	37538	605.47 MB
Dec 2014	4052	7716	97580	119452	1.70 GB
Jan 2015	6241	12474	89851	119690	1.70 GB
Feb 2015	5374	10216	54576	91202	1.27 GB
Mar 2015	4430	9142	24362	58252	1009.64 MB
Total 2014/5	39338	71519	383960	733795	20.11 GB

8.6.3 CRInet by Tim G Grout (CRI)

CRInet provides a good opportunity for growers to share opinions or ask questions on any technical citrus topic but it is mostly being used for dissemination of information from CRI or CGA. The 49 messages sent during the 2014 calendar year are close to the average of 52 per annum for the last 8 years (Table 8.6.3.1). There are currently 465 CRInet members.

Table 8.6.3.1. Numbers of messages circulated per month on CRInet.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2015	5	2	3										
2014	4	3	4	1	12	6	13	1	0	1	3	1	49
2013	1	15	0	7	3	0	2	4	6	13	1	6	58
2012	5	1	19	4	5	2	4	3	1	0	2	0	46
2011	14	3	5	2	8	24	2	3	3	2	2	2	70
2010	0	1	5	3	2	0	6	12	9	4	9	3	54
2009	1	7	3	6	11	0	6	8	4	2	1	2	51
2008	3	6	1	8	5	2	7	3	3	5	3	4	50
2007	5	2	7	1	1	2	4	2	5	4	3	3	39

8.6.4 CRI Cutting Edge by Tim G Grout (CRI)

Some growers consider the Cutting Edge to be the most valuable means of communication from CRI, perhaps because it always contains urgent information and is to the point. Past issues of the Cutting Edge can be downloaded from the member area of the CRI website. Topics covered in 2014/5 are given in Table 8.6.4.1.

Table 8.6.4.1. CRI Cutting Edge issues during 2014-15.

No.	Title	Issue	Author
178	Packing material for the 2014 season	April	D. Groenewald
179	Consumer Assurance Update	April	P. Hardman
180	Grain Chinch Bug warning	May	T.G. Grout
181	Standard operating procedure for dithiocarbamate testing for export citrus to Canada	June	P. Hardman

182	Consumer Assurance Update	June	P. Hardman
183	Assessing the incidence of imazalil resistance in a citrus packhouse environment	July	A. Erasmus, E. Basson, M. Kellerman, C. Savage, K. Lesar, C. Lennox & P. Fourie
184	Combination of post-harvest fungicide treatments with the ethephon test and identification of new black spot lesions on Valencias	June	G.C. Schutte
185	Update on Indian Import Requirements	July	P. Hardman
186	A cautionary note on management of pre-packhouse drench systems	July	A. Erasmus, P. Fourie, K. Lesar, M. Kellerman, C. Christie & M. Seyfferd
187	Consumer Assurance Update	Aug	P. Hardman
188a	CRI Citrus Research Symposium	Sept	H.F. le Roux
188b	CRI Citrus Research Symposium Questionnaire	Sept	H.F. le Roux
189	CBS Spray Programme	Oct	G.C. Schutte, H.F. le Roux & P. Fourie
190	Consumer Assurance Update	Nov	P. Hardman
191	National Road Traffic Act	Jan	M. Brooke
192	FCM Monitoring 2015 – of critical importance	Jan	S.D. Moore & V. Hattingh
192	FCM Monitoring – of critical importance: Addendum	Feb	S.D. Moore & V. Hattingh
193	Name change of the invasive fruit fly and update on its pest status in SA	Feb	<i>Bactrocera invadens</i> Steering Committee
194	Update on the Guazatine EU MRL	Mar	P. Hardman
195	Food Safety Update	Mar	P. Hardman

