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Citrus Research International, Nelspruit

## TABLE OF CONTENTS

	Page
1 MARKET ACCESS TECHNICAL COORDINATION	1
1.1 Summary	1
1.2 European Union (EU)	1
1.3 Japan	3
1.4 USA	4
1.5 China	4
1.6 South Korea	5
1.7 India	5
1.8 Thailand	5
1.9 Vietnam	6
1.10 The Philippines	6
1.11 eSwatini	6
1.12 Zimbabwe	6
1.13 Citrus one-pagers	7
1.14 Deviation for fruit age compliance on citrus that is intended for export	7
2 BIOSECURITY AND REGULATIONS	7
2.1 Summary	7
2.2 Develop and maintain a comprehensive Citrus industry biosecurity plan - to ensure overall mitigation of the Southern African Citrus industry's biosecurity risks	9
2.2.1 Project 1: Develop a Southern Africa Citrus industry biosecurity master plan	9
2.3 Biosecurity portfolio: Design, develop and oversee the implementation and operation of appropriate biosecurity structures, engagements, procedures, co-operations, resources, projects and other appropriate actions	9
2.3.1 Project 2: Identify, assess and initiate engagement with international funding providers, for future support of Southern African biosecurity projects	9
2.4 Networking and awareness: Obtain supportive participation of relevant stakeholders and interested parties	9
2.5 Ensure successful implementation of processes, procedures and interactions to ensure the timely identification and assessment of biosecurity threats facing the Southern African Citrus industry	10
2.5.1 Project 3: Develop and oversee implementation of Southern African Citrus industry pest-specific action plans for priority biosecurity pests	10
2.6 Ensure effective implementation of processes, procedures, interactions to advance actions required to successfully mitigate the risks and consequences of biosecurity incursions	10
2.6.1 Project 4: Develop and oversee implementation of a Southern African Citrus industry HLB action plan and safe tree production system	10
2.6.2 Project 5: Ensure that HLB and ACP surveillance is undertaken in Eastern Africa	11
2.6.3 Project 6: Facilitate initiation of an HLB eradication plan in Ethiopia	12
2.6.4 Project 7: Ensure, in close collaboration with relevant government officials, that regulations of relevance to biosecurity risk mitigation are appropriately updated and compliance effectively implemented	12
2.6.5 Project 8: Monitoring and control of Leprosis	13
2.6.6 Project 9: Phytosanitary Risk Forum	13
2.6.7 Project 10: Greening surveys (African greening - <i>Candidatus Liberibacter africanus</i> & Asiatic greening - <i>Candidatus Liberibacter asiaticus</i> )	13

## TABLE OF CONTENTS

		Page
3	PORTFOLIO: INTEGRATED PEST MANAGEMENT	15
3.1	Portfolio summary	15
3.2	Programme: False Codling Moth	18
3.2.1	Programme summary	18
3.2.2	FINAL REPORT: A comparison of <i>Thaumatotibia leucotreta</i> (Meyrick) (Lepidoptera: Tortricidae) in organic versus conventional citrus orchards	22
3.2.3	FINAL REPORT: Genetic analysis and field application of a UV-tolerant strain of CrleGV for improved control of <i>Thaumatotibia leucotreta</i>	42
3.2.4	PROGRESS REPORT: Impact of abbreviated and complete cold-treatment on survival and fitness of FCM larvae	76
3.2.5	PROGRESS REPORT: Identification and development of an attractant for monitoring FCM adult females	78
3.2.6	PROGRESS REPORT: Field trials for control of FCM	79
3.2.7	PROGRESS REPORT: Synergism between insecticides for improved control of FCM	80
3.2.8	PROGRESS REPORT: Identification and evaluation of male false codling moth pheromones and an investigation of the usefulness for monitoring of female moths	80
3.2.9	PROGRESS REPORT: Using the antennal response of the FCM larval parasitoid, <i>Agathis bishopi</i> , for identifying key volatiles indicative of FCM fruit infestation	81
3.2.10	PROGRESS REPORT: Improving understanding of mating disruption	82
3.2.11	PROGRESS REPORT: Sterile insect technique (SIT) and mating disruption (MD) in the control of FCM	83
3.2.12	PROGRESS REPORT: Comparing the performance of and mating disruption (MD) for FCM control in netted and open orchards	84
3.2.13	PROGRESS REPORT: Evaluation of potential repellents for false codling moth, <i>Thaumatotibia leucotreta</i> (Meyrick) (Lepidoptera: Tortricidae)	85
3.2.14	PROGRESS REPORT: Selected Ion Flow Tube Mass Spectrometry for postharvest detection of FCM infested fruit	86
3.2.15	PROGRESS REPORT: Investigating the appropriate timing for initiation of mating disruption against FCM in Limpopo Province	87
3.2.16	PROGRESS REPORT: Selection for a UV-resistant isolate of a nucleopolyhedrovirus for improved field persistence and efficacy against FCM	88
3.2.17	PROGRESS REPORT: Regional differences in sex pheromones and sexual attractiveness in FCM	89
3.2.18	PROGRESS REPORT: An investigation into the biological and genetic stability of UV-tolerant baculoviruses for improved control of FCM	90
3.2.19	PROGRESS REPORT: Accurate monitoring of FCM fruit infestation	91
3.2.20	PROGRESS REPORT: Evaluating hot air treatments for postharvest FCM control	92
3.3	Programme: Fruit Fly	92
3.3.1	Programme summary	92
3.3.2	FINAL REPORT: Efficacy of FCM partial cold treatments for fruit fly pests of citrus	94
3.3.3	FINAL REPORT: Understanding fruit fly trap efficiency: the role of physical and biotic variables	118
3.3.4	PROGRESS REPORT: The impact of interruptions on Medfly cold treatment efficacy	127
3.3.5	PROGRESS REPORT: Alternate hosts of the Oriental Fruit Fly, <i>Bactrocera dorsalis</i> , in the Sundays River Valley	127
3.3.6	PROGRESS REPORT: Fruit fly rearing	128

## TABLE OF CONTENTS

		Page
3.3.7	PROGRESS REPORT: Redefining dispersal potential for adequate fruit fly pest management (Diptera, Tephritidae)	129
3.3.8	PROGRESS REPORT: In-silico boosted, pest prevention and off-season focussed IPM against new and emerging fruit flies	130
3.3.9	PROGRESS REPORT: Rearing technology and taxonomy of the Cape fly <i>Ceratitis quilicii</i>	131
3.3.10	PROGRESS REPORT: Efficacy of fruit fly systems approach for export of citrus to EU	131
3.3.11	PROGRESS REPORT: Host status of citrus for <i>C. cosyra</i>	132
3.3.12	PROGRESS REPORT: F <sup>3</sup> (Fruit fly free)	133
3.3.13	PROGRESS REPORT: Utilization of <i>Fopius arisanus</i> for control of Oriental fruit fly	134
3.4	Programme: Other Pests	135
3.4.1	Programme summary	135
3.4.2	FINAL REPORT: Determine the primary cause for mealybug repercussions under netting	137
3.4.3	FINAL REPORT: Determining the LC <sub>50</sub> and LC <sub>90</sub> values for spinetoram on <i>Scirtothrips aurantii</i>	154
3.4.4	PROGRESS REPORT: Controlling mites on budwood	164
3.4.5	PROGRESS REPORT: IPM under nets in Mpumalanga Province	165
3.4.6	PROGRESS REPORT: Integrated pest management under nets in the Western Cape	166
3.4.7	PROGRESS REPORT: Chemical control of mealybug on Citrus	167
3.4.8	PROGRESS REPORT: Augmentation of <i>Aphytis melinus</i> DeBach (Hymenoptera: Aphelinidae) for the control of California red scale (Hemiptera: Diaspididae) in citrus	167
3.4.9	PROGRESS REPORT: A comparison of control of key citrus pests in orchards under nets, in a bio-intensive IPM programme and a conventional programme	168
3.4.10	PROGRESS REPORT: Investigating delivery systems for formulation and application of microbial control agents	170
3.4.11	PROGRESS REPORT: Identification and management of new lepidopteran pests on citrus	171
3.4.12	PROGRESS REPORT: Development of molecular detection tools for the identification of citrus pests and natural enemies.	173
3.4.13	PROGRESS REPORT: Monitoring and control techniques for Australian bug	174
3.4.14	PROGRESS REPORT: The influence of timing, insecticide residues and hyperparasitism on the efficacy of <i>Anagyrus vladimiri</i> augmentation for mealybug control	175
3.4.15	PROGRESS REPORT: New systemic insecticides for citrus	176
3.4.16	PROGRESS REPORT: Distinguishing <i>Diaphorina</i> spp. and <i>Trioza</i> spp. from other psyllids likely to be caught on yellow traps	177
3.4.17	PROGRESS REPORT: Development of novel monitoring and control tools for citrus psyllids	178
4	PORTFOLIO: DISEASE MANAGEMENT	179
4.1	Portfolio summary	179
4.2	Programme: Graft Transmissible Diseases	184
4.2.1	Programme summary	184
4.2.2	FINAL REPORT: Application of high-throughput sequencing (HTS) for routine virus and viroid detection in high value accessions.	185
4.2.3	PROGRESS REPORT: Comparison of shoot tip grafted citrus with field-cut (old clone) material	193

## TABLE OF CONTENTS

		Page
4.2.4	PROGRESS REPORT: Field testing of commercial or potentially important rootstock selections for viroid sensitivity	193
4.2.5	PROGRESS REPORT: Field evaluation of three single-strain CTV isolates on navel and soft citrus cultivars	194
4.2.6	PROGRESS REPORT: Field evaluation of two approved cross-protection sources for Grapefruit	195
4.3	Programme: Preharvest Diseases	196
4.3.1	Programme summary	196
4.3.2	FINAL REPORT: Epidemiology and management of <i>Botrytis cinerea</i> in citrus	202
4.3.3	FINAL REPORT: Influence of shade nets on Alternaria brown spot and citrus black spot: comparing epidemiological model output for covered (under shade nets) and uncovered (normal/open) orchards' weather datasets	216
4.3.4	FINAL REPORT: Management of pruning debris as part of the citrus black spot control strategy	225
4.3.5	FINAL REPORT: Epidemiology, inoculum potential and infection parameters of citrus black spot	231
4.3.6	FINAL REPORT: Development of a rapid molecular tool to detect benzimidazole resistance in field-collected <i>Phyllosticta citricarpa</i> isolates	239
4.3.7	FINAL REPORT: Unravelling the clonal distribution of <i>Phyllosticta citricarpa</i> through a Genotyping-By-Sequencing approach	250
4.3.8	PROGRESS REPORT: Evaluation of new spray programmes for the control of Alternaria brown spot in the summer rainfall regions of South Africa	270
4.3.9	PROGRESS REPORT: Development of new spray programmes for the control of citrus black spot	274
4.3.10	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	284
4.3.11	PROGRESS REPORT: Evaluation of alternative products for control of citrus nematode and <i>Phytophthora</i> spp. in citrus	285
4.3.12	PROGRESS REPORT: Characterization and management of Valley Bushveld Citrus Decline	301
4.3.13	PROGRESS REPORT: Further validation and improvements of CRI-Phytrisk	302
4.3.14	PROGRESS REPORT: New oomycete fungicide-based approaches for managing <i>Phytophthora nicotianae</i> in citrus orchards	303
4.3.15	PROGRESS REPORT: Effect of citrus rootstocks on phosphonate applications for <i>Phytophthora</i> disease management in citrus	303
4.3.16	PROGRESS REPORT: Sources of <i>Phytophthora</i> spp. infestation in citrus nurseries	304
4.3.17	PROGRESS REPORT: Phosphonate sensitivity of <i>Phytophthora nicotianae</i> in South African citrus orchards and nurseries	305
4.3.18	PROGRESS REPORT: Epidemiology and control of <i>Colletotrichum</i> species associated with anthracnose on citrus in South Africa	306
4.3.19	PROGRESS REPORT: Factors affecting results of citrus nursery <i>Phytophthora</i> Testing	307
4.3.20	PROGRESS REPORT: Further validation of the current CBS diagnostic protocols	308
4.3.21	PROGRESS REPORT: Sensitivity profiles of <i>Phyllosticta citricarpa</i> to quinone outside inhibitors and methyl benzimidazole carbamates	308
4.4	Programme: Postharvest Diseases	309
4.4.1	Programme summary	309

## TABLE OF CONTENTS

		Page
4.4.2	PROGRESS REPORT: Provision of an industry service whereby new packhouse treatments are comparatively evaluated, fungicide resistance is monitored and standardised recommendations are provided	311
4.4.3	PROGRESS REPORT: Optimising available alternative postharvest remedies as replacement for imazalil use on citrus exported to Europe	316
4.4.4	PROGRESS REPORT: Investigating rind aspects of mandarins, an important commercial citrus type, which affects efficacy of chemical application during postharvest treatments	317
4.4.5	PROGRESS REPORT: Increasing occurrence of saprophytic stem end growth - investigating the causes and species involved	318
4.4.6	PROGRESS REPORT: Management of postharvest diseases in the near-harvest period	319
4.4.7	PROGRESS REPORT: Evaluation of essential oil volatiles in degreening chambers for the management of <i>Penicillium</i> decay and Sour rot	319
4.5	Programme: Huanglongbing	320
4.5.1	Programme summary	320
4.5.2	FINAL REPORT: Application of CTV infectious clones to combat HLB	326
4.5.3	FINAL REPORT: Validation of LAMP diagnostics for in-field detection of HLB	336
4.5.4	FINAL REPORT: Studies to improve seed production of rootstock trees	343
4.5.5	PROGRESS REPORT: New systemic insecticides for citrus	405
4.5.6	PROGRESS REPORT: Evaluation of new University of Florida (UF) rootstocks	405
4.5.7	PROGRESS REPORT: Distinguishing <i>Diaphorina</i> spp. and <i>Trioza</i> spp. from other psyllids likely to be caught on yellow traps	406
4.5.8	PROGRESS REPORT: Development of novel monitoring and control tools for citrus psyllids	407
4.5.9	PROGRESS REPORT: Determination of genome diversity of citrus infecting ' <i>Candidatus Liberibacter africanus</i> ' species and subspecies	408
4.5.10	PROGRESS REPORT: Evaluation of the influence of CTV infection on ' <i>Candidatus Liberibacter africanus</i> ' titre	409
4.6	CRI Diagnostic Centre	410
5	PORTFOLIO: CITRICULTURE	413
5.1	Portfolio summary	413
5.2	Programme: Rind condition and cold chain	413
5.2.1	Programme summary	413
5.2.2	PROGRESS REPORT: Investigation of factors contributing toward the non-conformance of in-transit citrus container shipments to cold protocol markets	414
5.2.3	PROGRESS REPORT: An investigation into aspects affecting chilling injury of citrus	415
5.2.4	PROGRESS REPORT: Optimise 2,4-D applications and investigate alternatives for calyx retention	416
5.2.5	PROGRESS REPORT: Modelling citrus inland supply chains for improved handling practices	417
5.2.6	PROGRESS REPORT: Investigating the relationship between creep testing of corrugated cartons and current industry test methods	418
5.2.7	PROGRESS REPORT: Designing integrated packaging systems for enhanced cold treatments	418
5.2.8	PROGRESS REPORT: Optimisation of pallet base designs for the citrus industry	419
5.2.9	PROGRESS REPORT: Enhancing phytosanitary cold treatment capabilities of reefer refrigerated containers during citrus exports	420

## TABLE OF CONTENTS

		Page
5.3	Programme: Production and quality	421
5.3.1	Programme summary	421
5.3.2	FINAL REPORT: Studies to improve seed production of rootstock trees	422
5.3.3	FINAL REPORT: Adaptive nutrition management strategies for improved fruit quality	483
5.3.4	PROGRESS REPORT: PROGRESS REPORT: The influence of shade netting and rootstock choice on the oleocellosis incidence of citrus varieties, Navel oranges, and Eureka lemons	514
5.3.5	PROGRESS REPORT: The use of root growth restricting soil management practices to improve Valencia tree vigour, yield and fruit quality	515
5.3.6	PROGRESS REPORT: Evaluation of strategies to improve water use efficiencies in citrus production	516
5.3.7	PROGRESS REPORT: Water stress at different phenological stages of Nadorcott Mandarin trees in combination with two irrigation systems to mitigate water shortages in citrus growing areas	517
5.4	Programme: Cultivar evaluation	518
5.4.1	Programme summary	518
5.4.2	PROGRESS REPORT: Evaluation of Valencia selections in hot humid inland areas (Onderberg)	520
5.4.3	PROGRESS REPORT: Evaluation of Valencia selections in the hot dry inland areas (Letsitele and Hoedspruit)	525
5.4.4	PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the hot inland areas (Letsitele and Hoedspruit)	539
5.4.5	PROGRESS REPORT: Evaluation of Valencia selections in the hot dry production areas (Tshipise)	545
5.4.6	PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the intermediate production areas (Marble Hall)	554
5.4.7	PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the hot dry inland areas (Tshipise)	557
5.4.8	PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the intermediate production areas (Karino and Ngonini)	561
5.4.9	PROGRESS REPORT: Evaluation of Navel selections in the intermediate production areas (Karino)	566
5.4.10	PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the cool-inland production areas (Ngodwana, Orighstad and Burgersfort)	570
5.4.11	PROGRESS REPORT: Evaluation of Lemon selections in the hot dry production areas (Letsitele and Hoedspruit)	584
5.4.12	PROGRESS REPORT: Evaluation of Valencia selections in the intermediate production areas (Nelspruit)	586
5.4.13	PROGRESS REPORT: Evaluation of Mandarin Hybrid on different rootstocks in a cold production area (Buffeljagsrivier)	590
5.4.14	PROGRESS REPORT: Evaluation of Grapefruit on different rootstocks in a semi-desert production area (Kakamas)	594
5.4.15	PROGRESS REPORT: Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region (Sundays River Valley)	598
5.4.16	PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Sundays River Valley)	600
5.4.17	PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Gamtoos River Valley)	603
5.4.18	PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Western Cape)	605

## TABLE OF CONTENTS

### Page

5.4.19	PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (South West Cape)	609
5.4.20	PROGRESS REPORT: Cultivar characteristics and climatic suitability of navel oranges in a cold production region (Sundays River Valley)	612
5.4.21	PROGRESS REPORT: Cultivar characteristics and climatic suitability of experimental navel oranges in a cold production region (Gamtoos River Valley)	616
5.4.22	PROGRESS REPORT: Cultivar characteristics and climatic suitability of experimental navel oranges in a cold production region (Western Cape)	619
5.4.23	PROGRESS REPORT: Cultivar characteristics and climatic suitability of Valencia oranges in a cold production region (Sundays River Valley)	623
5.4.24	PROGRESS REPORT: Cultivar characteristics and climatic suitability of Valencia oranges in a cold production region (Citrusdal)	626
5.4.25	PROGRESS REPORT: Cultivar characteristics and climatic suitability of Clementine mandarins in a cold production region (Sundays River Valley)	629
5.4.26	PROGRESS REPORT: Cultivar characteristics and climatic suitability of Clementine mandarins in a cold production region (Western Cape)	631
5.4.27	PROGRESS REPORT: Cultivar characteristics and climatic suitability of Clementine mandarins in a cold production region (South West Cape)	633
5.4.28	PROGRESS REPORT: Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region (Western Cape)	635
5.4.29	PROGRESS REPORT: Studies into the high incidence of chimeras of Valencia orange cultivars, specifically Valencia Late	637
5.4.30	PROGRESS REPORT: Evaluation of new University of Florida (UF) rootstocks	638
6	CITRUS IMPROVEMENT SCHEME (CIS)	639
6.1	Introduction	639
6.2	Budwood	640
6.3	Seed	649
6.4	Production	650
6.5	Tree Certification	653
6.6	Nursery Certification	654
6.7	Statutory Improvement Scheme	655
6.8	Protective zone surrounding the Citrus Foundation Block	656
6.9	FINAL REPORT: Shoot tip grafting and CIS diagnostic services at CRI-Nelspruit	656
6.10	FINAL REPORT: Diagnostic and technical services for the Citrus Improvement Scheme by the ARC-TSC	666
7	VOORLIGTING / EXTENSION	675
7.1	Voorligtingoorsig	675
7.1.1	Terugblik op die 2021 seisoen	675
7.1.2	Blik op die 2022 seisoen	676
7.1.3	CRI Postharvest technical forum	676
7.1.4	Postharvest extension	679
7.1.5	Produksiestreke	680
7.1.5.1	Wes-Kaap Produksiestreek	680
7.1.5.2	Oos-Kaap Produksiestreek	681
7.1.5.3	Sentrale Produksiestreek	683
7.1.5.4	Noordelike Produksiestreek	684
7.1.6	Research priority meetings	686

## TABLE OF CONTENTS

	<b>Page</b>
7.1.7 Simposium terugvoer	687
7.1.8 CRI Geïntegreerde plaag- en siektebestuur werksinkels	687
7.1.9 Tegnieese besoek aan Namibia	687
7.1.10 CRI Na-oes werksinkels	687
7.1.11 11de CRI Sitrusnavorsings Simposium	688
7.2 VOORLIGTING DIVISIE	688
7.3 OTHER MEANS OF TECHNOLOGY TRANSFER	717
7.3.1 SA Fruit Journal	717
7.3.2 CRI website	718
7.3.3 CRI Cutting Edge	718
8 PUBLICATIONS IN 2021-22	719
8.1 Refereed Publications (or ISI ranked journals)	719

# 1 MARKET ACCESS TECHNICAL COORDINATION

By Vaughan Hattingh and Elma Carstens (CRI)

## 1.1 Summary

An amended protocol for citrus to China was signed which included improved shipping temperatures for lemons after the first negotiations between the two countries started in October 2015. Live video inspections were conducted for the first time in the South Africa citrus industry to enable China to approve the list of facilities (production units, packhouses, cold storage facilities and inspection points) for export in the 2021 season. Citrus was successfully shipped to the Philippines under the new protocol, after initial problems with issuing of permits had been resolved. Requirements to prepare fruit for export to South Korea were revised and resulted in a significant reduction in the rejections during pre-shipment inspections. Pre-clearance inspections for exports to USA, South Korea and Japan continued on the same basis as agreed upon between SA and the trading partners as for the 2020 citrus export seasons. Some longstanding market access issues such as wider access for USA exports, wider and improved conditions of access to Japan and improved conditions for access to India remained pending. After nine years of negotiations, the Vietnamese Authorities will conduct a site visit in June 2022. CRI continued to play a key role in preserving market access to the EU despite reports of interceptions of CBS and FCM. The FCM Systems Approach (FMS) was significantly strengthened after the 2021 season in support of sustained access to the EU market. The CBS Risk Management System was revised to align with the new EU CBS regulations.

## Opsomming

'n Gewysigde protokol vir sitrus na China is onderteken, wat verbeterde verskepingstemperature vir suurlemoene ingesluit het, nadat die eerste onderhandelinge tussen die twee lande in Oktober 2015 begin het. Regstreekse video-inspeksies is vir die eerste keer in die Suid-Afrikaanse sitrusbedryf gedoen, om China in staat te stel om die lys van fasiliteite (produksie-eenhede, pakhuis, koel-opbergingsfasiliteite en inspeksiepunte) vir uitvoer in die 2021-seisoen, goed te keur. Sitrus is onder die nuwe protokol, suksesvol na die Filippyne verskeep, nadat aanvanklike probleme met die uitreiking van perмите opgelos is. Vereistes om vrugte vir uitvoer na Suid-Korea voor te berei, is hersien, en het tot 'n aansienlike vermindering in afkeurings tydens vóór-verskeping-inspeksies gelei. Fitosanitiere inspeksies vir uitvoere na die VSA, Suid-Korea en Japan het op dieselfde basis voortgegaan as wat tussen SA en die handelsvennote ooreengekom is, soos vir die 2020-sitrusuitvoeriseisoen. Sommige jarelange marktoegangskwessies, soos wyer toegang vir VSA-uitvoere, wyer en verbeterde voorwaardes vir toegang tot Japan, en verbeterde voorwaardes vir toegang tot Indië, het hangende gebly. Ná nege jaar van onderhandelinge, sal die Viëtnamese owerhede die bedryf in Junie 2022 besoek. CRI het voortgegaan om 'n sleutelrol in die behoud van marktoegang tot die EU te speel, ten spyte van CBS en VKM onderskeppings. Die VKM-stelselbenadering (FMS) is ná die 2021-seisoen aansienlik versterk, ter ondersteuning van volgehoue toegang tot die EU-mark. Die CBS-risikobestuurstelsel is hersien om by die nuwe EU CBS-regulasies te pas.

## 1.2 European Union (EU)

### FCM

In accordance with the procedure as agreed with DALRRD, CRI coordinated a process of reviewing the FMS in light of the 2021 season results. The FMS Core Technical Team and the FMS Working Group met in October to discuss the proposed amendments to the FMS for 2022. A meeting with DALRRD took place on 10 November 2021 to discuss the amendments as approved by the FMS Working Group. The final FMS for 2022 was approved by DALRRD for implementation on 18 November 2021. CRI sent out a Cutting Edge to highlight the amendments to the FMS for the 2022 export season.

At the end of the 2021 citrus export season, South Africa had received notifications of 15 FCM interceptions in the EU. CRI coordinated the actions of the FMS Rapid Response group to conduct desk top investigations on the 15 interceptions and provided the reports to DALRRD. CRI also provided inputs on all the DALRRD investigation reports that were submitted to the EU.

The new EU draft regulations, as published on the WTO Portal for a 60-day consultation period and on the European Commission 'Have your Say', consist of a Commission Implementing Regulation that amends part of Regulation 2019/2072, specifically the part dealing with certain fruit types as hosts of FCM (citrus excluding lemons and West Indian Limes, pomegranates, peppers, peaches and nectarines), and a revision of Annex VII describing special requirements for FCM. Opportunity was provided for a response to the amended regulations by posting the comments on the WTO Portal by 11 April 2022 and on the European Commission 'Have your Say' by 10 March 2022.

Implementation issues with the new draft regulations entail: i) separate special requirements for oranges including navels and Valencias, ii) destructive sampling of 10% of the visually inspected fruit before export and iii) when cold treatment has been applied during transport, the consignment must be accompanied by a phytosanitary certificate and documentation extracted from the data loggers to prove the cold treatment. These issues were discussed with DALRRD on 23 February 2022. CRI provided a document to DALRRD in preparation for the meeting, detailing the implementation issues. DALRRD had similar meetings with the other implicated industries and will then engage with the European Commission.

After meetings with the other implicated industries, DALRRD provided a document to CGA and CRI which was used for their meeting with the EU on 17 March 2022. A further meeting took place on 24 March 2022 between DALRRD and the industry to clarify some of the issues. In this meeting DALRRD was requested to get clarification from the EU if the draft amended regulation provides for the type of treatment as in the FMS. This communication was sent to the EU on 25 March 2022. CRI provided inputs to DALRRD on SA's response to be submitted to the WTO Portal on the DRAFT COMMISSION IMPLEMENTING REGULATION AMENDING IMPLEMENTING REGULATION (EU) 2019/2072 IN RELATION TO MEASURES AGAINST *THAUMATOTIBIA LEUCOTRETA* (FCM). A meeting to discuss and finalise the response is scheduled for 6 April 2022.

CRI posted a response on the European Commission 'Have your Say' to call on the EU to withdraw the notified draft regulation as this draft proposal is without justification, arbitrary, disproportionate, unjustly discriminatory and ultimately unnecessary and to enter into bilateral discussion with South Africa to agree on a workable arrangement for the ongoing implementation of the effective systems approach that South Africa has been using for risk mitigation of *Thaumatotibia leucotreta* (FCM) in Citrus exports. At the end of this reporting period a new regulation for FCM had not as yet been published.

## **CBS**

At the end of the 2021 citrus export season, South Africa had received notifications of 43 CBS interceptions in the EU. CRI provided inputs on the 43 DALRRD investigation reports that were submitted to the EU. Additional measures to the current CBS RMS were introduced and communicated at the Annual Citrus Coordinating meeting (18 November 2021). CRI sent out a Cutting Edge to highlight the additional measures and also to urge producers to ensure that fruit is adequately protected as highly favourable weather conditions for CBS infections were experienced in all the production areas. CRI sent out Cutting Edge (No 329) to communicate the spray programmes for CBS management for 2021/2022 citrus season and the systems available to determine the associated risk.

CRI received the new draft Commission Implementing Regulation that amends the current EU emergency measures (Commission Implementing Decision (EU) 2016/715) for CBS on 01 February 2022 from DALRRD. The new draft temporary measures are for citrus from Argentina, Brazil, South Africa, Uruguay and Zimbabwe. The details for South Africa was presented in Annex III. The EU requested trading partners to provide feedback before 10 February 2022. CRI discussed the implications of the new draft regulations with DALRRD on 04 February 2022 and provided a document to DALRRD in preparation for the meeting, detailing the implementation issues. The problematic issues were requirements to: a) include an additional inspection (Phytosanitary) between packhouse and export, b) provide a list of approved orchards before the start of the export season and immediately provide updates related to the changes to that list, including the reason for those changes and c) verify that the orchard is CBS free when another orchard from the same PUC is detected.

A bilateral meeting took place between SA and the EU on 1 February 2022. DALRRD provided a document to Industry highlighting the discussion points. On 22 February 2022, CRI received the final CBS regulations from DALRRD. The final draft CBS regulations reflected all the changes proposed by SA – deletion of (a) and (c) and the need to provide the reasons for changes to the list.

A meeting between CBS RMS Working group and DALRRD took place in March 2022 to align the amendments of the Regulation with the CBS RMS and the changes were communicated during the DALRRD and PPECB Pre Season Workshop. The changes entail the following: Floating consignments – only fruit from orchards appearing on the list can be presented for import inspection in the EU. The relevant import operators should remove fruit from orchards implicated in CBS interceptions in SA and in the EU from consignments arriving in the EU. It is for the exporter and the relevant operator to decide how the implicated fruit should be handled; Exporter to ensure that requested information is on the phytosanitary certificate – last inspection date (DALRRD to indicate which date) and the number of boxes per orchard; Different hit system – the “hit” is now per orchard and not per PUC and Orchards implicated in 2021 in local CBS interceptions and in the EU are not allowed to export in 2022. The new “hit system” for 2022 - The threshold for interceptions during local CBS interceptions and EU interceptions combined, will be three (3) orchards per citrus type and five orchards per PUC. CRI sent out a Cutting Edge to highlight the amendments to the CBS RMS for the 2022 export season.

### **Fruit flies**

The EU reported two fruit fly interceptions on South African citrus in the 2021 export season. At the Annual Citrus Coordinating Meeting (18 November 2021), no changes were made to the FFMS for the 2022 citrus export season.

### **EFSA**

The European Commission (EC) requested EFSA to provide an opinion on the FCM systems approach used by South Africa. Meetings took place between DALRRD and CRI to prepare a response to the questionnaire received from EFSA. DALRRD submitted the completed questionnaire to EFSA in February 2021. On 29 June 2021, DALRRD forwarded a request from EFSA to CRI for additional information. CRI submitted the information on 2 July 2021 and DALRRD submitted the information to EFSA. EFSA released their opinion on the FMS in July 2021. CRI obtained a review of the FMS opinion from 2 expert reviewers. A meeting between CRI and DALRRD took place on 30 November 2021 to discuss the reviews. The documents were provided to DALRRD on 01 December 2021. Due to technical problems DALRRD did not receive the documents and the documents were resent on 14 January 2022. CRI and DALRRD had a meeting on 26 January 2022 in preparation for bilateral EU/SA meeting on 26 January 2022 to further discuss the CBS and FCM interceptions and the EFSA response. DALRRD submitted the SA response on 03 February 2022. By the end of this reporting period, a response from EFSA was still pending.

### **1.3 Japan**

In 2021 there was again no response from Japan-MAFF on the three long outstanding market access requests: access for all mandarins (except Satsumas), under the current protocol for Clementines (pending from November 2009); revision of the current cold treatment conditions for the export of all eligible citrus types to Japan by the inclusion a cold treatment of 1.4<sup>0</sup>C or lower for 16 consecutive days (pending from November 2009) and inclusion of all navel orange cultivars in the current protocol (pending from September 2016). They continued with their explanation - Japan-MAFF only work on one market access request from a country at a time and the protocol for South African Avocado exports to Japan has not been finalised yet.

DALRRD received a request from Japan-MAFF to review the current protocol and to include all changes since the protocol was signed between to two countries. A meeting took place between DALRRD, CRI and PPECB on 02 August 2021. CRI provided inputs and DALRRD submitted the information to Japan-MAFF on 16 November 2021. Japan-MAFF responded in February 2022 that they are examining the proposed changes to the current protocol. They also indicated that they are concerned about the multiple interceptions of quarantine

pests (mealybugs) on the citrus from South Africa. They requested that export inspections in South Africa must be conducted more carefully. During the CRI Workshops in January and February 2022, the industry was informed that PPECB and DALRRD will monitor the mealybug rejections at packhouses and in the ports and if the rejections are unacceptably high, correction actions will be implemented. They also approved that exports can continue for the 2022 export season.

#### 1.4 USA

A bilateral meeting between DALRRD and USDA-APHIS took place on 14<sup>th</sup> October 2021 to discuss all the long pending issues - the equivalence between USA domestic CBS regulations and USA import regulations (access for fruit from CBS areas in South Africa); recognition and access for CBS pest-free places of production in an area of low pest prevalence; and inclusion of other Western Cape magisterial districts in the export program; the updated pest list and an updated work plan. At this meeting, the USDA-APHIS indicated that they are not in a position to comment on the time line regarding the publication of the CBS Administrative rule that will allow for the importation of citrus fruit from CBS areas. DALRRD requested the USDA-APHIS to provide a time line for both sides to be prepared. USDA-APHIS confirmed that the CBS mitigations measures will be harmonized with the current regulations in place in the USA for the movement of citrus fruit from CBS areas to CBS free areas. These comments were also confirmed at the meeting of the Task Group that took place on 19<sup>th</sup> October 2021. By the end of this reporting period, none of the long outstanding issues were addressed.

CRI updated the CRI Production Guidelines for FCM Control on Citrus in February 2022 to include the new research that led to the development of an improved method to conduct pre-harvest fruit infestation monitoring for FCM. This development was necessitated to strengthen the FMS (for exports to EU). DALRRD approved that the updated version of the CRI Guidelines to be used during the 2022 orchard verification process. However, for the USDA-APHIS program, DALRRD must inform the USDA-APHIS of any changes to the guidelines, as the Checklists to determine whether pests are managed according to these guidelines, are attached to the Workplan. Currently, Appendix 19 (Checklist for FCM Control), the method for FRUIT DROP SURVEY is the 5 trees used as a data station, conducted on same day EVERY WEEK. DALRRD sent a letter to USDA-APHIS on 23 February 2022, with the request to include both fruit infestation monitoring methods in Appendix 19. On 15 March, USDA- APHIS responded positively and agreed that both fruit infestation monitoring methods can be included and used.

#### 1.5 China

DALRRD submitted the draft list of facilities that are registered by DALRRD for export to China to the GACC in March 2021. The GACC responded that due to the COVID 19 situation they will not be able to conduct on-site inspections for the new facilities on the list. They requested live video inspections to be conducted. The GACC selected the PUCs and packhouses to be visited for the inspections. Numerous problems were encountered before the final three packhouses were identified. The services of Lucentlands were contracted for the live video inspections as well as a private translator. The video inspections took place on 9<sup>th</sup> and 10<sup>th</sup> June 2021. One of the packhouse was in the Western Cape and the other two packhouses were in Limpopo. On 22 June 2021 DALRRD received an approved a list from GACC, but when the list was circulated it was found that some packhouses were not on the list. A meeting took place between CRI and DALRRD and the decision was taken that a phase in period will be allowed until 30 June 2021 for PPECB to use both the 2020 list and the approved 2021 list. On request from DALRRD, GACC then (29 June 2021) approved the full list of facilities as sent to them in March 2021.

Due to the COVID 19 situation, the SA DALRRD Minister signed the amended protocol on 18 June 2021 during a virtual meeting. This revision provides for an improved cold treatment for lemons. The signed document was couriered to China to be signed by the GACC. The amended citrus protocol was finally signed by both Ministers and South Africa received the signed protocol on 2 September 2021 for implementation after the first negotiations between the two countries started in October 2015. The amended protocol stipulated a new cold treatment for lemons at a temperature  $\leq 3^{\circ}\text{C}$  for 18 days. Apart from the amended shipping temperature for lemons, the amended protocol also included a change to the carton label. The new carton label should state -

“输往中华人民共和国” (Exported to the People’s Republic of China). DALRRD discussed with the GACC and a phase-in period was allowed. During the phase-in period, PPECB inspected cartons destined for China with the old carton label until 30 September 2021. From 1 October 2021, the new label was required. The GACC also indicated that the label can be in English or Chinese.

## 1.6 South Korea

In April 2021 DALRRD called for a meeting to discuss the rate of mealybug interceptions in the port and on 23 April 2021 a communication was sent to all role players in the South Korea's export programme. In the communication all stakeholders were urged to conduct a thorough review of the procedures agreed upon, as detailed in the following documents: 1) Requirements for the management of mealybugs on citrus fruit exported to South Korea; and 2) Corrective actions to safeguard the South Korean citrus export program's future. It was also highlighted that PPECB will be inspecting fruit with a strict requirement of no actionable mealybugs and placing consignments with unidentifiable mealybug on hold pending mealybug identification.

At the Annual Citrus Coordinating Meeting (18 November 2021), the decision was taken that the following rule will be added to the “Corrective actions” to safeguard the South Korean citrus export program's future - On the 5<sup>th</sup> interception of an actionable mealybug during PPECB packhouse inspections, the implicated orchard will be disqualified for export for the remainder of the season. The implicated orchard will be flagged on Phytclean as “NP” - Not permitted for export to South Korea. To further mitigate the risk of mealybug interceptions, a decision was taken that all mealybugs intercepted at the packhouse and at the port will be submitted to the laboratories (CRI’s Laboratory in Nelspruit or the DALRRD Laboratory in Stellenbosch) for identification. The consignment will be put on hold by PPECB or DALRRD pending the result. The exporter can decide to divert the fruit if they cannot wait for the laboratory results. In March a meeting was held to discuss the operations wrt identification of mealybugs intercepted by PPECB in the packhouses and DALRRD in the port. It was agreed that mealybugs parasitized with parasitoids will be regarded as dead and not actionable. DALRRD and PPECB were informed accordingly.

## 1.7 India

No response was received from the Indian Authorities since June 2020 and in July 2021 DALRRD-IR wrote letters to the High Commissioners. The High Commissioners acknowledged receipt and indicated that the letters will be sent under a Note Verbale to the High Commission in India and also forwarded to SA High Commission in Delhi for the attention of the High Commissioner. On 20 September 2021, DALRRD received a letter from India indicating that the citrus trial shipment sent in May 2018 was considered unsuccessful due to a logger failure and the detection of *Elsinoë australis*, a pest known not to occur in South Africa. In the letter it was indicated that the trial shipments of apples and pears were regarded as successful. A meeting took place between CRI and DALRRD. DALRRD undertook to take the matter up with the Indian authorities. On 11 October 2021, DALRRD indicated that the matter was discussed with the DTIC Attaché in India and he has undertaken to take the matter up with the Indian authorities. DALRRD also requested DALRRD-IR to take this matter up with the DIRCO Asia desk. In a courtesy meeting between the two countries the issue was also discussed. On 2 November 2021, DALRRD undertook to request for a bilateral meeting with Indian authorities to discuss this matter. The Indian authorities indicated that a survey will be conducted to determine the presence and spread of *Phyllosticta citricarpa* in India. DALRRD sent a letter to the Indian Authorities on 13 February 2022 to inquire about all the outstanding issues. No response was received from the Indian Authorities and on 2 March 2022, DALRRD sent a letter to the Indian Authorities to request for technical bilateral meeting to discuss all the outstanding matters. DALRRD also included DIRCO and DTIC in the communication sent to the Indian Authorities. The Indian authorities responded and a meeting is scheduled for 7 April 2022.

## 1.8 Thailand

Table grape and citrus containers were rejected in Thailand. Concerns about the reasons for the rejections resulted in exporters terminating further fruit exports to this market. On 12 April 2021 DALRRD sent a letter to

DOA to seek clarification on the interpretation of the cold treatment. On 21 June 2021, the DOA responded, confirming that there are misunderstandings and inviting further exchanges to resolve the situation. A meeting took place between DALRRD, CRI, HORTGRO, PPECB and SATI to discuss a response to DOA and CRI coordinated a consolidated position. In the response, information on the improved FCM cold treatments was included, as well as a request to remove the mandatory FCM cold treatment for lemons due to the non-hosts status. The consolidated response (Citrus and Table Grapes) was submitted to DALRRD on 06 October 2021. DALRRD submitted the consolidated response (Citrus and Table Grapes) to DOA on 07 October 2021 and the DOC acknowledged receipt on 18 October 2021. Feedback is still pending from DOA despite follow ups by DALRRD.

### 1.9 **Vietnam**

The two outstanding issues to complete the workplan for fresh citrus fruit exports to Vietnam, remained the incomplete Pest Risk Analysis and a verification visit to South Africa. As a way to initiate a response from the Vietnamese Plant Protection Department (PPD), CRI approached DALRRD on 11 June 2021 to consider requesting the PPD to accept video inspections of citrus orchards and packhouses to finalize the workplan. DALRRD submitted the request to the PPD for video inspections of citrus orchards and packhouses via the South African Embassy in Hanoi. On 29 July 2021 DALRRD received a response from the PPD. The PPD indicated that they don't have regulations to allow for video inspections of citrus orchards and packhouses to finalize the workplan and due to the COVID 19 situation they will not be able to visit South Africa. They also did not accept South Africa's proposals to finalize the list of quarantine pests, the cold treatment protocol for FCM and the packhouse procedures. CRI provided a response to DALRRD on 19 April 2022. In March 2022 a meeting took place between South Africa and Vietnam. In order to finalise the bilateral protocol for export for fresh oranges, the PPD needs to undertake a site inspection visit to South African orange orchards to the monitor both the pre-harvest and post-harvest operations. During this meeting, the PPD proposed that such a visit took place in June and July to inspect both the pre-harvest and post-harvest operations. A proposed itinerary for the visit was submitted to DALRRD on 31 March 2022. The PPD agreed with the proposed dates and the visit will take place from 26 June 2022 – 4 July 2022.

### 1.10 **The Philippines**

The first consignment destined for the Philippines was inspected on Tuesday, 8 June 2021 by DALRRD after the first engagements with the BPI to establish a bilateral protocol for the export of fresh citrus fruit started in 2009. Despite several follow ups and letters by DALRRD and various industry partners, problems continued with the issuing of import permits by the BPI. In the letters sent by DALRRD, they explained the different lists and requested the BPI not to link an exporter to a specific production unit and/or packhouse. On 22 July 2021 DALRRD received a response from the BPI indicating that an exporter can source fruit from any production unit and/or packhouse. A communication was sent to all exporters via FPEF to ensure that the name in which the import permit was issued, is reflected in all the accompanying documents to enable clearing of consignments.

### 1.11 **Eswatini**

CRI provided assistance to Eswatini in drafting a response to the new EU draft regulations, as published on the WTO Portal for a 60-day consultation period (due date 11 April 2022) and on the European Commission 'Have your Say' (due date 10 March 2022), which consist of a Commission Implementing Regulation that amends part of Regulation 2019/2072, specifically the part dealing with certain fruit types as hosts of FCM (citrus excluding lemons and West Indian Limes, pomegranates, peppers, peaches and nectarines), and a revision of Annex VII describing special requirements for FCM.

### 1.12 **Zimbabwe**

CRI provided assistance to Zimbabwe in drafting a response to the new EU draft regulations, as published on the WTO Portal for a 60-day consultation period (due date 11 April 2022) and on the European Commission 'Have your Say' (due date 10 March 2022), which consist of a Commission Implementing Regulation that

amends part of Regulation 2019/2072, specifically the part dealing with certain fruit types as hosts of FCM (citrus excluding lemons and West Indian Limes, pomegranates, peppers, peaches and nectarines), and a revision of Annex VII describing special requirements for FCM.

A bilateral protocol to export fresh citrus fruit from Zimbabwe to China was signed between the NPPO of Zimbabwe and the NPPO of China. In March 2022 training was provided to the NPPO of Zimbabwe and Zimbabwean growers to understand and implement the requirements of the protocol to enable them to successfully export citrus to China in 2022.

### 1.13 Citrus one pagers

CRI provided inputs to the PHYTOSANITARY REQUIREMENTS AND WORKING PROCEDURES FOR EXPORT OF CITRUS FROM RSA to China, Japan, Philippines, Taiwan and Thailand (Citrus One Pagers). The documents were circulated to the industry.

### 1.14 Deviation for fruit age compliance on citrus that is intended for export

In July 2021 and August 2021 several meetings were held with DALRRD, PPECB and Industry role players to address the problems caused wrt compliance with the fruit age protocols (CBS-RMS and fruit quality inspections) due the civil unrest in KZN and the Transnet Cyber-attack. The implementation of CBS-RMS fruit age inspections were waived from 12 July 2021 - 08 August 2021. The fruit quality inspections were waived from 12 July 2021 - 26 August 2021.

## 2 BIOSECURITY

By Solomon Gebeyehu, Wayne Kirkman, Elma Carstens and Vaughan Hattingh (CRI)

### 2.1 Summary

A draft Master Plan appropriate for the context of the southern African citrus industry was reviewed by the Biosecurity Advisory Committee (BAC), and inputs incorporated to a final revision. Following the publication of presence of HLB/Las in coastal Kenya in early 2020, DALRRD subsequently communicated with KEPHIS (NPPO of Kenya) in the course of 2021 and a proposal outlining objectives of a joint HLB/Las scoping exercise was prepared by CRI and DALRRD and sent to KEPHIS in November 2021. Official response from KEPHIS to the proposal is pending at the time of this report.

CGA Zimbabwe representing commercial citrus growers and the Ministry of Agriculture/ the NPPO have established a Joint Technical Forum to serve as a platform for engagements to pursue common objectives of supporting the citrus producers in the context of Government of Zimbabwe being focused on uplifting the Horticulture Industry, with more emphasis on the Citrus Industry. Accordingly, the Joint Technical Forum has embarked on revising/updating Zimbabwe's plant health regulations as a matter of priority. CRI engagements also continued with contacts in other African countries including Namibia, Mozambique, Tanzania, Botswana, Eswatini, Kenya, and Ethiopia in a bid to pursue collaboration and regional awareness relating to HLB and ACP.

The HLB Action Plan and HLB Safe System for nurseries were revised and subsequently sent to CRI's international contacts in the USA and Brazil for peer-review by experts with many years of experience dealing with HLB and ACP. Reports of the peer-review have been received and are currently being studied by the Biosecurity Advisory Committee (BAC). CRI arranged training at CRC in September 2021 for staff working on HLB and ACP detection surveys on the identification and taxonomy of Psyllids by Dr Daniel Burckhardt, a taxonomist in the superfamily Psylloidea (Order Hemiptera), based at the Naturhistorisches Museum in Switzerland.

Citrus Leprosis (CL), affected farms in the Sundays River Valley, Kleinplaas, Halaron, Bellevue, and Elim East were audited for compliance to the CLRP in July 2021, and will be audited again in July 2022. The audits

focused on pruning, spray programmes, weed control, mite presence, controlled movement of people and record keeping. Three new findings of leprosis were reported in the SRV in 2021, and all are on, or have ties to farms previously implicated. The Response plan has been implemented in all three new cases.

CRI continued its contribution to the Phyto Risk Forum. The EU had earlier in the year requested South Africa to provide proof that *Xylella fastidiosa* is not present in South Africa. Relevant industries submitted 5 samples to the DALRRD Diagnostic Laboratory for official testing which were negative. The negative results were communicated to the EU by DALRRD, and on 21 April 2021 the EU accepted South Africa's declaration as a country free from *Xylella fastidiosa*.

A survey of citrus trees or plantations was conducted in the region between the Keurbooms and Groot rivers, and the Uniondale Magisterial in 2021, to evaluate the suitability of this region as an African greening buffer zone between the citrus production regions of the Eastern and Western Cape provinces. A proposal for a new buffer zone was submitted to the HLB Steering Committee and the BAC for discussion and approval that includes the area between the Groot and Bloukrans Rivers, the northern part of the region between the Keurbooms and Groot Rivers (where no citrus occurs), and the Uniondale Magisterial District. The final decision on the structure of the new buffer zone is pending, and will be reported next year.

## Opsomming

'n Konsep Meesterplan wat vir die konteks van Suider-Afrika se sitrusbedryf geskik is, is deur die Biosekuriteitsadvieskomitee (BAC) hersien en insette is by 'n finale hersiening ingesluit. Na die publikasie van die teenwoordigheid van HLB/Las vroeg in 2020 aan die kus van Kenia, het DALRRD in die loop van 2021 met KEPHIS (NPPO van Kenia) gekommunikeer en 'n voorstel wat doelwitte van 'n gesamentlike HLB/Las omvangsbepaling uiteensit, is deur CRI en DALRRD voorberei en in November 2021 aan KEPHIS gestuur. Amptelike kommunikasie van KEPHIS op die voorstel is nog uitstaande.

CGA Zimbabwe, wat kommersiële sitrusprodusente verteenwoordig, en die Ministerie van Landbou/die NPPO, het 'n gesamentlike Tegniëse Forum gestig. Dit sal dien as 'n platform vir vergaderings om gemeenskaplike doelwitte na te streef, om die sitrusprodusente te ondersteun in die konteks van die regering van Zimbabwe wat daarop gefokus is om die Tuinboubedryf op te hef, met meer klem op die Sitrusbedryf. Gevolglik het die Gesamentlike Tegniëse Forum begin met die hersiening/bywerking van Zimbabwe se plantgesondheidsregulasies as 'n saak van prioriteit. CRI se samewerking met kontakte in ander Afrika-lande het ook voortgegaan. Lande sluit Namibië, Mosambiek, Tanzanië, Botswana, Eswatini, Kenia en Ethiopië in, in 'n poging om samewerking en streeksbewustheid met betrekking tot HLB en ACP na te streef.

Die HLB Aksieplan en HLB Veilige Stelsel vir kwekerye is hersien en daarna aan CRI se internasionale kontakte in die VSA en Brasilië gestuur vir eweknie-evaluering deur kundiges met baie jare se ondervinding met HLB en ACP. Verslae van die eweknie-beoordeling is ontvang en word tans deur die Biosekuriteitsadvieskomitee (BAC) bestudeer. CRI het in September 2021 opleiding by CRC vir personeel wat op HLB en ACP opsporing opnames werk, gereël oor die identifikasie en taksonomie van bladvlooië deur Dr Daniel Burckhardt, 'n taksonoom in die superfamilie Psylloidea (Orde Hemiptera), wat by die Naturhistorisches Museum in Switserland gebaseer is.

Sitrusleprose geaffekteerde plase in die Sondagsriviervallei, Kleinplaas, Halaron, Bellevue en Elim-Oos is in Julie 2021 vir voldoening aan die CLRP geoudit, en sal weer in Julie 2022 geoudit word. Die oudits het op snoei, bespuitingsprogramme, onkruidbeheer, aanwesigheid van myte, beheerde beweging van mense en rekordhouding, gefokus. Drie nuwe ontdekkings van leprose is in 2021 in die SRV aangemeld, en almal is op, of het bande met, plase wat voorheen geïmpliseer is. Die Reaksieplan is in al drie nuwe gevalle geïmplementeer.

CRI het sy bydrae tot die Phyto Risk Forum voortgesit. Die EU het vroeër vanjaar Suid-Afrika versoek om bewys te lewer dat *Xylella fastidiosa* nie in Suid-Afrika voorkom nie. Relevante bedrywe het 5 monsters by die DALRRD Diagnostiese Laboratorium vir amptelike toetsing ingedien, wat negatief was. Die negatiewe

resultate is deur DALRRD aan die EU gekommunikeer, en op 21 April 2021 het die EU Suid-Afrika se verklaring as 'n land vry van *Xylella fastidiosa*, aanvaar.

'n Opname van sitrusbome/ boorde is in die streek tussen die Keurbooms- en Grootrivier, en die Uniondale-landdrostdistrik in 2021 uitgevoer om die geskiktheid van hierdie streek as 'n Afrika-vergroeningsbuffersone tussen die sitrus produksiestreke van die Oos- en Wes-Kaap provinsies, te evalueer. 'n Voorstel vir 'n nuwe buffersone is aan die HLB-bestuurskomitee en die BAC vir bespreking en goedkeuring voorgelê, wat die gebied tussen die Groot- en Bloukransrivier, die noordelike deel van die streek tussen die Keurbooms- en Grootrivier (waar geen sitrus voorkom) en die Uniondale Landdrostdistrik insluit. Die finale besluit oor die struktuur van die nuwe buffersone is hangende.

## **2.2 Develop and maintain a comprehensive citrus industry biosecurity plan - to ensure overall mitigation of the Southern African citrus industry's biosecurity risks**

### **2.2.1 Project 1: Develop a Southern Africa citrus industry biosecurity master plan**

In a bid to develop a comprehensive citrus industry biosecurity master plan that would serve as a road map to guide industry in addressing biosecurity threats in the context of increasing threats from invasive pests and diseases, biosecurity strategic plans and published articles written by similar industries around the world were reviewed. A draft Master Plan appropriate for the context of the southern African citrus industry was compiled and sent to the Biosecurity Advisory Committee (BAC) for review and inputs. It was subsequently revised substantially after inputs were received from BAC. The final working version is under preparation to send to CRI BoD and the CGA to highlight progress being made by the Biosecurity Division to build a sound biosecurity system for the citrus industry.

## **2.3 Biosecurity portfolio: Design, develop and oversee the implementation and operation of appropriate biosecurity structures, engagements, procedures, co-operations, resources, projects and other appropriate actions**

### **2.3.1 Project 2: Identify, assess and initiate engagement with international funding providers, for future support of Southern African biosecurity projects**

Through engagement with the USDA/Pretoria office to provide financial support for a regional training workshop on Citrus Greening funding was secured in 2020. However due to Covid related travel restrictions the training could not go ahead in 2020 and also in 2021. The plan was to invite NPPO officials from selected countries in the region to share information about the status of Citrus Greening in Africa with emphasis on the highly destructive Asian Citrus Greening/HLB and its primary vector Asian citrus psyllid (ACP) and raise awareness. ICIPE was selected as the ideal institute for the training because both the HLB disease and the vector are present in Kenya, and because ICIPE scientists have been conducting research for several years. Discussion is underway with ICIPE and SA's NPPO/DALRRD if it would be more convenient to arrange a virtual workshop instead of an in-person training. Ultimately the aim is to share relevant information and raise awareness about the threat of HLB and ACP to the citrus value chain in Africa. It is also the aim of the workshop to highlight the need for early detection survey and monitoring efforts by respective NPPO's and promote collaborative research and information sharing.

## **2.4 Networking and awareness: Obtain supportive participation of relevant stakeholders and interested parties**

At the virtual bilateral meeting held between the NPPO of SA (DALRRD) and the NPPO of Kenya (KEPHIS) in September 2020, KEPHIS expressed commitment to better understand the status of HLB/Las in coastal Kenya where it was recently reported, and explore a proposed joint SA/Kenya scoping work to look into the possibility of eradication or containment. Several months later KEPHIS replied to International Relations Directorate of DALRRD with an update that a comprehensive strategy has been prepared to mitigate HLB, and welcomed technical collaboration with DALRRD to conduct HLB/Las scoping work. A proposal outlining the objectives of the proposed joint scoping exercise, and suggested time frame for the visit was prepared by CRI

and DALRRD and sent to KEPHIS in November 2021. Official response from KEPHIS to the proposal is being awaited at the time of compiling this report.

CGA Zimbabwe representing commercial citrus growers and the Ministry of Agriculture/ the NPPO have established a Joint Technical Forum to serve as a platform for engagements to pursue common objectives of supporting the citrus producers in the context of Government of Zimbabwe being focused on uplifting the Horticulture Industry, with more emphasis on the Citrus Industry. Accordingly, the Joint Technical Forum has embarked on revising/updating Zimbabwe's plant health regulations as a matter of priority. CRI has provided a copy of SA's regulations on HLB and ACP that were promulgated in February 2021, as well as SA's Action Plan to be used as reference as Zimbabwe develops its own regulatory framework as part of the foundational work to develop preparedness and response plans to mitigate the threat of HLB and ACP. CRI also provided Mr John Perrott of Zimbabwe CGA with a detailed response to questions he raised about various aspects related to the management of HLB and ACP risks. The attention being given in Zimbabwe to mitigate the risks of HLB and ACP is seen as a very positive development in support of regional biosecurity.

The Biosecurity Division has been engaging with the officials of Namibia's NPPO to advance discussions on collaboration in citrus biosecurity. Accordingly, a trip was made to Namibia during the week of October 11-16, 2021 by Wayne Kirkman and Hannes Bester. The main aim of this trip was to encourage the development of Namibia Citrus Association by meeting with NPPO officials and growers, with strong emphasis on biosecurity, particularly the importance of controlling the movement of plant material to mitigate the threat of HLB and ACP to existing citrus production areas and areas planned for new development or extension. The trip was also an opportunity to visit some of the major citrus production areas. The need to form a local industry organisation and associate with the CGA was emphasized as a prerequisite for ongoing CRI support. The meetings were attended by at least 18 growers, and representatives of the NPPO.

## 2.5 **Ensure successful implementation of processes, procedures and interactions to ensure the timely identification and assessment of biosecurity threats facing the Southern African citrus industry**

### 2.5.1 Project 3: Develop and oversee implementation of Southern African citrus industry pest-specific action plans for priority biosecurity pests

In order to ensure that the import conditions for Citrus are updated, a list of all the mites occurring on citrus has been compiled. Mites have been identified that comply with the definition of a quarantine pest – *A quarantine pest is a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled (ISPM 5, FAO)*. According to the list (list of mites that comply with definition of a q-pest) the 'dangerous mites' - mites known to cause economic losses and which can result in market access problems are already listed as part of our import conditions. Four new mites, not currently part of the import conditions, were identified that might be of quarantine importance to South Africa. A request was submitted to the BAC to advice on the inclusion of these four mites. Feedback was received from BAC and in July 2021 a request was sent to DALRRD to include the four mites identified by CRI as potential quarantine pests, along with the current list as part of the import conditions. A response is pending from DALRRD.

*Phytophthora palmivora*, currently listed as a quarantine pest for South Africa on several hosts including citrus, was detected in papaya samples in the Tzaneen and Malelane areas. A DALRRD/Industries meeting to discuss the way forward took place on 2 February 2021 and several workgroups were formed to draft documents needed to conduct follow up surveys. Inputs were made to **DIAGNOSTIC SAMPLING AND DETECTION PROTOCOL FOR PHYTOPHTHORA PALMIVORA IN SOUTH AFRICAN SOILS** and the **DELIMITING SURVEY, SAMPLING AND CONTAINMENT MEASURES FOR PHYTOPHTHORA PALMIVORA**

## 2.6 **Ensure effective implementation of processes, procedures, interactions to advance actions required to successfully mitigate the risks and consequences of biosecurity incursions**

### 2.6.1 Project 4: Develop and oversee implementation of a Southern African citrus industry HLB action plan and safe tree production system

The HLB Steering Committee continued to meet virtually in the course of the reporting period almost every three months, with the latest meeting held virtually on March 23, 2022. The HLB and ACP Action Plan that was revised to align the plan with the regulation on HLB and ACP that was enacted by DALRRD in February 2021 (Regulation No. 44188 of 12 Feb 2021) as well as the revisions to sections relating to detection delimitation surveys to make the plan practically feasible to implement were tabled, discussed and adopted by the Steering Committee. The HLB Safe System document that was also revised, to address concerns raised by the Executive Committee of SACNA while maintaining the core objectives of the system to mitigate HLB risk was discussed and adopted. Both the HLB action plan and safe tree production system documents were subsequently sent to CRI's international contacts in the USA and Brazil for peer-review by experts with many years of experience dealing with HLB and ACP. Reports of the peer-review have been received and are currently being studied by the Biosecurity Advisory Committee (BAC).

CRI arranged training at CRC in September 2021 for staff working on HLB and ACP detection surveys on the identification and taxonomy of Psyllids by Dr Daniel Burckhardt, a taxonomist in the superfamily Psylloidea (Order Hemiptera), based at the Naturhistorisches Museum in Switzerland. The aim of the training was to expose CRI Entomology researchers and Biosecurity staff on sampling protocols for Psyllids, their identification, and determination of their host plants. Psyllids are known to be host specific hence the need to know their host plants in these citrus environments. The training was instrumental in laying a good foundation for current CRI funded Entomology research projects on Psyllids and early detection survey efforts of *D. citri* as part of activities of the Biosecurity Division. The CRI personnel trained now have a good understanding on Psyllid sampling protocols and background on characteristics used for their identification. As part of the visit by Dr Burckhardt, was two weeks of psyllid collection by Dr D. Burckhardt accompanied by Dr Evans Mauda, CRI's Entomologist working on Psyllids in indigenous flora surrounding citrus production areas in Limpopo and Mpumalanga, with focus on Diaphorina species that have close resemblance to the target *D.citri*. Subsequently Drs Mauda, Manrakhan and Burckhardt visited the Psyllid collection at the Biosystematics Division of the Plant Health and Protection Department at the Agricultural Research Council in Pretoria to meet with the relevant specimen curators/researchers, deposit some of the freshly collected Psyllid specimens, as well as establish collaboration to advance research work on Diaphorina species going forward.

CRI appointed the third biosecurity field officer Mr Tshepang Makitla in January 2022 stationed in Tzaneen as part of the 4-Year Plan to increase capacity to expand early detection surveys and preparedness to respond to incursions by HLB and ACP.

#### 2.6.2 Project 5: Ensure that HLB and ACP surveillance is undertaken in Eastern and Southern Africa

During the reporting period, the following activities have taken place in various countries.

##### Angola

During the visit in 2019 by Dr Paul Fourie and Wayne Kirkman, Dr Fourie pledged CRI's ongoing support for citrus biosecurity initiatives in Angola, but stressed that CRI does not have the capacity to conduct the required surveillance throughout Angola. CRI has kept in regular contact with Drs Daniel Bassimba (Instituto de Investigación Agronómica, Chianga, Huambo) and Camillo Jose (PlantCare, Luanda), both trained plant pathologists, to receive updates on biosecurity issues discussed during the visit. Enquiries will be made shortly to Secretary of State for Agriculture, Jose Carlos Lopes da Silva Bettencourt, to determine if imports of citrus plant material from Brazil have been banned, as discussed in a meeting in 2019.

##### Mozambique

Previously surveys were conducted in Mozambique's southernmost province (Maputo province) was in May 2019, and Inhambane province in March 2020. No *D. citri* or *T. erythrae* were found at any of the sites by visual inspection, tap sampling or on traps.

In a bid to continue monitoring, two hundred ACP traps were given to a missionary who operates in the Cabo Delgado province. Placements of these traps has not been possible due to violent unrest in Cabo Delgado.

In March 2022, a survey for ACP and HLB was conducted in the Beira – Chimoio – Machipanda corridor. Sweep netting for vectors and inspection for disease symptoms were conducted at 81 sites in the corridor, and ACP traps were placed at each site. No greening symptoms were observed. One unknown *Diaphorina* sp individual was observed, but not captured. Traps from the survey have been evaluated, and no *D. citri* were found. A meeting was held with the owner of Proodusola nursery near to Chimoio, and a few traps were given to her for monitoring. One concern is that they are selling trees to Zimbabwe, and follow up investigations are ongoing. Armando Marcos Come, one of the collaborators, is in charge of Quarantine Services, and he welcomed the possibility of *Tamarixia radiata* being released when ACP is first found in Mozambique. He will investigate import requirements.

#### Tanzania

Initial contact has been made with the head of the NPPO of Tanzania, to discuss permission for researchers at Morogoro in Tanzania to arrange collection of ACP samples twice a year and send to CRI for testing for Las, as an indicator of the southward spread of HLB. Response from the NPPO is pending.

#### South Africa

CRI has placed about additional traps in the area between Komatipoort and Mananga on the Eswatini border, as this area was highlighted as a risk by the HLB/ACP heat risk map. In total, CRI now monitors 265 traps, and evaluates 182 from DALRRD, 60 from growers, 80 from CFB, 90 from nucleus blocks and 24 from Eswatini. In total 701 ACP traps are evaluated by CRI, excluding the traps periodically received from other countries.

#### Eswatini

The major citrus producing Ngonini and Tambuti Estates send ACP traps from time to time, which are read and reports generated by CRI. The Eswatini border with Mozambique has been prioritised as high risk by the heat map, and so a visit is planned in June to place traps at strategic points along this border, as well as northern KZN.

#### Botswana

Engagement with the NPPO of Botswana to raise awareness about citrus greening and the HLB threat in light of initiate early detection survey for ACP and HLB in major citrus growing areas has received a positive response. Plan is underway to arrange a trip to Botswana to meet with NPPO officials and setting up ACP trap networks in key production areas.

### 2.6.3 Project 6: Facilitate initiation of an HLB eradication plan in Ethiopia

On the basis of the results produced by CRI's diagnostics lab from the 2019 sampling of a citrus commercial citrus farm in Ethiopia owned by Africa Juice in which the presence of HLB/Las was confirmed, the Ethiopian collaborators from Wolayta Sodo University expanded the sampling exercise to the central and southern regions of Ethiopia and their results showed the presence of HLB and ACP in these parts of the country, indicating a more widespread presence than previously thought. The result of their finding was published in a peer reviewed paper: Detection of Huanglongbing, insect vectors and nutritional profile of citrus in Upper Awash, Ethiopia, *Journal of Plant Pathology (2022) 104:17–31*. The fact that local researchers have shown interest and pursued research on citrus greening and associated vectors is viewed as a positive development for regional citrus biosecurity efforts going forward.

### 2.6.4 Project 7: Ensure, in close collaboration with relevant government officials, that regulations of relevance to biosecurity risk mitigation are appropriately updated and compliance effectively implemented

This project will ensure that the relevant Acts and regulations are aligned with the relevant actions plans. Country specific import conditions for citrus propagation material and seeds were drafted and submitted to DALRRD. The document - The DETAILED PROTOCOL FOR THE IMPORTATION AND POST-ENTRY QUARANTINE MANAGEMENT OF PROPAGATION MATERIAL OF CITRUS SPP (BUDWOOD, TISSUE CULTURES AND SEED) was accepted by the work group on 5 May 2021.

Country specific import conditions for citrus propagation material and seeds were drafted and submitted to DALRRD. DALRRD submitted the import conditions to the specific countries. Comments were received from the USA. CRI provided inputs as requested by DALRRD.

*Ceroplastes floridensis* is listed as a quarantine pest for Citrus. Problems were encountered with the listing when pest information packages for deciduous fruit indicated that the pest is present in South Africa. They based their listing on the Crop Protection Compendium (CPC). CRI provided information to the editor of the CPC that the pest is not present in South Africa. The editor updated the datasheet and the current status for this pest is now - 'Absent, unconfirmed presence record'.

After consultation with DALRRD and growers, *Bactrocera dorsalis* has been declared established in the Sundays River Valley. This will be beneficial to the SRV, as lemons make up 40% of total citrus production in the region. Lemons are recognised a non-host, and so no control actions, including removal permits, other than monitoring, are required in lemon orchards. Orders have been issued, and removal permit applications are being processed. R110 will be updated accordingly. Inputs were provided to the notification submitted by the South African NPPO to the IPPC in December 2021 on the change of the status of *B. dorsalis* in the Eastern Cape Province of South Africa.

#### 2.6.5 Project 8: Monitoring and Control of Leprosis

The affected farms in the Sundays River Valley, Kleinplaas, Halaron, Bellevue, and Elim East were audited for compliance to the CLRP in July 2021, and will be audited again in July 2022. The audits focussed on pruning, spray programs, weed control, mite presence, controlled movement of people and record keeping. Halaron complied fully with all requirements of the CLRP, and it has been recommended that the Red1 status be downgraded. The affected orchards have subsequently been destroyed. There were once again no major non-compliances on Bellevue and Elim East. Bellevue was purchased by SRCC, and the Delta and Midnight Valencia orchards where leprosis was first discovered were removed and destroyed. The remaining affected orchards, Cara navels, are under good management and no new symptoms were detected. Once again the major finding at Kleinplaas was that the weeds and ground cover (Wandering Jew) had not been removed, and so the status of the farm remains Red-1. Three new findings of leprosis were reported in the SRV, and all are on, or have ties to farms previously implicated. The Response plan has been implemented in all three new cases.

#### 2.6.6 Project 9: Phytosanitary Risk Forum

The EU had earlier in the year requested South Africa to provide proof that *Xylella fastidiosa* is not present in South Africa. At the last meeting it was discussed and agreed that each relevant industry must submit 5 samples to the DALRRD Diagnostic Laboratory for official testing. The industries submitted the samples, the negative results were communicated to the EU by DALRRD, and on 21 April 2021 the EU accepted South Africa's declaration as a country free from *Xylella fastidiosa*.

#### 2.6.7 Project 10: Greening surveys (African greening - *Candidatus Liberibacter africanus* & Asiatic greening - *Candidatus Liberibacter asiaticus*)

##### **African greening surveys conducted in the buffer zone established on the border of the Western and Eastern Cape provinces including the African greening free Western Cape magisterial districts of Knysna and Uniondale**

In 2012 the Citrus Industry requested the Department of Agriculture, Land Reform and Rural Development (DALRRD) to amend the Control Measures R.110 of 27 January 1984 to provide for the establishment of two buffer zones to protect the Eastern Cape (EC) from African greening (*Candidatus Liberibacter africanus* (CLaf)). The following two buffer zones were identified to provide the Eastern Cape with an Accepted Level of Protection (ALOP) from CLaf.

- *The EC border region between the provinces of KZN and EC, including the EC district municipalities of Alfred Nzo and Oliver R Tambo, is recognised as an Area of Low Pest Prevalence (ALPP).* Detection surveys conducted from 2008 and onwards, had detected isolated occurrences of CLaf-infected trees in these two magisterial districts of the EC close to the border of KwaZulu-Natal (KZN) province, but the remainder of the EC has remained free from CLaf.
- *The Western Cape (WC) border region adjoining the Eastern Cape, recognised as a Pest Free Area (PFA).* CLaf is known to occur in some magisterial districts of the WC, but the region adjoining the Eastern Cape, including the WC magisterial districts of Uniondale and Knysna were considered free from African greening.

In 2014, Control Measures R.110 of 27 January 1984 was amended by means of R.442 of 06 June 2014, to include the two buffer zones.

Phytosanitary measures for the WC and EC buffer zones include prohibited movement of plant material of Citrus and related genera into the buffer zones from areas where CLaf occurs, as well as prohibited movement from the buffer zones to any PFA (including the EC province). Maintenance of the buffer zone requires periodic detection surveys for CLaf and mandatory eradication of CLaf-infected trees.

Maintenance detection and delimitation surveys were conducted in the WC buffer zone from 2017 to 2020. These surveys included commercial orchards and home gardens in the Knysna magisterial district, including the towns of Knysna, Belvedere, Reenendal, Sedgefield, Wittedrif, Harkerville and Karatara, as well as the Uniondale magisterial district. From these surveys 130 samples from the Knysna magisterial district were submitted to DALRRD's laboratory in Stellenbosch and 27 of the 130 samples tested positive for CLaf. DALRRD issued orders to the implicated owners and the trees were removed. None of the samples taken from the Uniondale magisterial district tested positive for CLaf.

CLaf was detected in all of the suburbs in Knysna and Belvedere where surveys were conducted, so it is evident that the disease is established in this area. During the surveys access was gained to only about 25% of the homesteads, due to owners being away or denying access, so the survey is an underestimate of the occurrence of CLaf-infected trees in the area. The abundance of host trees in the area makes continued eradication unmanageable. It is therefore considered impractical to pursue eradication of CLaf from the two towns (Knysna and Belvedere).

There are several deep river valleys and gorges in the region east of the Keurbooms River that could provide a suitable geographic demarcation of a new buffer zone, and the region is less populated than Knysna and Belvedere. The initial proposed new buffer zone was the area between the mouths of the Keurbooms and Groot River (Natures Valley) along the coast, stretching inland to around the N2 highway.

A survey of citrus trees or plantations was conducted in the region between the Keurbooms and Groot rivers between 8 and 10 September 2021, to evaluate the suitability of this region as an African greening buffer zone between the citrus production regions of the Eastern and Western Cape provinces. The survey was conducted by following as many roads with open access in the time available, and plotting all visible citrus trees or plantations. Due to the preliminary nature of the survey, no access was sought into private property or where gates were closed/locked. It was the opinion of the surveyors that this region could be utilised as a CLaf-free buffer zone and would be more easily and effectively managed than the Knysna area. Thirty-one sites containing citrus were identified without accessing any private property, so an intensive survey could reveal more. Many of the sites contained a single tree, but some contained between 4 and 10 trees, and only one orchard was observed.

The villages of Keurboomsstrand and Natures Valley were briefly visited, with 6 sites being identified at Keurboomsstrand. An added benefit would be that Groot River, which constitutes the Eastern border of this proposed buffer zone, forms an extremely deep and wide gorge, making the likelihood of an infected psyllid crossing this almost zero. In summary, the region could be utilised as an effective buffer zone, as there is enough citrus in the region to constitute such a zone, but not too much to make CLaf eradication impossible.

After internal CRI discussions, due to the many citrus trees found in the area between Keurboomsstrand and Natures Valley, it was decided to conduct a further exploratory survey in the region between the Groot and Bloukrans Rivers as a possible buffer zone. This survey was conducted in October 2021. Only three citrus trees were located and DALRRD took samples which tested negative for CLAs. This makes this region suitable for a buffer zone, as it has very little citrus and has extremely deep river gorges on either side, which would be virtually impossible for infected *T. erythrae* to traverse.

A survey was also conducted in the Uniondale Magisterial in November 2021. Many citrus trees were located, especially in the town itself, but no greening symptoms or presence of vectors were observed. No commercial orchards were found in the district. Some samples were taken but tested negative for CLAs. This indicates that the Uniondale Magisterial District appears to be CLAs free, and is still suitable for being part of a greening free buffer zone. Therefore, the following proposal for a new buffer zone was submitted to the HLB Steering Committee and the BAC for discussion and approval:

- The area between the Groot and Bloukrans Rivers
- The northern part of the region between the Keurbooms and Groot Rivers, where no citrus occurs, but links the first region to the Uniondale Magisterial District.
- The Uniondale Magisterial District

The final decision on the structure of the new buffer zone will be included in the next report as both meetings took place in April 2022.

### **African greening surveys conducted in East London, part of African greening free Eastern Cape Province**

In March 2022, a positive "African Greening" *Trioza* was found in a DALRRD surveillance trap in East London. The trap was near the East London port and next to a residential area. A delimiting survey was conducted by DALRRD and CRI in the week of 21 March 2022. Samples were taken from the only two citrus trees found in the residential area next to the trap. Samples were also taken from a neglected orchard about 50 km from the trap and a lemon orchard about 20 km from the trap. Samples were submitted to CRI Laboratory in Nelspruit and DALRRD's laboratory in Stellenbosch. Results were not available by the end of this reporting period.

## **3 PORTFOLIO: INTEGRATED PEST MANAGEMENT**

### **3.1 PORTFOLIO SUMMARY**

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

CRI is now in its first year of the dramatically increased levy. Consequently, the IPM Research Portfolio, as with other portfolios and divisions within CRI, has grown significantly. Not only has the number of projects grown relative to the previous year, but the number of collaborators has also increased. Consequently, we have been able to take on far more research than previously, something that is still very much in its growing phase. Fortunately, the impediments that the Covid-19 pandemic threw at us are something of the past. However, these seemed to be a greater problem for our collaborators, particularly the universities, who were subject to direct bureaucratic restrictions. A further challenge has been that of load-shedding, which can interject cold treatment trials and damage sensitive and expensive laboratory equipment. Despite these challenges, progress with research within the IPM Portfolio has been good.

Within the IPM Portfolio, there are three research programmes: FCM, Fruit Fly and Other Pests. FCM remains the biggest of the three programmes, reflective of the quarantine status of this pest for several markets, most prominently, the European Union. However, the other two programmes have also grown notably.

Research within the FCM programme has focussed on both preharvest and postharvest management of FCM, with a good balance between research that produces rapid applied results and more long-term high-tech research. Highlights in this programme include the following. It was demonstrated that a granulovirus isolate

(CrleGV) selected for its resistance to UV-irradiation retained its genetic integrity when passaged twice through FCM, indicating that it may be possible to produce this isolate in vivo, without reversion to the original state of UV-sensitivity. It was demonstrated in a field trial that both Coragen and Exirel have a significant mating disruption effect on FCM, in addition to their insecticidal effect, thus effectively increasing the efficacy of both products against FCM. Two separate studies showed a synergistic effect between yeast and CrleGV, and between Neem oil and CrleGV, thus potentially significantly improving the efficacy of a product like Cryptogran. A range of potential repellents to FCM oviposition were also identified. In a comparison of FCM on organic and conventional farms, it was determined that lower levels of FCM in organic orchards could be attributed to several factors, including differences in fruit composition and soil-dwelling generalist predators.

Research within the Fruit Fly Programme, like the FCM programme, also consisted of a good balance between preharvest and postharvest studies. This programme also included three international collaborative studies that were externally funded. Highlights in the programme include the following. Two new cold disinfestation treatments were developed for fruit flies, being 3.5°C for 24 days and 5°C for 27 days. These were based on the finding that Medfly is the most cold-tolerant of the fruit fly species and therefore all cold studies can simply be conducted with this species, with relevance for all of the others. It was also determined that the Cape fly, *Ceratitis quilicii* is more widely distributed throughout South Africa than the Natal fly, *C. rosa*, emphasising the importance of further studies on the former species, even though it has not yet been shown to infest citrus in the field. In a study on the development of a systems approach for fruit flies, it was shown that good agricultural practices in the field and sorting during and post-harvest, were effective in limiting or preventing fruit fly infestation.

In the Other Pests Programme, a wide range of entomological issues on citrus were studied. The one that probably received the most attention was various aspects of psyllids in preparation for the arrival of the Asian citrus psyllid (ACP) that vectors the devastating bacterial disease, Huanglongbing or Asian greening. Various studies were also conducted on citrus thrips, mealybug, red scale, Australian bug, various Lepidoptera, and the effect of nets on pest management in general. Highlights in this programme include the following. It was determined that there is no sinister reason why mealybug levels are higher under nets, such as compromised biocontrol. It is simply that nets create a more favourable and protected environment. A benchmark for the efficacy of spinetoram for thrips was determined in the Eastern Cape, with no indication of thrips resistance to this compound. It was determined that early season releases of *Anagyrus vladimiri* can be effective for controlling mealybug, but that this can be unpredictably undermined by hyperparasitoid activity. Several *Diaphorina* species have been discovered in and around citrus orchards, which closely resemble ACP. This is important preparatory work for the arrival of ACP, so that any confusion is avoided.

In order to increase the capacity of the CRI IPM team, in line with the increased research portfolio, we have appointed three new researchers (Dr Evans Mauda, Dr Tammy Marsberg and Dr Davina Saccaggi) and three new technicians (Courtney Morris, Leani Serfontein and Luke Cousins) in the last few years. It is good to see new blood coming into the industry at a high level of scientific expertise. Furthermore, we have established collaborative relationships with entities with whom we have not worked in the past or with whom we have done very little, such as the University of Mpumalanga, University of North-West, University of Venda and internationally, the University of Leuven. Sean Moore and Aruna Manrakhani have also been recognised for their contributions to Rhodes University and Stellenbosch University, respectively, by being awarded professorships by these institutions.

After a hiatus from in-person meetings during the more than two-year Covid pandemic lockdown, IPM researchers have resumed addressing in-person industry workshops and grower study groups. However, virtual meetings have remained a valuable addition to our communication tools – one of the few positives that came out of the pandemic. International travel is also resuming, with some researchers starting to participate in international scientific conferences. Several valuable peer-reviewed scientific and semi-popular publications have also been published by our entomologists, as well as making periodic contributions to the Cutting Edge. We look forward to showcasing our research findings to the industry at the upcoming Citrus Research Symposium, after an unprecedented four-year break.

We also look forward to a series of new studies and collaborations that will be initiated during the 2022/23 citrus season and will strive to continue our contribution to the technical competitiveness of the southern African citrus industry.

## PORTEFEULJE OPSOMMING

CRI is nou in sy eerste jaar van die dramaties verhoogde heffing. Gevolglik het die IPM Navorsingsportefeulje, soos met ander portefeuljes en afdelings binne CRI, aansienlik gegroei. Nie net het die aantal projekte relatief tot die vorige jaar gegroei nie, maar die aantal medewerkers het ook toegeneem. Gevolglik kon ons baie meer navorsing as voorheen aanpak, iets wat nog rereg in sy groeifase is. Gelukkig is die struikelblokke wat die Covid-19-pandemie voor ons gegooi het iets van die verlede. Dit blyk egter 'n groter probleem te wees vir ons medewerkers, veral die universiteite, wat onderworpe was aan direkte burokratiese beperkings. 'n Verdere uitdaging was dié van beurtkrag, wat kouebehandelingsproewe kan onderbreek en sensitiewe en duur laboratoriumtoerusting kan beskadig. Ten spyte van hierdie uitdagings was vordering met navorsing binne die IPM-portefeulje goed.

Binne die IPM Portoflio is daar drie navorsingsprogramme: VKM, Vrugtevlieg en Ander Plaag. VKM bly die grootste van die drie programme, wat die kwarantynstatus van hierdie plaag weerspieël vir verskeie markte, veral die Europese Unie. Die ander twee programme het egter ook merkbaar gegroei.

Navorsing binne die VKM-program het gefokus op beide voor-oes en na-oes bestuur van VKM, met 'n goeie balans tussen navorsing wat vinnig toegepaste resultate lewer en meer langtermyn hoë-tegnologie navorsing. Hoogtepunte in hierdie program sluit die volgende in. Dit is gedemonstreer dat 'n granulovirus-isolaat (CrleGV) wat geselekteer is vir sy weerstand teen UV-bestraling, sy genetiese integriteit behou het wanneer dit twee keer deur VKM gevoer is, wat aandui dat dit moontlik is om hierdie isolaat in vivo te produseer, sonder om terug te keer na die oorspronklike toestand van UV-sensitiwiteit. Dit is in 'n veldproef gedemonstreer dat beide Coragen en Exirel 'n beduidende paringontwrigtingseffek op VKM het, benewens hul insekdodende effek, wat dus die doeltreffendheid van beide produkte teen VKM verhoog. Twee afsonderlike studies het 'n sinergistiese effek tussen gis en CrleGV, en tussen Neem olie en CrleGV getoon, wat die doeltreffendheid van 'n produk soos Cryptogran moontlik aansienlik kan verbeter. 'n Reeks potensiele afweermiddels vir VKM-eierlegging is ook geïdentifiseer. In 'n vergelyking van VKM op organiese en konvensionele plase, is dit vasgestel dat laer vlakke van VKM in organiese boorde toegeskryf kan word aan verskeie faktore, insluitend verskille in vrugsamestelling en grondbewonende algemene roofdiere.

Navorsing binne die Vrugtevlieg-program, soos die VKM-program, het ook bestaan uit 'n goeie balans tussen voor- en na-oes studies. Hierdie program het ook drie internasionale samewerkende studies ingesluit wat eksterne befonds is. Hoogtepunte in die program sluit die volgende in. Twee nuwe koue ontsmettingsbehandelings is vir vrugtevlieë ontwikkel, naamlik 3.5°C vir 24 dae en 5°C vir 27 dae. Dit was gebaseer op die bevinding dat Medvlieg die mees koue-verdraagsame van die vrugtevlieg spesies is en daarom kan alle koue studies eenvoudig met hierdie spesie uitgevoer word, met relevansie vir al die ander. Daar is ook vasgestel dat die Kaapse vlieg, *Ceratitis quilicii* wyer oor Suid-Afrika versprei is as die Natalse vlieg, *C. rosa*, wat die belangrikheid van verdere studies oor eersgenoemde spesie beklemtoon, al is dit nog nie getoon dat dit sitrus in die veld besmet nie. In 'n studie oor die ontwikkeling van 'n stelselbenadering vir vrugtevlieë, is dit getoon dat goeie landboupraktyke in die veld en sortering tydens en na-oes doeltreffend was om vrugtevliegbesmetting te beperk of te voorkom.

In die Ander Plaag-program is 'n wye reeks entomologiese kwessies oor sitrus bestudeer. Die een wat waarskynlik die meeste aandag gekry het, was verskeie aspekte van psilloïede ter voorbereiding van die koms van die Asiatiese sitrus bladvlooi (ACP), wat die vernietigende bakteriese siekte, Huanglongbing of Asiatiese vergroening, dra. Verskeie studies is ook gedoen oor sitrusblaaspootjies, witluis, rooidopluis, Australiese wolluis, verskeie Lepidoptera, en die effek van nette op plaagbestuur in die algemeen. Hoogtepunte in hierdie program sluit die volgende in. Daar is vasgestel dat daar geen sinistere rede is waarom witluisvlakke hoër onder nette is nie, soos gekompromitteerde biologiese beheer. Dit is eenvoudig dat nette 'n gunstiger en beskermende omgewing skep. 'n Maatstaf vir die doeltreffendheid van spinetoram vir blaaspootjies is in die Oos-Kaap bepaal, met geen aanduiding van blaaspootjie weerstandbiedend teen hierdie verbinding is nie.

Daar is vasgestel dat vroeë seisoen vrylatings van *Anagyrus vladimiri* doeltreffend kan wees vir die beheer van witluis, maar dat dit onvoorspelbaar ondermyn kan word deur hiperparasitiese aktiwiteit. Verskeie *Diaphorina*-spesies is in en om sitrusboorde ontdek, wat baie soos ACP lyk. Dit is belangrike voorbereidingswerk vir die koms van ACP, sodat enige verwarring vermy kan word.

Om die kapasiteit van die CRI IPM-span te verhoog, in ooreenstemming met die verhoogde navorsingsportefeulje, het ons drie nuwe navorsers (dr Evans Mauda, dr Tammy Marsberg en dr Davina Saccaggi) en drie nuwe tegnici (Courtney Morris, Leani Serfontein en Luke Cousins) die afgelope paar jaar aangestel. Dit is goed om te sien dat nuwe bloed op 'n hoë vlak van wetenskaplike kundigheid in die bedryf inkom. Verder het ons samewerkende verhoudings gevestig met entiteite met wie ons nie in die verlede gewerk het nie of met wie ons baie min gedoen het, soos die Universiteit van Mpumalanga, Universiteit van Noordwes, Universiteit van Venda en internasionaal, die Universiteit van Leuven. Sean Moore en Aruna Manrakhan het ook erkenning gekry vir hul bydraes tot onderskeidelik Rhodes Universiteit en Universiteit van Stellenbosch deurdat hulle as professoraat deur hierdie instellings toegeken is.

Na 'n onderbreking van persoonlike vergaderings tydens die meer as twee jaar lange Covid-pandemie-inperkings, het IPM-navorsers hervat om in-persoon bedryfswerkswinkels en produsentstudiegroepe toe te spreek. Virtuele vergaderings het egter 'n waardevolle toevoeging tot ons kommunikasie-instrumente gebly - een van die min positiewe aspekte wat uit die pandemie gekom het. Internasionale reis word ook hervat, met sommige navorsers wat aan internasionale wetenskaplike konferensies begin deelneem. Verskeie waardevolle eweknie-geëvalueerde wetenskaplike en semi-gewilde publikasies is ook deur ons entomoloë gepubliseer, sowel as periodieke bydraes tot die Snyant. Ons sien daarna uit om ons navorsingsbevindinge aan die bedryf by die komende Sitrusposium ten toon te stel, na 'n ongekende vier jaar lange breek.

Ons sien ook uit na 'n reeks nuwe studies en samewerking wat gedurende die 2022/23-sitrusseisoen begin sal word en sal streef om aanhoudend ons bydrae tot die tegniese mededingendheid van die Suider-Afrikaanse sitrusbedryf voort te sit.

### 3.2 **PROGRAMME: FALSE CODLING MOTH**

Programme coordinator: Sean D Moore (CRI)

#### 3.2.1 **Programme summary**

With the increased levy, the scope and extent of projects within the FCM Programme have increased notably. Furthermore, we are now able to address certain high-tech projects with new collaborators, which was not previously possible. Within the programme, 19 projects were tackled during the last research cycle. Fifteen of these focussed on issues related to preharvest management of FCM and the remaining four researched postharvest management issues.