9 PUBLICATIONS IN 2014-15

9.1 REFEREED PUBLICATIONS (OR ISI RANKED JOURNALS)

- Brits D., Ridgeway J.A., Timm, A.E. 2015. Laboratory evaluation of temperature effects on the efficacy of *Cryptophlebia leucotreta* granulovirus (CrleGV-SA) on fourth-instar false codling moth larvae. *African Entomology* 23. doi: 10.4001/003.023.0106
- Bownes, A, Moore, S.D. & Villet, M.H. 2014. My enemy's enemy: recruiting hemipteran-tending generalist ants for biological control in citrus orchards by spatial partitioning of foraging webs. *African Entomology* 22(3): 519-529.
- Cook, G., van Vuuren, S.P., Breytenbach, J.H.J., Burger J.T. & Maree, H.J. 2015. Expanded strain-specific RT-PCR assay for differential detection of currently known *Citrus tristeza virus* strains: a useful screening tool. *Journal of Phytopathology* (in Press).
- Coombes, C.A., Hill, M.P, Moore, S.D., Dames, J.F. & Fullard, T. 2015. *Beauveria* and *Metarhizium* against false codling moth (Lepidoptera: Tortricidae): A step towards selecting isolates for potential development of a mycoinsecticide. *African Entomology* 23(1): 00–00 (2015).
- Defraeye Thijs, Rutger Lambrecht, Mulugeta Admasu Delele, Alemayehu Ambaw Tsige, Umezuruike Linus Opara, P.J.R. Cronje, Pieter Verboven, Bart Nicola. 2014. Forced-convective cooling of citrus fruit: Cooling conditions and energy consumption in relation to package design. *Journal of Food Engineering* 121: 118-127.
- Erasmus, Arno, Cheryl L. Lennox, Lise Korsten, Keith Lesar, Paul H. Fourie. 2015. Imazalil resistance in *Penicillium digitatum* and *P. italicum* causing citrus postharvest green and blue mould: impact and options. *Postharvest Biology and Technology* 107: 66-76.
- Erasmus, Arno, Cheryl L. Lennox, Lise Korsten, Ncumisa S. Njombolwana, Keith Lesar, Paul H. Fourie. 2015. Curative control of citrus green mould by imazalil as influenced by infection age, wound size, fruit exposure time, solution pH and fruit brushing after treatment. *Postharvest Biology and Technology* 101: 26-36.