Of the preharvest projects, three looked at methods and technologies for improved monitoring of FCM, four pursued a better understanding of and improvement in mating disruption technology, three addressed various aspects of baculovirus usage, two investigated general aspects of FCM control, and one project each investigated FCM ecology, repellents for FCM and semiochemicals in general.

The first monitoring project investigated potential attractants for female moths (3.2.3). The only compound that appeared to trigger a response in the moths was  $\beta$ -caryophyllene. However, all compounds must still be tested against mated moths. The second monitoring project investigated male pheromones (3.2.8), as male moths are known to possess three androconial glands that play a role in mating. So far, four compounds have been identified from the androconia. However, these are also present in the insect's cuticle. An electron microscopy study of the glands is now underway. The final project exploring an aspect of monitoring is one that is developing an improved preharvest infestation monitoring protocol for FCM (3.2.19). Although the 5-data tree monitoring system was shown to be adequately accurate and sensitive, a new method, sampling sanitation fruit, was developed, as such a system is considered to be less susceptible to data tampering.

The first mating disruption project is exploring a better understanding of release rates of the various products and the influence of temperature (3.2.10). Thus far, the project has been dedicated to methodology development, which has proven more challenging than anticipated, but progress has been made, which will enable release rate trials to be initiated shortly. The second mating disruption project is investigating a combination of mating disruption and the sterile insect technique (SIT) (3.2.11). Data collected at the first trial site were not sufficiently enlightening. This was followed by the grower subverting the trial by deploying his own mating disruption dispensers and consequently, the trial has been re-initiated at a new site. The third mating disruption project is comparing the efficacy of this technology between open and netted orchards (3.2.12). To date, no difference in trap catches has been recorded. A new site was recently selected to initiate a new trial. The final mating disruption project is being conducted in Letsitele, to determine if efficacy of the technology is improved, by initiating it much earlier in the season, as winter breaks earlier in the warm northern parts of the country (3.2.15). The project will run until September this year when the late Valencias are harvested. It is thus too early to draw any conclusions.

The first of the baculovirus projects determined that an isolate of the FCM granulovirus, selected for its UV-tolerance, retained its genetic integrity when passaged twice through FCM larvae, indicating that the UV-tolerance may well be stable (3.2.3). However, this could not yet be demonstrated in bioassays. The second baculovirus project was based on the findings of the first project, but applied to the litchi moth nucleopolyhedrovirus (NPV), which is also virulent against FCM (3.2.16). Unfortunately, contamination with a granulovirus in the NPV preparation held up progress with this project, but UV-exposure cycles are now underway. The last of the baculovirus projects is investigating the biological and genetic stability of UV-tolerant baculoviruses, as contamination with another virus or reversion to UV-susceptibility might occur with *in vivo* passaging through larvae, as is the normal mode of virus production (3.2.18). A series of preliminary bioassays have been conducted to provide a benchmark for future virulence comparisons.

Of the two projects addressing more general aspects of FCM management, one is a catch-all for all field trials (3.2.6). In this project, a series of semi-field bioassays were conducted, mainly to test whether the addition of gut-borne yeasts of FCM enhance the efficacy of a granulovirus spray, as was previously recorded in laboratory trials. Treatments with yeasts added did indeed perform better than those without yeasts. The second field trial determined that in addition to their insecticidal effect, Coragen and Exirel also have a mating disruption effect on FCM. The second project on FCM management investigated synergism between various compounds for improved control of FCM (3.2.7). A high degree of synergism was demonstrated between Neem oil and the FCM granulovirus, even at extremely low doses of Neem oil.

The ecological project investigated differences in FCM incidence on organic versus conventional farms and the reasons for these differences (3.2.2). It was confirmed that FCM incidence is indeed lower on organic farms. This is attributed to several factors, including a greater preference of moths for conventional fruit, related to higher levels of certain important nutritional compounds in the fruit, and a higher abundance of generalist predators in organic orchard soils.

In the second to last preharvest management project within the FCM programme, the repellent effect of a range of compounds against FCM was determined, measured by a reduction in oviposition (3.2.13). Two essential oils (lavender and peppermint), two plant crude extracts (garlic and marigold), and three chemicals (Delegate, Coragen, and Warlock) showed a significant repellent effect.

In the final preharvest FCM management project, regional differences in sex pheromones and sexual attractiveness in FCM were investigated (3.2.17). This project follows on from a previous similar study, in order to answer some important unanswered questions. So far, females from five populations were shown to attract Addo males, indicating no potential chemical signalling barriers that may trigger speciation. However, males from Nelspruit were not attracted to females from Citrusdal. The findings of this project may have important implications for regional efficacy of semiochemical-based technologies, such as monitoring, mating disruption, attract and kill and SIT.

Two of the postharvest technology projects explored disinfestation treatments for FCM and the other two investigated infestation detection technologies.

A long running cold treatment project (3.2.4) examined several important aspects of cold treatment. For example, it was determined that after 28 days at 4°C, all larvae were dead. It was also tentatively determined that FCM in several cultivars of fruit were more susceptible to FCM in artificial diet, when inoculated in the laboratory, indicating that artificial diet can be used for cold trials without risk of overestimating efficacy. Lastly, larval flaccidness, as opposed to turgidity, was identified as a highly reliable characteristic, indicating imminent mortality of larvae surviving cold treatment. Work is being conducted to enable accurate quantification of these factors. The other postharvest treatment project is exploring the use of vapour heat for disinfestation of fruit (3.2.20). Efficacy of temperatures up to 47°C against third and fifth instars are being tested and thus far mortalities approaching 90% have been recorded. However, the research is ongoing.

In the first of the postharvest infestation detection projects, the antennal response of the FCM larval parasitoid, *Agathis bishopi*, is being used for identifying key volatiles indicative of FCM fruit infestation (3.2.9). This is being done using Coupled Gas Chromatography-Flame Ionisation Detector-Electroantennographic Detection (GC-FID-EAD). Preliminary experiments yielded antennal responses to six compounds in headspace samples from infested oranges. One of these compounds has tentatively been identified as an isomer of limonene oxide. In the final project, Selected Ion Flow Tube Mass Spectrometry is being used for postharvest detection of FCM infested fruit. So far, only physical injury experiments have been conducted. Discrimination between injured and healthy fruit was achieved.

### **Program-opsomming**

Met die verhoogde heffing het die omvang van projekte binne die VKM-program aansienlik toegeneem. Verder is ons nou in staat om sekere hoëtegnologieprojekte met nuwe medewerkers aan te spreek, wat voorheen nie moontlik was nie. Binne die program is 19 projekte gedurende die laaste navorsingsiklus aangepak. Vyftien hiervan het gefokus op kwessies wat verband hou met vooroesbestuur van VKM en die oorblywende vier na-oesbestuurskwessies wat nagevors is.

Van die vooroesprojekte het drie gekyk na metodes en tegnologieë vir verbeterde monitering van VKM, vier het 'n beter begrip van en verbetering aan paringsontwrigtingstegnologie nagestreef, drie het verskeie aspekte van bakulovirusgebruik aangespreek, twee het algemene aspekte van VKM-beheer ondersoek, en een projek elk VKM-ekologie, afweermiddels vir VKM en semiochemikalieë in die algemeen ondersoek.

Die eerste moniteringsprojek het potensiële lokmiddels vir wyfie motte ondersoek (3.2.3). Die enigste verbinding wat blykbaar 'n reaksie by die motte veroorsaak het, was  $\beta$ -kariofilleen. Alle verbindings moet egter steeds teen gepaarde motte getoets word. Die tweede moniteringsprojek het mannetjie feromone ondersoek (3.2.8), aangesien dit bekend is dat mannetjiemotte drie androkoniese kliere besit wat 'n rol speel in paring. Tot dusver is vier verbindings uit die androkonia geïdentifiseer. Dit is egter ook teenwoordig in die insek se kutikula. 'n Elektronmikroskopiëstudie van die kliere is nou aan die gang. Die finale projek wat 'n aspek van monitering ondersoek, is een wat 'n verbeterde vooroes besmetting moniteringsprotokol vir VKM ontwikkel (3.2.19). Alhoewel daar getoon is dat die 5-data boom moniteringstelsel voldoende akkuraat en sensitief is, is 'n nuwe metode, monsterneming van sanitasievrugte, ontwikkel, aangesien so 'n stelsel as minder vatbaar vir datapeutering beskou word.

Die eerste paringsontwrigtingsprojek is om 'n beter begrip van vrystellingstempo's van die verskillende produkte en die invloed van temperatuur te ondersoek (3.2.10). Tot dusver is die projek gewy aan metodologie-ontwikkeling, wat meer uitdagend was as wat verwag is, maar vordering is gemaak, wat dit moontlik sal maak om vrystellingstempo-proewe binnekort te begin. Die tweede paringsontwrigting projek ondersoek 'n kombinasie van paringsontwrigting en die steriele insek tegniek (SIT) (3.2.11). Data wat by die eerste proefperseel ingesamel is, was nie voldoende verhelderend nie. Daarna het die produsent die proef ondermyn deur sy eie paringsontwrigting vrystellers te ontplooi en gevolglik is die proef weer by 'n nuwe perseel begin. Die derde paringsontwrigtingsprojek is om die doeltreffendheid van hierdie tegnologie tussen oop en genette boorde te vergelyk (3.2.12). Tot op hede is geen verskil in lokvalvangste aangeteken nie. 'n Nuwe perseel is onlangs gekies om 'n nuwe proef te begin. Die finale paringsontwrigtingsprojek word in Letsitele uitgevoer om te bepaal of die doeltreffendheid van die tegnologie verbeter word, deur om dit baie vroeër in die seisoen te

begin, aangesien die winter vroeër in die warm noordelike dele van die land breek (3.2.15). Die projek duur tot September vanjaar wanneer die laat Valencias geoes word. Dit is dus te vroeg om enige gevolgtrekkings te maak.

Die eerste van die bakulovirusprojekte het vasgestel dat 'n isolaat van die VKM-granulovirus, geselekteer vir sy UV-verdraagsaamheid, sy genetiese integriteit behou het wanneer dit twee keer deur VKM-larwes gevoer was, wat aandui dat die UV-verdraagsaamheid wel stabiel kan wees (3.2.3). Dit kon egter nog nie in biotoetse gedemonstreer word nie. Die tweede bakulovirusprojek was gebaseer op die bevindinge van die eerste projek, maar is op die lietsjiemot-nukulêreopolihedrovirus (NPV) uitgevoer, wat ook virulent is teen VKM (3.2.16). Ongelukkig het kontaminasie met 'n granulovirus in die NPV-voorbereiding vordering met hierdie projek vertraag, maar UV-blootstellingsiklusse is nou aan die gang. Die laaste van die bakulovirusprojekte is om die biologiese en genetiese stabiliteit van UV-verdraagsame bakulovirusse te ondersoek, aangesien kontaminasie met 'n ander virus of terugkeer na UV-gevoeligheid kan voorkom met *in vivo* produksie in larwes, wat wel die normale manier van virusproduksie is (3.2.18). 'n Reeks voorlopige biotoetse is uitgevoer om 'n maatstaf vir toekomstige virulensievergelykings te verskaf.

Van die twee projekte wat meer algemene aspekte van VKM-bestuur aanspreek, is een 'n vangplek vir alle veldproewe (3.2.6). In hierdie projek is 'n reeks semi-veld biotoetse uitgevoer, hoofsaaklik om te toets of die byvoeging van dermgedraagde giste van VKM die doeltreffendheid van 'n granulovirusbespuiting verhoog, soos voorheen in laboratoriumproewe aangeteken is. Behandeling met bygevoegde giste het inderdaad beter gevaar as dié sonder giste. Die tweede veldproef het vasgestel dat, benewens hul insekdodende effek, Coragen en Exirel ook 'n paringsontwrigting effek op VKM het. Die tweede projek oor VKM-bestuur het sinergisme tussen verskeie verbindings vir verbeterde beheer van VKM ondersoek (3.2.7). 'n Hoë mate van sinergisme is tussen Neem-olie en die VKM-granulovirus gedemonstreer, selfs teen uiters lae dosisse Neem-olie.

Die ekologiese projek het verskille in VKM-voorkoms op organiese teenoor konvensionele plase en die redes vir hierdie verskille ondersoek (3.2.2). Dit is bevestig dat VKM-voorkoms inderdaad laer is op organiese plase. Dit word toegeskryf aan verskeie faktore, insluitend 'n groter voorkeur van motte vir konvensionele vrugte, wat verband hou met hoër vlakke van sekere belangrike voedingsverbindings in die vrugte, en 'n groter voorkoms van algemene roofdiere in organiese boordgrond.

In die naaslaaste vooroesbestuursprojek binne die VKM-program, is die afstotende effek van 'n reeks verbindings teen VKM bepaal, gemeet deur 'n vermindering in eierlegging (3.2.13). Twee essensiële olies (laventel en peperment), twee ru-plantekstrakte (knoffel en gousblom) en drie chemikalieë (Delegate, Coragen en Warlock) het 'n beduidende afwerende effek getoon.

In die finale vooroes-VKM-bestuursprojek is streeksverskille in seksferomone en seksuele aantreklikheid in VKM ondersoek (3.2.17). Hierdie projek volg op 'n vorige soortgelyke studie, om 'n paar belangrike onbeantwoorde vrae te beantwoord. Tot dusver is getoon dat wyfies uit vyf bevolkings Addo-mannetjies lok, wat geen potensiële chemiese seinversperrings aandui wat spesiasie kan veroorsaak nie. Mannetjies van Nelspruit was egter nie aangetrokke tot wyfies van Citrusdal nie. Die bevindinge van hierdie projek kan belangrike implikasies hê vir die streeksdoeltreffendheid van semiochemies-gebaseerde tegnologieë, soos monitering, paringsontwrigting, lok en vrek en SIT.

Twee van die na-oes tegnologieprojekte het ontsmettingsbehandelings vir VKM ondersoek en die ander twee het besmettings-opsporingstegnologieë ondersoek.

'n Langlopende kouebehandelingsprojek (3.2.4) het verskeie belangrike aspekte van kouebehandeling ondersoek. Daar is byvoorbeeld vasgestel dat na 28 dae by 4°C, alle larwes dood was. Daar is ook tentatief vasgestel dat VKM in verskeie vrugtekultivars meer kouegevoelig was as VKM in kunsmatige dieet, wanneer hulle in die laboratorium ingeënt is, wat aandui dat kunsmatige dieet vir koue proewe gebruik kan word sonder die risiko om doeltreffendheid te oorskakel. Laastens is larwe slapheid, in teenstelling met turgiditeit, geïdentifiseer as 'n hoogs betroubare eienskap, om aan te dui dat larwes wat koue behandeling oorleef, wel sal doodgaan. Werk word gedoen om akkurate kwantifisering van hierdie faktore moontlik te maak. Die ander

na-oes behandelingsprojek ondersoek die gebruik van damphitte vir ontsmetting van vrugte (3.2.20). Doeltreffendheid van temperature tot 47°C teen derde en vyfde instars word getoets en tot dusver is mortaliteit naby aan 90% aangeteken. Die navorsing is egter aan die gang.

In die eerste van die na-oes besmetting opsporing projekte, word die antennale reaksie van die VKM larwe parasitoïed, *Agathis bishopi*, gebruik vir die identifisering van sleutel vlugtige stowwe wat dui op VKM vrug besmetting (3.2.9). Dit word gedoen met behulp van gekoppelde gaschromatografie-vlamionisasie-detektor-elektroantennografiese opsporing (GC-FID-EAD). Voorlopige eksperimente het antennale reaksies op ses verbindings in kopspasiemonsters van besmette lemoene opgelewer. Een van hierdie verbindings is voorlopig geïdentifiseer as 'n isomeer van limoneenoksied. In die finale projek word geselekteerdeioonvloei buismassaspektrometrie gebruik vir na-oes opsporing van VKM besmette vrugte. Tot dusver is slegs fisiese beseringseksperimente uitgevoer. Diskriminasie tussen beseerde en gesonde vrugte is bereik.

### 3.2.2 FINAL REPORT: A comparison of *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) in organic versus conventional citrus orchards

Project 1253 (2019-2022) by Luke Cousins, Candice Coombes, Mellissa Peyper, Sean Moore (CRI), Martin Hill (RU) and Antoinette Malan (SU)

#### Summary

Citrus packhouses in South Africa report lower infestation of false codling moth (FCM), *Thaumatotibia leucotreta* (Meyr) (Lepidoptera: Tortricidae), in fruit from organically farmed orchards than conventionally farmed orchards. Field surveys and laboratory studies were conducted to determine if, and why FCM infestation was lower on organic citrus farms. In field surveys, wild FCM numbers were significantly higher in conventionally farmed Palmer Navel and Newhall Navel orchards compared to matching orchards on neighbouring organic farms. Pitfall trapping recorded significantly higher arthropod generalist predator abundance and species richness on organic farms. Organic orchards had a mean of 7.3 ( $\pm$  0.6) distinct predatory species per trap whereas in conventional orchards, 3.9 ( $\pm$  0.4) species were recorded per trap. Ants, rove beetles, pseudoscorpions and crickets were significantly more abundant in organic orchards. No significant difference was found in spider numbers between organic and conventional citrus orchards. Soil from conventional orchards did not reveal a significantly different incidence of entomopathogenic nematodes (EPN) and entomopathogenic fungi (EPF) isolates compared to the soil from organic orchards. There was, however, a difference in the dominant species of EPF and EPNs in organic and conventional orchard soils. The EPF, *Metarhizium anisopliae* was the dominant species in organic soils (81% of isolates) while *Beauveria bassiana* was dominant in conventional soils (66% of isolates). Of the few EPN isolates obtained, the majority of isolates in conventional soils (87% of isolates) were *Heterorhabditis zealandica*, while in organic soils the majority of isolates (75% of isolates) were identified to be *Heterorhabditis bacteriophora*. In fruit nutrient analyses, conventional fruit were found to have significantly higher concentrations of magnesium, boron and nitrogen, but significantly lower concentrations of copper and thinner peels. FCM oviposition choice and no-choice trials recorded small but statistically significant oviposition preference in favour of conventional fruit. Farm management surveys showed that spray regimes on conventional citrus farms are significantly less compatible with integrated pest management (IPM), based on a rating system and have less vegetation on the orchard floor. Poor farm IPM ratings were positively correlated with wild FCM catches. Results of this study demonstrate the importance of natural enemy conservation as part of an IPM strategy and show how fruit nutrient composition could potentially impact FCM ecology in citrus orchards.

#### Opsomming

Sitruspakhuse in Suid-Afrika rapporteer laer besmetting van valskodlingmot (VKM), *Thaumatotibia leucotreta* (Meyr) (Lepidoptera: Tortricidae), in vrugte van organies gekweekte boorde as konvensioneel gekweekte boorde. Veldopnames en laboratoriumstudies is gedoen om vas te stel of, en waarom VKM-besmetting laer was op organiese sitrusplase. In veldopnames was wilde VKM-getalle aansienlik hoër in konvensioneel gekweekte Palmer Navel- en Newhall Navel-boorde in vergelyking met ooreenstemmende boorde op naburige organiese plase. Slaggatvangery het 'n beduidende hoër aantal geleedpotige algemene roofdiere en spesierikheid op organiese plase aangeteken. Organiese boorde het 'n gemiddeld van 7.3 ( $\pm$  0.6) afsonderlike

rooiespesies per lokval gehad terwyl in konvensionele boorde 3.9 ( $\pm$  0.4) spesies per lokval aangeteken is. Miere, kewers, pseudoskerpioene en krieke was aansienlik meer volop in organiese boorde. Geen betekenisvolle verskil is gevind in spinnekopgetalle tussen organiese en konvensionele sitrusboorde nie. Grond van konvensionele boorde het nie 'n betekenisvol verskillende voorkoms van entomopatogeniese nematode (EPN) en entomopatogeniese swam (EPF) isolate getoon in vergelyking met die grond van organiese boorde nie. Daar was egter 'n verskil in die dominante spesies van EPF en EPNs in organiese en konvensionele boordgrond. Die EPF, *Metarhizium anisopliae* was die dominante spesie in organiese gronde (81% van isolate) terwyl *Beauveria bassiana* dominant was in konvensionele gronde (66% van isolate). Van die min EPN-isolate wat verkry is, was die meerderheid isolate in konvensionele gronde (87% van isolate) *Heterorhabditis zealandica*, terwyl in organiese gronde die meerderheid isolate (75% van isolate) as *Heterorhabditis bacteriophora* geïdentifiseer is. In vrugtevoedingstofontledings is gevind dat konvensionele vrugte aansienlik hoër konsentrasies magnesium, boor en stikstof het, maar aansienlik laer konsentrasies koper en dunner skille gehad het. VKM-eierleggings-keuse en geen-keuse proewe het klein maar statisties beduidende eierleggings voorkeur aangeteken ten gunste van konvensionele vrugte. Plaasbestuuroopnames het getoon dat spuitregimes op konvensionele sitrusplase aansienlik minder versoenbaar is met geïntegreerde plaagbestuur (IPM), gebaseer op 'n graderingstelsel en minder plantegroei op die boordvloer het. Swak plaas IPM-graderings was positief gekorreleer met wilde VKM-vangste. Resultate van hierdie studie demonstreer die belangrikheid van natuurlike vyand bewaring as deel van 'n IPM-strategie en wys hoe vrugtevoedingstofsamesstelling potensieel 'n impak kan hê op VKM-ekologie in sitrusboorde.

## Introduction

Sundays River Citrus Company, which packs both conventionally and organically produced citrus fruit, has recorded a significantly lower level of FCM infestation in fruit from organic than fruit from conventional orchards, in packhouse assessments conducted over many years (Christo Theron, pers. comm.). Conventional wisdom might conclude that naturally occurring biological control would be greater on an organic farm. However, this cannot simply be assumed; for example, Goble *et al.* (2010) recorded very similar levels and diversity of entomopathogenic fungi (EPF) on conventional and organic farms. Consequently, biological control in the two environments needs to be measured. Additionally, natural biological control of FCM is diverse, with several parasitoids, predators and pathogens recorded as being potentially effective. For example, the egg parasitoid, *Trichogrammatoidea cryptophlebiae*, has been reported to parasitise almost 100% of FCM eggs where conditions are favourable (Moore & Fourie, 1999; Moore & Richards, 2000, 2001 & 2002; Moore & Hattingh 2012). The larval parasitoid, *Agathis bishopi*, can parasitise up to 34% of larvae at any one time (Sishuba 2003; Zimba *et al.* 2016).

Furthermore, biocontrol agents occurring in orchard soils can have a significant impact on soil-dwelling life stages of FCM. Infection of planted FCM pupae in orchard soil infested with the entomopathogenic nematode, *Heterorhabditis zealandica*, was more than 90% higher than in soil where the EPNs had been chemically suppressed. This led to an almost 50% reduction in FCM infestation of fruit in the orchard (Manrakhan *et al.* 2014). Although it has not yet been possible to measure the impact of naturally occurring EPF in orchard soils, application of both *Beauveria bassiana* and *Metarhizium anisopliae* to orchard soils reduced FCM infestation of fruit by more than 80% for the full duration of a growing season, with a single application in spring (Coombes *et al.* 2016). Additionally, the previously mentioned extensive survey conducted by Goble *et al.* (2010) revealed a high level of natural occurrence of EPF in citrus orchards soils. Sixty-two potentially useful entomopathogenic fungal isolates belonging to four genera were collected from 288 soil samples, an occurrence frequency of 21.53%. Although there was no significant difference in recovery of fungal isolates from organically and conventionally managed soils, this survey was conducted 10 years ago, in the early days of organic citrus production in the Sundays River Valley and things may well have changed over this time.

Apart from these biological factors, biochemical factors may also be playing a role. For example, Albertyn (2017) found that fruit from juvenile orchards were significantly more susceptible to FCM infestation than fruit from mature orchards, in controlled choice and no-choice laboratory trials. This correlated with considerably higher ash levels and also notably higher protein levels in fruit from the juvenile orchards. Consequently, similar types of differences may be recorded in biochemical/nutritional constitution between organic and conventional fruit. Generally lower nitrogen levels have been recorded in organically grown fruit (Worthington 2001); this

may have an effect on attractiveness of organic fruit or their susceptibility to FCM. Similarly, terpene levels, known to deter herbivores, including insects, may differ between organic and conventional plants, the main terpenes in citrus being limonene and linalool (Haleva-Toledo *et al* 1999).

Finally, management practices will differ dramatically on organic and conventional farms. Far more control options will be available to conventional farmers. However, this may not necessarily mean that management of the pest is better on conventional farms. Sanitation practices, which can be extremely effective (Ullyett and Bishop 1938; Stofberg 1954; Moore and Kirkman 2008), may differ in the two farming systems. Refer to the thesis titled; “A comparison of *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) in organic versus conventional citrus orchards”, for more introductory information

## **Objectives**

The ultimate objective is to identify factors leading to reduced incidence of FCM on organic farms and to determine how any of these influential factors can be transferred to conventional farming. In order to do this, the following objectives have been pursued:

- A. Compare FCM moth activity and fruit infestation in organically and conventionally managed citrus orchards
- B. Compare above-ground biological control of FCM in organically and conventionally managed citrus orchards
- C. Compare biological control agents, which could attack FCM, in soils in organically and conventionally managed citrus orchards
- D. Compare management practices, including control programmes and orchard sanitation, in organically and conventionally managed citrus orchards
- E. Compare attractiveness and susceptibility of organically and conventionally produced citrus fruit to FCM in laboratory trials and relate this to biochemical differences between the fruit

## **Materials and methods**

### **Study Site**

Monitoring of 10 orchards across two pairs of neighbouring organic and conventional farms in the Sundays River Valley commenced in October 2019. Five organic orchards five conventional orchards of two different cultivars (Palmer Navel and Delta Valencia) were initially monitored. This monitoring continued uninterrupted until October 2020, when one of the organic orchards was removed (Hippo Pools orchard 14). In the same month two new organic orchards and two new conventional orchards of two different cultivars (Nadorcott Mandarin and Newhall Navels) on three new neighbouring farms were added to existing study sites. These 13 orchards were monitored until September 2021. It was ensured that on each set of neighbouring farms, there were corresponding orchards of the same cultivar, age, rootstock, and irrigation system.

### **Objective A**

In each orchard, a single Chempac® (Suider-Paarl, South Africa) yellow delta trap was hung in the South Western corner where possible, in order to record FCM moth catches (Moore 2017). In scenarios where the South Western corner of the orchard was too close to the trap of a neighbouring orchard, a different corner had to be chosen. The traps were hung on the 5th tree of the 5th row of each orchard at approximately head height. The yellow delta traps each contained a Chempac sticky pad and Chempac female FCM pheromone lure. As per recommended protocol, pheromone lures were replaced every 12 weeks and sticky pads were replaced every 4 to 6 weeks depending on rate of weathering. The FCM caught by the traps were recorded weekly. It was ensured that wild and sterile moths were carefully differentiated from one another. Once fruit had begun to develop sufficiently, the orchard floor underneath the same 10 data trees was inspected for dropped fruit weekly. For the 2021 season, a simple barrier was made using danger tape and wooden stakes to better demarcate the canopy floor under the data trees in an attempt to avoid accidental fruit pick-up by orchard sanitation staff. Dropped fruit was picked up and carefully dissected to examine for larvae or signs of larval damage. Any larvae found within a fruit was collected and stored in a 30 ml glass vial with artificial diet

(Moore *et al.* 2014) obtained from river bioscience (Addo, South Africa). Sterile cotton wool plugs were used to close off the vials, acting as a substrate for pupation once the larvae had developed

### **Objective B**

Larva from infested fruit were kept in vials were check regularly for moth and larval parasitoid emergence as well as signs of virus. Every second week for three weeks in 2020 and 2021, the fruit on the data trees were scouted for eggs and egg parasitism. 10 fruit on 10 individual data trees per orchard were used for inspection. Normal non-parasitized eggs are dark brown, pink or cream, while parasitized eggs are black (Georgala 1969, Daiber 1979a, Newton 1998). The sample trees were the first 10 normal/healthy trees in a row after the tree in which the yellow delta trap was situated.

To record and compare the communities of ground-dwelling arthropods in each orchard, the pitfall trapping method was used. Single holes were dug between the first and second data tree as well as the ninth and tenth data tree in order to house 500 ml plastic tubs with a diameter of 15 cm. The tubs were embedded in the ground so that their openings were level with the surrounding soil surface. Square corrugated plastic sheets of 50cm by 50cm were used to create a roof over each pitfall in order to prevent excess debris and rain from entering the traps. 15cm building nails with were used to pin the roofs into the soil and foam spacers kept the roof height at approximately 5cm from the surface of the ground. Propylene glycol used to fill the tubs up to the 2cm mark, acting as the trapping and preserving liquid. A drop of Breakthru was added to the solution as a surfactant, breaking the surface tension of the water, to avoid escape of trapped organisms. The pitfall traps were left for a period of 1 week to collect organisms until the tubs were removed. Two pitfall traps were place in each orchard, and the trapping process was conducted twice in 2020 citrus season. Captured organisms viewed in the lab under a dissection microscope and taxonomic keys were used to identify the organisms up to the family level of classification.

### **Objective C**

Soil samples were collected every second month from November 2019 until July 2021, in every orchard that was monitored. The sample area for all orchards was based on size of smallest orchard. Starting from one side of orchard, leaving one buffer row of trees, soil samples were taken from every second row for five rows, resulting in five soil samples per orchard. Each soil sample consisted of five subsamples, taken from every second tree in the row for five trees, leaving the first tree in the row as a buffer. Loose debris and topsoil was cleared under each tree and a garden spade was used to extract one shovelful of soil approximately 150g and 15 cm deep into the soil profile for each subsample. Subsamples were taken approximately 1m from the tree trunk, directly under the canopy. All subsamples were placed together in a plastic zip lock bag, transported to the laboratory and baited.

The insect baiting technique was used to recover entomopathogenic fungi and nematodes from the soil samples. Soil samples were mixed thoroughly within the plastic zip lock bags before being processed through a metal sieve with a mesh size of 2mm. Sieved soil from each sample was used to fill single 250 ml curry tubs. Three mealworms were then added to each curry tub and breathable lid was used to close the containers. Sample were stored in the dark at 25 °C, within a large plastic container. Containers were inverted every day for the first three days to increase exposure of the mealworms to the soil. Every week, for three weeks, soil samples were inspected carefully for dead mealworms. If a dead mealworm was found, it was initially subjected to a cleaning process to remove any opportunistic scavenger nematodes and avoid the growth of opportunistic fungi. Firstly, the dead insect was surface sterilised by dipping it in a 70% ethanol solution, after which it was dipped in 1:1 ratio of sodium hypochlorite (3.5%) and distilled water. Finally, the cadaver was rinsed in distilled water and placed into a modified white trap to capture emerging EPN as well as provide a suitable environment for EPF development. To create a white trap, a thin layer of water was added to a 250 ml curry tub, onto which an open petri dish with moistened filter paper was placed. When EPNs emerged, they were able to crawl across the moist filter paper and out of the petri dish, into the thin layer of water from where they were harvested. Isolation of fungi was carried out using a selective growth medium, similar to that described by Meyling (2007) (See thesis for more details). Once and EPF infected insect cadaver showed signs of sporulation, a small portion of these spores were removed and transferred to the growth medium using the

sterile technique. Petri dishes containing fungi were then closed, sealed with Parafilm and incubated at 25°C in the dark until sporulation, after which plates were stored in a fridge between 5°C and 10°C until genetic identification. EPF and EPNs extracted from soil samples were first tested to confirm fulfilment of Koch's postulates for pathogenicity, before genetic identification (See thesis for more information)

#### **Objective D**

Soil samples from each orchard were collected and sent to Bemlab (Strand, South Africa), to test for soil pH and texture. Ratios of stone, clay, silt and sand were recorded in order to determine the overall composition of the sample from each orchard.

Information on farm management practices was obtained directly from the growers. Growers were asked to provide pesticide spray records for each orchard for the duration that the orchard was monitored in the study. The active ingredients of each spray were obtained from the product label, along with the target pests that the product is registered for on citrus. Integrated pest management (IPM) ratings for each spray were obtained from Grout & Hattingh (2019). Sprays that had not been assessed by Grout & Hattingh (2019) were given a base IPM rating of "1", rather than not rating them, in an attempt to reduce underestimation. Furthermore, all such products were considered relatively "soft" on beneficials, despite the absence of specific non-target effect assays. Growers were also asked to provide information on orchard sanitation practices, including frequency and method of fruit disposal. Additionally, irrigation regimes for each farm were obtained from growers, including the type, duration and frequency of irrigation. Methods of weed control were also obtained from farmers, and put into three categories; "Only mechanical", "mainly chemical with some mechanical" and "mainly mechanical with some chemical". The vegetation on the floors of each orchard was visually estimated to assign to a category based on the abundance of weeds. These categories included "low", "medium" and "high" abundance of vegetation.

#### **Objective E**

FCM egg sheets obtained from river bioscience were placed into glass jars with breathable fabric mesh lids and kept at 25°C. Fruit used for susceptibility trials was used within 24 hours of being picked. When the eggs begun to hatch, the mesh lid was replaced with a metal one to allow convenient removal of neonate larvae. Only larvae which were able to crawl up onto the underside of the lid were used, thus ensuring the relative homogeneity of neonate fitness. A paintbrush was used to carefully remove neonate larvae from the lid and place six onto the surface of each fruit. It was ensured that each larva was able to move once placed and had not been injured. Between 12 and 15 fruit per orchard were infested and stored at 25 °C for two weeks (14 days), allowing the larvae to enter the fruit, feed and develop. On the 14th day, the fruit was dissected and carefully inspected for live larvae to record FCM infestation. The number and instar of larvae per fruit was recorded. Susceptibility trials were conducted both early and late in the 2020 and 2021 seasons.

After being extracted from cotton wool plugs or corrugated cardboard sheets, pupae were placed individually into 40 ml plastic pharmaceutical vials. The pupae were allowed to eclose, after which the moths were sexed using morphological features. Unmated male and female moths were paired up in single vials within 24 h of emergence. Each vial that contained a pair of moths was fitted with a cotton wool ball, moistened with water. Moths were allowed to mate over two nights at room temperature. Laboratory trials took place in medium-sized mesh oviposition cages (60 x 40 x 40 cm) at a temperature of approximately 25 °C. For the oviposition choice trials, 10 fruit from a single organic orchard placed into a cage with another 10 fruit from a corresponding conventional orchard (neighbouring farms and matching orchards). Fruit were arranged in a consistent alternating pattern within the cage. For the oviposition no-choice trials, 10 fruit from a single orchard were placed in a cage. In the evening, five pairs of moths were released in each cage for both the choice and no-choice experiments. The following morning, the moths were removed, as this provided sufficient time for oviposition to occur, as shown by Love *et al.* (2014). The fruit were inspected before hatching could occur and the egg counts for each fruit were recorded. Choice and no-choice experiments were conducted using fruit harvested both early and late in the 2020 and 2021 growing seasons. At each point in the season, the trials were repeated three times for all orchards. Corresponding pairs of orchards in the choice trials were swapped after each repetition and the oviposition cage positions were randomly shifted.

Standard internal fruit quality tests were performed to determine Fruit acid content (g per 100 ml citric acid), brix (sugar percentage), the ratio of brix/acid, juice percentage and peel mass (g) were determined using standard internal fruit quality tests (Anonymous 1946, Anonymous 1999). Twelve fruit were taken from every orchard. Prior to juicing, the fruit size and peel thickness was recorded. For each orchard, the twelve fruit were weighed together, after which each fruit was cut in half and juiced. After juicing, the fruit were weighed once more to determine the peel mass and juice percentage. The extracted juice was then mixed thoroughly in preparation for determining brix and acid percentage. A refractometer was used to record brix percentage. 20 ml of juice was titrated with sodium hydroxide to determine acid content (Anonymous 1999). Fruit quality tests were conducted once early in the season and once near harvest for every orchard.

Nutritional composition differences between fruit from organic and conventional farms was determined by cutting 20 segments of equal weight (approximately 55 g) from 20 individual fruit for each orchard. The fruit segments were then desiccated in an oven at 60°C. The dried fruit samples were sent to CAL Laboratories (Roodepoort, South Africa) to be analysed for ash, lignin, protein, fat and calcium content. Fruit was harvested and sent to CAL only once in the season (approximately 1 week before harvest). Another 20 fruit from each orchard were picked and sent (without any preparation) to Bemlab (Strand, South Africa), to be analysed for nitrogen, phosphorus, potassium, calcium, magnesium, sodium, manganese, iron, copper, zinc, boron, silica and moisture content. Bemlab analysis was repeated twice each season; once early in the season (May) and once late in the season (approximately 1 week before harvest).

## Results and discussion

### Task Table

Objective / Milestone	Achievement
A. FCM ecology and infestation	Achieved: Significantly higher FCM catches in conventional Palmer Navels and Newhall Navels
B. Comparison of above ground natural enemies of FCM	Achieved: Significantly higher counts for a variety of general predators as well as a significantly higher species richness of predators in organic orchards
C. Comparison of below ground (soil-dwelling) natural enemies	Achieved: No difference between EPF and EPN abundance but a in the dominant genus of EPF.
D. Farming management practices	Achieved: Organic farms overall have a significantly lighter spray program, more compatible with IPM. Lighter spray programs seem to be linked to lower FCM occurrence.
E. Fruit Oviposition preference, susceptibility and fruit nutritional characteristics	Achieved: Significantly higher oviposition preference shown in some cases towards conventional fruit. No significant difference in fruit susceptibility. Significantly higher magnesium, boron and nitrogen concentration in conventional fruit. Differences in nutrients are greater earlier in the season.

### Objective A (Chapter 2): FCM ecology and infestation

There was a highly significant difference in adult male FCM catches recorded between the organic and conventional Palmer Navel orchards in the Kirkwood area ( $t = 7.51$ ,  $df = 207$ ,  $P < 0.001$ ). The mean catches per week in the conventional Palmer Navel orchards was  $0.500 (\pm 0.065)$ , while the mean catches for the organic orchards was  $0.006 (\pm 0.006)$  per week. Significantly more adult male FCM were caught in conventional Newhall Navel orchards compared to organic orchards ( $t = 2.64$ ,  $df = 49$ ,  $P = 0.01$ ):  $0.18 (\pm 0.07)$  moths were caught per week in conventional orchards, while no wild moths were caught in the organic Newhall Navel orchard. No significant differences were found in moth catches between conventional and organic orchards, for Palmer Navels in the Barclay Bridge area, and for Nadorcott Mandarins and Delta Valencias (all

T-test  $P$  values  $> 0.05$ ). However, wild moth catches were notably higher in conventional Delta Valencia and Nadorcott Mandarin orchards. The Palmer Navels in the Barclay Bridge area were the only group that had higher wild moth catches in the organic orchards, but the difference was small and insignificant (Table 3.2.2.1).

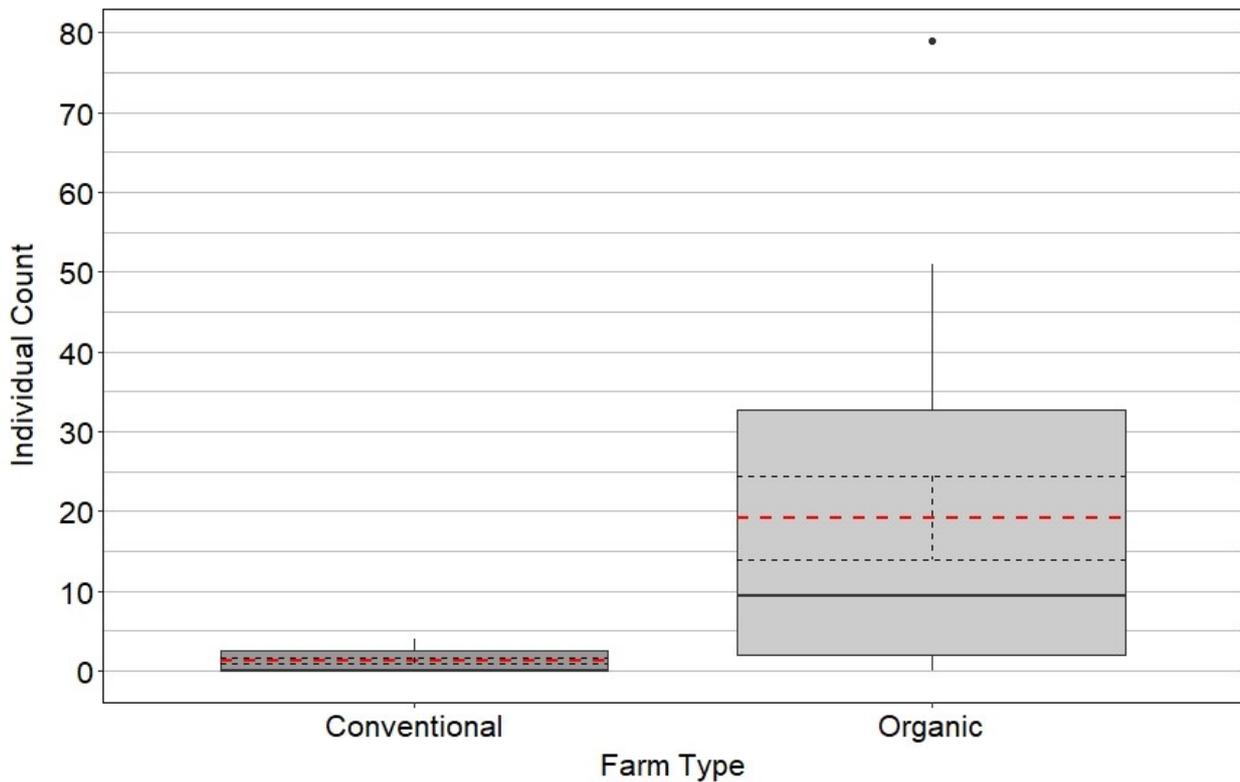
**Table 3.2.2.1.** Mean weekly delta trap catches of wild FCM males for all organic and conventional cultivars that were monitored in each location. Standard error and  $P$  values included. Significant  $P$  values are highlighted using bold font.

Cultivar	Location	Moth catches per week per orchard				$P$ value
		Organic		Conventional		
		Mean	SE	Mean	SE	
Palmer Navel	Kirkwood	0.006	0.006	0.500	0.065	<b>&lt;0.001</b>
Palmer Navel	Barclay Bridge	0.108	0.034	0.029	0.022	0.053
Newhall Navel	Riverbend	0.000	0.000	0.180	0.068	<b>0.011</b>
Delta Valencia	Barclay Bridge	0.049	0.017	0.069	0.026	0.521
Nadorcott Mandarin	Riverbend	0.140	0.070	0.220	0.082	0.461

A significantly higher number of infested fruit were collected from under the conventional Palmer Navel data trees in Kirkwood compared to organic ( $t = 2.26$ ,  $df = 203$ ,  $P = 0.03$ ). A mean of  $0.025 (\pm 0.011)$  infested fruit per week were picked up from under the conventional Palmer Navels for the duration of monitoring, while no fruit were picked up under organic Palmer Navel data trees. There were no significant differences between any other cultivars in other locations. Overall, there was a scarcity of infested fruit under data trees across all study sites. In total, over approximately two years of monitoring, 12 infested fruit were picked up from all orchards monitored. Nine infested fruit in total were picked up from conventional orchards, and three in total were obtained from organic orchards (see thesis for more figures).