- Kellerman, Mareli, Arno Erasmus, Paul J. Cronje, Paul H. Fourie. 2014. Thiabendazole residue loading in dip, drench and wax coating applications to control green mould and chilling injury on citrus fruit. *Postharvest Biology and Technology* 96: 78-87.
- Knox, C.M., Moore, S.D. Luke, G.A. & Hill, M.P. 2015. Baculovirus-based strategies for the management of insect pests: A focus on research and development in South Africa. *Biocontrol, Science and Technology* 25(1): 1-20.
- Lado, J., Rodrigo, M-J., Cronje, P.J.R., Zacarias, L. 2014. Involvement of lycopene in the induction of tolerance to chilling injury in grapefruit. *Postharvest Biology and Technology* 100: 176-186.
- Love, C.N., Hill, M.P. & Moore, S.D. 2014. *Thaumatotibia leucotreta* and the Navel orange: ovipositional preferences and host susceptibility. *Journal of Applied Entomology* 138: 600-611.
- Magarey, Roger D., Hong, Seung Cheon, Paul H. Fourie, David N. Christie, Andrew K. Miles, Gerhardus C. Schutte, Timothy R. Gottwald. 2015. Prediction of *Phyllosticta citricarpa* using an hourly infection model and validation with prevalence data from South Africa and Australia. *Crop Protection* 75: 104-114.
- Magwaza, L.S., Opara, U.L. Cronje, P.J.R., Landahl, S., Nieuwoudt, H.H., Mouazen, M., Nicolai, B.M., Terry, L.A. 2014. Assessment of rind quality of 'Nules Clementine' mandarin during postharvest storage: 1. Vis/NIRS PCA models and relationship with canopy position. *Scientia Horticulturae* 165: 410-420.
- Magwaza, L.S., Opara, U.L., Cronje, P.J.R., Landahl, S., Nieuwoudt, H.H., Mouazen, A.M., Nicolai, B.M., Terry, L.A. 2014. Assessment of rind quality of 'Nules Clementine' mandarin fruit during postharvest storage: 2. Robust Vis/NIRS PLS models for prediction of physico-chemical attributes. *Scientia Horticulturae* 165: 421-432.
- Manrakhan, A., Kilian, J. Daneel, J-H. & Mwatawala, M.W. 2015. 2014. Sensitivity of *Bactrocera invadens* (Diptera: Tephritidae) to methyl eugenol. *African Entomology* 22(2):445-447.
- Marsberg, T., Hill, M.P., Moore, S.D. & Timm, A.E. 2015. DNA-based identification of Lepidoptera associated with citrus in South Africa. *African Entomology* 23(1): 165-171.
- Moore, S.D., Kirkman, W., Richards, G.I. & Stephen, P. 2014. The *Cryptophlebia leucotreta* granulovirus – 10 years of commercial field use. *Viruses* 2014, 6 : 1-x manuscripts; doi:10.3390/v60x0000x (in press).
- Opoku-Debrah, J., Hill, M., Knox, C.M. & Moore, S.D. 2014. Comparison of the biology of geographically distinct populations of the citrus pest, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) in South Africa. *African Entomology* 22(3): 530-537.
- Read, D.A. & Pietersen, G. 2015. Genotypic diversity of *Citrus tristeza virus* within red grapefruit in a field trial site in South Africa. *European Journal of Plant Pathology*, 142(3): 531-545.
- Ridgeway J.A., Timm, A.E. (2015) Reference Gene Selection for Quantitative Real-Time PCR Normalization in Larvae of Three Species of Grapholitini (Lepidoptera: Tortricidae). *PLoS ONE* 10(6): e0129026. doi:10.1371/journal.pone.0129026.
- Roberts, R., Steenkamp, E.T. & Pietersen, G. 2015. Three novel lineages of '*Candidatus Liberibacter africanus*' associated with native rutaceous hosts of *Trioza erytrae* in South Africa. *International Journal of Systematic and Evolutionary Microbiology* 65: 723-731.
- Stander, O.P.J., Theron, K.I., Cronje, P.J.R. 2014. Foliar 2,4-D application after physiological fruit drop reduces fruit splitting of mandarin. *HortTechnology* 24(6), pp. 717-723.
- Taylor, N.J., W. Mahohoma, J.T. Vahrmeijer, M.B. Gush, R.G. Allen, J.G. Annandale. Crop coefficient approaches based on fixed estimates of leaf resistance are not appropriate for estimating water use of citrus. *Irrigation Science* (2015) 33:153-166.
- Van Zyl, J. Gideon, Ewald G. Sieverding, David J. Viljoen, Paul H. Fourie. 2014. Evaluation of two organosilicone adjuvants at reduced foliar spray volumes in South African citrus orchards of different canopy densities. *Crop Protection* 64: 198-206.

9.2 SEMI-SCIENTIFIC PUBLICATIONS (other than SA Fruit Journal)

None.

10 PRESENTATIONS AT SOCIETAL AND INTERNATIONAL CONGRESSES

- Abdulkadir, F., Know, C.M., Marsberg, T., Hill, M.P., Moore, S.D. (2014) Genetic and biological characterisation of a novel South African *Plutella xylostella* granulovirus, PxyGV-SA. In: Proceedings of the 47th Annual Meeting of the Society for Invertebrate Pathology, Mainz, Germany, 3-7 Aug 2014.
- Carstens, E. The global population structure and reproductive biology of the fungal pathogen, *Phyllosticta citricarpa* Kiely. Stellenbosch University, Department Plant Pathology, 2014.
- Chambers, C.B., Moore, S.D., Hill, M.P., Know, C.M. (2014). Production of the *Cydia pomonella* granulovirus (CpGV) in a heterologous host. In: Proceedings of the 47th Annual Meeting of the Society for Invertebrate Pathology, Mainz, Germany, 3-7 Aug 2014.

- Coombes, C.A., Hill, M.P., Moore, S.D., Dames, J.F. (2014) Entomopathogenic fungi for control of false codling moth in South African citrus orchards. In: Proceedings of the 47th Annual Meeting of the Society for Invertebrate Pathology, Mainz, Germany, 3-7 Aug 2014.
- Fourie, P.H., V. Hattingh, E. Carstens, G.C. Schutte, H. le Roux, Li Hongye, A. Miles, M.B. Sposito, M. Dewdney. 2014. Citrus Black Spot – a global perspective. Invited presentation at C.L.A.M. General Assembly, Agrotechnical Commission, Madrid, 17 October 2014.
- Fourie, P.H., V. Hattingh, E. Carstens, G.C. Schutte, H. le Roux, L. Korsten, Li Hongye, A. Miles, M.C. Sposito, M. Dewdney. 2015. Citrus Black Spot: a scientific and political conundrum. Keynote presentation at 49th Congress of the South African Society for Plant Pathology, Bloemfontein, 18-21 January 2015.
- Jukes, M.J., Know, C.M., Moore, S.D., Hill, M.P. (2014) Isolation, genetic characterisation and evaluation of biological activity of a novel South African *Phthorimaea operculella* granulovirus (PhopGV). In: Proceedings of the 47th Annual Meeting of the Society for Invertebrate Pathology, Mainz, Germany, 3-7 Aug 2014.
- Kotze, C., J.M. van Niekerk & M.C. Pretorius. Trunk and dry root rot of citrus observed in some South African orchards. 49th Congress of the southern African Society for Plant Pathology, 18-21 January 2015.
- Lado, L., P.J.R. Cronje, M-J. Rodrigo, L. Zacarias. 2014. Resistance to chilling injury in red, lycopene-accumulating tissue of cold-stored grapefruits. Lemesos (Cyprus). 10-13 June 2014.
- Love, C.N., Hill, M.P. and Moore, S.D. (2014, October). The biology, behaviour and survival of pupating false codling moth under varying soil environments. Presented at the Postgraduate Symposium, Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa.
- Manrakhan, A., Grout, T., Venter, J.H., Grove, T., Weldon, C. 2014. Use of Male Annihilation Technique for control of pest species in the *Bactrocera* group on Mainland Africa presented at the Ninth International Symposium on Fruit Flies of Economic Importance, 12-16 May 2014, Bangkok, Thailand.
- Moore, S.D. (2014) Story of an African firm: 10 years in the biopesticide business – lessons learnt along the way. (Plenary address). In: Proceedings of the 47th Annual Meeting of the Society for Invertebrate Pathology, Mainz, Germany, 3-7 Aug 2014.
- Opoku-Debrah, J.K., Hill, M.P., Moore, S.D., Knox, C.M. (2014) Studies on existing and new isolates of *Cryptophlebia leucotreta* granulovirus (CrleGV) on FCM populations from a range of geographic regions in South Africa. In: Proceedings of the 47th Annual Meeting of the Society for Invertebrate Pathology, Mainz, Germany, 3-7 Aug 2014.
- Stander, O.P.J. Suggestion of flower induction inhibition as an important factor pertaining to alternate bearing in Mandarin (*Citrus reticulata*). Horticultural Society Workshop, January 2015.
- Van Niekerk, J.M., E. Basson, C. Kotze, M.C. Pretorius & A. McLeod. Identification of *Pythium* spp. in South African citrus nurseries. 49th Congress of the southern African Society for Plant Pathology. 18-21 January 2015.
- Van Zyl, J.G. Schutte, G.C., Grout, T.G., Fourie, P.H. 2015. Understanding and optimising fungicide spray application in citrus orchards through plant pathology research. 49th Congress of the southern African Society for Plant Pathology, Bains Lodge, Bloemfontein, Free State, South Africa (19-21 January 2015).