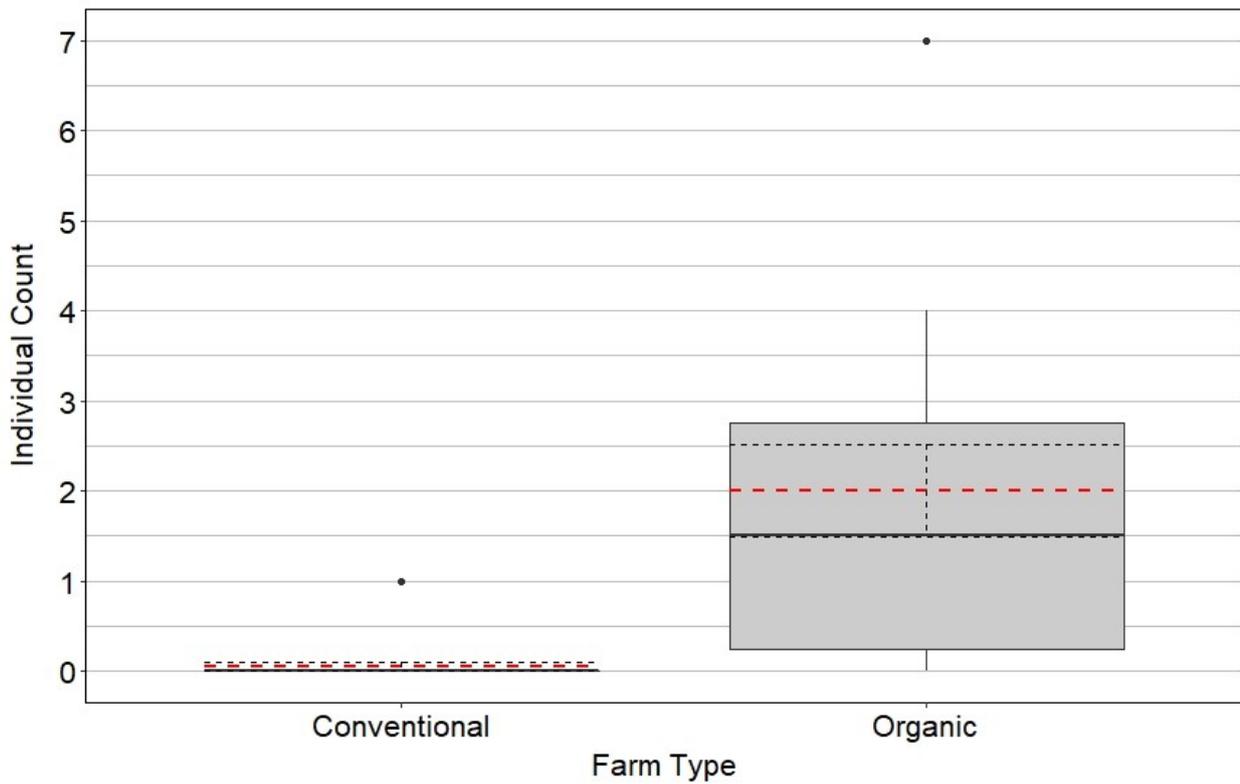
### **Objective B (Chapter 2): Comparison of above ground natural enemies of FCM**

A significantly higher number of ants was recorded on organic farms ( $X^2=32.8$ ,  $df= 1$ ,  $P<0.001$ ) (nbinom glmmTMB Chisq III) (Farming type \* Location + (1| rep)). A mean of  $19.2 (\pm 5.2)$  individuals per trap were found in organic orchards, while  $1.2 (\pm 0.363)$  per trap were found in conventional orchards (Fig. 3.2.2.1).



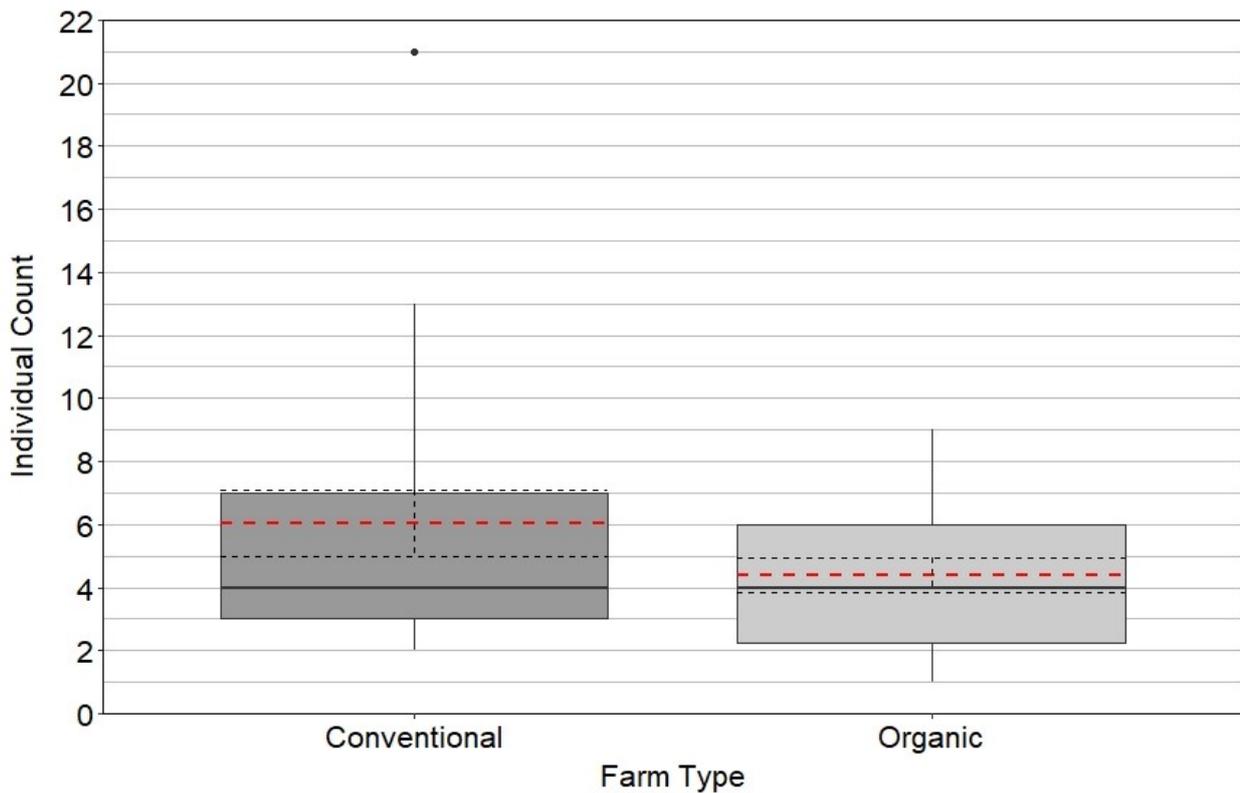
**Figure 3.2.2.1.** Mean number of individual ants (of any species) caught in pitfall traps in organic and conventional orchards. Organic orchards differed significantly from conventional orchards ( $P=0.001$ ). The dashed red line represents the mean, the dashed black lines represent the standard error, while the horizontal solid black line on each box represents the median. The box length represents the interquartile range, while black dots represent outlier values. Vertical solid black line represents the range of values within one and a half times the interquartile range.

Number of predatory rove beetles were significantly higher in organic orchards ( $X^2 = 9.11$ ,  $df = 1$ ,  $P = 0.003$ ) (nbinom glmmTMB Chisq III) (Farming type \* Location + (1| rep). A mean of 2.00 ( $\pm 0.51$ ) individuals were recorded per trap in organic orchards, while a mean of 0.05 ( $\pm 0.05$ ) individuals per trap were recorded in conventional orchards (Fig. 3.2.2.2).



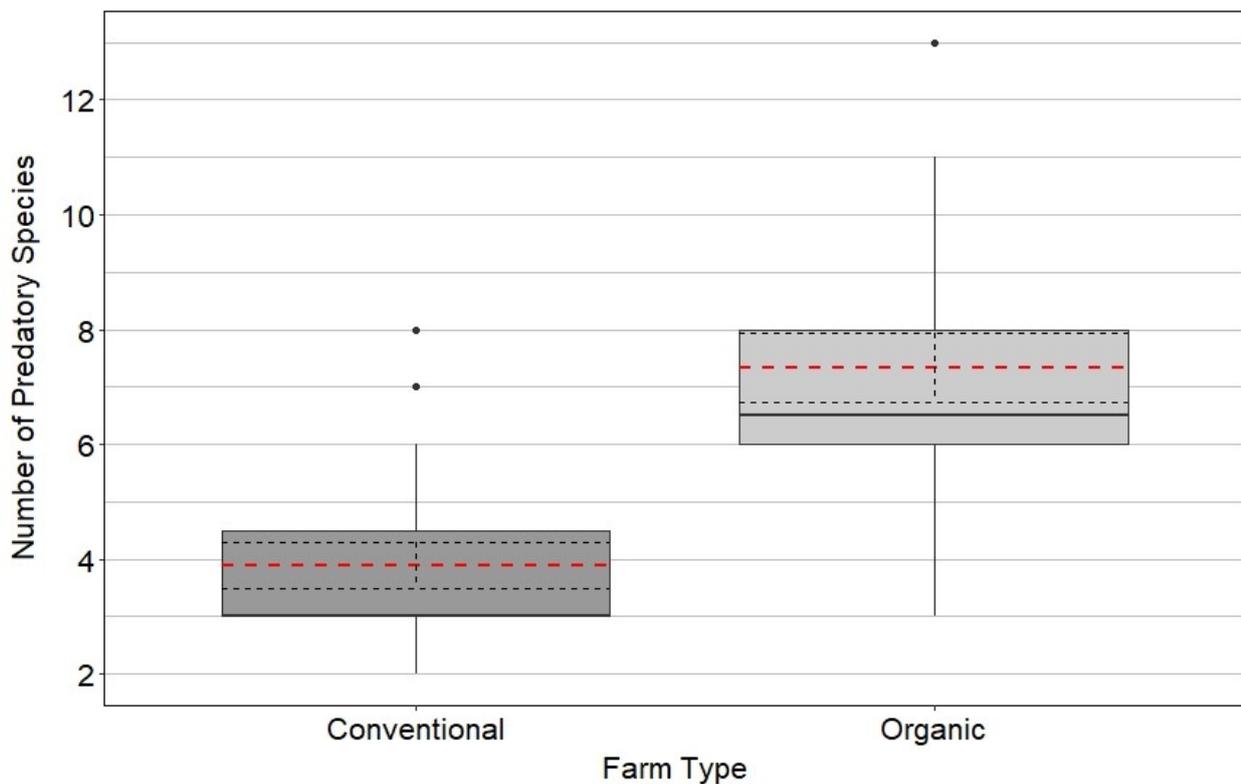
**Figure 3.2.2.2.** Mean number of individual rove beetles (of any species) caught in pitfall traps in organic and conventional orchards. Organic orchards differed significantly from conventional orchards ( $P = 0.003$ ).

There was no significant difference between the number of spiders found in organic and conventional orchards ( $X^2 = 2.53$ ,  $df = 1$ ,  $P = 0.11$ ) (nbinom glmmTMB Chisq III) (Farming type \* Location). There was a notably higher number of spiders recorded on conventional farms. A mean of  $6.1 (\pm 1.1)$  spiders per trap were recorded in conventional orchards and  $4.4 (\pm 0.6)$  spiders per trap in organic orchards (Fig. 3.2.2.3). No notable difference in spider species richness was found, with approximately three distinct spider species in each trap for organic and conventional farming.



**Figure 3.2.2.3.** Mean number of individual spiders (of any species) caught in pitfall traps in organic and conventional orchards. Organic orchards did not differ significantly from conventional orchards ( $P = 0.11$ ).

A significantly higher number of crickets were found on organic farms ( $X^2 = 5.32$ ,  $df = 1$ ,  $P = 0.02$ ) (nbinom glmmTMB Chisq III) (Farming type \* Location). The mean number of crickets recorded per trap was  $1.4 (\pm 0.4)$  in organic orchards and  $0.2 (\pm 0.2)$  in conventional orchards. The number of species (of the chosen potential predators) found per trap was significantly higher for organic farms ( $X^2 = 6.82$ ,  $df = 1$ ,  $P = 0.009$ ) (nbinom glmmTMB Chisq III) (Farming type \* Location). A mean of  $7.3 (\pm 0.6)$  predatory species were found per trap in organic orchards, whereas traps in conventional orchards recorded  $3.9 (\pm 0.4)$  species per trap (Fig. 3.2.2.4).

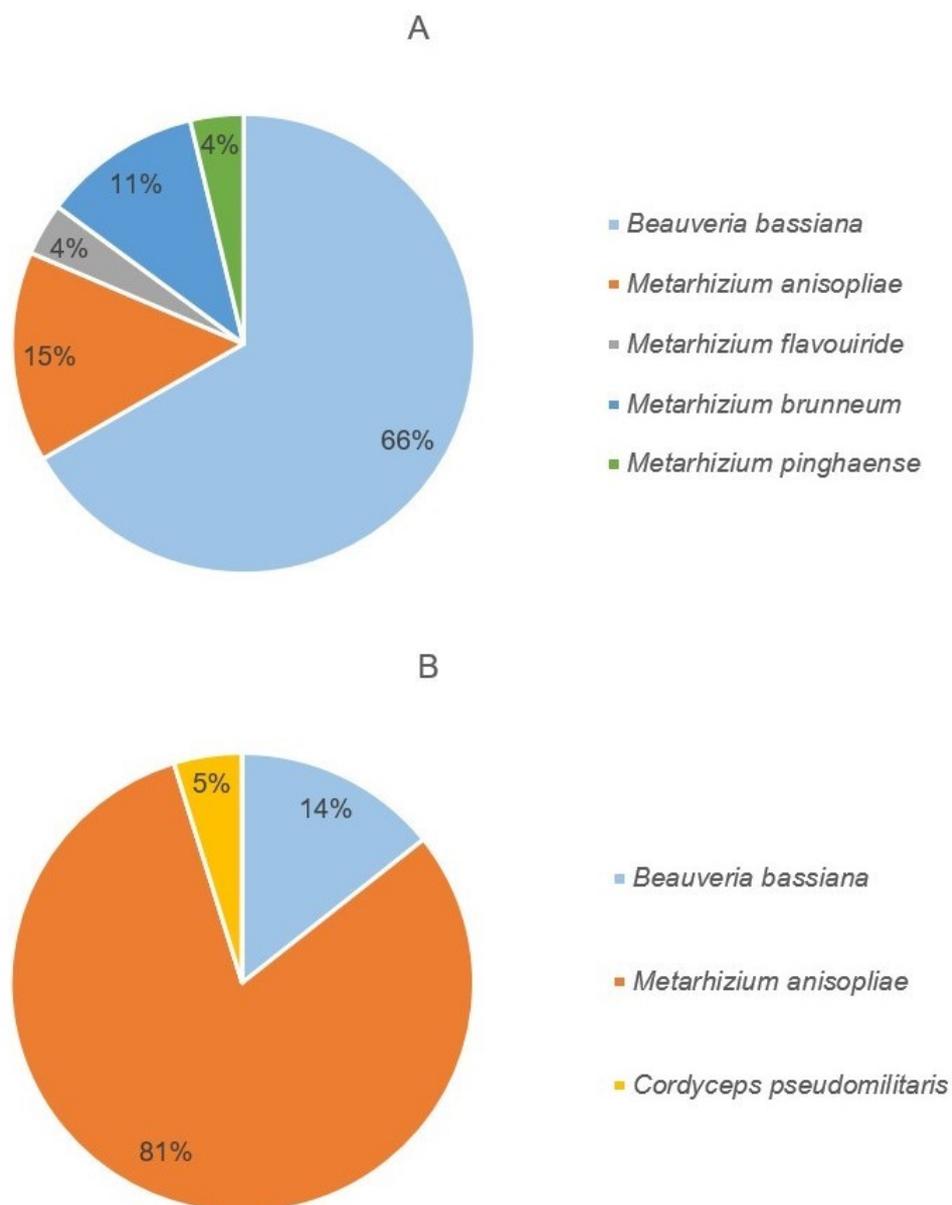


**Figure 3.2.2.4.** Mean number of distinct species in the chosen potential predator groups. Organic orchards differed significantly from conventional orchards ( $P = 0.009$ ).

**Objective C (Chapter 2): Comparison of below ground (soil-dwelling) natural enemies**

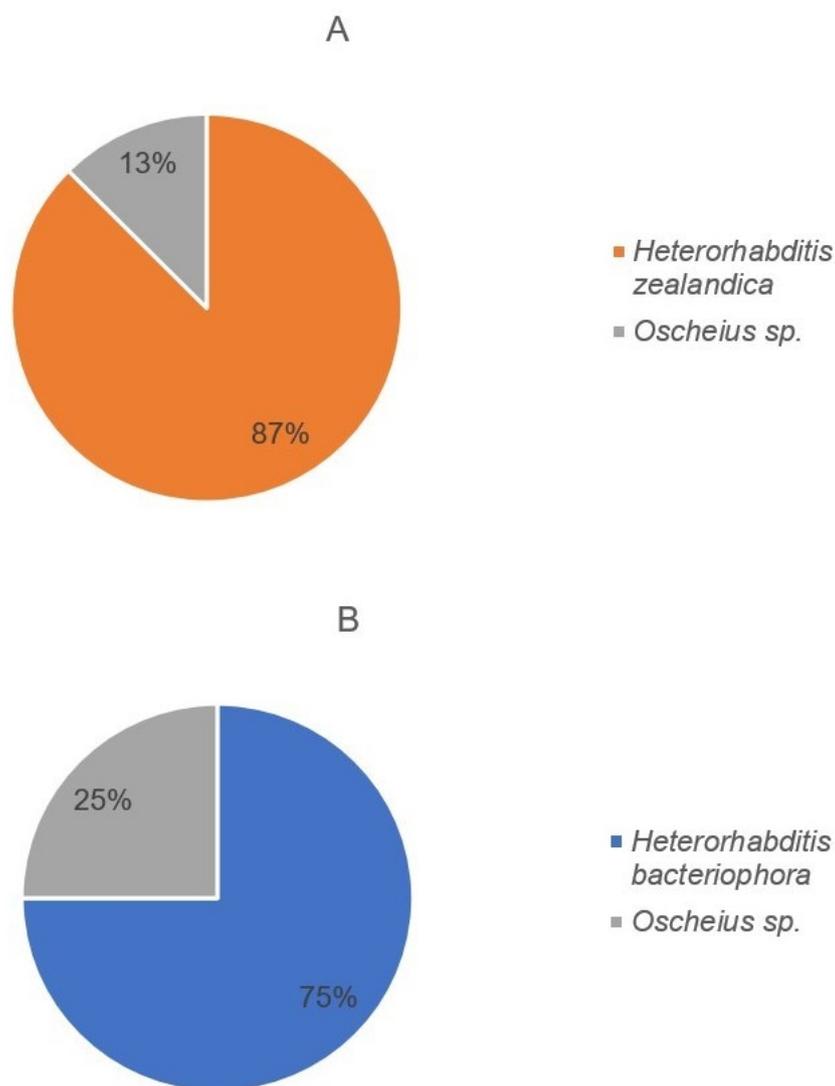
No significant difference was found between the relative abundance of EPF in organic and conventional citrus orchards ( $t = -0.13$ ,  $df = 71.90$ ,  $P = 0.90$ ). Out of the 411 soil samples collected, 48 EPF were isolated. A mean of 10% ( $\pm 2.5\%$ ) of conventional soil samples contained EPF, while a mean of 10.5% ( $\pm 3.18\%$ ) of organic samples contained EPF (See thesis for figures).

There was a notable difference in EPF species composition between organic and conventional citrus orchards. In conventional orchards (Fig. 3.2.2.5 A), the dominant species was *Beauveria bassiana* (66%), while in organic orchards (Fig. 3.2.2.5 B), the majority of isolates were *Metarhizium anisopliae* (81%). Three distinct EPF species were isolated from organic orchards and five distinct species were harvested from conventional orchards.



**Figure 3.2.2.5 A.** Relative species composition of entomopathogenic fungi isolated from all conventional orchards. **B:** Relative species composition of entomopathogenic fungi isolated from organic orchards.

The overall abundance of entomopathogenic nematodes was not significantly different between organic and conventional orchard soil ( $t = 0.31$ ,  $df = 37.92$ ,  $P = 0.76$ ). A total of 16 isolates were obtained. 7.9% ( $\pm 2.7\%$ ) of conventional soil samples and 6.7% ( $\pm 2.6\%$ ) of organic soil samples contained EPN isolates (See thesis for figures)). Although there was no significant difference between abundance of EPNs between organic and conventional soils, there was a clear difference in species composition (Fig. 3.2.2.6). For conventional farms (Fig. 3.2.2.6 A), the majority of isolates (87%) were *Heterorhabditis zealandica*, while in organic soils (Fig. 3.2.2.6 B), the majority of isolates (75%) were identified to be *Heterorhabditis bacteriophora*. No isolates of *H. zealandica* were found in organic soils, and no isolates of *H. bacteriophora* were found in conventional soils. A small proportion of soil samples contained *Oscheius sp.* in conventional and organic soils, 13% and 25%, respectively.



**Figure 3.2.2.6 A.** Relative species composition of entomopathogenic nematodes isolated from all conventional orchards. **B:** Relative species composition of entomopathogenic nematodes isolated from all organic orchards

#### Objective D (Chapter 4): Farming management practices

No significant difference was found between the soil structure of organic and conventional orchards ( $P > 0.05$ ) (See thesis for soil structure table). The only notable difference in soil structure was between the different areas in which farms were located. Soils from the Greater Addo area were classified as sandy-clay-loam due to their relatively high clay content. Soils from organic and conventional farms in the Barclay Bridge and Kirkwood areas were mostly classified as fine sandy-loam. No significant difference in soil pH was recorded between organic and conventional soils ( $P > 0.05$ ).

Soil pH for all farms ranged between 7.2 and 7.7.

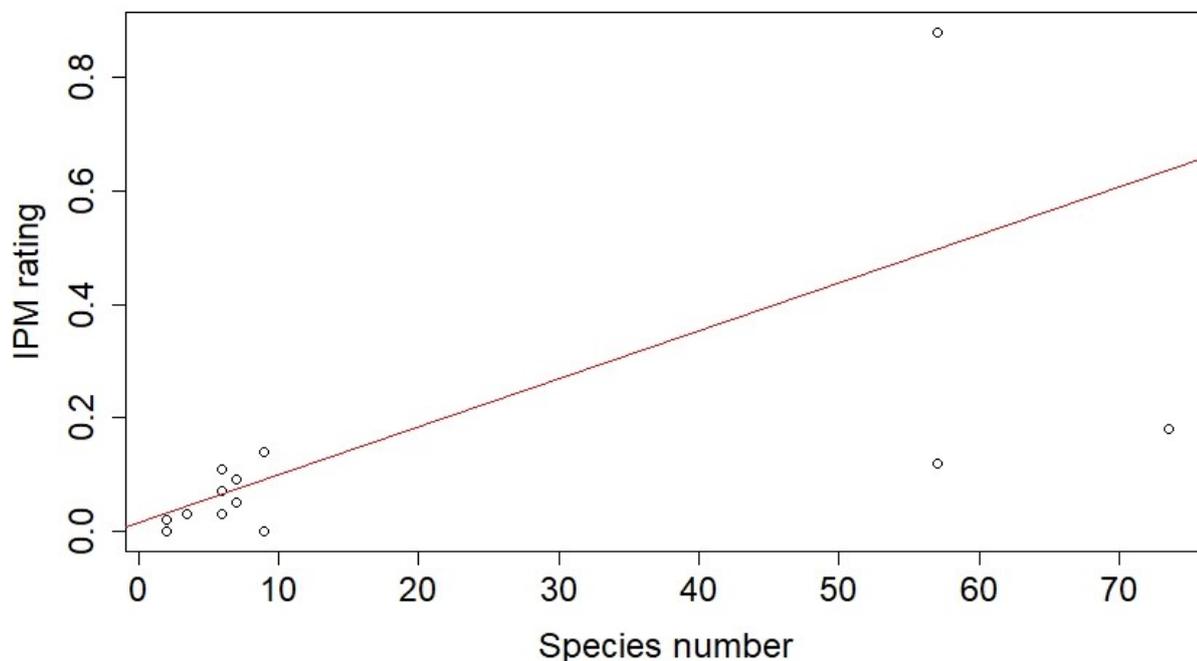
All except for one orchard in the study made use of micro-sprinkler irrigation (See thesis for tabulated management practice information). There did not seem to be a large difference in irrigation practice between organic and conventional farms. Irrigation duration and frequency did not seem to depend on whether the farm was organically or conventionally managed. All orchards received between 4-9 hours of irrigation at least once a week depending on the time of year. Five out of Seven farms mulched sanitised fruit after removing them from the orchards, taking them to a composting or dumping area far from the orchard. Two of the farms, one conventionally managed and one organically managed, mulched the sanitised fruit and left them to dry out in the sun on the roads next to the orchards. All farms conducted orchard sanitation at least once per week. The organic or conventional status of farms did not seem to influence orchard sanitation practices.

Organic farms used fewer pesticide sprays than conventional farms in most cases (See thesis for tabulated pesticide programs relating to each orchard). Between three and five sprays in total were used per year on each organic farm orchard, whereas total number of sprays ranged between five and thirteen per conventional farm orchard. Total IPM ratings for organic farms ranged from four to nine and total IPM ratings for conventional farms ranged between six and forty eight. An independent two-group *T*-test revealed a significantly poorer IPM rating for conventional farms ( $t = 2.72$ ,  $df = 6.23$ ,  $P = 0.03$ ).

Organic farms only employed mechanical methods of weed control, whereas conventional farms always used a combination of chemical and mechanical control (See thesis for tabulated farm management practices). Conventional farms differed from one another in the dominant mode of weed removal, with some using more mechanical control than chemical and vice versa. Abundance of orchard floor vegetation ranged from medium to high for organic farms and low to medium for conventional farms. Conventional farms generally had less live vegetation on the orchard floor.

Packhouse infested fruit interceptions from neighbouring pairs of farms in Kirkwood (Farm B (conventional) and Farm A (organic)) were several times higher on the conventional farms compared to the organic farms for Navels and late Navels (See thesis for tabulated information). FCM infested Navel interception was approximately 6 times higher from the conventional farm: 0.028% of the Navel and Late Navel samples from the conventional farm was infested whereas 0.005% of the organic Navel sample was infested. For each matching set of conventional and organic orchards, the orchards with the highest IPM rating (i.e. least IPM friendly) and/or the lowest vegetation abundance, generally had the highest FCM catches.

There was a significant positive correlation between the total IPM rating for each orchard and the mean weekly FCM catches per orchard ( $t = 2.51$ ,  $df = 12$ ,  $P = 0.03$ ). The orchard which had the highest number of sprays that were not compatible with IPM had the highest number of wild moth catches per week (Fig. 3.2.2.7).

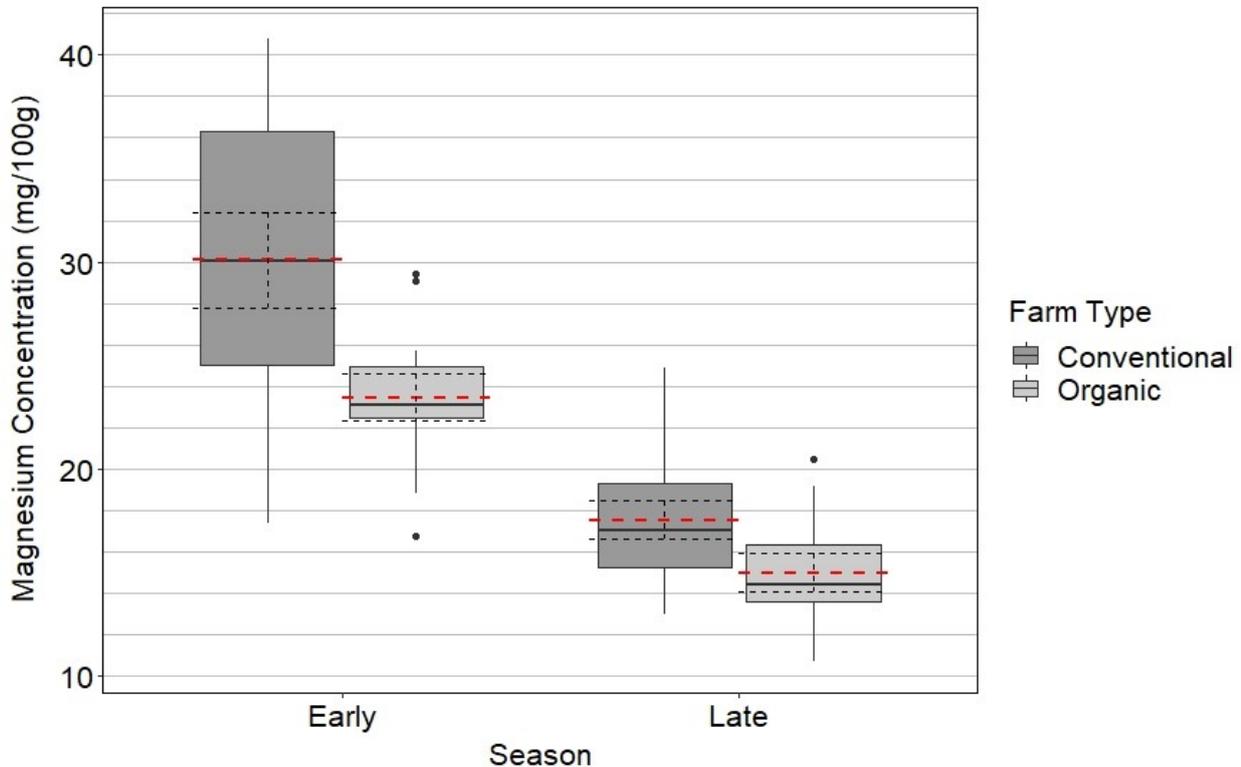


**Figure 3.2.2.7.** Linear regression between IPM rating (Grout & Hattingh, 2019) and mean weekly moth catches (Refer to Table 4.6 for individual values and standard errors).

**Objective E (Chapter 3): Fruit Oviposition preference, susceptibility and fruit nutritional characteristics**

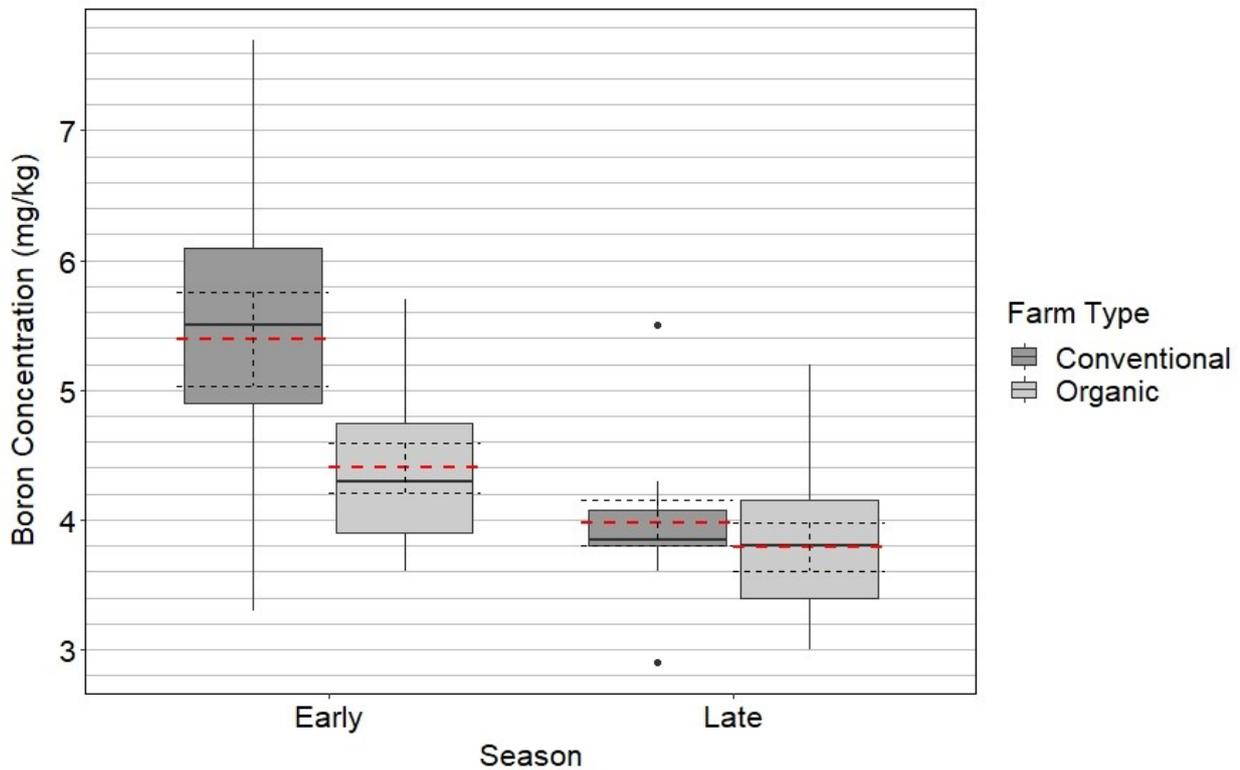
Significantly higher magnesium concentration was recorded in conventional fruit across all cultivars, over the 2020 and 2021 seasons ( $X^2 = 6.76$ ,  $df = 1$ ,  $P = 0.01$ ) (GLMM Chisq III) (Farming type \* time of season + (1 |

orchard)). The difference between magnesium in conventional and organic fruit was larger early in the season compared to later in the season. For unripe conventional fruit, the mean magnesium concentration was 30.1 mg/100 g ( $\pm 2.3$ ), compared to 23.5 mg/100 g ( $\pm 1.1$ ) for unripe organic fruit. Mean magnesium concentration for ripe conventional fruit was 17.6 mg/100 g ( $\pm 0.9$ ) compared to 15 mg/100 g ( $\pm 0.9$ ) for ripe organic fruit. Magnesium was 28% higher in conventional fruit early in the season and 17% higher later in the season (Fig. 3.2.2.8).



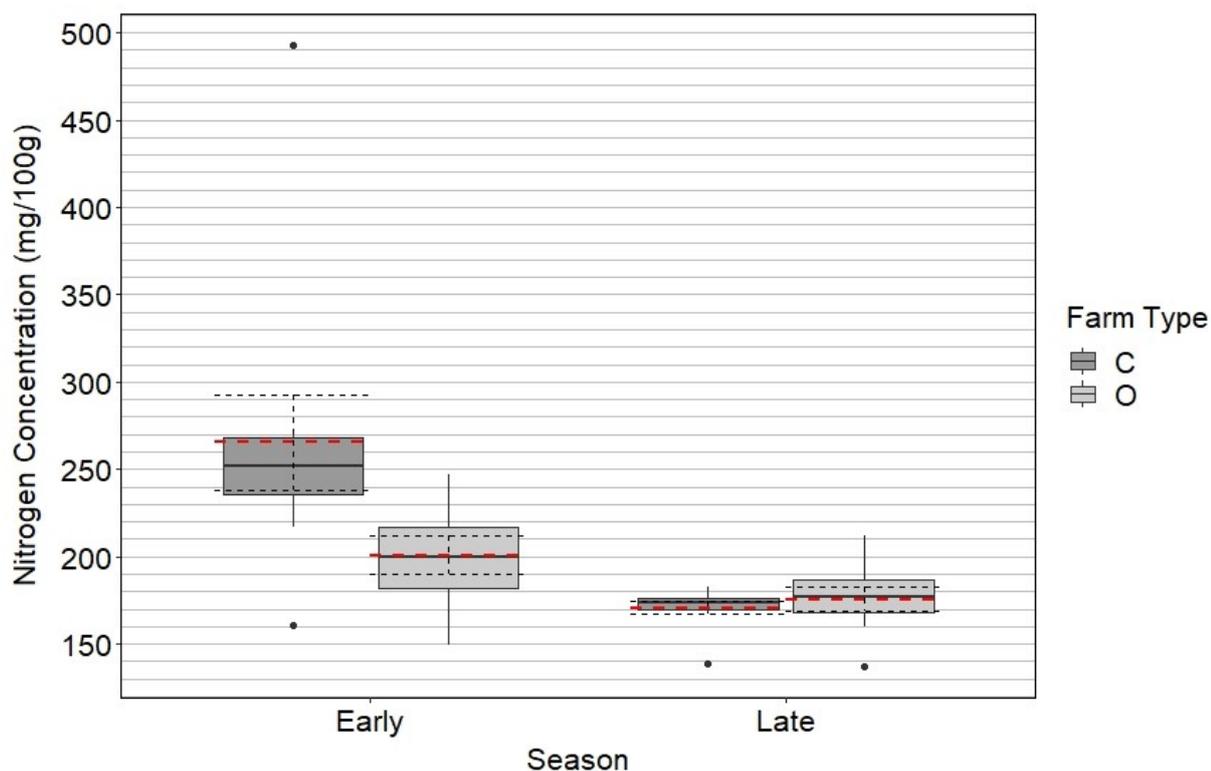
**Figure 3.2.2.8.** Mean magnesium concentration in all four organic and conventional citrus cultivars combined, both early and late in the growing season, spanning two seasons. Overall, Magnesium concentration was significantly higher in conventional fruit ( $P = 0.01$ ). The dashed red lines represent the mean, the dashed black lines represent the standard error, while the horizontal solid black line on each box represents the median. The box length represents the interquartile range, while black dots represent outlier values. Vertical solid black lines represent the range of values within one and a half times the interquartile range.

Boron concentration was significantly higher in conventional fruit. ( $X^2 = 8.17$ ,  $df = 1$ ,  $P < 0.01$ ) (Gaussian GLM LR III)(Farming type \* time of season). The mean boron concentration for unripe conventional fruit was 5.4 mg/kg ( $\pm 0.4$ ), compared to 4.4 mg/kg ( $\pm 0.2$ ) in unripe organic fruit. Later in the season, mean boron concentration in conventional fruit was 4.0 mg/kg ( $\pm 0.2$ ) compared to 3.8 mg/kg ( $\pm 0.9$ ) in organic fruit. Once again, the difference between concentrations earlier in the season were larger than later in the season. Boron concentration in unripe conventional fruit was approximately 23% higher than in organic fruit, whereas later in the season, it was only approximately 5% higher (Fig. 3.2.2.9).



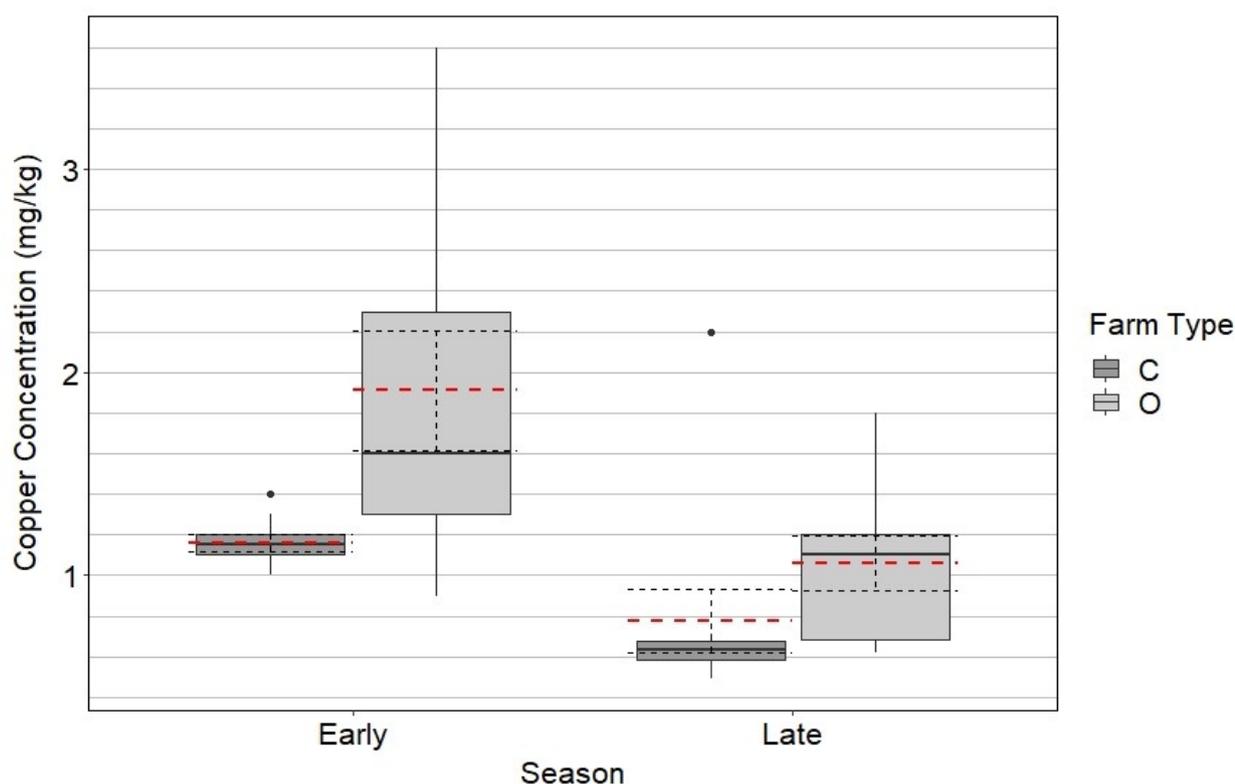
**Figure 3.2.2.9.** Mean boron concentration in all four organic and conventional citrus cultivars combined, both early and late in the growing season, spanning two seasons. Overall boron concentration was significantly higher in conventional fruit ( $P < 0.01$ ).

Early in the season, there was a significantly higher concentration of nitrogen in Delta Valencias and Palmer Navels ( $X^2 = 5.08$ ,  $df = 1$ ,  $P = 0.02$ ) (GLMM Chisq III) (Farming type \* cultivar + (1 | orchard)). Newhall Navels and Nadorcott Mandarins were excluded from this analysis as they had just one data point per cultivar for each farming practice type early in the season. Conventional fruit had a mean nitrogen concentration of 265 mg/100 g ( $\pm 27$ ) dry mass, which was approximately 31% higher than the 201 mg/100 g recorded for organic Palmer Navels and Delta Valencias (Fig. 3.2.2.10)



**Figure 3.2.2.10.** Mean nitrogen concentration in Palmer Navels and Delta Valencia combined, early and late in the growing season. Nitrogen concentration was significantly higher in conventional fruit early in the season ( $P = 0.02$ )

Copper concentration was significantly higher in organic Palmer Navels and Delta Valencia ( $X^2 = 31.7$ ,  $df = 1$ ,  $P < 0.001$ ) (Gaussian GLM LR III)(Farming type \* time of season \* cultivar). Nadorcott Mandarins and Newhall Navels were excluded from statistical analysis as there were too few data points for cultivar, season and farming practice combination. Mean copper concentration in unripe organic fruit was 1.79 mg/kg ( $\pm 0.25$ ), approximately 58% higher than in conventional fruit, which had a mean concentration of 1.13 mg/kg ( $\pm 0.04$ ). Late in the season, mean copper concentration in organic fruit was 1.03 mg/kg ( $\pm 0.11$ ) approximately 36% higher than organic fruit, had a mean of 0.76 mg/kg ( $\pm 0.13$ ) (Fig. 3.2.2.11).



**Figure 3.2.2.11.** Mean copper concentrations for Palmer Navels and Delta Valencias combined, early and late in the growing season. Organic fruit had a significantly higher copper concentration overall ( $P < 0.001$ )

No significant differences were found for concentrations of phosphorous, potassium, calcium, sodium, manganese, iron and zinc between organic and conventional fruit. (All GLM/GLMM modelled resultant  $P$  values  $> 0.05$ ) (See thesis for tabulated results). No significant differences between organic and conventional fruit were recorded for protein, ash, lignin, crude fat, calcium and moisture (All GLM/GLMM modelled resultant  $P$  values  $> 0.05$ ) (See thesis for tabulated results).

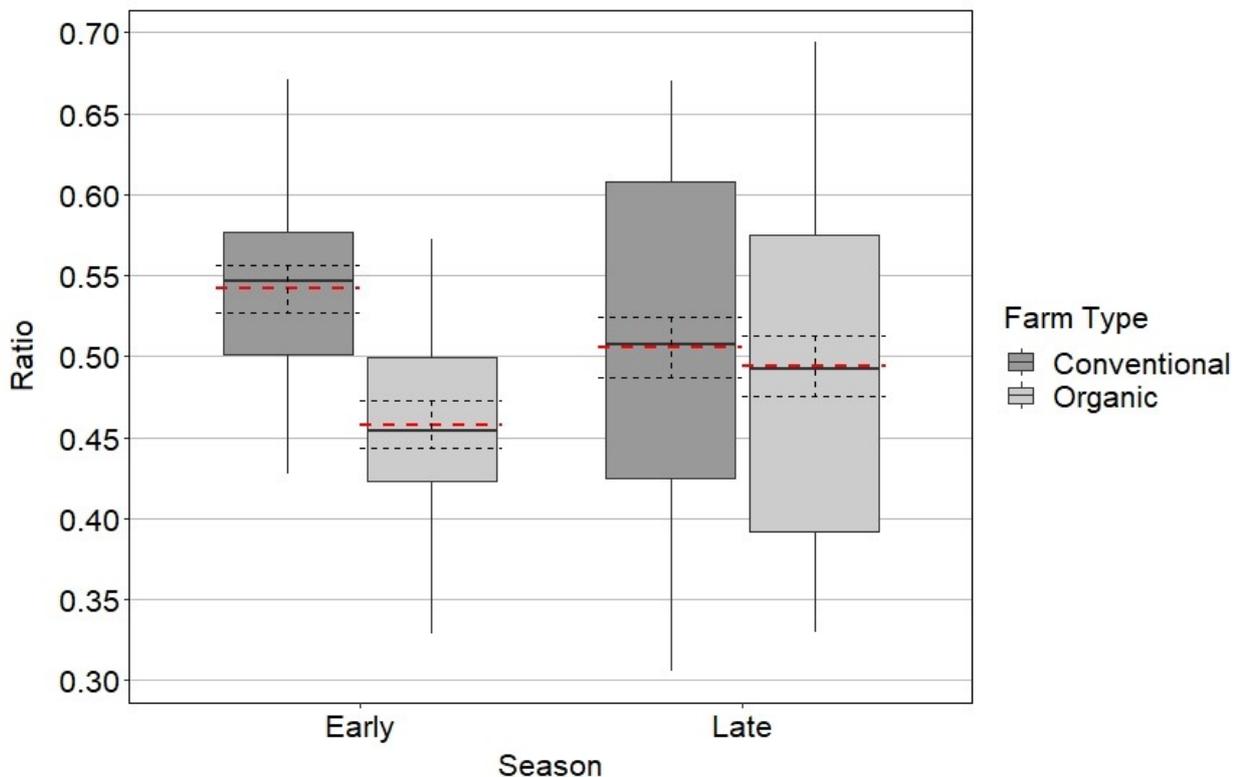
### Fruit Physical Parameters

As to be expected, fruit size increase with time in the season and differs between cultivars. Organic fruit are slightly but statistically significantly larger than conventional fruit ( $X^2 = 5.26$ ,  $df = 1$ ,  $P = 0.0218$ ). (GLMM Chisq III) (Farming type \* time of season \* cultivar + (1|orchard)). Overall, conventional fruit are approximately five percent larger than organic fruit (See thesis for figures). There was no significant difference in peel thickness across all cultivars ( $X^2 = 0.6$ ,  $df = 1$ ,  $P = 0.4$ ) (Gaussian GLM LR III) (Farming type \* time of season \* cultivar). A Welch Two Sample t-test revealed a significantly thicker peel for organic Palmer Navels compared to conventional Palmer Navels ( $t = -4.96$ ,  $df = 241$ ,  $P < 0.001$ ), as well as significantly thicker peels for organic Newhall Navels compared to conventional Newhall Navels ( $t = -4.71$ ,  $df = 43$ ,  $P < 0.001$ ). Overall, organic fruit peels were approximately 7% thicker than conventional fruit, when measured early in the season and approximately 11% thicker later in the season (see thesis for figures). As expected, sugar to acid ratio increased with time in the season, however there was no significant difference between sugar ratios of organic and conventional fruit at different points in the season ( $X^2 = 0.1$ ,  $df = 1$ ,  $P = 0.7$ ) (GLMM Chisq III) (Farming type \* time of season + (1|Orchard)) (See thesis for figures). While juice percentage naturally increases as the season progresses and differs between cultivars, there was no significant difference in juice percentage of conventional and organic fruit, both early and late in the season ( $X^2 = 1.6$ ,  $df = 1$ ,  $P = 0.2$ ) (GLMM Chisq III) (Farming type \* time of season + (1|Orchard)). Organic fruit had slightly higher juice percentages than conventional fruit early in the season (41% and 37% respectively) (See thesis for figures).

### FCM fitness related to fruit

There was a slight oviposition preference recorded in favour of conventional fruit for the no-choice oviposition trials, although this difference was not significant ( $X^2 = 0.9$ ,  $df = 1$ ,  $P = 0.3$ ) (glmmTMB Chisq III) (Farming type \* time in season + (1 | orchard/rep) + dispersion model for time of the season). There was a significantly higher preference towards oviposition on conventional fruit cultivars early in the 2021 season. ( $X^2 = 11.1$ ,  $df = 1$ ,  $P < 0.001$ ) (glmmTMB Chisq III) (Farming type \* cultivar + (1 | orchard/rep) + dispersion model for cultivar). Mean egg counts were almost twice as high when fruit were unripe, with a mean of 47.1 ( $\pm 2.0$ ) and 42.1 ( $\pm 1.8$ ) eggs recorded for conventional and organic fruit respectively, compared to 26.3 ( $\pm 1.4$ ) and 22.7 ( $\pm 1.4$ ) later in the season (See thesis for figures). This finding was of statistical significance ( $X^2 = 24.16$ ,  $df = 1$ ,  $P < 0.001$ ).

A significantly higher number of eggs were laid on conventional fruit in the choice oviposition trials ( $X^2 = 4.27$ ,  $df = 1$ ,  $P = 0.03$ ). The choice trials for early in the 2020 season were excluded due to a change in experimental method. There was a larger difference in egg-laying towards the beginning of the season compared to later in the growing season. For unripe fruit, 54% of eggs were laid on conventional fruit. Later in the season, near harvest, only 51% of eggs laid on conventional fruit. Although the difference was small, it was statistically significant overall (Fig. 3.2.2.12).



**Figure 3.2.2.12.** Mean egg count ratios for the choice oviposition trials, on fruit from all four organic and conventional citrus cultivars combined, both early and late in the 2021 growing season as well as late in the 2020 growing season. A significant higher number of eggs were laid on conventional fruit overall ( $P = 0.03$ )

There was no significant difference in fruit susceptibility between organic and conventional fruit. ( $X^2 = 0.1$ ,  $df = 1$ ,  $P = 0.7$ ). (Gaussian GLM LR III) (Farming type \* time of season). All fruit were significantly more susceptible later in the season compared to early ( $X^2 = 100$ ,  $df = 1$ ,  $P < 0.001$ ) (Gaussian GLM LR III) (Farming type \* time of season) (See thesis for figures). There was a notably higher mean number of survivors from diet jars supplemented with conventional fruit, however this difference was not significant when compared using a paired Ttest ( $t = 1.3$ ,  $df = 10.7$ ,  $P = 0.2$ ). There was no significant difference in the sex ratio of the pupae from diet jars supplemented with organic or conventional fruit powder (See thesis for figures). While there was a notably higher weight of pupae from jars supplemented with organic fruit powder, the difference was not significant ( $X^2 = 4.1$ ,  $df = 2$ ,  $P = 0.1$ ). This may be related to the lower number of pupae in organic jars, rather than any nutritional advantage. As expected, female pupae weighed significantly more than male pupae. ( $X^2$

= 108, df = 1,  $P < 0.001$ ) (GLMM Chisq III) (Farming type \* sex of pupa + (1 | orchard)). There was no significant difference in fecundity between female moths reared on diet supplemented with conventional or organic fruit powder ( $X^2 = 0.323$ , df = 1,  $P = 0.570$ ). (glimmTMB Chisq III) (Farming type \* cultivar + (1 | orchard)). The mean egg sheet counts were almost exactly the same, at 95.9 ( $\pm 4.4$ ) and 94.1 ( $\pm 5.5$ ) eggs for conventional and organic cultivars respectively (See thesis for figures)

## Conclusions to date

The ecology of any organism is complex. Many factors can dictate how an organism interacts with its environment and with other organisms around it. In the case of FCM in citrus, it seems that no one isolated factor is responsible for the infestation differences observed between organic and conventional farms. By determining the most likely reasons that FCM is higher in conventional than organic citrus farms, one can catch a glimpse into some of the ecological processes at play in an agricultural ecosystem. The better we understand these processes, the better our ability to control the pest species effectively and sustainably. This study demonstrates the importance of natural enemy conservation and the potential impact of fruit nutritional composition on pest abundance. Continued work needs to be conducted on the relationship between fruit nutrition and the fitness of FCM. Conclusive findings might guide farmers to strategize the timing and quantity of chemical nutrient inputs as part of their FCM management decision-making. The ripeness of the fruit used in trials has been shown to be important, as differences in fruit nutritional properties between organic and conventional orchards diminish towards the end of the growing season, as the fruit ripens. This body of work also demonstrates the extent to which management methods on farms can differ within conventional citrus farming, ranging from IPM-friendly practices that are very similar to organic farms, to practices that are much less compatible with IPM. For many years now, farmers have been encouraged to follow chemical spray regimes and management practices that are not harmful to beneficial organisms. This study adds to the ever-increasing body of literature on which those recommendations are based.

## Technology Transfer

- 22nd Biennial Congress of the Entomological Society of Southern Africa, 28<sup>th</sup> June – 1<sup>st</sup> July 2021, Oral presentation (Virtual)
- Citrus Research Symposium, June 2020, written presentation (one-page summary)
- Private meeting with SOGA (Sundays River Organic Growers Association), 10<sup>th</sup> September 2020, Oral presentation

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**3.2.3 FINAL REPORT: Genetic analysis and field application of a UV-tolerant strain of CrleGV for improved control of *Thaumatotibia leucotreta***  
 Project 1263 (2020 – 2021/22) by T T Bennett, M Hill, M Jukes and C Knox (RU) and S D Moore (CRI)

**Summary**

*Thaumatotibia leucotreta* is a serious pest of the citrus industry in South Africa and is controlled using an integrated pest management (IPM) programme. One of the components in the IPM programme is *Cryptophlebia leucotreta* granulovirus (CrleGV-SA), the active ingredient of Cryptogran® (River Bioscience (Pty) Ltd, South Africa) which has been used successfully in the field for many years. One of the main factors

influencing baculovirus insecticides such as Cryptogran® and its application in the field is UV irradiation. The DNA of the virus is damaged by exposure to UV light and this decreases the efficiency of the biopesticide. Thus there is a need to improve resistance of the OBs to UV. In a recent study, a UV-resistant virus strain of CrleGV-SA which differed genetically and biologically from the wildtype virus was selected after repeated exposure of viral OBs to UV-irradiation (Mwanza, 2019). This UV-resistant strain has potential as a biocontrol agent for improving the control of *T. leucotreta* in the field. It is important to determine whether the genetic and biological stability of this strain is maintained when passaged through the host for commercial purposes.

## Opsomming

*Thaumatotibia leucotreta* is 'n ernstige plaag in die sitrusbedryf in Suid-Afrika en word beheer met behulp van 'n geïntegreerde plaagbestuursprogram (IPM). Een van die komponente in die IPM-program is Cryptophlebia leucotreta granulovirus (CrleGV-SA), die aktiewe bestanddeel van Cryptogran® (River Bioscience (Pty) Ltd, Suid-Afrika) wat jare lank suksesvol in die veld gebruik is. Een van die belangrikste faktore wat bakulovirus-insekdoders beïnvloed, soos Cryptogran®, en die toepassing daarvan in die veld, is UV-bestraling. Die DNA van die virus word beskadig deur blootstelling aan UV-lig en dit verminder die doeltreffendheid van die bioplaagdoder. Daar is dus 'n behoefte om die weerstand van die okklusie partikels (OPs) teen UV te verbeter. In 'n onlangse studie is 'n UV-weerstandige virus isolaat van CrleGV-SA geselekteer, na herhaalde blootstelling van virus OPs aan UV-bestraling wat geneties en biologies van die wildtipe-virus verskil (Mwanza, 2019). Hierdie UV-weerstandige isolaat het potensiaal as 'n bioplaagdoder om die beheer van *T. leucotreta* in die veld te verbeter. Dit is belangrik om vas te stel of die genetiese en biologiese stabiliteit van hierdie isolaat gehandhaaf word wanneer dit deur kommersiële doeleindes deur die gasheer oorgedra word.

## Introduction

One of the major drawbacks with the use of baculoviruses as biopesticides is their susceptibility to ultraviolet radiation (UVA and UVB) from the sun. Exposure to UV light damages the virus DNA and thus kills the virus. The effect of sunlight increases with duration of exposure (Mwanza, 2015). In the field, this leads to abbreviated residual efficacy of the baculovirus biopesticide. Over the last four years, Mwanza (2019) has conducted a study to select for a strain of UV-resistant virus, using Cryptophlebia leucotreta granulovirus (CrleGV), the active ingredient of Cryptogran. CrleGV-SA resistant to UV was indeed successfully isolated by repeated exposure of virus to UV in a Q-SUN Xe-3HC Xenon Test Chamber that simulates normal sunlight, followed by propagation in FCM fifth instars for a total of five exposure cycles. Bioassays with control and UV exposure cycles 1- 5 showed more than 1000-fold improvement in virulence (LC<sub>50</sub>) on exposure to UV light after 5 selection cycles, indicating selection of UV-resistant virus. UV damage to the virion in the original CrleGV-SA sample was observed by TEM imaging. After 5 cycles of selection only 11% of virus showed UV damage, with the remainder appearing intact. The 1st and 5th cycle virus genomes were sequenced and compared with the original CrleGV-SA sequence. Mutations in the genome of UV-tolerant CrleGV were indeed observed. Non-synonymous single nucleotide polymorphisms (SNPs) were observed and considered likely to be responsible for enabling UV-resistance and improving the virulence of the UV-tolerant population. Although a handful of previous studies have also demonstrated successful selection for UV resistance, it has never been to the extent demonstrated by Mwanza (2019) which must be considered a very exciting breakthrough. Furthermore, this study was also the first to identify the genetic changes associated with, and probably responsible for, the development of UV resistance. Consequently, it is important that this study be taken further towards the end of developing an improved CrleGV biopesticide with greater persistence and thereby, improved overall efficacy. For a more detailed report of the study, the MSc thesis for this study, by Tahnee Bennett, is available from Rhodes University.

## Objectives

- A. To identify and compare variations between the CrleGV-SA-C5 and the CrleGV-SA genome sequences.
- B. To develop screening methods to distinguish between CrleGV-SA-C5 and CrleGV-SA isolate regions.
- C. To determine whether the genetic stability of the CrleGV-SA-C5 isolate is retained when bulked up *in vivo*.

- D. To compare the UV-tolerance of the CrleGV-SA-C5 and CrleGV-SA isolates under natural UV irradiation via detached fruit bioassays.

## Materials and methods

### Objective A

#### De novo assembly and genome alignment

The Illumina sequence data obtained from the CrleGV-SA-C5 isolate sequence was provided by Mwanza (2019). Duplicates within the sequence reads were removed using the Duplicate Read Remover function, and the reads were error corrected using Geneious R11 (v11.1.5) (Biomatters, New Zealand). The complete CrleGV-SA genome sequenced by van der Merwe *et al.* (2017) was used as the reference sequence for mapping the CrleGV-SA-C5 *de novo* assembly against it. In Geneious R11 (v11.1.5), a *de novo* assembly was run using the contigs and the *de novo* assembly function, with the assembler set to Geneious and the sensitivity set to medium sensitivity/fast and a single consensus sequence was generated. A pairwise alignment was performed on the consensus sequence and the reference CrleGV-SA sequence using the ClustalW method. The find SNPs/Variants tool in Geneious R11 was used to search for SNPs between the CrleGV-SA and the CrleGV-SA-C5.

#### Oligonucleotide design targeting variable regions

After a pairwise alignment between the CrleGV-SA reference sequence (van der Merwe *et al.*, 2017) and the CrleGV-SA-C5 was performed, three sets of oligonucleotides were designed to bind to target regions of the genome where SNPs were identified. Primer3 v0.4.0 (Untergasser *et al.*, 2012) was used to design the oligonucleotide sets. An *in silico* test was conducted in Geneious R11 to determine if the sets of oligonucleotides bind to the target regions correctly. The sets of oligonucleotides were synthesized by Inqaba Biotechnical Industries (Pty) Ltd (South Africa) (Table 3.2.3.1).

**Table 3.2.3.1.** Oligonucleotides targeting variable regions identified in CrleGV-SA-C5

Gene	Oligonucleotide Name	Sequence 5'- 3'	Position	Tm (°C)	% GC	Product size (bp)
<i>pif-2</i>	Pif-2 F	GACGATACGCTCCATTGCAT	38101→38120	58.5	50.0	348
	Pif-2 R	TCAAGTTACCTTCTCCCGCA	38448→38429	58.7	50.0	
<i>HP</i>	HypoP F	CATATGTAGGGTTCGCGTCA G	79632→79652	58.6	52.4	302
	HypoP R	AAACGACACCTATTACTTT GC	79933→79911	57.6	39.1	
<i>lef-8</i>	Lef-8/HP F	CCAACCAAGTACCAATAACGA C	104295→1043 16	57.3	45.5	311
<i>HP</i>	Lef-8/HP R	TTCGACGGATAGCATGTTTCG	104605→1045 86	58.2	50.0	

Pif-2: Per os infectivity factor-2, HP: Hypothetical Protein CDS, lef-8: Late expression factor-8

#### Virus samples, occlusion body purification and TEM

Purified CrleGV-SA-C5 OBs were provided by Nelson Mandela University, South Africa. CrleGV-SA OBs were purified from infected larval cadavers following the protocol described in Jukes (2017), originally adapted from Hunter-Fujita *et al.* (1998) and Moore (2002). Briefly, 6 ml of 0.1 % SDS (sodium dodecyl sulphate) was used to homogenise 0.75 g of the cadavers using a mortar and pestle. The homogenate was filtered into a beaker using cheese cloth and then equally divided into two JA-20 centrifuge tubes. The tubes were filled with ddH<sub>2</sub>O and centrifuged at 7840 ×g for 30 min at 4 °C in a Beckman Coulter Avanti® J-E centrifuge. The supernatant was discarded with the pellet resuspended using ddH<sub>2</sub>O and centrifuged again at 7840 ×g for 30 min at 4 °C. The supernatant was again discarded, and the pellet was resuspended with 1.5 ml ddH<sub>2</sub>O. Continuous 30-80 % (v/v) glycerol gradients were prepared in two ultracentrifuge tubes using 0.1 % SDS. 1.5 ml of the substrate was pipetted on top of the glycerol gradient, and this was centrifuged at 27783 ×g (15000 rpm) for 15 min in a

Beckman Coulter Optima™ L-90K Ultracentrifuge. The whitish brown OB forming bands were collected and pipetted into two new clean JA-20 tubes, ddH<sub>2</sub>O was used to fill each tube to the top and this was centrifuged at 7840 ×g for 30 min at 4 °C. The supernatant was discarded and ddH<sub>2</sub>O was used to resuspend the pellets. The centrifugation step at 7840 ×g for 30 min at 4 °C was repeated. Thereafter, the supernatant was discarded, the pellets were combined into one JA-20 tube, resuspended with ddH<sub>2</sub>O, and centrifuged for a final time at 7840 ×g for 30 min at 4 °C. The pellet was resuspended in 750 µl ddH<sub>2</sub>O and transferred into a 1.5 ml microcentrifuge tube. The purified OBs were used for subsequent experiments.

Purified CrleGV-SA and CrleGV-SA-C5 OBs were used for TEM. The preparation of transmission electron microscope grids was done following the method described by Opoku-Debrah *et al.* (2013). 5 µl of the purified OBs was pipetted onto a carbon formvar grid and left for 60 s. The excess liquid was removed using filter paper, followed by 5 µl 1 % uranyl acetate (w/v) pipetted onto the grid for 60 s to stain the grid. Filter paper was used to remove the excess uranyl acetate and the grid was left at room temperature to dry overnight. A Libra 120 (Zeiss, Germany) TEM was used to view the grid and OB images were captured. The size of 30 OBs was measured and data was analysed in Microsoft Excel® 2016.

## **Objective B**

### **Recombinant plasmids**

Six recombinant plasmids containing amplicons generated using Pif-2, HypoP and Lef-8/HP oligonucleotides were constructed, as seen in Table 3.2.3.2.

**Table 3.2.3.2.** Details of the recombinant pJET1.2/blunt plasmids following ligation of the Pif-2, HypoP and Lef-8/HP inserts, indicating the insert size, SNPs encompassed and the resulting size of each recombinant plasmid.

<b>Plasmid name</b>	<b>PCR amplicon cloned in pJET1.2/blunt vector</b>	<b>Insert size</b>	<b>Plasmid backbone</b>	<b>SNPs encompassed</b>	<b>Size of recombinant plasmid</b>
pC5_Pif-2	CrleGV-SA-C5 Pif-2	348		UV_2 & UV_3	3322
pSA_Pif-2	CrleGV-SA Pif-2	348			3322
pC5_HypoP	CrleGV-SA-C5 HypoP	302	pJET1.2/blunt	UV_5	3276
pSA_HypoP	CrleGV-SA HypoP	302	vector		3276
pC5_Lef-8/HP	CrleGV-SA-C5 Lef-8/HP	311		UV_6 & UV_7	3285
pSA_Lef-8/HP	CrleGV-SA Lef-8/HP	311			3285

### **Ligation of amplicons into pJET1.2/blunt and transformation into *E. coli***

Ligations were performed following the manufacturer's protocol for the CloneJET PCR Cloning Kit (Thermo Fisher Scientific, USA). The blunting enzyme mixture was set-up on ice in a total volume of 18 µl. In a 1.5 ml microcentrifuge tube, 10 µl of 2 × reaction buffer, 6 µl ddH<sub>2</sub>O, 1 µl PCR product (20 ng.µl) and 1 µl DNA blunting enzyme was added. The tube was gently mixed and incubated for 5 min at 70 °C. Thereafter the tube was placed back on ice and 1 µl pJET1.2/blunt cloning vector and 1 µl T4 Ligase was added to the blunting enzyme mixture, having a new total volume of 20 µl. The microcentrifuge tube was gently mixed and briefly centrifuged for 5 s, followed by incubation for 5 min at room temperature. This process was repeated for each of the six PCR amplicons (Table 3.2.3.2). The ligation mixtures were then used directly to perform transformation.

For each ligation reaction, competent TOP10 *E. coli* cells were thawed on ice and all the ligation mixture was added to 100 µl of the competent cells in a 1.5 ml microcentrifuge tube. A control was used for the reaction setup by adding 100 µl ddH<sub>2</sub>O to 100 µl of the competent cells. The tube was then gently mixed and then incubated on ice for 15 min. The mixture was then subjected to heat shock for 45 s at 42 °C and incubated on ice for 2 min. 900 µl Luria Broth (LB) (1.25 g NaCl, 1.25 g Yeast Extract, 2.5 g Tryptone and 250 ml ddH<sub>2</sub>O, autoclaved) was added, and the mixture was then incubated for 30 min at 37 °C. An initial 100 µl of the sample was spread-plated on Luria agar (LA) (1.25 g NaCl, 1.25 g Yeast Extract, 2.5 g Tryptone, 5 g Agar and 250 ml ddH<sub>2</sub>O, autoclaved) (with 100 µg.ml<sup>-1</sup> Ampicillin) plate, and the tube was then centrifuged for 3 min at 1000 ×g. The supernatant was gently poured out, leaving ~100 µl sample in the tube. The pellet was gently re-

suspended and the remaining cells were spread-plated on an LA (with 100 µg.ml<sup>-1</sup> Ampicillin) plate, followed by incubation overnight at 37 °C. The transformation process was repeated for each of the six ligation mixtures.

#### Colony PCR

Following the manufacturers protocol of the CloneJET PCR Cloning kit (Thermo Fisher Scientific, USA), colony PCR reactions were set-up in PCR tubes as described in Table 3.2.3.3. A distinct colony was selected for each transformation and transferred into separate PCR mixtures using a toothpick. A SimpliAmp™ Thermal Cycler (Thermo Fisher Scientific, USA) was used to carry out the PCR amplification, which consisted of an initial denaturation period of 3 min at 95 °C, followed by 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min, all for 25 cycles each and a final elongation at 72 °C for 2 min. Amplicons were visualised by 1 % agarose gel electrophoresis with ethidium bromide staining in 1 × TAE buffer (1 mM EDTA, 20 mM acetic acid, 40 mM Tris-acetate) run at 80 V for 30 min.

**Table 3.2.3.3.** Reaction set-up for colony PCR of recombinant plasmids in transformed TOP10 *E. coli* cells.

Reagents	Sample	NTC
2 × Amplicon <i>Taq</i>	10 µl	10 µl
pJET1.2 Forward primer (10 µM)	0.4 µl	0.4 µl
pJET1.2 Reverse primer (10 µM)	0.4 µl	0.4 µl
ddH <sub>2</sub> O	9.2 µl	9.2 µl
Transformed colony (Template DNA)	✓	—
<b>Total</b>	<b>20 µl</b>	<b>20 µl</b>

#### Plasmid extraction

Colonies identified as positive by colony PCR were inoculated in 5 ml LB containing ampicillin (100 µg.ml<sup>-1</sup>) followed by incubation with shaking at 37°C overnight. Plasmids were extracted from 3 ml of the cultured cells following the manufacturer's protocol of the GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific, USA). All centrifugation steps were carried out at 12000 ×g, and plasmid DNA was eluted with 50 µl Elution buffer. Samples were stored at -20°C.

#### Restriction enzyme digestion

Restriction enzyme digestion reactions were setup on ice in 0.2 ml tubes according to Table 3.2.3.4, using FastDigest restriction enzymes (Thermo Fisher, USA). The reaction was gently mixed and then centrifuged for ~5 s, followed by incubation at 37 °C for 15 min. Digests were visualised by 1 % agarose gel electrophoresis with ethidium bromide staining in 1 × TAE buffer at 80 V for 30 min. A GeneRuler™ 1 kb DNA ladder (Thermo Fisher, USA) was used to estimate band sizes. A ChemiDoc™ XRS+ System with Image Lab™ Software (Bio-Rad Laboratories, USA) was used to visualise the agarose gel.

**Table 3.2.3.4.** Reaction setup for restriction digestion of recombinant plasmid DNA

Components	Volume
ddH <sub>2</sub> O	14 µl
10 × FastDigest Buffer	2 µl
Plasmid DNA (51.0 – 159.9 ng/µl)	2 µl
FastDigest <i>Xba</i> I (10 U/µl)	1 µl
FastDigest <i>Xho</i> I (10 U/µl)	1 µl
<b>Total Volume</b>	<b>20 µl</b>

#### Plasmid sequencing and sequence alignment

Successfully extracted plasmids were sent for sequencing by Inqaba Biotechnical Industries (Pty) Ltd (South Africa). All plasmids were sequenced in the forward direction only using the pJET1.2 forward oligonucleotide. The CrleGV-SA Lef-8/HP PCR product was also sequenced in both the forward and reverse direction using the Lef-8/HP F and Lef-8/HP R oligonucleotides. A pairwise alignment was performed using the forward and reverse CrleGV-SA Lef-8/HP PCR sequences and the consensus sequence from the PCR product alignment was used for the alignment against the CrleGV-SA and CrleGV-SA-C5 sequences. A multiple alignment was

performed using the resulting sequence data, the reference CrleGV-SA (Accession number: MF974563.1) sequence and the CrleGV-SA-C5 sequence using the ClustalW method in Geneious R11.

#### Melt curve analysis

The CrleGV-SA-C5 HypoP and CrleGV-SA HypoP PCR products were used for the melt curve analysis. Each 20 µl SYBR melt curve reaction was setup according to Table 3.2.3.5. No Template controls (NTC) were used for each reaction setup, replacing the template DNA with ddH<sub>2</sub>O. The cycle parameters had an initial denaturation set at 95°C for 3 min followed by the melt curve generation starting at 65 °C increasing up to 95°C with a 0.5°C increment and 5 s hold at each temperature. The samples were setup in triplicate. Melt curve analysis was viewed using the CFX Manager™ Software v3.1 (Bio-Rad Laboratories, USA).

**Table 3.2.3.5.** SYBR Melt Curve reaction setup

Reagents	Setup	NTC
SYBR <i>Taq</i>	10 µl	10 µl
PCR Product template (12.5 ng/µl)	1 µl	-
ddH <sub>2</sub> O	9 µl	10 µl
<b>Total Volume</b>	<b>20 µl</b>	<b>20 µl</b>

#### Objective C

##### Occlusion body enumeration

Light microscopy was used to determine the concentration of the OBs from the CrleGV-SA-C5 sample and the bulked up CrleGV-SA-C5 samples. A 1:5 dilution of the virus suspension was made by adding 10 µl of the purified OBs to 40 µl of ddH<sub>2</sub>O in a 1.5 ml tube, making a total volume of 50 µl, and this was followed by mixing to homogenise the suspension. 200 µl of SDS (0.07 % w/v) solution was added to the 50 µl virus suspension making a further 1:5 dilution (total 25 × dilution). The suspension was mixed until homogenous. The sample was sonicated at 60 Hz for four 15 s pulses (ear protection was worn). A further four dilutions, were prepared using the sonicated suspension in four 2 ml tubes, whereby 1975.0, 1971.4, 1966.7 and 980.0 µl ddH<sub>2</sub>O was added to 25.0, 28.6, 33.3 and 20.0 µl of the sonicated suspension, respectively, resulting in dilutions ranging from 1:80 to 1:50 (total 1:2000 to 1:1250). The suspensions were mixed to ensure homogenous dilutions. A Thoma bacterial counting chamber with a 0.02 mm depth (Marienfeld, Germany) and cover slip were cleaned using 70 % ethanol solution and tissue paper. The cover slip was partially placed over the counting chamber for the virus suspension to be pipetted. The dilutions were tested, starting with the most diluted virus suspension, and working up until the most concentrated. The virus suspension that had ± 7 OBs per small square (0.0025 mm<sup>2</sup>) of the grid was used for counting and 5 µl was pipetted onto the counting chamber. The cover slip was placed completely over the counting chamber. The slide was then left to stand for 5 min prior to counting. This allows Brownian movement of non-virus particles to settle. The CrleGV-SA-C5 OBs were counted under the light microscope at 400 × magnification by phase contrast. The bulk-up 1 (CrleGV-SA-C5\_BU1) and bulk-up 2 OBs (CrleGV-SA-C5\_BU2) were observed at 400 × magnification using dark field. A Helber counting chamber with a Thoma ruling and a 0.02 mm depth (Hawksley, United Kingdom) was used for the dark field microscopy. The OBs in the top right and left, bottom right and left and a central large square of the main 4 × 4 grid (each square consisting of 16 smaller squares of 0.0025 mm<sup>2</sup>) were counted. Equation 1 was used to calculate the concentration of OBs.

Equation 1: Equation to determine the concentration of the virus sample using a counting chamber

$$\text{Concentration (OBs per ml)} = (D \times x) \div (N \times V)$$

Where D = dilution factor, x = Average number of OBs counted, N = Number of small squares and V= volume

##### *Thaumatotibia leucotreta* diet preparation

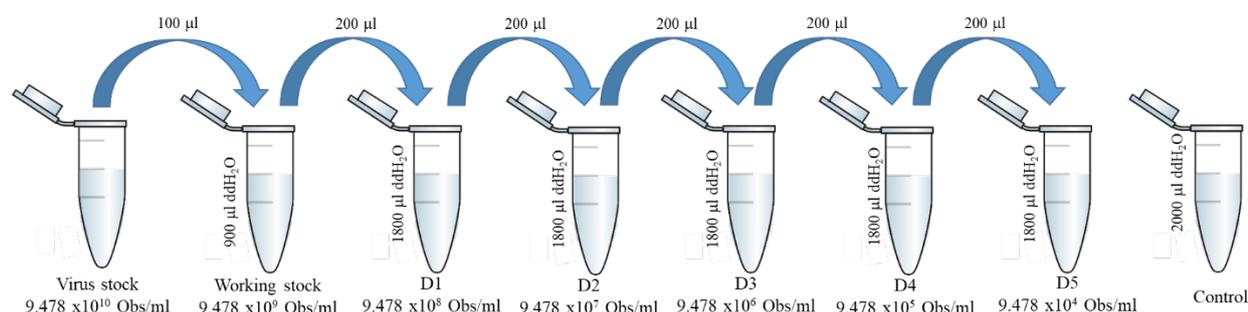
In a baking tray, 200 g of the dry FCM diet (210 g maize meal, 36.5 g milk powder, 100 g Brewer's yeast, 200 g wheat germ, 6.5 g sorbic acid and 15 g nipagin) (Moore et al., 2014) was homogenised in 200 ml ddH<sub>2</sub>O and baked for 20 min at 200 °C.

### Rearing of *Thaumatotibia leucotreta* larvae

*Thaumatotibia leucotreta* eggs laid on wax paper were provided by the Centre for Biological Control (CBC), Department of Zoology and Entomology, Rhodes University and were reared on artificial *T. leucotreta* diet. *Thaumatotibia leucotreta* diet was baked in a baking tray and placed in a laminar flow hood to cool down. In the laminar flow hood, the eggs were sterilised with 5 % formaldehyde and then placed on the surface of the diet. A small piece of paper towel was placed on the surface of the diet to reduce condensation. A glass cover was placed on the baking tray and secured with crocodile clips. The baking tray was incubated for ~11 days at 25 °C with a 16h:8h (D:N) photoperiod, until larvae reached fourth instar.

### Surface dose-response bioassays to determine the lethal concentration estimates

Surface-dose bioassays were performed in 24-well bioassay plates to estimate specific LCs. *Thaumatotibia leucotreta* diet was prepared and baked in a Pyrex dish and placed under a laminar flow hood to cool down. A modified 10 ml syringe was used to cut the diet, then pressed down into each well and covered with bioassay plate covers. A 10-fold dilution series was conducted with five concentrations made from the CrleGV-SA-C5 OB stock in ddH<sub>2</sub>O (Figure 3.2.3.1). 50 µl of each virus dilution or the control was evenly pipetted on the surface of the diet in each well and a glass lid was used to cover the plates. A single fourth instar larva was placed on the surface of the diet in each well. A sample of 10 larvae from the insect batch was collected, the larval head capsule width was measured using the Leica EZ4 D microscope (Leica Microsystems) and Hofmeyr *et al.* (2016) was used for reference to confirm the larval instar. The mean head capsule width of the larvae used was 0.8416 mm (n=10) which confirmed the larvae were 4<sup>th</sup> instar. Bioassay plates were securely closed with masking tape and incubated at 25 °C. After 7 days, diet was carefully dissected and larval survival was recorded. The data from the dose-response bioassays were subjected to probit analysis in ToxRat Professional to determine the LC<sub>50</sub> and LC<sub>90</sub>.



**Figure 3.2.3.1.** Dilution series of CrleGV-SA-C5 for surface dose-response bioassays against *T. leucotreta* fourth instars to determine LC<sub>90</sub>.

### CrleGV-SA-C5 bulk-up in fourth instar *T. leucotreta*

CrleGV-SA-C5 was bulked up using a modified protocol described by Mwanza (2019). CrleGV-SA-C5 was bulked up in fourth instar *T. leucotreta* larvae carried out in 24-well bioassay plates. *Thaumatotibia leucotreta* diet was prepared and baked in a Pyrex dish and placed under a laminar flow hood to cool down. A modified 10 ml syringe was used to cut the diet, then pressed down into each well and covered with bioassay plate covers. The surface of the diet in each well was inoculated with 50 µl CrleGV-SA-C5 with an adjusted LC<sub>90</sub> concentration of  $8.75 \times 10^9$  OBs/ml. A single fourth instar larva (mean head capsule width: ~0.875 mm, n=10) was placed on the surface of the diet in each well. Bioassay plates were securely closed with masking tape and incubated at 25 °C. Bioassay plates were inspected after 7 days for infected or dead larvae, and larvae were collected in a microcentrifuge tube. Occlusion bodies were extracted from larval cadavers as described in below. Purified virus was stored at -20 °C for future use in subsequent bulk-up. The purified virus from bulk-up 1 was used to conduct a second bulk-up in fourth instar *T. leucotreta* larvae (mean head capsule width: ~0.863 mm, n=10). Virus was extracted and purified from larval cadavers collected from bulk-up 2 and stored at -20 °C. Three bioassay plates were used for bulk-up 1 and ten bioassay plates were used for bulk-up 2.

### Crude purification of occlusion bodies

Purification of OBs from larval cadavers collected from bulk-up 1 and from bulk-up 2 was conducted following the protocol described in Jukes (2017), originally adapted from Wennmann & Jehle (2014). Into sterile 2 ml tubes, larval cadavers were homogenised in 1 ml 0.1 % SDS (w/v) using a sterile mortar, followed by vortexing for 1-2 min. Homogenised samples were centrifuged for 60 s at 100 ×g and the supernatants were collected in a new 2 ml tube. 1 ml of 0.1 % SDS was used to resuspend the pellets followed by centrifugation for 60 s at 100 ×g. The supernatants were collected and pooled together, followed by centrifugation at 2500 ×g for 5 min. The supernatants were discarded, and the pellet was resuspended in 1 ml ddH<sub>2</sub>O. A final centrifugation was performed at 12000 ×g for 5-10 min and the supernatant was removed. The pellets were resuspended in 500 and 1500 µl ddH<sub>2</sub>O for bulk-up 1 and 2 respectively and stored at -20°C for future use.

#### Genomic DNA extraction

100 µl of purified OBs was mixed with 45 µl Na<sub>2</sub>CO<sub>3</sub> followed by incubation for 30 min at 37°C. The pH was neutralised by the addition of 55 µl 1M Tris-HCl (pH 6.8). Thereafter DNA was extracted following the manufacturer's instructions of the Quick-DNA™ Miniprep Plus kit (Zymo Research, USA). All centrifugations were performed at 12000 × g. DNA was eluted in 50 µl pre-heated (70°C) DNA elution buffer and stored at -20 °C for future use.

#### Polymerase chain reaction of target regions

Each 25 µl PCR reaction was set up as follows: Template DNA with concentration between 40.9 – 64.8 ng/µl was used. No template controls (NTC) were used for each PCR, replacing the template gDNA with ddH<sub>2</sub>O. Positive controls were used for each PCR, using the plasmid DNA from pC5\_Pif-2, pC5\_HypoP and pC5\_Lef-8/HP as template DNA for the respective PCR reaction set ups. The PCR products were analysed by 1% agarose gel electrophoresis (AGE) with ethidium bromide staining in 1 × TAE buffer (1 mM EDTA, 20 mM acetic acid, 40 mM Tris-acetate) for 30 min at 80V. The GeneRuler™ 1 kb DNA ladder (Thermo Fisher, USA) was used to estimate amplicon size. A ChemiDoc™ XRS+ System with Image Lab™ Software (Bio-Rad Laboratories, USA) was used to visualise the agarose gel.

#### Sanger sequencing of polymerase chain reaction amplicons

Amplicons from bulk-up 2 were sent for sequencing by Inqaba Biotechnical Industries (Pty) Ltd (South Africa) in both the forward and reverse direction using the Pif-2, HypoP and Lef-8/HP oligonucleotide sets. A pairwise alignment was conducted using the ClustalW method, aligning the forward and reverse sequences for each of the three regions generating a consensus sequence. A pairwise alignment was then performed using the generated consensus sequence and the CrleGV-SA-C5 sequence using the ClustalW method in Geneious R11.

### **Objective D**

#### Collection and preparation of oranges and virus treatment preparation

Oranges were collected in the Sundays River Valley, Eastern Cape Province. Oranges were thoroughly washed, to remove any residue or contaminants on the orange, in water with a small amount of dishwashing liquid, followed by a rinse in water, wiped down and left to dry overnight. Three treatments, two of which were the viruses CrleGV-SA-C5 and CrleGV-SA, and a ddH<sub>2</sub>O control were used for the two detached fruit bioassays set-ups, the UV exposure (UV\_DFB) and Non-UV exposure control (Non-UV\_DFB) detached fruit bioassays (

Table 3.2.3.6). Each virus treatment was applied at a concentration of 5 × 10<sup>5</sup> OBs/ml (being the registered concentration for Cryptogran) in a total of 1 litre.

**Table 3.2.3.6.** Treatments applied to oranges for each UV\_DFB and Non-UV\_DFB set-up to determine the UV tolerance of the CrleGV-SA-C5 isolate in comparison to the CrleGV-SA isolate.

Treatments	Concentration (OBs/ml)	Number of infected oranges per treatment
☞ > ☞ × ddH <sub>2</sub> O Control	—	30

	CrleGV-SA-C5		
	CrleGV-SA	$5 \times 10^5$	
Non-UV Exposure	ddH <sub>2</sub> O Control	—	
	CrleGV-SA-C5		30
	CrleGV-SA	$5 \times 10^5$	

#### Detached fruit bioassay

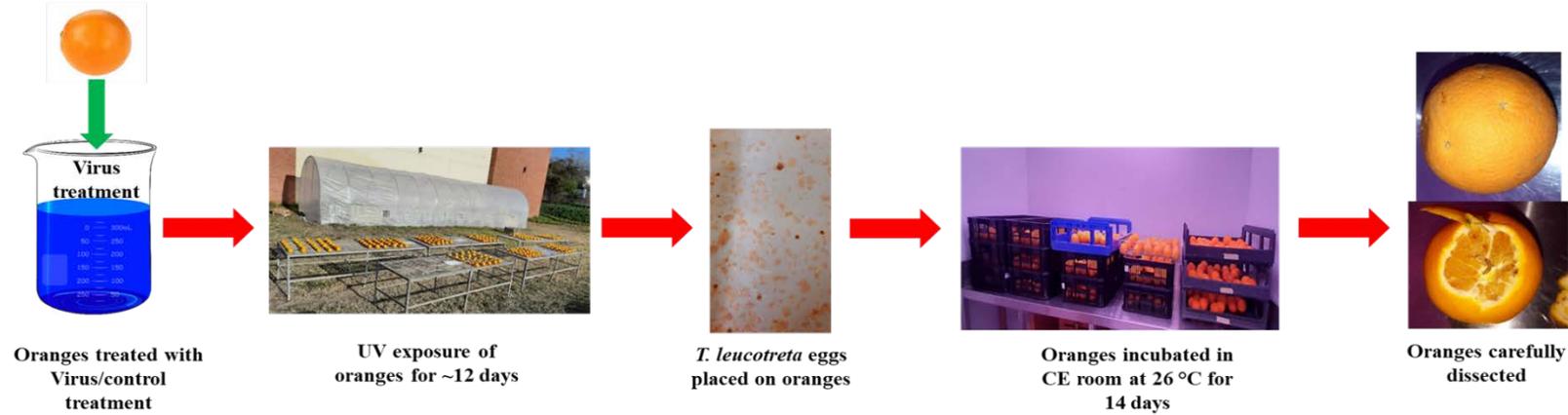
Two detached fruit bioassay set-ups, a UV\_DFB and Non-UV\_DFB were used (Figure 3.2.3.2). The same virus treatment and ddH<sub>2</sub>O control were used for each detached fruit bioassay set-up and a total of 30 oranges was used per virus treatment and for the control treatment (

Table 3.2.3.6). Oranges were dipped into the treatment for full coverage, thereafter treated oranges for the UV\_DFB set-up were placed onto galvanized steel tables that were placed outside for natural UV exposure from sunlight. Oranges were exposed to UV for 48 h per hemisphere, equating to oranges being exposed for a total of 96 h and ~8 h exposure per day (total ~12 days), therefore oranges were taken outside in the morning and taken indoors in the late afternoon. Oranges treated for the Non-UV\_DFB set-up were placed on galvanized steel tables placed indoors and left for ~12 days.

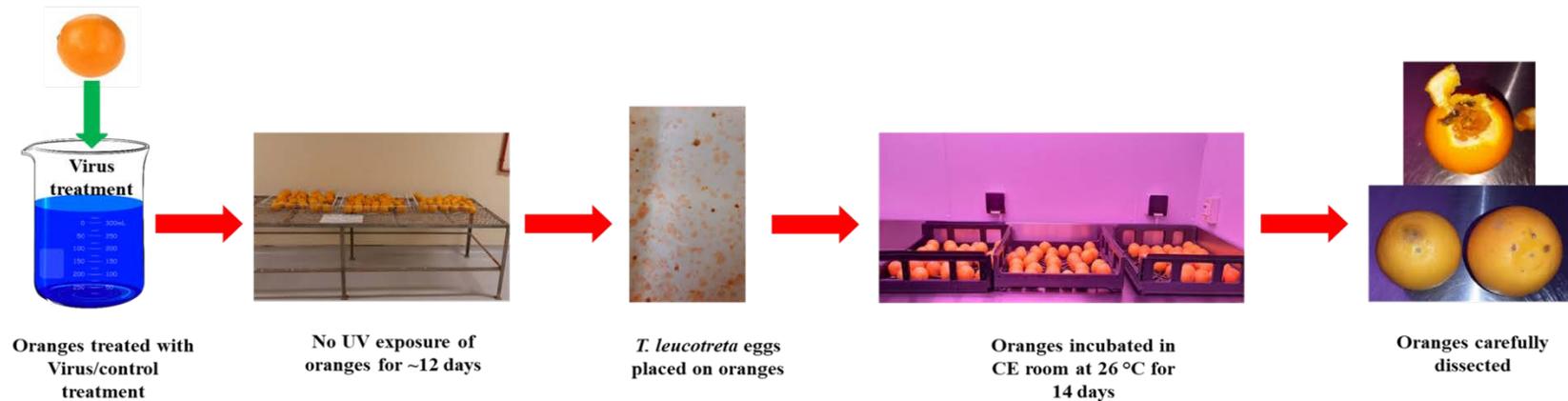
*Thaumatotibia leucotreta* egg sheets were provided by River Bioscience (Pty) Ltd. Ten *T. leucotreta* eggs were placed onto each of the treated oranges from both bioassay set-ups, oranges were placed into crates and incubated in a CE room set to 26 °C for 14 days. Following incubation, oranges were carefully dissected and inspected for the presence of live *T. leucotreta* larvae. The infestation of *T. leucotreta* was identified by the presence of the larva. Each detached fruit bioassay set-up, UV\_DFB and Non-UV\_DFB set-up was replicated three times.

Weather data for each day of UV exposure for the UV\_DFB was collected from the Rhodes University Geography Department weather station (33°18'00" S, 26°30'00" E) which was approximately 500 m from the study site. GraphPad Prism v 9.3.1 (GraphPad Software, San Diego, California USA) was used to perform all statistical analyses. A Kruskal-Wallis nonparametric test was performed on the data from both UV\_DFB and Non-UV\_DFB. A Mann-Whitney test was performed on the data from the control treatments for both the UV\_DFB and Non-UV\_DFB.

### UV Exposure Detached Fruit Bioassay Set-up (UV\_DFB)



### Non-UV Exposure Detached Fruit Bioassay Set-up (Non-UV\_DFB)



**Figure 3.2.3.2.** A schematic flow diagram of the UV exposure and Non-UV Exposure detached fruit bioassay setup.

## Results and discussion

### Task table

Objective / Milestone	Achievement
A.	
A.1. Bioinformatic analysis	Complete: Several SNPs were identified in the CrleGV-SA-C5 72 hour isolate.
A.2. Oligo design	Complete: Oligonucleotides were designed which targeted several of the SNPs identified in objective A.1.
A.3. TEM	Complete: Images of CrleGV-SA-C5 were obtained
B.	
B.1. DNA extraction	Complete: DNA was extracted from CrleGV-SA-C5 and WT isolates
B.2. PCR	Complete: Three regions were successfully amplified from CrleGV-SA-C5 and WT DNA using the oligos designed in A.2.
B.3. Cloning	Complete: The amplicons were cloned into the pJet1.2. vector and sequenced. Several SNPs were detected in the target regions. Attempts to differentiate the target regions by RT-PCR were unsuccessful.
B.4. Melt Curve	Complete: May have been successful as it supports the sequencing results shown that there was no difference between the CrleGV-SA-C5 HypoP and CrleGV-SA HypoP regions.
C.	
C.1. OB Enumeration	Complete: The CrleGV-SA-C5 and WT stocks and subsequent extractions were enumerated.
C.2. Surface dose Bioassays	Complete: Surface dose against L3 <i>T. leucotreta</i> larvae were completed and LC <sub>50</sub> and LC <sub>90</sub> values were determined
C.3. Bulk Up	Complete: Virus was produced via the infection of L3 <i>T. leucotreta</i> larvae. This was conducted via two successive infections enabling the production of sufficient virus for downstream experiments.
C.4. Sanger Sequencing	Complete: PCR amplicons from CrleGV-SA-C5_BU2 were Sanger sequenced, confirming the stability of the SNPs.
D.	
D.1. Detached fruit bioassays	Complete: There was no significant difference between the virus treatments in both UV_DFB and Non-UV_DFB.
Thesis outcome	Thesis is submitted and under examination

### **Objective A**

#### De novo assembly and genome alignment

The genome sequence of the CrleGV-SA-C5 was assembled *de novo* from previously generated NGS data and aligned against the full-length genome of CrleGV-SA. The length of the generated CrleGV-SA-C5 sequence was determined to be 111334 bp. A percentage identity of 99.99 was shown after a pairwise alignment between the CrleGV-SA-C5 with the reference genome for CrleGV-SA. The length of the assembled consensus CrleGV-SA-C5 sequence is consistent with the length of the genome sequence assembled by van der Merwe *et al.* (2017), both being 111334 bp in length. Both the current study and the study by Mwanza (2019) obtained the same pairwise identity after sequence comparison with the published CrleGV-SA. Following alignment, seven SNPs were identified between the CrleGV-SA and the CrleGV-SA-C5 (Table 3.2.3.7).

**Table 3.2.3.7.** Single polymorphisms identified in the genome sequence of CrleGV-SA-C5

Name	Nucleotide Position	Change	Codon Change	Amino Acid Change	Protein	Protein Effect	Polymorphism Type	ORF numbers
UV_1	21222	G → C					SNP (Transversion)	
*UV_2	38194	T → C	ATT→ACT	I → T	Pif-2	Substitution	SNP (Transition)	ORF 45
UV_3	38366	G → A	GAG→GAA		Pif-2		SNP (Transition)	ORF 45
*UV_4	45853	C → T	GTG→ATG	V → M	39K	Substitution	SNP (Transition)	ORF 57
*UV_5	79840	T → G	TTG→GTG	L → V	HP	Substitution	SNP (Transversion)	ORF 94
UV_6	104395	G → A	AAG→AAA		Lef-8		SNP (Transition)	ORF 119
*UV_7	104574	T → C	ATG→ACG	M → T	HP	Start Codon Loss	SNP (Transition)	ORF 120

\*SNPs identified are the same as those identified from Mwanza (2019)

Pif-2: Per os infectivity factor-2, 39K: 39K protein, HP: Hypothetical protein, lef-8: Late expression factor-8

Amino Acid Change: I = Isoleucine, T = Threonine, V = Valine, M = Methionine, L = Leucine

Nucleotide: T = Thymine, C = Cytosine, G = Guanine, A = Adenine

ORF = Open reading frame

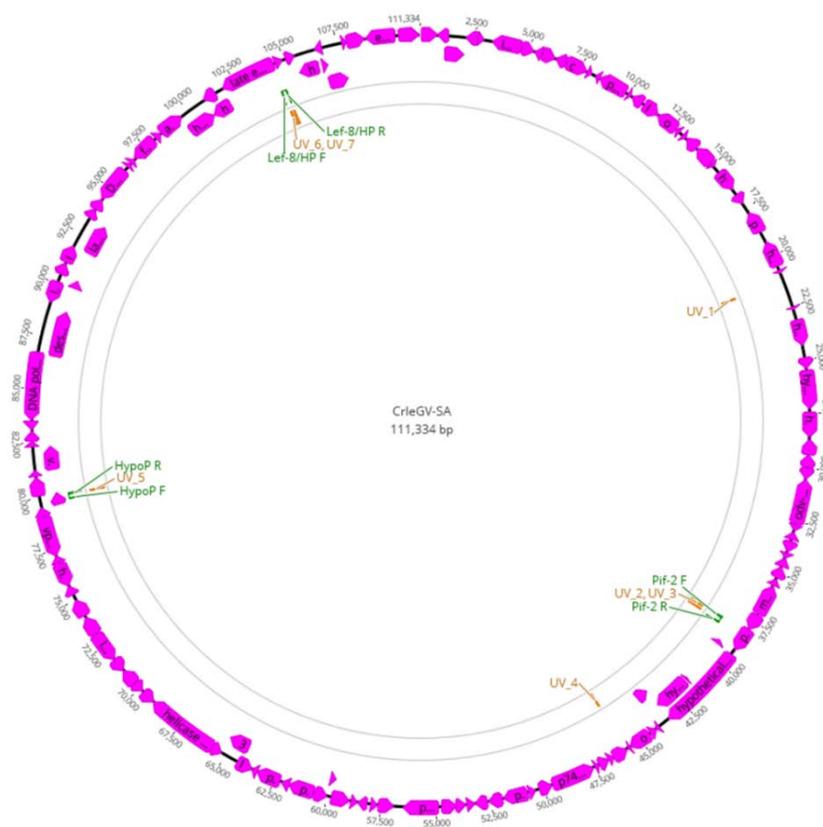
Three of the SNPs identified did not result in amino acid changes and these were at position 21222, 38366 and 104395. The four SNPs that resulted in amino acid changes were at position 38194, 45853, 79840 and 104574. Additionally, four out of the seven identified SNPs were identical to those identified by Mwanza (2019) and Mwanza *et al.* (2022), as indicated in Table 3.2.3.7; these were at position 38194, 45853, 79840 and 104574. Two out of the seven SNPs detected were unique to the current study and the seventh SNP detected was previously reported by van der Merwe *et al.* (2017). The remove duplicate, error correcting and *de novo* assembly step of the data analysis may have resulted in a slightly different portion of the NGS data set being selected, compared to Mwanza (2019). Therefore, the consensus sequence generated after *de novo* assembly may have been slightly different to the consensus sequence generated by Mwanza (2019), as demonstrated by the unique SNPs detected.

The identified SNPs in the previous and current studies are possibly introduced due to the UV radiation. It is known that UV radiation induces mutations (Shibai *et al.*, 2017). UV radiation also results in the inactivation of OBs (Beas-Catena *et al.*, 2014). DNA molecules absorb UV light, which as a result, causes pyrimidine dimers in the DNA chain (Yoon *et al.*, 2000). Additionally, the SNPs detected could have been introduced due to the UV radiation selecting for a different genotype of the same virus. Baculoviruses exist as mixtures of different genotypes (Herniou & Jehle, 2007; Opoku-Debrah *et al.*, 2013). Baculovirus isolates consist of variation in the genetic variants which are caused by multiple mechanisms such as horizontal gene transfer, point mutations, deletion, and insertions. Opoku-Debrah *et al.* (2013) bioprospected for new CrleGV isolates, with the use of overcrowding of host populations as an induction method for latent infections. The host populations of *T. leucotreta* were geographically distinct and five new isolates of CrleGV were recovered from these populations. Each isolate comprised of a mixture of greater than one CrleGV-SA genotype, which was evidence of genotypic variation between samples.

A study by van der Merwe *et al.* (2017) generated seven full genome sequences of the isolate CrleGV-SA selected over 15 years. These isolates were compared with one another to investigate the genetic stability of CrleGV-SA since 2000 when it was first produced and applied in the field. The SNP detected at position 21222 in the current study is the same nucleotide observed in the 2000, 2003, 2005, 2007, 2009, and 2012 sequences by van der Merwe *et al.* (2017). The SNP at position 38194 in the current study, is the same as the nucleotide observed by van der Merwe *et al.* (2017) in the 2012 sequence reported. The SNPs detected at these positions in the CrleGV-SA-C5 isolate sequence, therefore, may not be mutations introduced due to exposure to UV radiation, but rather a selection of a different pre-existing genotype of the virus, which exhibits improved tolerance to UV stress. Such genetic variation may naturally exist within baculovirus populations, offering improved fitness to changing environmental conditions.

#### Oligonucleotide design and *in silico* testing

Three sets of oligonucleotides were designed targeting the regions where the SNPs have been identified between the CrleGV-SA-C5 and CrleGV-SA isolates (Table 3.2.3.1). The three sets of oligonucleotides were tested *in silico* (Figure 3.2.3.3). The first oligonucleotide set, Pif-2, incorporated two SNPs (UV\_2 and UV\_3) at position 38194 and 38366. The HypoP oligonucleotide set encompassed one SNP (UV\_5) at position 79840 and the Lef-8/HP oligonucleotide set encompassed two SNPs (UV\_6 and UV\_7) at positions 104395 and 104574.

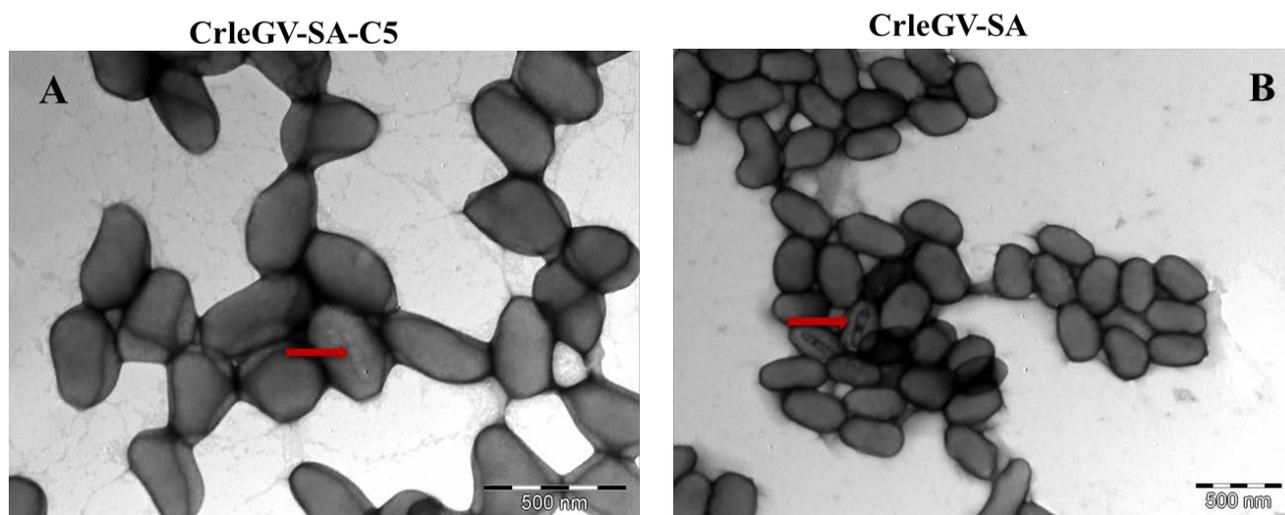


**Figure 3.2.3.3.** *In silico* test conducted on the CrleGV-SA using the three sets of oligonucleotides designed targeting five different SNPs identified. The binding sites are shown in green, with the various SNPs indicated in yellow. The CrleGV-SA genes are shown in purple.

Oligonucleotides were designed flanking SNPs that were in close proximity to each other, instead of flanking the SNPs individually. The *Pif-2* set flanked two SNPs that were 172 bp apart and the *Lef-8/HP* set flanked two SNPs that were 179 bp apart. The oligonucleotide sets were successfully tested *in silico*, encompassing the correct SNPs.

### Transmission electron microscopy

Transmission Electron Microscopy was used to examine the OBs of the CrleGV-SA-C5 virus sample and the CrleGV-SA virus sample to determine the purity of each. Image results are shown in Figure 3.2.3.4 for both the CrleGV-SA-C5 and the CrleGV-SA sample. Little debris was observed for both virus samples and the OBs were roughly oval shaped. For the CrleGV-SA-C5 sample the average length of the OB was  $344.7 \pm 49.4$  nm and average width  $205.7 \pm 32.3$  nm (n=30). For the CrleGV-SA sample, the average OB length was  $364.9 \pm 30.1$  nm and average width  $215 \pm 26.5$  nm (n=30).



**Figure 3.2.3.4.** Transmission Electron Micrograph of A: CrleGV-SA-C5 and B: CrleGV-SA. The red arrow is indicating the nucleocapsid within the OB (CrleGV-SA TEM images courtesy of Thuthula Mela).

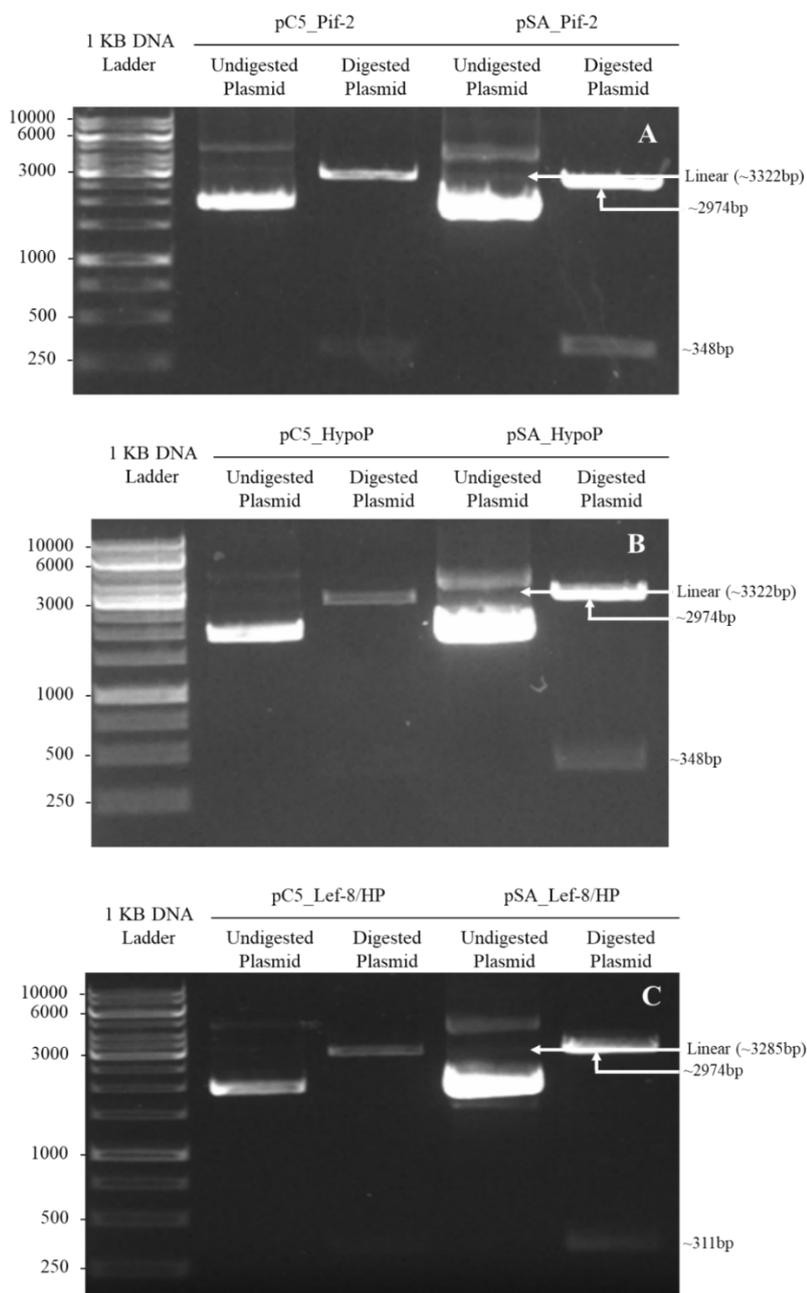
Transmission electron microscopy results showed CrleGV OBs that were consistent with the size and morphology of GV OBs as described in Herniou *et al.* (2011), who state that the diameter of GV OBs range from 120-300 nm and the length of GV OBs range from 300-400 nm. Therefore, the measured CrleGV-SA-C5 and CrleGV-SA OBs fall within the size range of GV OBs. The shape of GVs is ovoid (Ackermann & Smirnov, 1983), which is consistent with the results observed in the current study.

### **Objective B**

#### Plasmid extraction and restriction digestion

The CrleGV-SA-C5 and CrleGV-SA Pif-2, HypoP, and Lef-8/HP PCR amplicons were PCR amplified using three oligonucleotide sets. The PCR amplicons were successfully cloned into pJET1.2/blunt cloning vector. Plasmid maps were successfully constructed and used to determine the expected colony PCR amplicon sizes. Colony PCR was performed to screen for plasmids containing the cloned inserts, with the resulting AGE images showing the successful amplification and the ligated inserts producing amplicons of the expected size for each cloned insert (Data not shown).

To further confirm the presence of the insert, recombinant plasmids were extracted and digested with the FastDigest *XhoI* and *XbaI* restriction enzymes. The undigested along with digested plasmids were then analysed by agarose gel electrophoresis (Figure 3.2.3.5, panels A, B and C). In the undigested samples, in lanes 1 and 3, the different conformations of the plasmid were observed with the open circular, linear and supercoiled conformations all visible. The size of the linear bands for these plasmids were 3322 bp for pC5\_Pif-2 and pSA\_Pif-2, 3276 bp for pC5\_HypoP and pSA\_HypoP and 3285 bp for pC5\_Lef-8/HP and pSA\_Lef-8/HP. For the digested samples in lanes 2 and 4, a large band with vector backbone and a smaller band of the insert was observed. Bands of approximately 2974 bp and 348 bp for plasmids pC5\_Pif-2 and pSA\_Pif-2, 2974 bp and 302 bp for plasmids pC5\_HypoP and pSA\_HypoP and 2974 bp and 311 bp for plasmids pC5\_Lef-8/HP and pSA\_Lef-8/HP were visible and these included the insert which resolved lower down (Figure 3.2.3.5).



**Figure 3.2.3.5.** Agarose gel electrophoresis (1 %) with ethidium bromide staining showing the results with undigested and digested plasmids. A) pC5\_Pif-2 and pSA\_Pif-2. B) pC5\_HypoP and pSA\_HypoP. C) pC5\_Lef-8/HP and pSA\_Lef-8/HPs.

All six plasmids were successfully extracted, undigested plasmids were analysed by AGE and the amplicon size of the linear band produced was of the expected size for all the constructed plasmids. Plasmids were further analysed by restriction digestion to identify the presence of the inserts. The restriction enzymes *XhoI* and *XbaI* were selected for restriction digestion because the restriction sites of these enzymes are located on either end of where the PCR product was ligated into the pJET1.2/blunt vector. All restriction digest fragments were of the expected sizes, suggesting that ligation of inserts into pJET1.2/blunt vector were successful.

#### Sequencing of recombinant plasmids

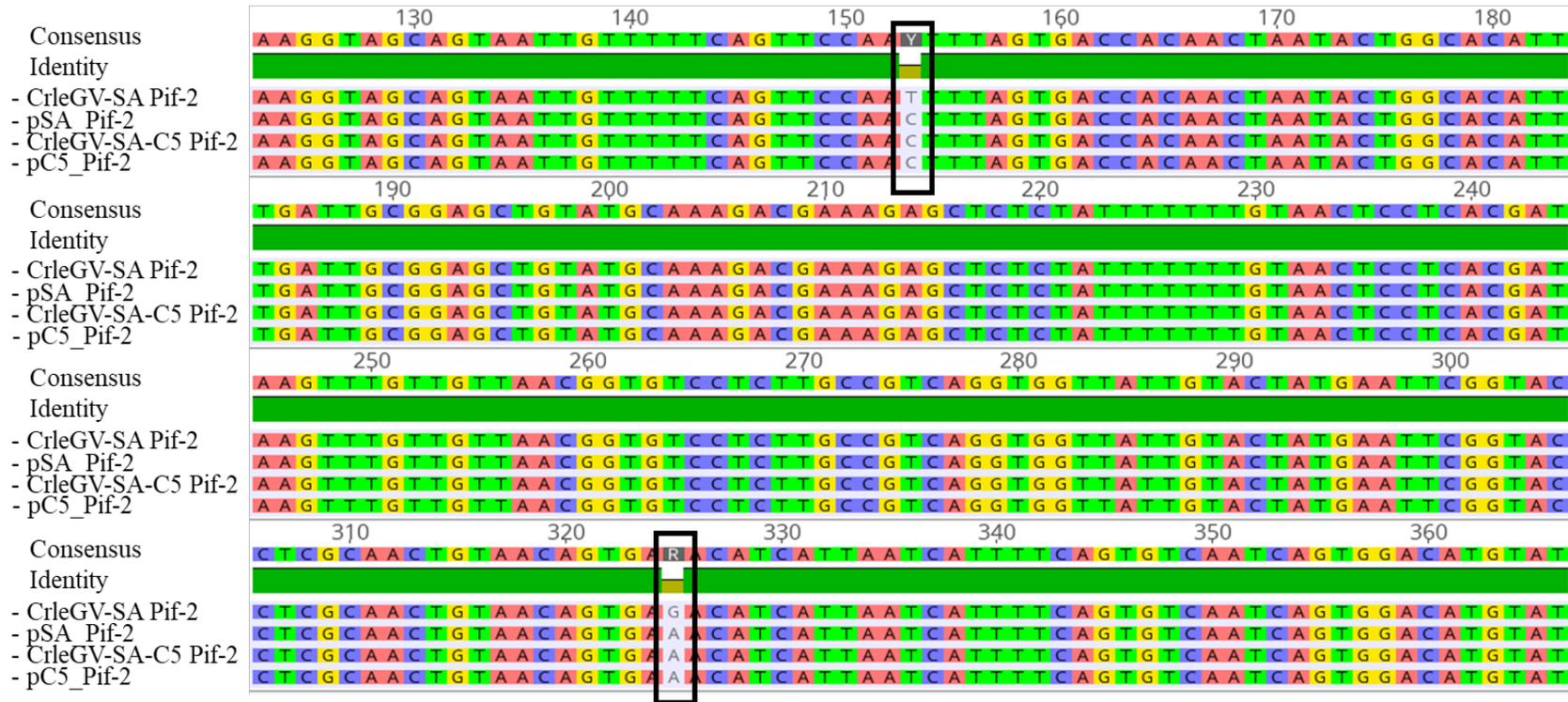
##### Multiple alignment of Pif-2 region

Six recombinant plasmids were constructed, confirmed for the presence of the insert by colony PCR and restriction enzyme digestion and then Sanger sequenced. Plasmids pC5\_Pif-2 and pSA\_Pif-2 were aligned to the CrleGV-SA-C5 Pif-2 and CrleGV-SA Pif-2 genes, this is shown in Figure 3.2.3.6. The first SNP (UV\_2) was

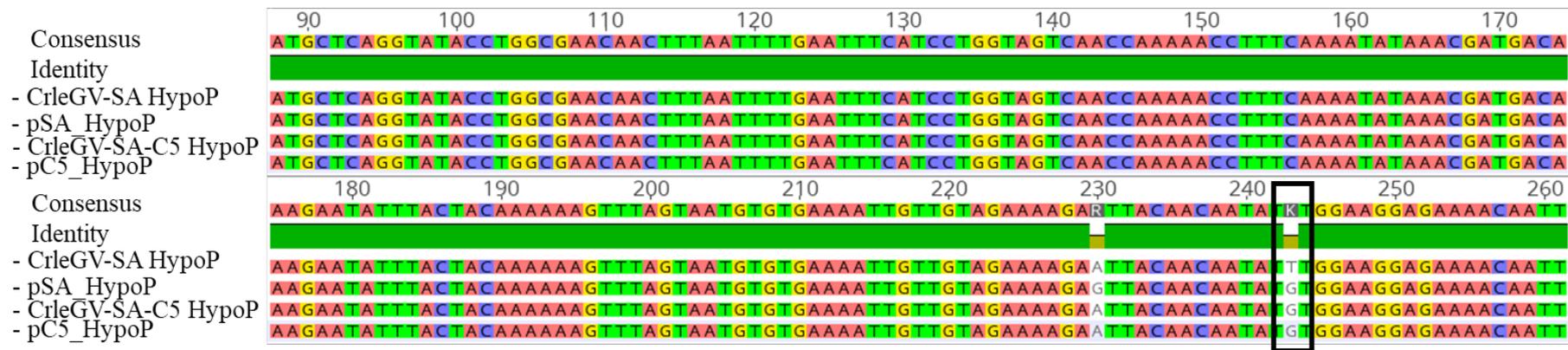
identified at position 153, this was a Thymine to Cytosine and the second SNP (UV\_3) was identified at position 325, this was a Guanine to Adenine change, as observed in the alignment, which were both identified in the initial bioinformatics analysis.

#### Multiple alignment of the HypoP region

The sequence results from plasmid pC5\_HypoP and pSA\_HypoP were aligned against the HypoP gene from CrleGV-SA-C5 genome and the HypoP gene from CrleGV-SA genome with the results shown in Figure 3.2.3.7. The SNP can be seen at position 243, with a Thymine to Guanine change present, this SNP represents UV\_5 as identified during the initial bioinformatics analysis.



**Figure 3.2.3.6.** Multiple sequence alignment of the CrleGV-SA-C5 and CrleGV-SA Pif-2 regions against the plasmid sequence of pC5\_Pif-2 and pSA\_Pif-2. The targeted SNPs are highlighted by the black box.



**Figure 3.2.3.7.** Multiple Sequence alignment of the CrleGV-SA-C5 and CrleGV-SA HypoP region against the plasmid sequence of pC5\_HypoP and pSA\_HypoP. The targeted SNP is highlighted by the black box.

#### Multiple alignment of the Lef-8/HP region

Sequences of pC5\_Lef-8/HP and the CrleGV-SA Lef-8/HP PCR amplicon were aligned to the CrleGV-SA-C5 Lef-8/HP and CrleGV-SA Lef-8/HP reference gene sequences, the result is shown in Figure 3.2.3.8. The SNP can be observed at position 362, with a Thymine to Cytosine change present. This represents SNP UV\_7 as was identified during the initial bioinformatics analysis. The SNP UV\_6 identified during the initial bioinformatics analysis was a Guanine to Adenine change, which is not observed in the sequence alignment at position 183.

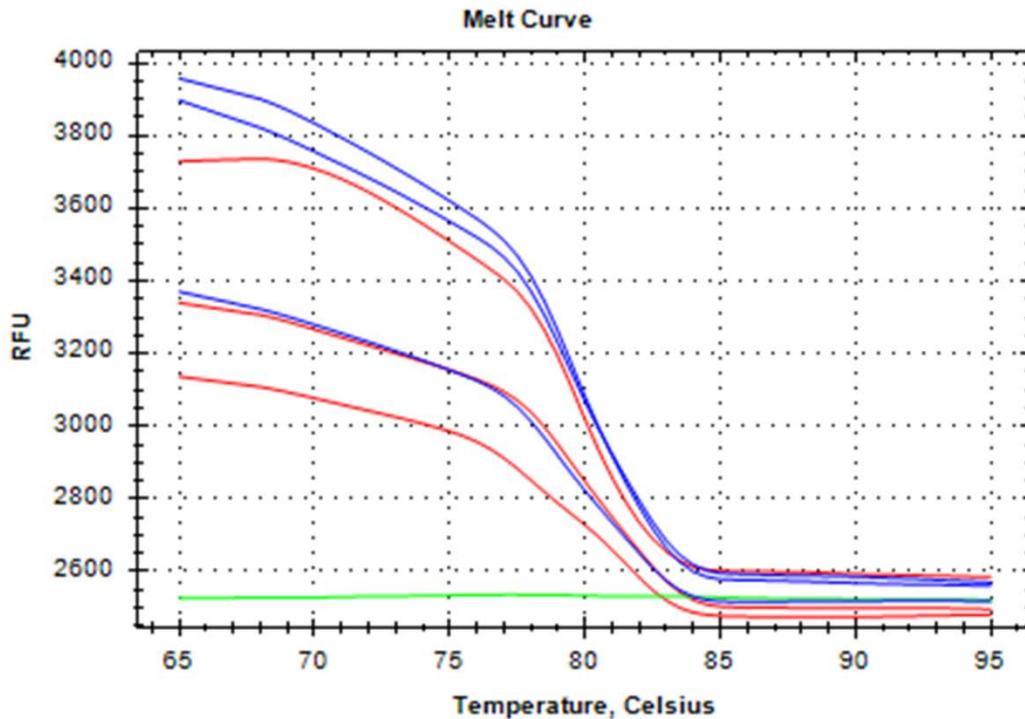


The successfully generated recombinant plasmids were then sent for Sanger sequencing. Five out of the six plasmids were successfully sequenced. After sequencing of the pSA\_Lef-8/HP plasmid, the sequence data generated was not satisfactory (data not shown) and therefore the CrleGV-SA Lef-8/HP PCR amplicon was sequenced directly. A vital limitation of Sanger sequencing automation is that the sequences within the first 15-40 bp is of low-quality because of primer binding and the inability of single base pair differences being distinguishable in longer segments (Crossley *et al.*, 2020). The loss of sequence data due to these limitations of Sanger sequencing was of concern when directly sequencing the PCR products, as the sequence results may be affected. Furthermore, the reverse sequence read was of low quality in this region and as a result, the SNP was poorly identified. To mitigate this, the CrleGV-SA Lef-8/HP PCR amplicon was sequenced in both the forward and reverse directions to overcome these limitations and to ensure that the SNPs were accurately identified. Whereas the entire sequence of the insert was successfully generated when sequencing from the plasmid DNA with only the forward direction required. An advantage of cloning the PCR amplicons into the pJET1.2/blunt vector followed by sequencing, is that the vector consists of sequencing sites that flank the insert region and would therefore provide additional nucleotides on either side of the insert for the machine when sequencing. Four out of the five targeted SNPs remained detectable in the C5 genome, with the SNP UV\_6 in the Lef-8/HP region not being detected.

It has been observed within various baculovirus species that there is considerable genotypic variation (López-Ferber *et al.*, 2003). The SNPs present in the CrleGV-SA isolate could be due to genotypic variation within the sample. The SNP at position 153 in the pSA\_Pif-2 is the same as the nucleotide identified at position 38194 in van der Merwe *et al.* (2017). The SNPs UV\_2, UV\_3, and UV\_5 were identical to the SNPs at the same position identified in the pSA\_Pif-2 and pSA\_HypoP sequences respectively, and UV\_6 was the same as the SNP identified at the same position in the CrleGV-SA Lef8/HP sequence which may be a variation that is unstable. This therefore indicates that UV\_2, UV\_3, UV\_5 and UV\_6 are not ideal markers to use for differentiating between the CrleGV-SA-C5 and CrleGV-SA isolates. The SNP UV\_7 was the only SNP that was different from the CrleGV-SA sequences, resulting in the only suitable marker to differentiate between the CrleGV-SA-C5 isolate and the CrleGV-SA isolate. Therefore, four of the five SNPs did not render markers that could be selected and UV\_7 is the only marker that will be used in next chapter to differentiate between the CrleGV-SA-C5 and CrleGV-SA isolates.

#### SYBR melt curve analysis

The CrleGV-SA-C5 and CrleGV-SA HypoP PCR products were used for qPCR Melt curve analysis. The results are presented in Figure 3.2.3.9, the average  $T_m$  for the CrleGV-SA-C5 samples was 79.3°C ( $\pm 0.28$ , n=3) and the  $T_m$  for the CrleGV-SA samples were 79.5°C ( $\pm 0.0$ , n=2).



**Figure 3.2.3.9.** Melt Curve generated using the CrleGV-SA-C5 HypoP PCR product and the CrleGV-SA HypoP PCR Product. The blue curves represent the CrleGV-SA-C5 HypoP PCR products, the red curves represent the CrleGV-SA HypoP PCR products, and the green line the NTC reaction.

Unfortunately, the melt curve was not successful at differentiating between the two isolate regions. The melt curve analysis does however show potential, but the technique may need to be optimised, either by decreasing the size of the amplicon, or using a machine with a high-resolution melt analysis. Due to the CrleGV-SA-C5 and CrleGV-SA HypoP isolates not being differentiated by melt curve analysis, this method was not conducted for the Lef-8/HP and Pif-2 PCR amplicons. It was later identified upon sequencing analysis that the melt curve may have been successful as it supports the sequencing results shown that there was no difference between the CrleGV-SA-C5 HypoP and CrleGV-SA HypoP regions as UV\_5 was identical to the SNPs at the same position identified in the pSA\_HypoP sequences. This technique would need further evaluation.

### **Objective C**

#### Occlusion body enumeration

The OB enumeration of CrleGV-SA-C5 was determined by phase contrast on a light microscope. The concentration of the stock of CrleGV-SA-C5 was  $9.478 \times 10^{10}$  OBs/ml. The OB enumeration of the CrleGV-SA-C5\_B1 was determined by dark field microscopy and the concentration of the stock was  $4.075 \times 10^{11}$  OBs/ml.

#### Surface dose-response bioassays to determine the lethal concentration estimates

Surface dose bioassays were performed in triplicate using CrleGV-SA-C5 OBs against fourth instar *T. leucotreta* larvae. Each bioassay setup consisted of a control treatment using ddH<sub>2</sub>O and five dose treatments ranging from  $9.478 \times 10^8$  OBs/ml to  $9.478 \times 10^4$  OBs/ml. The percentage larval mortality, regression probit and percentage corrected mortality conducted for the first two bioassay replicates are shown in Table 3.2.3.8 and for all three bioassay replicates are shown in Table 3.2.3.9.

**Table 3.2.3.8.** *Thaumotobia leucotreta* larval mortality in surface dose-response bioassay after 7 days for the first two bioassay replicates

Treatment (OBs/ml)	Mortality (%)	Corrected Mortality (%)	Reg. Probit
D1 ( $9.478 \times 10^8$ )	85.4	82.5	0.911

D2 ( $9.478 \times 10^7$ )	70.8	65.0	0.527
D3 ( $9.478 \times 10^6$ )	68.8	62.5	0.143
D4 ( $9.478 \times 10^5$ )	50.0	40.0	-0.240
D5 ( $9.478 \times 10^4$ )	37.5	25.0	-0.624
Control	16.7	0.0	—

**Table 3.2.3.9.** *Thaumatotibia leucotreta* larval mortality in surface dose-response bioassay after 7 days for all three bioassay replicates

Treatment (OBs/ml)	Mortality (%)	Corrected Mortality (%)	Reg. Probit
D1 ( $9.478 \times 10^8$ )	80.6	76.7	0.750
D2 ( $9.478 \times 10^7$ )	69.4	63.3	0.430
D3 ( $9.478 \times 10^6$ )	66.7	60.0	0.110
D4 ( $9.478 \times 10^5$ )	52.8	43.3	-0.211
D5 ( $9.478 \times 10^4$ )	38.9	26.7	-0.531
Control	16.7	0.0	—

The mortality for the third bioassay replicate was lower than 90 % and for the first two replicates the mortality was greater than 90 %. An  $LC_{90}$  could not be determined when probit analysis was conducted on all three bioassay replicates and was only determined with the analysis conducted on the first two bioassay replicates. A linear regression was performed using the first two bioassay replicate data, resulting in a chi-squared value of 1.57627 and a  $p(\text{Chi}^2)$  value of 0.665 and a statistically significant concentration  $p(F)$  of 0.004. The probit analysis indicated a  $LC_{50}$  of  $4.01 \times 10^6$  OBs/ml, a  $LC_{75}$  of  $2.29 \times 10^8$  OBs/ml and a  $LC_{90}$  of  $8.75 \times 10^9$  OBs/ml (Table 3.2.3.10).

**Table 3.2.3.10.** The  $LC_{50}$  and  $LC_{90}$  for the CrleGV-SA-C5 bioassay against fourth instar *T. leucotreta* larvae

Lethal Concentration	Concentrations (OBs/ml)	95 % Confidence limits	
		Upper	Lower
$LC_{50}$	$4.01 \times 10^6$ OBs/ml	$1.10 \times 10^7$	$1.25 \times 10^6$
$LC_{90}$	$8.75 \times 10^9$ OBs/ml	n.d.	$1.29 \times 10^9$

The study by Mwanza (2019) indicated the  $LC_{50}$  and  $LC_{90}$  to be  $1.73 \times 10^6$  and  $6.22 \times 10^9$  OBs/ml respectively for the CrleGV-SA-C5. These are 2.3-fold and 1.4-fold lower in comparison to the current study despite the bioassays conducted by Mwanza (2019) being against neonates. This difference may be due to the use of different *T. leucotreta* colonies from which larvae were sourced, or minor differences in bioassay technique. Variation between separate insect batches may also occur (Jones, 2000). According to Burges & Thompson (1971) assays conducted by different researchers and in different laboratories can cause variation between assays.

#### CrleGV-SA-C5 bulk-up 1 and bulk-up 2

The CrleGV-SA-C5 was bulked up twice in fourth instar *T. leucotreta* larvae. Three 24-well bioassay plates were used for the first bulk-up to get enough virus to use for the second bulk-up. The second bulk-up was conducted in 10 24-well bioassay plates to obtain sufficient virus to conduct semi-field trials reported below. Table 3.2.3.11 shows the results of the bulk-up after crude purification of the larvae collected from each bulk-up procedure.

**Table 3.2.3.11.** CrleGV-SA-C5 bulk-up results showing the amount of tissue from which the OBs were extracted from, the concentration of the OBs and the final volume obtained.

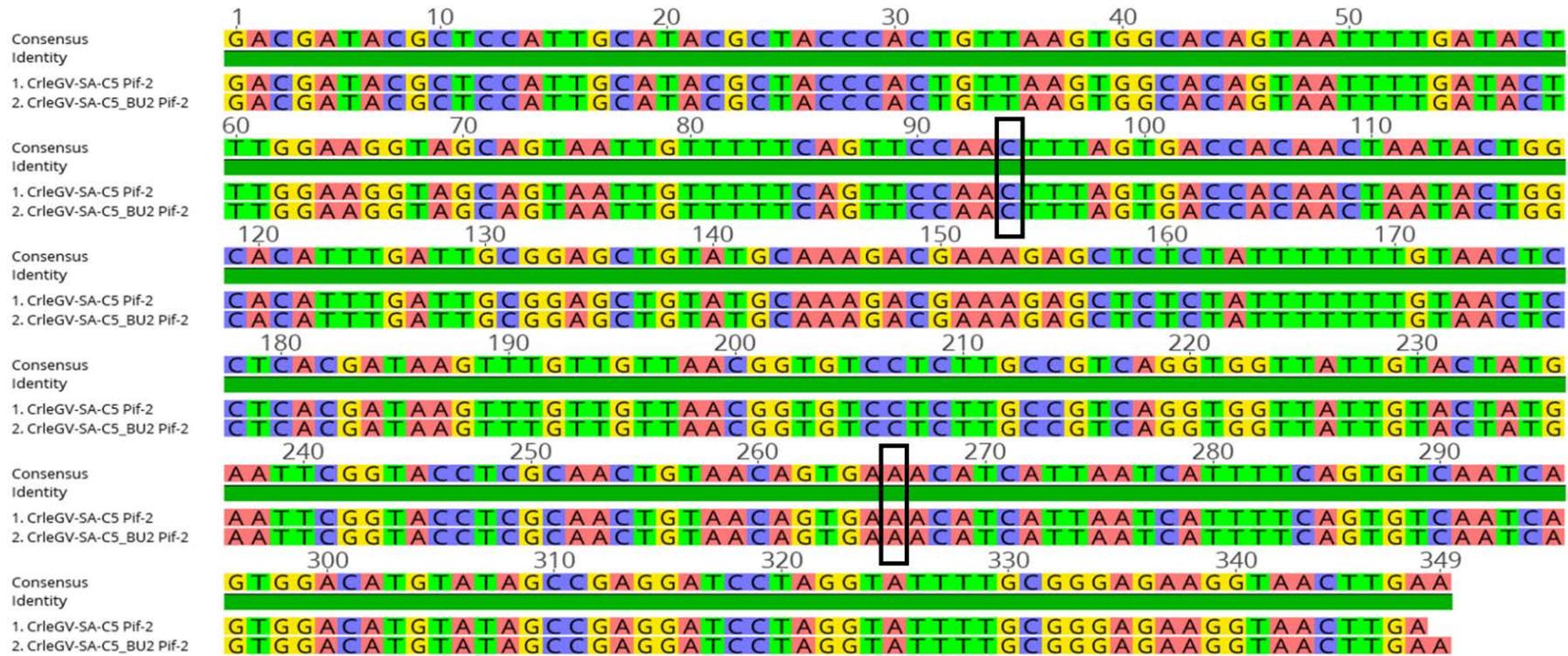
Sample	Number of larvae collected	Tissue (g)	Concentration (OBs/ml)	Volume ( $\mu$ l)
<b>CrleGV-SA-C5_BU1</b>	72	0.995	$4.075 \times 10^{11}$	500
<b>CrleGV-SA-C5_BU2</b>	240	2.510	$1.02835 \times 10^{12}$	1500

Sanger sequencing of polymerase chain reaction amplicons

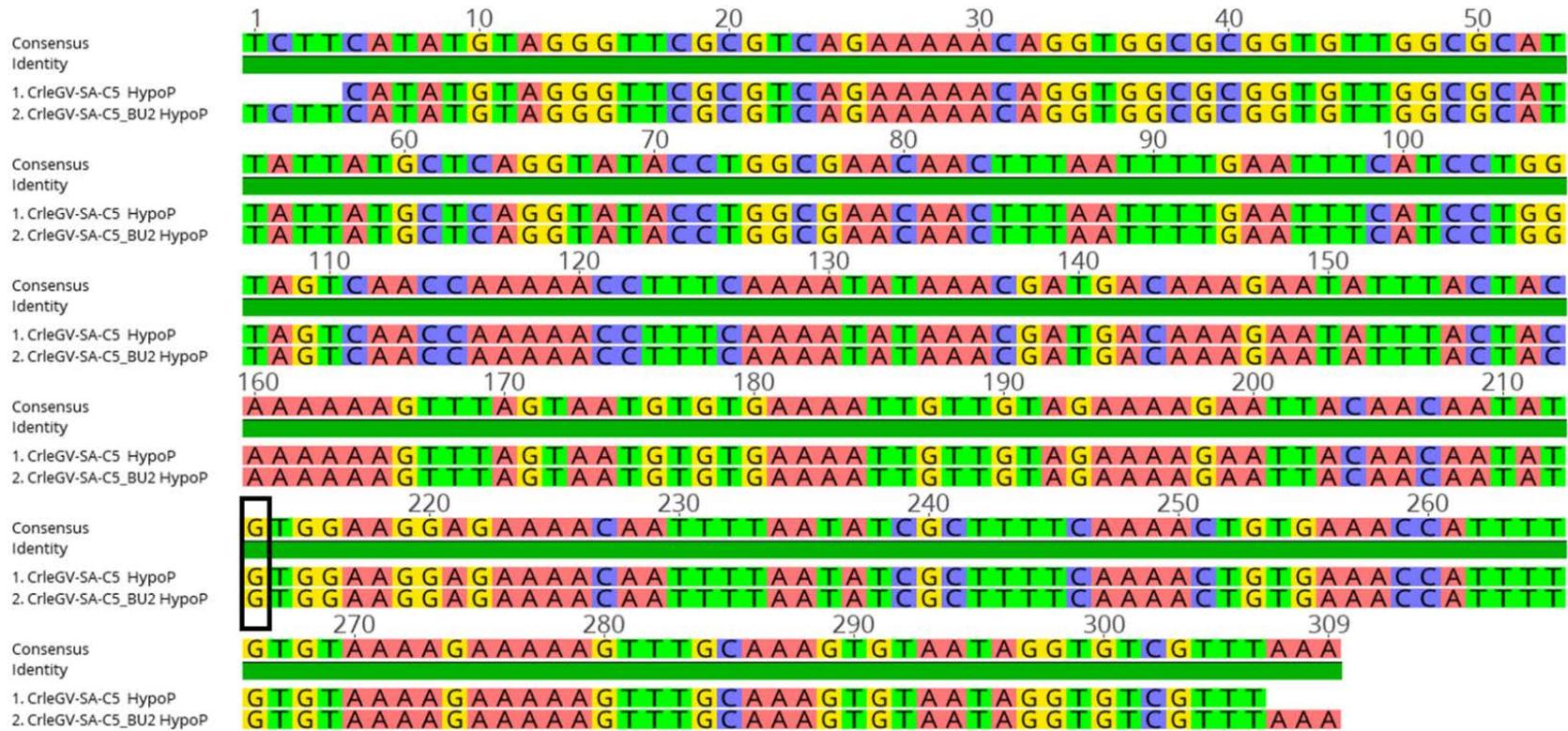
Polymerase chain reaction amplicons from CrleGV-SA-C5\_BU2 were Sanger sequenced. The consensus sequence, from the alignment of the CrleGV-SA-C5\_BU2 Pif-2 forward and reverse sequences, was aligned to the CrleGV-SA-C5 *Pif-2* gene, shown in Figure 3.2.3.10. The first SNP (UV\_2) was identified at position 94, which was a Cytosine and the second SNP (UV\_3) was identified at position 266, which was an Adenine change; both were identified in the initial bioinformatics analysis.

The sequence results from CrleGV-SA-C5\_BU2 HypoP forward and reverse sequences were aligned generating a consensus sequence. The consensus sequence was aligned to the CrleGV-SA-C5 *HypoP* gene; the result is shown in Figure 3.2.3.11. The SNP UV\_5 was identified at position 213, this was a Thymine to Guanine change as identified during the initial bioinformatics analysis.

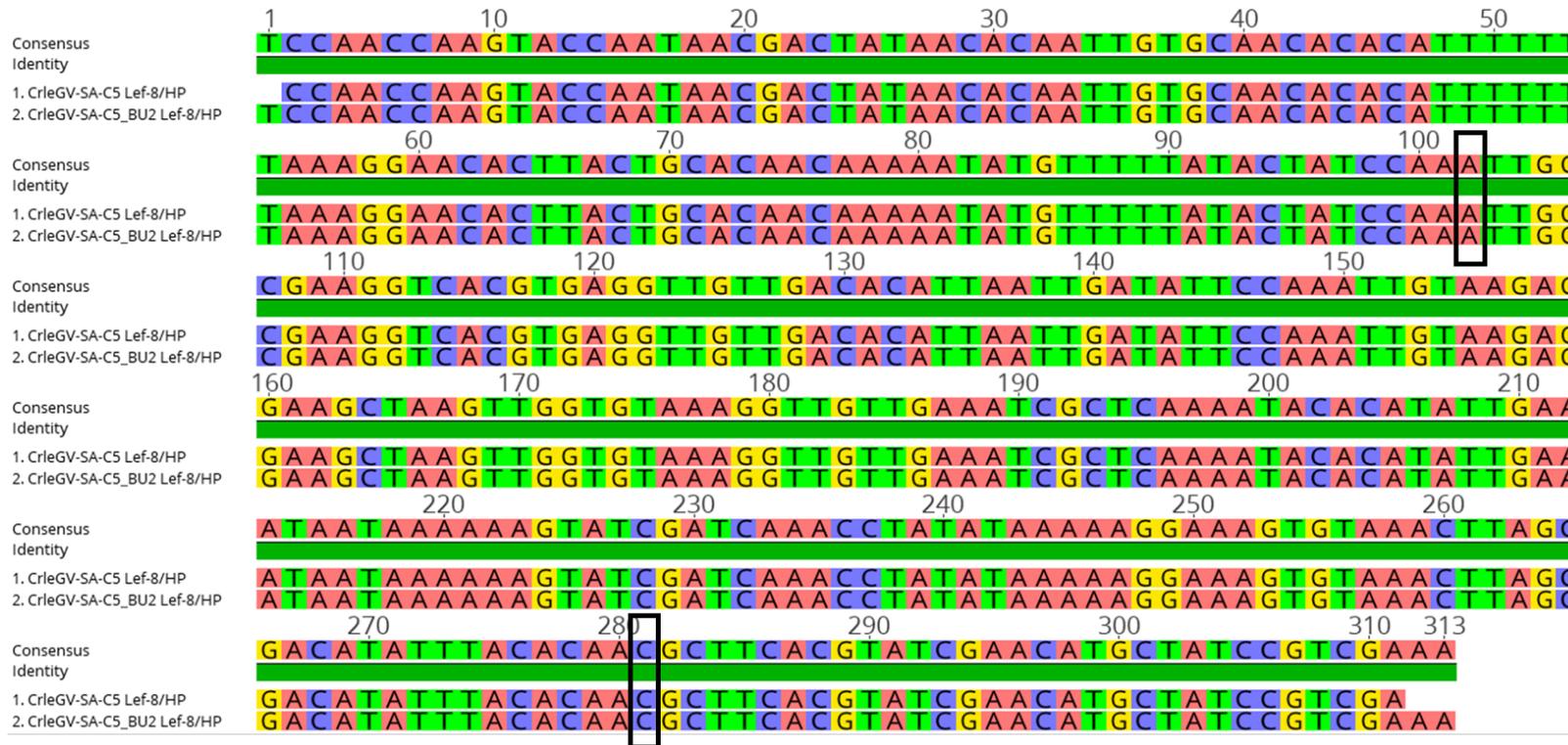
The consensus sequence generated from the pairwise alignment of the CrleGV-SA-C5 Lef-8/HP forward and reverse sequences was aligned to the CrleGV-SA-C5 *Lef-8/HP* genes, as observed in Figure 3.2.3.12. The SNPs UV\_6 and UV\_7 were observed at positions 102 and position 281 respectively. These were identified during the initial bioinformatics analysis, where the first SNP was an Adenine, and the second SNP was a Cytosine.



**Figure 3.2.3.10.** Pairwise sequence alignment of the consensus sequences generated of the CrleGV-SA-C5\_BU2 Pif-2 PCR amplicons against the CrleGV-SA-C5 *Pif-2* gene. The targeted SNPs are highlighted within the black boxes.



**Figure 3.2.3.11.** Pairwise sequence alignment of the CrleGV-SA-C5\_BU2 HypoP consensus sequence, aligned against the CrleGV-SA-C5 *HypoP* gene. The targeted SNP is highlighted within the black box.



**Figure 3.2.3.12.** Pairwise sequence alignment of the CrleGV-SA-C5\_BU2 Lef-8/HP consensus sequence aligned against the CrleGV-SA-C5 *Lef-8/HP* genes. The black boxes highlight the targeted SNPs.

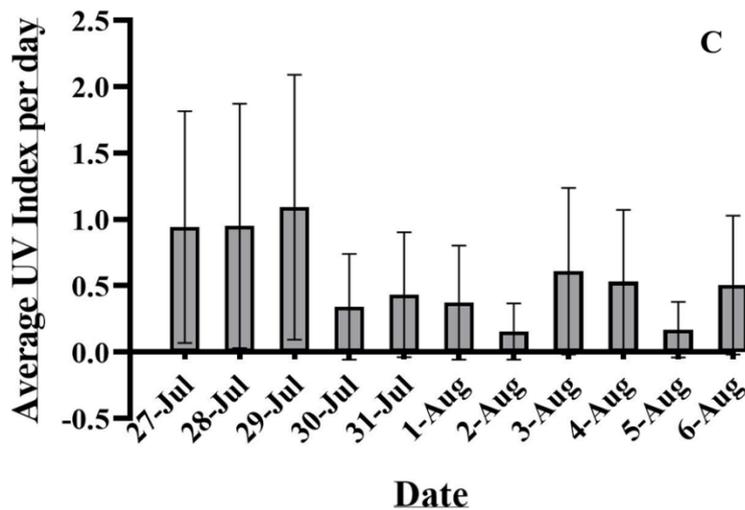
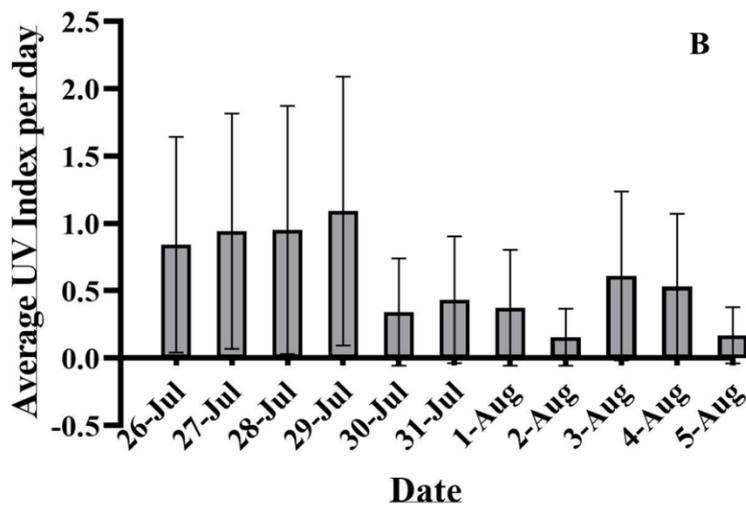
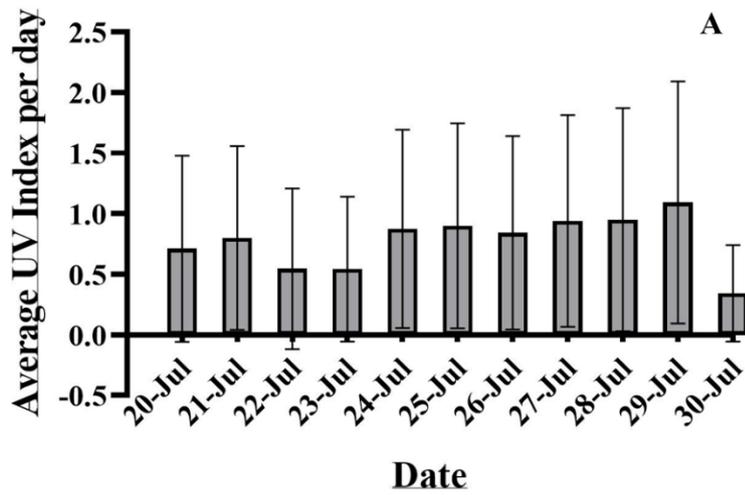
The CrleGV-SA-C5 was successfully bulked up in fourth instar *T. leucotreta* larvae and sufficient virus was obtained which was used in detached field trials. The CrleGV-SA-C5\_BU1 and CrleGV-SA-C5\_BU2 OBs were successfully purified, and the extraction of the genomic DNA was successful for both the CrleGV-SA-C5\_BU1 and CrleGV-SA-C5\_BU2 gDNA. All PCR amplifications were successful, and the amplicons were of the expected sizes (Data not shown). The next objective was Sanger sequencing of the PCR amplicons in both the forward and reverse direction. Sequencing was successful and results confirmed the stability of the SNPs after virus bulk-up. Although all SNPs were present after virus bulk-up, as previously discussed, only UV\_7 can be used as a genetic marker to differentiate between the CrleGV-SA-C5 and the CrleGV-SA isolate. It was previously shown that the two approaches, cloning the PCR products followed by sequencing and sequencing PCR products directly, were both able to detect the SNPs. However, sequencing of the PCR products directly would require sequencing in both the forward and reverse direction to mitigate limitations of Sanger sequencing.

A UV-tolerant baculovirus pesticide with longer field persistence may potentially be developed with the existence of a UV-tolerant strain. As a result, the potential of the pathogen against the pest may be enhanced and the cost of UV protectants and the number of applications required may be reduced (Jeyarani et al., 2013). For the commercial production of a virus into a biopesticide, large quantities of the virus need to be obtained, likely requiring multiple passages of the virus through susceptible host larvae (Grzywacz et al., 2003). It is not known if the identified SNPs directly contribute to the isolate being UV-tolerant. The current results indicate that the CrleGV-SA-C5 isolate is genetically stable after two bulk-ups, suggesting that the CrleGV-SA-C5 isolate has the potential to be used on a commercial scale, particularly if these genetic changes do indeed directly contribute to the UV tolerance measured by Mwanza (2019). However, further analysis would have to be conducted to determine if the CrleGV-SA-C5 isolate will remain stable after bulking the virus up multiple times.

#### **Objective D**

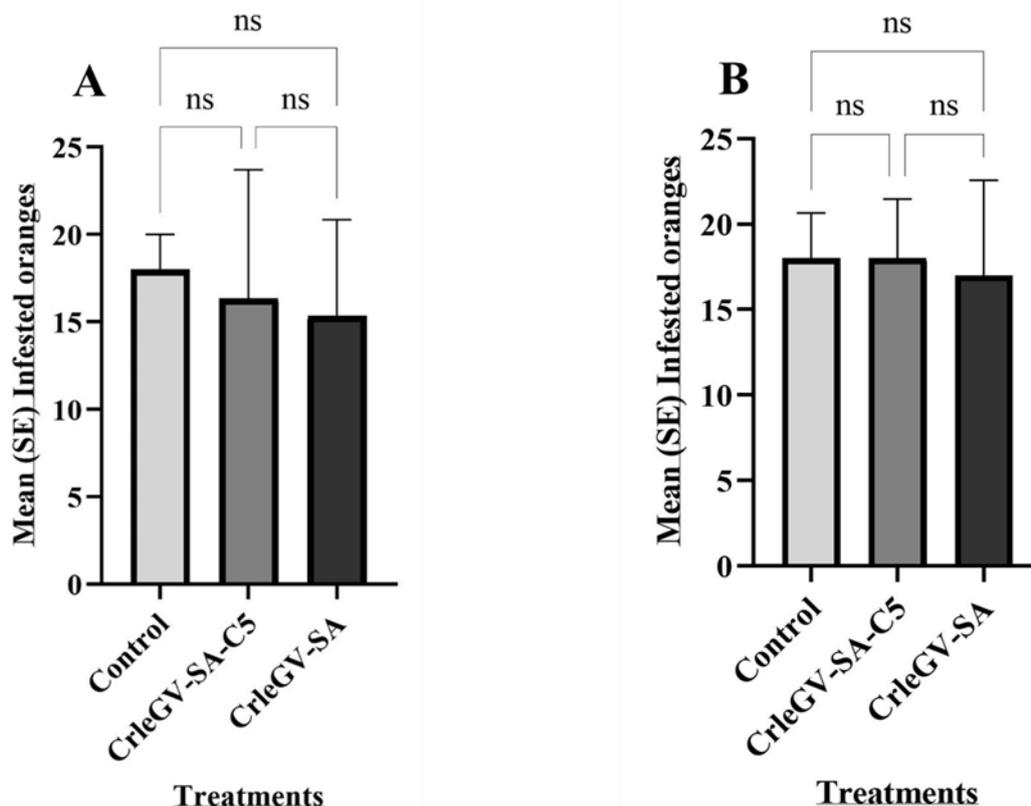
##### **Detached fruit bioassay**

The weather data for the duration of the UV\_DFB for all three replicates was obtained from Rhodes University Geography Department. The temperature, rain and UV Index was recorded for each day. For UV\_DFB replicate 1, the highest temperature was 25.5°C on the 8<sup>th</sup> day and rainfall was recorded on the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> day of 0.002, 0.030, and 0.009 mm respectively. The highest temperature was 25.8°C recorded for UV\_DFB replicate 2 and UV\_DFB replicate 3. Rainfall of 0.006 mm was recorded on the 8<sup>th</sup> and 7<sup>th</sup> day for UV\_DFB replicate 2 and replicate 3 respectively. The highest UV Index recorded for UV\_DFB replicate 1, replicate 2 and replicate 3 was 1.1 recorded on the 10<sup>th</sup> day, on the 4<sup>th</sup> day and on the 3<sup>rd</sup> day respectively (Figure 3.2.3.13).



**Figure 3.2.3.13.** Average (SE) UV Index recorded per day for each UV exposure detached fruit bioassay replicate set-up. A: UV Index data from bioassay replicate 1, B: UV Index data from bioassay replicate 2 and C: UV Index data from bioassay replicate 3.

The UV\_DFB bioassay was conducted by exposing the treated oranges to UV irradiation from sunlight to determine the efficacy of the CrleGV-SA-C5 isolate when exposed to UV compared to the CrleGV-SA isolate. The Non-UV\_DFB bioassay was conducted by not exposing the treated oranges to UV irradiation, this setup was a non-exposure control. There was no significant difference in larval infestation of fruit between CrleGV-SA-C5 and CrleGV-SA for the UV\_DFB ( $p = 0.9036$ ). For the Non-UV\_DFB there was also no significant difference in infestation between CrleGV-SA-C5 and CrleGV-SA treated fruit ( $p > 0.9999$ ) (Figure 3.2.3.14).



**Figure 3.2.3.14.** The oranges that were not infested by *T. leucotreta* larvae from both UV\_DFB and Non-UV\_DFB were also recorded (data not shown). There was no significant difference between the UV\_DFB and Non-UV\_DFB Control treatments ( $p > 0.9999$ )

The outcome was not as anticipated, as statistical analysis indicated that there was no significant difference between the virus treatments in both UV\_DFB and Non-UV\_DFB. The lack of difference between treatments in UV\_DFB could theoretically be as a result of UV irradiation, rainfall, virus OBs not attaching to the oranges or low virus concentration and therefore warrant investigation.

One of the major drawbacks affecting the persistence of baculoviruses in the field is UV irradiation. UV irradiation inactivates baculoviruses, resulting in short residual activity (Cory & Evans, 2007; Shapiro, 1995). DNA molecules actively adsorb UV light and as a result induce pyrimidine dimer formation in DNA chains (Yoon et al., 2000). When OBs are still wet they are more susceptible to UV inactivation as a result of UV being able to refract onto the OB, which is located in the droplet (Grzywacz & Moore, 2017). After dipping the oranges in the relevant treatments, the oranges were not completely dry when placed outside for UV exposure or when placed indoors to be subjected to no UV exposure. As a result, the poor efficacy of the virus treatments in UV\_DFB could have been due to UV possibly refracting onto the OBs in the droplet on the orange rind making the OBs more susceptible to UV inactivation. UV irradiation may have inactivated the virus OBs when the treated oranges were subjected to UV exposure, contributing to the CrleGV-SA-C5 and CrleGV-SA isolates' poor efficacy in the UV\_DFB. However, given the lack of a significant difference between the UV and Non-UV treatments and their controls, UV irradiation may not have affected the efficacy of the virus.

For each bioassay replicate in the UV\_DFB, during the period of UV exposure, as observed from the weather data, there was at least one day where rainfall was recorded. It was emphasised by Evans (1994) that products need to be tested under laboratory and under field conditions. Despite there being rain during the period of UV exposure, oranges were still left outside to expose them to the most natural environmental conditions as possible. It is therefore necessary to explore the possibility that rain could have washed the virus off the oranges and therefore be a possible contributing factor to the outcome in results. However, Kirkman (2007) conducted laboratory bioassays to determine the degree of rainfastness of Cryptogran. The study found that rainfall did not have any detrimental effect on the virus efficacy. Therefore, given the fact that Kirkman (2007) showed that Cryptogran has a high degree of rainfastness and the detached fruit bioassay results in the current study showed that there was no difference between the UV\_DFB and Non-UV\_DFB, rain is not likely to have been a contributing factor affecting the outcome of the results.

According to Silva & Moscardi (2002), biological products used during the 1993/94 soybean season were reported to have low quality and efficacy, which could have been in relation to various factors affecting the efficacy and stability under field conditions. Some of the factors include: relative humidity and precipitation (Jaques, 1977), solar radiation (Silva, 1987), temperature (Ignoffo, 1985), age and population intensity of the host insects (Silva, 1987), pH of the aqueous viral suspension in the spray tanks (Batista, 1997) and viral formulation, equipment and application technology (Silva, 1986). Therefore, the low efficacy of the virus treatment in both Non-UV\_DFB and UV\_DFB could have been due to complications in the preparation of the virus treatments or the application of the treatments.

The concentration of the virus used in the current study could have been too low, therefore resulting in the lack of significant efficacy. Studies by van der Merwe (2021) and Moore *et al.* (2011) conducted detached fruit bioassays using CrleGV-SA respectively. Detached fruit bioassays conducted by van der Merwe (2021) had CrleGV-SA included in treatments at a LC<sub>50</sub> concentration of  $9.31 \times 10^7$  OBs/ml and those conducted by Moore *et al.* (2011) had five CrleGV-SA concentrations prepared as a series of two-fold dilutions, with the concentration of the stock suspension of the virus being  $5.4 \times 10^{11}$  OBs/ml. In comparison to the current study, the virus concentrations used by van der Merwe (2021) and Moore *et al.* (2011) were higher. The concentration of the treatments used in the current study was the concentration registered for Cryptogran. A 70 % reduction in *T. leucotreta* infestation over a 17-week period was recorded in a semi-commercial block trial conducted using Cryptogran to combat pre-harvest fruit loss. A similar level of infestation was expected in the current study.

Another possibility is that the virus OBs may not have adhered to the oranges, thus contributing to the poor results recorded. It was shown that the efficacy of Cryptogran is enhanced and improved by the addition of molasses (Moore *et al.*, 2004). Molasses acts as a feeding attractant for *T. leucotreta* larvae and improves virus adhesion, thus improving the efficacy of the biopesticide (Kirkman, 2007). It was also shown that the medial lethal exposure time (LET<sub>50</sub>) for neonate codling moth, *Cydia pomonella*, larvae was dramatically decreased by the incorporation of 15 % cane molasses in a formulation of purified CpGV and it was also found that the damage caused by codling moth in field trials, where molasses was added to the virus, was reduced (Ballard *et al.*, 2000). Additionally, it has been shown that the efficacy of the virus may be significantly improved with the addition of surfactants and adjuvants into the CrleGV-SA formulations, such as BREAK-THRU® S 240 (250 g/L Polyether, 750 g/L Polyether modified trisiloxane) (Evonik Industries AG, Germany) (Moore *et al.*, 2015). Cryptogran is registered to be applied with molasses and an adjuvant. Neither were used in this study and consequently their addition in future trials may improve the outcome.

## Conclusion and future research

In conclusion, the overall aim of this study was to determine the biological and genetic stability of the UV-tolerant CrleGV-SA-C5 isolate, which can potentially improve *T. leucotreta* control. Variations were successfully identified between the CrleGV-SA-C5 and CrleGV-SA isolates. Six amplicons were PCR amplified, targeting three regions of the CrleGV-SA-C5 genome which encompass five SNPs. The construction of six recombinant plasmids were successful and the presence of the SNPs originally detected in the regions of the CrleGV-SA-C5 genome were confirmed by Sanger sequencing, with one suitable marker identified. Surface dose response bioassays and virus bulk-up were conducted, and the presence of the target SNPs in

the genome of the CrleGV-SA-C5\_BU2 virus was confirmed by Sanger sequencing. This led to testing the UV tolerance of the CrleGV-SA-C5 isolate by detached fruit bioassays. Results confirmed that there was no significant difference in the treatments in both the Non-UV\_DFB and UV\_DFB. Future research into the UV tolerance of the CrleGV-SA-C5 isolate is required for the development of this isolate into a biopesticide formulation for the control of *T. leucotreta*.

The ultimate objective of the study was to evaluate the CrleGV-SA-C5 isolate, which could be incorporated into novel formulations of CrleGV-SA for improved field efficacy and enhanced control of *T. leucotreta* in IPM programmes. According to Moore (2002), extensive field trials need to be conducted to assess a control agent's efficacy and persistence against the abiotic and biotic factors encountered in the field, prior to the potential incorporation into IPM programmes. Therefore, future research projects could conduct extensive field trials testing the UV-tolerance of the CrleGV-SA-C5 isolate, with the possibility of incorporating the isolate into the *T. leucotreta* IPM programme. A repeat of the detached fruit bioassay with adaptations to the current methodology, such as the addition of surfactants and adjuvants such as BREAK-THRU® S 240 and molasses, the testing of pH for all prepared solutions, and an increase in the virus concentrations used, should be considered.

Massive parallel sequencing techniques are the most generally applied methods established for the fragmentation of genomic DNA, the selection of fragment size, PCR amplification followed by large scale sequencing, and as a result millions of short sequenced reads are generated (Wennmann *et al.*, 2020). In studying genotypic composition, the position and number of SNPs from data obtained by sequencing are generally used as suitable markers (Wennmann *et al.*, 2020). Therefore, future studies can investigate intraspecific genetic variability in the CrleGV-SA-C5 isolate with the identification of other possible SNPs present within the genome that can be used as suitable markers to differentiate it from other isolates such as CrleGV-SA, as having only one suitable marker is not adequate. Additionally, the role of the identified SNPs in baculovirus tolerance to UV should be investigated in future studies.

Desiccation of the CrleGV-SA-C5 virus before inoculating the oranges with *T. leucotreta* eggs during the detached fruit bioassay may have affected the efficacy of the virus. Future research could potentially assess the effect of desiccation by adding a potential control setup for the detached fruit bioassays. Potential methodology could include the dipping of oranges into the virus treatments and add larvae to fruit immediately after fruit have dried, to ensure the treatments are working.

Commercial baculovirus insecticides such as Cryptogran with CrleGV-SA and Madex with CpGV-M are only formulated with a single isolate. As a result of the registration requirements and/or the production guidelines, the specific genotypes that these insecticides are comprised of remain consistent. Mixtures of isolates, distinct virus species or genotypes have been shown by several studies to result in interactions giving rise to improved activity (Carballo *et al.*, 2017; Jukes *et al.*, 2017). Future research could potentially assess and compare the effects of the UV-tolerance of a mixture of CrleGV-SA-C5 and CrleGV-SA viruses with the UV-tolerance of each virus alone.

## **Technology transfer**

Results will be reviewed for publication in a suitable journal.

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

Project1039 (April 2012 – March 2022): Sean Moore, Wayne Kirkman, Peter Stephen, Sean Thackeray, Mellissa Peyper, Kim Stolz, Luke Cousins, Tim Grout and Vaughan Hattingh (CRI)

#### **Summary**

As of 1 January 2018, FCM was regulated as a phytosanitary organism by the European Union. The regulation requires citrus fruit to be sourced from an FCM-free area or place of production, or to receive a cold treatment or any other treatment that can ensure the exported consignment is free of FCM. Cold disinfestation of citrus fruit from South Africa to Europe is not feasible, due to the relatively short shipping time, particularly from Cape Town port, but more importantly due to the large volumes of fruit exported to Europe and the inadequate infrastructure to facilitate cold treatment of these large volumes. Additionally, many cultivars would suffer unacceptable chilling injury at the temperatures required. Consequently, a multi-step systems approach has been developed as an alternative treatment to cold sterilisation. The final stage in the systems approach is a time-temperature shipping protocol. Although this is a cold treatment, it is not a complete disinfestation treatment. The precise level of mortality required by this treatment is determined by the measured efficacy of the preceding steps in the systems approach. The mortality of the most cold-tolerant larval stages of FCM, using several time-temperature combinations, have been determined.

Since complete mortality for 4°C and above were not achieved in any of the 26 day trial periods, trials at extended durations were conducted, achieving 100% mortality after 28 days for the same trial is being done at 4.5°C.

Two further replicates were conducted, comparing cold efficacy against larvae in four different cultivars (Star Ruby grapefruit, Valencias, Navels and Nadorcotts) and in artificial diet. However, only Valencias and Nadorcotts were available for the last replicate. Overall results showed that artificial diet can be used for such trials without overestimating the efficacy of cold treatment against larvae in fruit. This trial will be repeated in the new season, until we have sufficient data.

A trial was conducted to compare the efficacy of cold treatment against a new FCM culture (established from field collected larvae) and an old laboratory culture to determine if laboratory reared FCM becomes less tolerant over time. One replicate was conducted at 1°C for 5 to 19 days. No significant difference was recorded between the two cultures. A similar trial will be conducted, however, this time comparing the cold susceptibility of a wild-augmented culture with an old non-augmented culture.

Lower lethal temperatures experiments found that temperatures in excess of -14.265°C for a 2-hour duration would likely kill 99.9% of insects from all these laboratory-maintained populations. For guaranteed results, temperatures in excess of -16.133°C (the lower 95% CI) are to be tested. Lethal temperatures were also tested for the 5 different cultures from the different regions. The Nelspruit culture could survive significantly colder conditions than the Old Colony L population. Although not significantly so, the LLTs of the Addo population were slightly higher than those of the Nelspruit population, while Citrusdal insects could tolerate slightly colder conditions compared to the Old Colony L insects.

Characteristics of larvae surviving cold treatment were diagnosed and recorded, after which larvae were placed onto artificial diet to record whether they continued to survive and develop or if they died. Consequently, characteristics reliably indicative of possible survival or imminent death, were identified. Turgidity or flaccidness were identified as the most important characteristics for predicting this. Informed by this preliminary work CRI then began a collaboration with eNtsa, which is an engagement institute within the Nelson Mandela University, to develop a more quantitative, scientifically robust system by which to accurately measure the relevant larval characteristics. The latest development through eNtsa is a vision system, which measures displacement of

larvae through compression. The vision system might prove to be a workable method with some more trial runs and further development. This will ultimately enable accurate prediction of whether a larva surviving a cold treatment might continue to survive or will definitely die, ensuring that inspectors do not make false calls on the risk of larval findings.

## Opsomming

Vanaf 1 Januarie 2018 is VKM as gereguleer as 'n fitosanitêre organisme deur die Europese Unie. Die regulasie vereis dat sitrus vrugte vanaf 'n VKM vrye-area of plek van produksie verkry word, of 'n koue behandeling ontvang of enige ander behandeling wat kan verseker dat die versending vry van VKM is. Koue ontsmetting van sitrus vrugte vanaf Suid-Afrika na Europa is nie haalbaar nie, as gevolg van die relatiewe kort verskepingstydperk, veral vanaf die Kaapstad hawe, maar meer belangrik as gevolg van die hoë volumes vrugte wat uitgevoer word na Europa en die onvoldoende infrastruktuur om die koue behandelings te fasiliteer van hierdie groot volumes. Boonop sal baie kultivars onaanvaarbare verkoelingsbeserings opdoen by die vereiste temperature. Gevolglik is 'n multi-stap stelselsbenadering as 'n alternatiewe behandeling vir koue sterilisasie ontwikkel. Die finale stap in die stelselsbenadering is 'n tyd-temperatuur verskepings protokol. Al is hierdie wel 'n koue behandeling, is dit nie 'n volledige ontsmettings behandeling nie. Die presiese vlak van mortaliteit wat deur hierdie stap benodig word, word bepaal deur die gemete doeltreffendheid van die vorige stappe in die stelselsbenadering. Tot op hede is die mortaliteit van die mees kouetolerante lewensstadiums van VKM deur gebruik van verskeie tyd-temperatuur kombinasies bepaal.

Aangesien volledige mortaliteit vir 4°C en hoër nie in enige van die 26 dae proefperiodes bereik is nie, is proewe met verlengde duur uitgevoer, wat 100% mortaliteit behaal het na 28 dae vir dieselfde proef wat by 4.5°C gedoen is.

Twee verdere herhalings is uitgevoer, met vergelyking van koue doeltreffendheid teen larwes in vier verskillende kultivars (Star Ruby pomelo, Valencias, Navels en Nadorcotts) en in kunsmatige dieet. Slegs Valencias en Nadorcotts was egter beskikbaar vir die laaste herhaling. Algehele resultate het getoon dat kunsmatige dieet vir sulke proewe gebruik kan word sonder om die doeltreffendheid van kouebehandeling teen larwes in vrugte te oorskakel. Hierdie proef sal in die nuwe seisoen herhaal word totdat ons voldoende data het.

'n Proef is uitgevoer om die doeltreffendheid van kouebehandeling te vergelyk met 'n nuwe VKM-kultuur (gevestig uit veld-versamelde larwes) en 'n ou laboratoriumkultuur om te bepaal of laboratorium-geteelde VKM mettertyd minder verdraagsaam word. Een herhaling is vir 5 tot 19 dae by 1°C uitgevoer. Geen betekenisvolle verskil is tussen die twee kulture aangeteken nie. 'n Soortgelyke proef sal egter uitgevoer word, hierdie keer om die koue vatbaarheid van 'n wild-aangevulde kultuur met 'n ou nie-aangevulde kultuur te vergelyk.

Eksperimente met laer dodelike temperature het bevind dat temperature van meer as -14.265°C vir 'n 2-uur duur waarskynlik 99.9% van insekte van al hierdie laboratoriumonderhoude populasies sal doodmaak. Vir gewaarborgde resultate moet temperature van meer as -16.133°C (die onderste 95% CI) getoets word. Dodelike temperature is ook getoets vir die 5 verskillende kulture uit die verskillende streke. Die Nelspruit-kultuur kan aansienlik kouer toestande oorleef as die Ou Kolonie L-bevolking. Alhoewel dit nie betekenisvol was nie, was die LLT's van die Addo-bevolking effens hoër as dié van die Nelspruit-bevolking, terwyl Citrusdal-insekte effens kouer toestande kon verdra in vergelyking met die Ou Kolonie L-insekte.

Eienskappe van larwes wat kouebehandeling oorleef is gediagnoseer en aangeteken, waarna larwes op kunsmatige dieet geplaas is om aan te teken of hulle aanhou oorleef en ontwikkel het en of hulle dood is. Gevolglik is eienskappe wat betroubaar aandui van moontlike oorlewing of dreigende dood, geïdentifiseer. Turgiditeit of slapheid is geïdentifiseer as die belangrikste kenmerke om dit te voorspel. Op die hoogte van hierdie voorlopige werk het CRI toe 'n samewerking met eNtisa, wat 'n betrokkenheidsinstituut binne die Nelson Mandela Universiteit is, begin om 'n meer kwantitatiewe, wetenskaplik robuuste stelsel te ontwikkel om die relevante larwe-eienskappe akkuraat te meet. Die jongste ontwikkeling deur eNtisa is 'n visiestelsel, wat die verplasing van larwes deur kompressie meet. Die visiestelsel kan dalk 'n werkbare metode wees met nog 'n paar proeflopies en verdere ontwikkeling. Dit sal uiteindelik akkurate voorspelling moontlik maak van of 'n

larwe wat 'n koue behandeling oorleef kan aanhou oorleef of beslis sal sterf, om te verseker dat inspekteurs nie vals oproepe maak oor die risiko van larwe-bevindings nie.

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

Project 1162 (April 2017 – March 2022): Sean Moore, Wayne Kirkman, Mellissa Peyper and Tamryn Marsberg (CRI)

#### **Summary**

FCM is currently monitored in the field using traps baited with synthesised female moth sex pheromone, thus attracting only male moths. However, such a system can never be adequately accurate, as it is the females (not the males) that lay eggs on the fruit, leading to the larvae that do the damage. Additionally, male moths are believed to fly greater distances than females and are therefore not necessarily representative of the female population in the area. Identification of an attractant for females would enable a far more accurate monitoring technique. Previous work identified a few volatile compounds emitted by citrus fruit, and blends of compounds, that may have potential for attracting adult female FCM. Recently two yeast species were identified as being female attractants. These will also be tested. A field net trial conducted to test the attractiveness of these compounds resulted in only one trap catching one female. We purchased a glass wind tunnel to test the volatiles in a controlled environment. Hexane which is used to dilute the volatiles had no effect on the moths and can therefore be used to flush the cage and room once a replicate is completed. Thus far, three volatiles were tested in the wind tunnel. They are D-limonene, Ocimene and  $\beta$ -caryophyllene. The first two chemicals had no effect on the moths, whereas  $\beta$ -caryophyllene had some effect where five FCM females flew out the small cage and sat on the sides of cages where they intermittently walked, sat and flew around. None of the moths flew close to, or touched the dispenser in which the volatile was placed. Thus far, tests have only been conducted with virgin females. Once this is complete, tests will be repeated with mated females.

#### **Opsomming**

VKM word tans in die veld gemonitor deur gebruik van lokvalle met 'n lokaas van gesintetiseerde wyfie mot seksferomoon, en lok dus net mannetjie motte. So 'n stelsel kan egter nooit akkuraat genoeg wees nie, want dit is die wyfies (nie mannetjies nie) wat eiers op die vrugte lê, wat lei tot die larwes wat die skade doen. Daarbenewens, blyk dit dat mannetjie motte groter afstande as wyfie motte vlieg en is daarom nie noodwendig verteenwoordigend van die wyfie populasie in die area nie. Identifikasie van 'n lokmiddel vir wyfies sal 'n meer akkurate moniterings tegniek moontlik maak. Vorige werk het 'n paar vlugtige stowwe en mengsels van verbindings geïdentifiseer wat belofte inhou vir aanlokking van volwasse wyfie VKM. Onlangs is twee gis spesies geïdentifiseer as wyfie lokmiddels. Hierdie sal ook getoets word. 'n Veldnetproef wat uitgevoer is om die aantreklikheid van hierdie verbindings te toets, het daartoe gelei dat slegs een lokval een wyfie gevang het. Ons het 'n glas windtonnel gekoop om die vlugtige stowwe in 'n beheerde ongewing te toets. Heksaan wat gebruik word om die vlugtige stowwe te verdun het geen effek op die motte gehad nie en kan dus gebruik word om die hok en die kamer uit te spoel sodra 'n replikaat voltooi is. Tot dusver is drie vlugtige stowwe getoets in die windtonnel. Hulle is D-limoneen, Ocimene en  $\beta$ -kariofilleen. Die eerste twee chemikalieë het geen effek op die motte gehad nie, terwyl  $\beta$ -kariofilleen 'n effek gehad het waar vyf VKM wyfies uit die klein hokkie gevlieg het en aan die kante van hokke gesit het waar hulle kort-kort geloop, gesit en rondvlieg het. Nie een van die motte het naby die vrystellers waarin die vlugtige stowwe geplaas is gevlieg of aangeraak nie. Tot dusver is toetse uitsluitlik met ongepaarde wyfies gedoen. Sodra hierdie voltooi is, sal toetse met gepaarde wyfies herhaal word.

### 3.2.6 PROGRESS REPORT: Field trials for control of FCM

Project 1225 (April 2019 – March 2022) by Sean Moore, Wayne Kirkman, Mellissa Peyper, Tammy Marsberg (CRI), Luke Cousins, Marcel van der Merwe and David Taylor (RU)

#### Summary

A trial was conducted to compare three methods for inoculation of fruit in semi-field bioassays. The first method was to paste eggs on a small square of wax paper onto the fruit. Egg numbers per fruit ranged from 51 to 145. For the second method, five pairs of moths were then placed into a cage with 10 fruit, and removed after 48 hours. For the third method, eight neonate larvae were placed onto each fruit. After two weeks at  $\pm 27^{\circ}\text{C}$ , fruit were dissected and the number of larvae in each fruit counted. Method 1 had 100% infestation of fruit, with an average of 14.36 larvae per fruit; method two had an 80% larval infestation with an average of 3.56 larvae per fruit; method 3 had a 42% infestation with 0.7 larvae per fruit. Thereafter, a trial was conducted to determine the optimal number of eggs or larvae to place per fruit, ranging from 10 to 50 for each method. We concluded that the best method to inoculate fruit was to place approximately 30 eggs per fruit, as this gave us 100% infestation with an average of 2.9 larvae per fruit.

One semi-field bioassay trial was completed in June when fruit started to colour up. The following treatments were compared: Untreated control, Cryptogran + BreakThru, Cryptogran + molasses + BreakThru, Cryptogran + yeast 1 + BreakThru, Cryptogran + yeast 1 + molasses + BreakThru, Cryptogran + yeast 2 + BreakThru, Cryptogran + yeast 2 + molasses + BreakThru, Cryptogran + CrpeNPV + molasses + BreakThru, CrpeNPV + molasses + BreakThru. Both yeast sprays with molasses performed the best with a 49% reduction in infestation. These trials will be repeated next season.

Another mating disruption trial was completed to establish if anthranilic diamides have a mating disruption effect on FCM adults. Chlorantraniliprole (Coragen™) and cyantraniliprole (Exirel® 100 SE) were used to test these effects, with the addition of Delegate to ensure that we are not interpreting insecticidal effect as a mating disruption effect. Results are still being analysed.

#### Opsomming

'n Proef is uitgevoer om drie metodes vir die inenting van vrugte in semi-veld bioassays te vergelyk. Die eerste metode was om eiers op 'n klein vierkant waspapier op die vrugte te plak. Eiergetalle per vrug het gewissel van 51 tot 145. Vir die tweede metode is vyf pare motte in 'n hok met 10 vrugte geplaas en na 48 uur verwyder. Vir die derde metode is agt neonaat larwes op elke vrug geplaas. Na twee weke by  $\pm 27^{\circ}\text{C}$  is vrugte gedissekteer en die aantal larwes in elke vrug getel. Metode 1 het 100% besmetting van vrugte gehad, met 'n gemiddeld van 14,36 larwes per vrug; metode twee het 'n 80% larwale besmetting gehad met 'n gemiddeld van 3,56 larwes per vrug; metode 3 het 'n 42% besmetting gehad met 0,7 larwes per vrug. Daarna is 'n proef uitgevoer om die optimale aantal eiers of larwes per vrug te plaas, wat wissel van 10 tot 50 vir elke metode, te bepaal. Ons het tot die gevolgtrekking gekom dat die beste metode om vrugte in te ent was om ongeveer 30 eiers per vrug te plaas, aangesien dit ons 100% besmetting gegee het met 'n gemiddeld van 2,9 larwes per vrug.

Een semi-veld bioassay is in Junie voltooi toe vrugte begin verkleur. Die volgende behandelings is vergelyk: Onbehandelde kontrole, Cryptogran + BreakThru, Cryptogran + melasse + BreakThru, Cryptogran + gis 1 + BreakThru, Cryptogran + gis 1 + melasse + BreakThru, Cryptogran + gis 2 + BreakThru, Cryptogran + gis 2 + melasse + BreakThru, Cryptogran + CrpeNPV + melasse + BreakThru, CrpeNPV + melasse + BreakThru. Beide gisbespuitings met melasse het die beste gevaar met 'n 49% vermindering in besmetting. Hierdie proewe sal volgende seisoen herhaal word.

Nog 'n paringsontwrigting proef is voltooi om vas te stel of antraniliese diamiede 'n paringsontwrigting effek op VKM volwassenes het. Chlorantraniliprole (Coragen™) en cyantraniliprole (Exirel® 100 SE) is gebruik om hierdie effekte te toets, met die byvoeging van Delegate om te verseker dat ons nie insekdodende effek interpreteer as 'n paringsontwrigting effek nie. Resultate word nog ontleed.

### 3.2.7 **PROGRESS REPORT: Synergism between insecticides for improved control of FCM**

Project 1226: (April 2021 – March 2022): Tamryn Marsberg and Sean Moore (CRI)

#### **Summary**

Synergism was investigated between *Cryptophlebia leucotreta* granulovirus (CrleGV) and an insect growth regulator (IGR), methoxyfenozide. The IGR was selected for synergism as it binds to the ecdysone receptor complex in lepidopteran larvae and mimics insect moulting, resulting in early death. This is similar to that achieved by deletion of the ecdysteroid glycosyl transferase (*egt*) gene in baculoviruses i.e. moulting, suppressed by the *egt* gene, is restored, resulting in a more rapid death. Synergism between CrleGV and the IGR was examined using surface dose bioassays against neonate *Thaumatotibia leucotreta* (false codling moth) larvae. Previous results with the selected IGR showed an antagonistic relationship for the majority of the treatments. Alternative insecticides were then selected, Neem oil and emamectin benzoate. Bioassay results with emamectin benzoate and CrleGV showed to be mostly antagonistic. Results with various low concentration of Neem oil and CrleGV were extremely promising, with the majority of the treatments showing a synergistic relationship. Further studies will focus on laboratory fruit bioassays and eventually testing various concentrations of Neem oil or azadirachtin and CrleGV in the field. Various other chemical and natural insecticides will also be identified and tested for synergism with CrleGV.

#### **Opsomming**

Sinergisme is ondersoek tussen *Cryptophlebia leucotreta* granulovirus (CrleGV) en die insekgroeireguleerder (IGR), methoxyfenozide. Die IGR is gekies vir sinergisme aangesien dit aan die ekdysoonreseptorkompleks in Lepidoptera larwes bind en insekverveling naboots, wat lei tot vroeë dood. Dit is soortgelyk aan dié wat bereik word deur die verwydering van die ekdisteroïed glikosieltransferase (*egt*) geen in bakulovirusse, d.w.s. vervelling, onderdruk deur die *egt* geen, word herstel, wat tot 'n vinniger dood lei. Sinergisme tussen CrleGV en die IGR is ondersoek met behulp van oppervlak dosis biotoetse teen pasuitgebroeide *Thaumatotibia leucotreta* (valskodlingmot) larwes. Vorige resultate met die geselekteerde IGR het 'n antagonistiese verwantskap vir die meerderheid van die behandelings getoon. Alternatiewe insekdoders is daarna geselekteer, Neem olie en emamektienbenzooat. Biotoets resultate met emamektienbenzooat en CrleGV het getoon om meestal antagonisties te wees. Resultate met verskeie lae konsentrasies Neem olie en CrleGV was uiters belowend, met die meerderheid van die behandelings wat 'n sinergistiese verwantskap getoon het. Verdere studies sal fokus op laboratoriumvrugte-biotoetse en uiteindelik verskeie behandelings van Neem olie of azadirachtin en CrleGV in veldproewe. Verskeie ander chemiese en natuurlike insekdoders sal ook geïdentifiseer en getoets word vir sinergisme met CrleGV.

### 3.2.8 **PROGRESS REPORT: Identification and evaluation of male false codling moth pheromones and an investigation of the usefulness for monitoring of female moths**

Project 1256 (2020/4 – 2022/12) by Adam Shuttleworth, Anne Heiduk, Steve Johnson (UKZN) and Sean Moore (CRI)

#### **Summary**

Male false codling moths are known to produce pheromones from three androconia, and at least one of these is likely involved in mating. Identification of these pheromones and understanding their role in mating could allow for the development of female attractants for monitoring FCM in the field. Current trap monitoring relies on the female pheromone to attract male moths, but this is suboptimal for predicting the threat that FCM poses to the crop as the males tend to disperse more widely than the females which actually cause the damage. Isolation and identification of the male pheromones would open the door for their use in the management of FCM, including development of a more accurate monitoring technique and possibly also enhancement of the efficacy of the sterile insect technique. Solvent extracts (dichloromethane and hexane) have been collected from all three androconia plus non-androconial body parts (as a control for non-pheromone cuticular components) and run on GC-MS. GC-FID-EAD experiments with female FCM and androconial extracts remain ongoing. So far, these experiments have identified four active compounds, tentatively identified as nonadecane, hexadecanoic acid, heneicosane and linoleic acid. However, these compounds are also present

in cuticle controls and thus unlikely to represent a unique male pheromone. To better understand the structure of the three androconia, a morphological study of the androconia using Environmental Scanning Electron Microscopy has been initiated and remains ongoing. SEM images of androconia, in conjunction with existing information regarding the courtship behaviour of FCM, will be used to inform and optimise our pheromone extraction protocols. The possibility that pheromone release is stimulated by exposure to female moths is also being explored.

## Opsomming

Dit is bekend dat mannetjie valkodingmotte feromone van drie androkonie produseer, en ten minste een hiervan is waarskynlik betrokke by paring. Identifikasie van hierdie feromone en begrip van hul rol in paring kan die ontwikkeling van wyfie lokmiddels vir die monitering van VKM in die veld moontlik maak. Huidige lokvalmonitering maak staat op die wyfieferomoon om mannetjie motte aan te trek, maar dit is suboptimaal om die bedreiging wat VKM vir die gewas inhou te voorspel aangesien die mannetjies geneig is om wyer te versprei as die wyfies wat eintlik die skade veroorsaak. Isolering en identifikasie van die mannetjie feromone sal die deur oopmaak vir hul gebruik in die bestuur van VKM, insluitend die ontwikkeling van 'n meer akkurate moniteringstegniek en moontlik ook die verbetering van die doeltreffendheid van die steriele insektegniek. Oplosmiddeleksrakte (dichloormetaan en heksaan) is van al drie androkonie plus nie-androkoniese liggaamsdele (as 'n kontrole vir nie-feromoon kutikulêre komponente) versamel en op GC-MS uitgevoer. GC-FID-EAD eksperimente met wyfie VKM en androkoniese ekstrakte bly aan die gang. Tot dusver het hierdie eksperimente vier aktiewe verbindings geïdentifiseer, voorlopig geïdentifiseer as nonadekaan, heksadekaansuur, heneikosaan en linoleïensuur. Hierdie verbindings is egter ook teenwoordig in kutikula-kontroles en sal dus waarskynlik nie 'n unieke mannetjie feromoon verteenwoordig nie. Om die struktuur van die drie androkonie beter te verstaan, is 'n morfologiese studie van die androkonie met behulp van Omgewings-skanderende Elektronmikroskopie geïnisieer en word nog voortgesit. SEM-beelde van androkonie, in samewerking met bestaande inligting rakende die paringsgedrag van VKM, sal gebruik word om ons feromoon-ekstraksieprotokolle in te lig en te optimaliseer. Die moontlikheid dat feromoonvrystelling deur blootstelling aan wyfie motte gestimuleer word, word ook ondersoek.

### 3.2.9 PROGRESS REPORT: Using the antennal response of the FCM larval parasitoid, *Agathis bishopi*, for identifying key volatiles indicative of FCM fruit infestation

Project 1260 (2020/4 – 2022/3) by Adam Shuttleworth, Anne Heiduk, Steve Johnson (UKZN), Mellissa Peyper, Sean Moore and Luke Cousins (CRI)

## Summary

FCM has an effective larval parasitoid, *Agathis bishopi*, which has the ability to locate and parasitise first instar FCM shortly after the larva has penetrated the fruit. If we can identify the volatile cue leading the parasitoid to the larva in infested fruit, we may be able to exploit this for non-destructive identification of FCM infested fruit at an early stage of infestation. This study aims to use Coupled Gas Chromatography-Flame Ionisation Detector-Electroantennographic Detection (GC-FID-EAD) experiments to identify the key volatile/s indicative of infestation, by recording the antennal responses of *A. bishopi* to volatiles collected from infested fruit, FCM larvae or larval frass. To date, headspace volatiles have been collected from FCM infested and healthy fruit and from FCM larvae and larval frass (on both fruit and artificial diet). *Agathis bishopi* have been collected from the field and a small culture initiated, but due to low numbers of *Agathis* in the field only three females have been available for GC-FID-EAD experiments. Preliminary experiments with these individuals yielded antennal responses to six compounds in headspace samples from infested oranges. Three of these peaks yielded responses across all runs. One of these compounds has tentatively been identified as an isomer of limonene oxide, while the remainder are likely terpenoids or aliphatic acids that remain to be firmly identified pending replication of the EAD results. GC-FID-EAD experiments with extracts from (1) FCM larvae and (2) larval frass remain to be conducted. GC-FID-EAD experiments will continue sporadically as and when *Agathis* females become available for experiments. The results of this study could ultimately lead to the development of a device such as an electronic nose or a differential mobility spectrometry (DMS) device, which could be programmed to detect the identified key volatiles, rather than depending on the live parasitoid to do this.

## Opsomming

VKM het 'n effektiewe larwe-parasitoïed, *Agathis bishopi*, wat die vermoë het om eerste instar VKM op te spoor en te parasiteer kort nadat die larwe die vrug binnegedring het. As ons die vlugtige leidraad kan identifiseer wat die parasitoïed na die larwe in besmette vrugte lei, kan ons dit dalk ontgin vir nie-vernietigende identifikasie van VKM besmette vrugte in 'n vroeë stadium van besmetting. Hierdie studie het ten doel om gekoppelde gaschromatografie-vlamionisasie-detektor-elektroantennografiese opsporing (GC-FID-EAD) eksperimente te gebruik om die sleutelvlugtige stof of stowwe wat dui op besmetting te identifiseer, deur die antennale reaksies van *A. bishopi* op vlugtige stowwe wat van besmette vrugte, VKM larwes of larwe mis versamel is, aan te teken. Tot op hede is vlugtige stowwe in die kopruimte versamel van VKM-besmette en gesonde vrugte en van VKM-larwes en larwe-mis (op beide vrugte en kunsmatige dieet). *Agathis bishopi* is van die veld af versamel en 'n klein kultuur is geïnisieer, maar as gevolg van lae getalle *Agathis* in die veld was slegs drie wyfies beskikbaar vir GC-FID-EAD eksperimente. Voorlopige eksperimente met hierdie individue het antennale reaksies op ses verbindings in kopspasiemonsters van besmette lemoene opgelewer. Drie van hierdie pieke het reaksies oor alle lopies gelewer. Een van hierdie verbindings is voorlopig geïdentifiseer as 'n isomeer van limoneenoksied, terwyl die res waarskynlik terpenoïede of alifatiese sure is wat nog met sekerheid geïdentifiseer moet word hangende die replikasie van die EAD-resultate. GC-FID-EAD-eksperimente met ekstrakte van (1) VKM-larwes en (2) larwe-mis moet nog uitgevoer word. GC-FID-EAD-eksperimente sal sporadies voortgaan soos en wanneer *Agathis*-wyfies vir eksperimente beskikbaar word. Die resultate van hierdie studie kan uiteindelik lei tot die ontwikkeling van 'n toestel soos 'n elektroniese neus of 'n differensiële mobiliteit spektrometrie (DMS) toestel, wat geprogrammeer kan word om die geïdentifiseerde sleutel vlugtige stowwe op te spoor, eerder as om afhanklik te wees van die lewende parasitoïed om dit te doen .

### 3.2.10 PROGRESS REPORT: Improving understanding of mating disruption

Project 1262 (2020/4 – 2023/3) by Adam Shuttleworth, Anne Heiduk, Steve Johnson (UKZN) and Sean Moore (CRI)

#### Summary

Mating disruption for FCM was successfully introduced in South Africa in the late 1990s. Currently, there are four mating disruption products registered and commercially available for FCM. Data on the relative release rates and hence relative pheromone densities of the four products is absent. Additionally, other than for Isomate, adequate data on the influence of temperature on release rate are not available. This is important, as we need to know when, during the season, mating disruption loses its efficacy. Lastly, the optimal (or minimal) density of pheromone in the environment to induce mating disruption and the relationship between pheromone density and reduction in mating events is unknown. This study aims to investigate these important aspects in the successful application of mating disruption for FCM. Progress on this project was delayed initially by the pandemic lockdown and then by the discovery of unanticipated variation in the trapping effectiveness of the adsorbent headspace traps that were intended to be used for these experiments. We have been unable to resolve this variation, despite extensive efforts testing different variables in the sampling process. We have therefore developed an alternative protocol to measure the amount of pheromone remaining in dispensers at different intervals. This method is adapted from the method described by Stelinski *et al* (2009, Journal of Economic Entomology 102: 315-323). This new protocol has been tested, and found effective, for Isomate and X-mate dispensers, and the protocol is currently being tested for Checkmate and Splat dispensers (which are applied as a gel or spray as opposed to the other dispenser types). Release rate trials will commence in the lab as soon as the protocol has been optimised for each dispenser type.

#### Opsomming

Paringsontwrigting vir VKM is van die laat 1990s suksesvol in Suid-Afrika toegepas. Tans is daar vier paringsontwrigting produkte geregistreer en kommersieel beskikbaar vir VKM. Data oor die relatiewe vrystellingstempo en dus relatiewe feromoondigthede van die vier produkte is afwesig. Daarbenewens, behalwe vir Isomate, is voldoende data oor die invloed van temperatuur op vrystellingstempo nie beskikbaar nie. Dit is belangrik, aangesien ons moet weet wanneer, gedurende die seisoen, paringsontwrigting sy doeltreffendheid verloor. Laastens is die optimale (of minimale) digtheid van feromoon in die omgewing om

paringsontwrigting te veroorsaak en die verband tussen feromoondigtheid en vermindering in paringsgevalle onbekend. Hierdie studie het ten doel om hierdie belangrike aspekte in die suksesvolle toepassing van paringsontwrigting vir VKM te ondersoek. Vordering met hierdie projek is aanvanklik vertraag deur die pandemiese inperking en toe deur die ontdekking van onverwagte variasie in die vangdoeltreffendheid van die adsorberende hoofruimte-valle wat bedoel was om vir hierdie eksperimente gebruik te word. Ons was nie in staat om hierdie variasie op te los nie, ten spyte van uitgebreide pogings om verskillende veranderlikes in die monsternemingsproses te toets. Ons het dus 'n alternatiewe protokol ontwikkel om die hoeveelheid feromoon wat in vrystellers oorbly met verskillende intervalle te meet. Hierdie metode is aangepas vanaf die metode beskryf deur Stelinski *et al* (2009, *Journal of Economic Entomology* 102: 315-323). Hierdie nuwe protokol is getoets en effektief gevind vir Isomate en X-mate vrystellers, en die protokol word tans getoets vir Checkmate en Splat vrystellers (wat as 'n gel of spuit toegedien word in teenstelling met die ander vrysteller tipes). Vrystellingtempo-proewe sal in die laboratorium begin word sodra die protokol vir elke vrystellertipe geoptimaliseer is.

### 3.2.11 **PROGRESS REPORT: Sterile insect technique (SIT) and mating disruption (MD) in the control of FCM**

Project 1282 (2020/21 – 2021/22) by Davina Saccaggi and Courtney Morris (CRI)

#### **Summary**

False Codling Moth (FCM) is a key pest in the South African citrus industry. This project examined the effect of combining SIT and MD products to control FCM. Two MD products, Checkmate and Isomate, and SIT sterile male releases were compared together or separately to determine their impact. Adult FCM were monitored using yellow delta traps with Chempac FCM lure and fruit were inspected for larval infestation. Traps were monitored fortnightly in April and May 2021, and again from October to November 2021.

Average trap catches were 0.8 wild and 1.9 sterile FCM per trap per fortnight over the monitoring periods. Wild FCM trap catches were lowest in the Isomate-only treatment and highest in the SIT-only treatment. However, due to low numbers caught and the large gap in monitoring, no definitive differences between treatments could be seen. No signs of FCM infestation were detected in fruit. However, since the fruit monitoring was not systematic and the number of fruit inspected was not recorded, the confidence in this result cannot be calculated.

Trap catches from mid-November declined to zero in all treatment blocks. Upon further enquiry, we found that the farm manager had applied an additional FCM mating disruption product in all blocks, effectively nullifying our trial from that point forwards. We were thus forced to abandon the Breede River Valley trial site for this project and project 1283 in December 2021.

In January 2022 a new trial site was identified near Citrusdal. In February 2022 new treatment blocks were laid out, combining the current 1282 and 1283 projects into a large-scale project examining the interaction of SIT, MD and netting on FCM control.

#### **Opsomming**

Valskodlingmot (VKM) is 'n sleutelplaag in die Suid-Afrikaanse sitrusbedryf. Hierdie projek het die effek van die kombinasie van SIT- en MD-produkte ondersoek om VKM te beheer. Twee MD produkte, Checkmate en Isomate, en SIT steriele mannetjie vryslatings is saam of afsonderlik vergelyk om hul impak te bepaal. Volwasse VKM is gemonitor deur gebruik van geel delta lokvalle met Chempac FCM lokmiddel en vrugte is geïnspekteer vir larwe besmetting. Lokvalle is tweeweekliks in April en Mei 2021 gemonitor, en weer van Oktober tot November 2021.

Gemiddelde lokvalvangste was 0.8 wilde en 1.9 steriele VKM per lokval per twee weke oor die moniteringsperiodes. Wilde VKM lokval vangste was die laagste in die Isomate-alleen behandeling en die hoogste in die SIT-alleen behandeling. As gevolg van lae getalle gevang en die groot gaping in monitering, kon geen definitiewe verskille tussen behandelings egter gesien word nie. Geen tekens van VKM-besmetting

is in vrugte opgespoor nie. Aangesien die vrugtemonitoring egter nie sistematies was nie en die aantal vrugte wat geïnspekteer is nie aangeteken is nie, kan die vertroue in hierdie resultaat egter nie bereken word nie.

Vangste vanaf middel November het in alle behandelingsblokke tot nul gedaal. By verdere navraag het ons gevind dat die plaasbestuurder 'n bykomende VKM-paringsontwrigtingsprodukt in alle blokke toegedien het, wat ons proef van daardie stadium af effektief tot niet gemaak het. Ons was dus gedwing om die Breederiviervallei-proefferrein vir hierdie projek en projek 1283 in Desember 2021 te verlaat.

In Januarie 2022 is 'n nuwe proefperseel naby Citrusdal geïdentifiseer. In Februarie 2022 is nuwe behandelingsblokke uitgelê, wat die huidige 1282 en 1283 projekte kombineer in 'n grootskaalse proef wat die interaksie van SIT, MD en nette op VKM-beheer ondersoek.

### **3.2.12 PROGRESS REPORT: Comparing the performance of and mating disruption (MD) for FCM control in netted and open orchards**

Project 1283 (2020/21 – 2021/22) by Davina Saccaggi and Courtney Morris (CRI)

#### **Summary**

False Codling Moth (FCM) is a key pest in the South African citrus industry. This project examined the efficacy of FCM MD products in netted and open orchards. Two MD products, Checkmate and Isomate, were compared in netted and open blocks on a site in the Breede River Valley growing Nules Clementines. All treatment blocks were also part of the FCM SIT programme. Adult FCM were monitored using yellow delta traps with Chempac FCM lure. Fruit were inspected ad hoc during routine monitoring for any signs of infestation of FCM larvae. Traps were monitored fortnightly from March to early June 2021, and again from October to November 2021.

Average trap catches were 1.11 wild and 5.25 sterile FCM per trap per fortnight over the monitoring periods. Trap catches were not different between netted and open sites for the same treatment. Between treatments, trap catches were highest in the two SIT-only sites, but not vastly different between the two MD products. No signs of FCM infestation were detected in fruit. However, since the fruit monitoring was not systematic and the number of fruit inspected was not recorded, the confidence in this result cannot be calculated.

Trap catches from mid-November declined to zero in all treatment blocks. Upon further enquiry, we found that the farm manager had applied an additional FCM mating disruption product in all blocks, effectively nullifying our trial from that point forwards. We were thus forced to abandon the Breede River Valley trial site for this project and project 1282 in December 2021.

In January 2022 a new trial site was identified near Citrusdal. In February 2022 new treatment blocks were laid out, combining the current 1282 and 1283 projects into a large-scale project examining the interaction of SIT, MD and netting on FCM control.

#### **Opsomming**

Valskodingmot (VKM) is 'n sleutelplaag in die Suid-Afrikaanse sitrusbedryf. Hierdie projek het die doeltreffendheid van VKM MD produkte in nette en oop boorde ondersoek. Twee MD-produkte, Checkmate en Isomate, is vergelyk in nette en oop blokke op 'n perseel in die Breederiviervallei wat Nules Clementines verbou. Alle behandelingsblokke was ook deel van die VKM SIT-program. Volwasse VKM is gemonitor deur gebruik van geel delta lokvalle met Chempac VKM lokmiddel. Vrugte is ad hoc geïnspekteer tydens roetine-monitoring vir enige tekens van besmetting van VKM-larwes. Lokvalle is tweeweekliks gemonitor van Maart tot vroeg in Junie 2021, en weer van Oktober tot November 2021.

Gemiddelde lokvalvangste was 1.11 wilde en 5.25 steriele VKM per lokval per twee weke oor die monitoringsperiodes. Vangste het nie tussen nette en oop terreine vir dieselfde behandelings verskil nie. Tussen behandelings was vangste die hoogste in die twee SIT-alleen-persele, maar nie baie verskillend tussen die twee MD-produkte nie. Geen tekens van VKM-besmetting is in vrugte opgespoor nie. Aangesien die

vrugtemonitoring egter nie sistematies was nie en die aantal vrugte wat geïnspekteer is nie aangeteken is nie, kan die vertroue in hierdie resultaat egter nie bereken word nie.

Vangste vanaf middel November het in alle behandelingsblokke tot nul gedaal. By verdere navraag het ons gevind dat die plaasbestuurder 'n bykomende VKM-paringsontwrigtingsprodukt in alle blokke toegedien het, wat ons proef van daardie stadium af effektief tot niet gemaak het. Ons was dus gedwing om die Breederiviervallei-proefperseel vir hierdie projek en projek 1282 in Desember 2021 te verlaat.

In Januarie 2022 is 'n nuwe proefperseel naby Citrusdal geïdentifiseer. In Februarie 2022 is nuwe behandelingsblokke uitgelê, wat die huidige 1282 en 1283 projekte kombineer het in 'n grootskaalse proef wat die interaksie van SIT, MD en nette op VKM-beheer ondersoek.

### 3.2.13 **PROGRESS REPORT: Evaluation of potential repellents for false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae)**

Project 1291 (2021 – 2022/23) by K Dambuza, C Coombes, M Hill (RU), and S Moore (CRI)

#### **Summary**

False codling moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) is a key phytosanitary pest in citrus orchards across South Africa. The mere presence of a single moth in consignments destined for overseas markets may result in the rejection of the entire shipment. For this reason, multiple control strategies have been implemented to reduce FCM populations in South Africa. Such strategies include the use of semiochemicals as insect pest disruptants, repelling males from finding females. Consequently, this study assessed FCM oviposition repellence in plants, essential oils, and other common commercial products used for FCM control. Repelling oviposition could improve FCM control, since the larval stage is considered the only damaging stage of its life cycle. Repellence trials were conducted in complete darkness in a controlled environment room. Oranges treated with solutions/suspensions of potential repellents were placed one by one into a cage of gravid FCM females for four hours, with oviposition being recorded every hour. Seven potential repellents were identified from the initial trials i.e. two essential oils (lavender and peppermint), two plant crude extracts (garlic and marigold), and three chemicals (Delegate, Coragen, and Warlock). Additionally, most of these repellents have maintained their repellent effect in ongoing dose-response trials, and Warlock has been identified to have dual action (both repellent and ovicidal properties). The efficacy of these repellents can be further tested in field trials, and they may be valuable for dispensing in an orchard or for spraying onto trees. Results from this study have the potential of improving FCM control in current integrated pest management programmes.

#### **Opsomming**

Valskodlingmot (VKM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is 'n belangrike fitosanitêre plaag in sitrusboorde regoor Suid-Afrika. Die blote teenwoordigheid van 'n enkele mot in besendings wat na oorsese markte bestem is, kan lei tot die afkeuring van die hele besending. Om hierdie rede is veelvuldige beheerstrategieë geïmplementeer om VKM-bevolkings in Suid-Afrika te verminder. Sulke strategieë sluit die gebruik van semiochemikalieë as insekplaag ontwrigters wat verhoed dat mannetjie motte wyfie motte vind. Gevolglik het hierdie studie VKM-eierleggings-afstoting in plante, essensiële olies en ander algemene kommersiële produkte wat vir VKM-beheer gebruik word, beoordeel. Afstoting van eierlegging kan VKM-beheer verbeter aangesien die larwestadium as die enigste skadelike stadium van sy lewensiklus beskou word. Afweerproewe is in algehele duisternis in 'n beheerde omgewingskamer uitgevoer. Lemoene wat met oplossings/suspensies van potensiële afweermiddels behandel is, is vir vier uur een vir een in 'n hok van bevrugte VKM-wyfië geplaas, met eierlegging wat elke uur aangeteken is. Sewe potensiële afweermiddels is uit die aanvanklike proewe geïdentifiseer, naamlik twee essensiële olies (laventel en peperment), twee ru-plantekstrakte (knoffel en goudbloem), en drie chemikalieë (Delegate, Coragen en Warlock). Boonop het die meeste van hierdie afweermiddels hul afstotende effek behou in deurlopende dosis-respons proewe, en daar is geïdentifiseer dat Warlock 'n dubbele werking het (beide afstotende en eierdodende eienskappe). Die doeltreffendheid van hierdie afweermiddels kan verder in veldproewe getoets word, en hulle kan moontlik

waardevol wees vir vrystelling in 'n boord of vir bespuiting op bome. Resultate van hierdie studie het die potensiaal om VKM-beheer in geïntegreerde plaagbestuursprogramme te verbeter.

### 3.2.14 **PROGRESS REPORT: Selected Ion Flow Tube Mass Spectrometry for postharvest detection of FCM infested fruit**

Project 1293 (Nov 2021 to March 2022) by Cecilia Cardinez, Maarten Hertog, Bart Nicolai (KU Leuven) and Sean Moore (CRI)

#### **Summary**

False codling moth (FCM) is a phytosanitary pest indigenous to various regions in Africa. It poses a threat to the production and marketing of many agricultural products, including citrus fruits. FCM is labelled as an A2 quarantine insect pest by the European and Mediterranean Plant Protection Organization (EPPO) which restricts the international trade of produce which FCM can potentially infest. Various preharvest control options can be applied in the field to manage FCM infestation, however these controls do not ensure 100% effectivity. Therefore, postharvest screening is also necessary to reduce the risk of exporting already infested commodities. In this way, the detection of FCM postharvest is crucial. A potential way to detect FCM infestation in fruits is through the identification of volatile organic compounds unique to the infested produce. In this regard, the use of Selected Ion Flow Tube- Mass Spectrometry which is a non-destructive, rapid, and quantitative method for detecting volatiles is a promising approach. The project aims to test the ability of SIFT-MS to accurately and rapidly detect FCM infested citrus fruit in a range of cultivars and at various stages after infestation. Ultimately, the aim is to be able to develop a postharvest tool based on SIFT-MS which can be applied for the packhouse detection of FCM infested fruit.

Initially, experiments with non-infested citrus fruits were done to test the ability of SIFT-MS to detect injury. Since FCM is a quarantine pest, the laboratory in KU Leuven had to first apply for a permit to import the FCM eggs. Therefore, injured citrus fruits were used to establish the parameters of the SIFT-MS. The objective of the initial experiment was to determine the accumulation time where ample volatiles are present in the headspace such that discrimination between an uninjured and injured fruit can be made. Various citrus fruits (mandarin, clementine, navelina, white grapefruit, red grapefruit) were injured with a 0.5 mm needle to simulate the form of injury that a larva will inflict onto the fruit. Afterwards, the fruit was placed in an airtight respiration jar and incubated at 25°C for different times (0,5,10,15, 20, 30 min). Measurements of uninjured and injured fruits were done with the SIFT-MS. Multivariate analyses (Principal Component Analysis and Partial Least Squares Regression) of the results were performed with Unscrambler. The results showed no proper discrimination was achieved between uninjured and injured citrus for the different accumulation times. A second experiment was conducted wherein certain parameters were changed, most notably the accumulation time was extended and set to 1 h. Results from the multivariate analysis showed that with the modified parameters, discrimination between the uninjured and injured fruit were observed. A follow-up experiment will be performed to determine if discrimination can still be achieved with a shorter accumulation time with the new parameters.

So far, only injury experiments were done. However, as of May 2022, the application has been finalised and the document required to legally import the FCM eggs to Belgium is underway. It is expected that experiments with the FCM infested citrus fruits will be carried out in the following months.

#### **Opsomming**

Valskodlingmot (VKM) is 'n fitosanitêre plaag wat inheems aan verskeie streke in Afrika is. Dit hou 'n bedreiging in vir die produksie en bemarking van baie landbouprodukte, insluitend sitrusvrugte. VKM word deur die Europese en Mediterreense Plantbeskermingsorganisasie (EPPO) as 'n A2-kwarantyn-insekplaag gemerk, wat die internasionale handel van produkte wat VKM moontlik kan besmet, beperk. Verskeie vooroesbeheeropsies kan in die veld toegepas word om VKM-besmetting te bestuur, maar hierdie beheermaatreëls verseker nie 100% doeltreffendheid nie. Daarom is na-oes ondersoek ook nodig om die risiko van uitvoer van reeds besmette kommoditeite te verminder. Op hierdie manier is die opsporing van VKM na-oes van kardinale belang. 'n Potensiële manier om VKM-besmetting in vrugte op te spoor is deur die

identifisering van vlugtige organiese verbindings wat uniek is aan die besmette produkte. In hierdie verband is die gebruik van geselekteerde ionvloeibuismassaspektrometrie, wat 'n nie-vernietigende, vinnige en kwantitatiewe metode is om vlugtige stowwe op te spoor, 'n belowende benadering. Die projek het ten doel om die vermoë van SIFT-MS te toets om VKM-besmette sitrusvrugte akkuraat en vinnig op te spoor in 'n reeks kultivars en op verskeie stadiums na besmetting. Uiteindelik is die doel om 'n na-oes-instrument gebaseer op SIFT-MS te ontwikkel wat toegepas kan word vir die pakhuisopsporing van VKM besmette vrugte.

Aanvanklik is eksperimente met nie-besmette sitrusvrugte gedoen om die vermoë van SIFT-MS om beserings op te spoor, te toets. Aangesien VKM 'n kwarantynplaag is, moes die laboratorium in KU Leuven eers aansoek doen om 'n permit om die VKM-eiers in te voer. Daarom is beseerde sitrusvrugte gebruik om die parameters van die SIFT-MS vas te stel. Die doel van die aanvanklike eksperiment was om die akkumulasietyd te bepaal waar genoeg vlugtige stowwe in die kopspasie teenwoordig is, sodat onderskeid tussen 'n onbeseerde en beseerde vrug gemaak kan word. Verskeie sitrusvrugte (mandaryne, clementine, navelina, wit pomelo, rooi pomelo) is met 'n 0.5 mm naald beseer om die vorm van besering wat 'n larwe aan die vrug sal toedien na te boots. Daarna is die vrugte in 'n lugdigte respirasiefles geplaas en by 25°C vir verskillende tye (0, 5, 10, 15, 20, 30 min) geïnkubeer. Metings van onbeseerde en beseerde vrugte is met die SIFT-MS gedoen. Meerveranderlike ontledings (Hoofkomponentanalise en Gedeeltelike Kleinste Kwadrate-regressie) van die resultate is met Unscrambler uitgevoer. Die resultate het getoon dat geen behoorlike diskriminasie tussen onbeseerde en beseerde sitrus vir die verskillende ophopingtye bereik is nie. 'n Tweede eksperiment is uitgevoer waarin sekere parameters verander is, veral die akkumulasietyd is verleng en op 1 uur gestel. Resultate van die meerveranderlike analise het getoon dat met die gewysigde parameters, diskriminasie tussen die onbeseerde en beseerde vrugte waargeneem is. 'n Opvolgekperiment sal uitgevoer word om te bepaal of diskriminasie steeds bereik kan word met 'n korter akkumulasietyd met die nuwe parameters.

Tot dusver is slegs beseringseksperimente gedoen. Vanaf Mei 2022 is die aansoek egter gefinaliseer en die dokument wat nodig is om die VKM-eiers wettiglik na België in te voer, is aan die gang. Daar word verwag dat eksperimente met die VKM-besmette sitrusvrugte in die volgende maande uitgevoer sal word.

### **3.2.15 PROGRESS REPORT: Investigating the appropriate timing for initiation of mating disruption against FCM in Limpopo Province**

Project 1294 (March 2021 – March 2023) by Steve Oosthuysen (HortResearch) and Sean Moore (CRI)

#### **Summary**

In a previous research project, it was determined that night temperatures in Letsitele and Hoedspruit begin increasing from early in July, after winter. Temperatures increased to levels sufficient to allow increased FCM activity and consequently such an increase in FCM activity was recorded. Consequently, it is postulated that the initiation of mating disruption only in October in the warm northern regions, as is standard practice, may be later than ideal. Therefore, a trial is being conducted to compare the efficacy of a mating disruption programme initiated in July with a mating disruption programme initiated in October. This is being done with all four registered mating disruption products, Isomate, Splat, Checkmate and X-Mate, and will continue until after harvest in August/September 2022. The idea is that not only will the first FCM generation of the season be suppressed by the early introduction of mating disruption, but the late Valencias will be protected against FCM during the last couple of months before harvest. At one of the trial sites, Isomate and Checkmate were applied. Unfortunately, FCM pressure has been so low that no fruit infestation has been recorded yet (from July 2021 to date). However, moths catches were generally higher in the control and where mating disruption was only initiated in October. Splat and X-Mate were applied at the other trial site. FCM pressure between Splat orchards was too uneven to draw any conclusions yet. However, for X-Mate, moths were only caught in the control block and fruit infestation was highest in the control and lowest where mating disruption was initiated early.

#### **Opsomming**

In 'n vorige projek was it bepaal dat nag temperature in Letsitele en Hoedspruit van vroeg in Julie, na die winter, begin toeneem het. Temperature het tot vlakke toegeneem wat voldoende was om 'n verhoging in VKM

aktiwiteit toe te laat en gevolglik is so 'n verhoging in VKM aktiwiteit aangeteken. Daarom is dit gepostuleer dat inisieering van paringsontwrigting eers in Oktobermaand in die warm noordelike streke, wat standaard praktyk is, dalk later kan wees as wat ideaal is. Daarom is 'n proef aan die gang om die doeltreffendheid van 'n paringsontwrigting program wat in Julie geïnisieer is met 'n Oktober geïnisieerde program te vergelyk. Hierdie word tans gedoen met al vier geregistreerde paringsontwrigting produkte, Isomate, Splat, Checkmate en X-Mate, en sal voortduur tot oestyd in Augustus/September 2022. Die beginsel is dat die eerste VKM generasie van die seisoen onderdruk sal word deur die vroeë instelling van paringsontwrigting, maar die laat Valencias sal ook teen VKM besmetting beskerm word gedurende die laaste paar maande voor oes. By een van die proefpersele is Isomate en Checkmate toegedien. Ongelukkig was VKM-druk so laag dat geen vrugtebesmetting nog aangeteken is nie (vanaf Julie 2021 tot op datum). Motvangste was egter oor die algemeen hoër in die kontroleblok en waar paringsontwrigting eers in Oktober begin is. Splat en X-Mate is by die ander proefperseel toegedien. VKM-druk tussen Splat-boorde was te ongeluk om nog enige gevolgtrekkings te maak nie. Vir X-Mate is motte egter slegs in die kontroleblok gevang en vrugtebesmetting was die hoogste in die kontrole en die laagste waar paringsontwrigting vroeg begin is.

### 3.2.16 **PROGRESS REPORT: Selection for a UV-resistant isolate of a nucleopolyhedrovirus for improved field persistence and efficacy against FCM**

Project 1295 (2021/2 – 2022/3) by M van der Merwe (CRI), M Jukes, C Knox, M Hill (RU), S Moore (CRI)

#### **Summary**

Biological control agents are one of the main strategies used in integrated pest management programmes. They are environmentally friendly, have no detrimental effects on consumers or applicators and are generally target specific. Entomopathogenic viruses (EPV) are one of the main agents used in biological control. Baculoviruses are the predominant EPV used, as they are species-specific, have a narrow host spectrum, are environmentally friendly and leave no residues on the fruit. However, one of their greatest shortcomings is their sensitivity to ultraviolet radiation, leading to their rapid breakdown in the field. A study conducted at Nelson Mandela University, selected for a UV-resistant isolate of CrleGV by exposing the virus to UV irradiation in an environmental test chamber, simulating natural sunlight. This led to the selection of a UV-resistant isolate of CrleGV that had about a 1000-fold improvement in virulence after just five exposure cycles, relative to the wild-type isolate used in CRYPTOGRAN™, after UV-exposure. We propose to apply the same approach to the newly discovered litchi moth virus CrpeNPV, which is also highly virulent against the false codling moth. Additionally, various UV protectants will also be tested alongside the newly selected UV resistant CrpeNPV isolate in laboratory trials to enhance its persistence in the field further. Finally, semi-field trials are also planned to examine whether the UV resistance observed in the laboratory is carried over into the field. Various technical difficulties were experienced with the initial setup of the UV chamber, but these issues have been resolved and the correct parameters for mimicking UV irradiation in the field have been determined. It was also observed that a GV was present within purified CrpeNPV samples; this contaminating GV was later determined to be CrleGV-SA. Surface dose bioassays have been conducted and indicated that litchi moth larvae are susceptible to CrleGV-SA but only at extremely high doses. Thus, in future surface dose bioassays where CrleGV is a contaminant, there should be no significant effect on litchi moth larval mortality. The first round of CrpeNPV OBs have been exposed to UV irradiation. However, virus counts done afterwards showed that the overall survival was extremely low and not high enough to conduct surface dose bioassays. A higher initial starting concentration of CrpeNPV OBs is required when exposing them to UV irradiation.

#### **Opsomming**

Biologiese beheermiddels is een van die hoofstrategieë wat in geïntegreerde plaagbestuursprogramme gebruik word. Hulle is omgewingsvriendelik, het geen nadelige uitwerking op verbruikers of toedieners nie en is oor die algemeen teikenspesifiek. Entomopatogeniese virusse (EPV) is een van die hoofmiddels wat in biologiese beheer gebruik word. Bakulovirusse is die oorheersende EPV wat gebruik word aangesien hulle spesie-spesifiek is, 'n smal gasheerspektrum het, omgewingsvriendelik is en los geen residue op die vrugte nie. Een van hul grootste tekortkominge is egter hul sensitiwiteit vir ultravioletstraling, wat tot hul vinnige ineenstorting in die veld lei. 'n Studie wat by die Nelson Mandela-Universiteit gedoen is, het vir 'n UV-bestande

isolaat van CrleGV geselekteer deur die virus bloot te stel aan UV-bestraling in 'n omgewingstoetskamer, wat natuurlike sonlig simuleer. Dit het gelei tot die seleksie van 'n UV-weerstandige isolaat van CrleGV wat ongeveer 'n 1000-voudige verbetering in virulensie gehad het na net vyf blootstellingsiklusse, relatief tot die wildtipe isolaat wat in CRYPTOGRAN™ gebruik is, na UV blootstelling. Ons stel voor om dieselfde benadering toe te pas op die nuut ontdekte lietsjiemotvirus, CrpeNPV, wat ook hoogs virulent is teen die valskodlingmot. Daarbenewens sal verskeie UV-beskerende middels ook getoets word saam met die nuut geselekteerde UV-bestande CrpeNPV-isolaat in laboratoriumproewe om die volharding daarvan in die veld verder te verbeter. Laastens word semi-veldproewe ook beplan om te ondersoek of die UV-weerstand wat in die laboratorium waargeneem word, na die veld oorgedra word. Verskeie tegniese probleme is ondervind met die aanvanklike opstelling van die UV-kamer, maar hierdie probleme is opgelos en die korrekte parameters om UV-bestraling in die veld na te boots is bepaal. Daar is ook waargeneem dat 'n GV teenwoordig was binne gesuiwerde CrpeNPV monsters. Hierdie kontaminerende GV is later bepaal as CrleGV-SA. Oppervlak-dosis biotoetse is uitgevoer en het aangedui dat lietsjiemotlarwes vatbaar is vir CrleGV-SA maar slegs teen uiters hoë dosisse. Dus, behoort daar in toekomstige oppervlak dosis biotoetse waar CrleGV 'n kontaminant is, geen beduidende effek op lietsjiemotlarwe mortaliteit te wees nie. Die eerste rondte CrpeNPV OBs is aan UV-bestraling blootgestel. Virustellings wat daarna gedoen is, het egter getoon dat die algehele oorlewing uiters laag was en nie genoeg om oppervlakdosis biotoetse uit te voer nie. 'n Hoër aanvanklike aanvangskonsentrasie van CrpeNPV OBs word vereis wanneer hulle aan UV-bestraling blootgestel word.

### 3.2.17 **PROGRESS REPORT: Regional differences in sex pheromones and sexual attractiveness in FCM**

Project: 1296 (2021 – 2023) by Pascal Aigbedion-Atalor, Candice Coombes, Martin Hill (RU), Sean Moore (CRI), Annemarie Heiduk, and Adam Shuttleworth (UKZN)

#### **Summary**

This is an ongoing project, and the rationale is based on reports indicating that FCM males in South Africa prefer mating with females from their own populations. Previous reports of these findings are incomplete, requiring further investigations. Specifically, the project aims to (objective 1) analyse and compare the relative blend of isomers in female FCM pheromones in populations from different regions, (objective 2) determine whether male moths' preferences for females from their own populations are retained when not given a choice, and (objective 3) devise solutions in the event that these preferences are retained (e.g. universally attractive pheromone blend and universally attracted FCM culture for SIT). For objective 1, methodological optimisation fundamental for pheromone collection from female moths during scotophase is ongoing at UKZN with Dr. Annemarie Heiduk and Dr. Adam Shuttleworth as principal investigators. Objective 2 is progressing well. Repetitive no-choice tests of males and females from five geographical locations: Addo, Citrusdal, Marble Hall, Nelspruit, Old colony are ongoing in an outdoor tunnel at Waainek, Rhodes University. So far, results have shown that females from all five populations attract Addo males, indicating no potential chemical signalling barriers that may trigger speciation. However, males from Nelspruit were not attracted to females from Citrusdal. These findings are still preliminary, and further repetitions will provide more insights. The third objective (i.e. objective 3) has not begun, as it is nested and dependent on the overall outcomes of objectives 1 and 2.

#### **Opsomming**

Hierdie is 'n deurlopende projek, en die rasionaal is gebaseer op verslae wat aandui dat VKM-mannetjies in Suid-Afrika verkies om met wyfies uit hul eie bevolkings te paar. Vorige verslae van hierdie bevindings is onvolledig, wat verdere ondersoek vereis. Die projek het spesifiek ten doel om (objektief 1) die relatiewe mengsel van isomere in wyfie VKM-feromone in populasies van verskillende streke, (objektief 2) te ontleed en te vergelyk of mannetjiemotte se voorkeure vir wyfies uit hul eie populasies behou word wanneer hulle nie 'n keuse gegee word nie, en (objektief 3) oplossings ontwikkel in die geval dat hierdie voorkeure behou word (bv. universeel aantreklike feromoonmengsel en universeel aangetrekte VKM-kultuur vir SIT). Vir objektief 1 is metodologiese optimalisering fundamenteel vir feromoonversameling van wyfie motte tydens skotofase aan die gang by UKZN met Dr. Annemarie Heiduk en Dr. Adam Shuttleworth as hoofondersoekers. Objektief 2 vorder goed. Herhalende geenkeusetoeetse van mannetjies en wyfies vanaf vyf geografiese streke: Addo,

Citrusdal, Marble Hall, Nelspruit en 'n Ou kolonie is aan die gang in 'n buitetonnel by Waainek, Rhodes Universiteit. Tot dusver het resultate getoon dat wyfies uit al vyf bevolkings Addo-mannetjies lok, wat geen potensiële chemiese seinversperrings aandui wat spesiasie kan veroorsaak nie. Mannetjies van Nelspruit was egter nie aangetrokke tot wyfies van Citrusdal nie. Hierdie bevindings is nog voorlopig, en verdere herhalings sal meer insigte verskaf. Die derde doelwit (m.a.w. objektief 3) het nie begin nie, aangesien dit genesteeer is en afhanklik is van die algehele uitkomst van objektiewe 1 en 2.

### 3.2.18 **PROGRESS REPORT: An investigation into the biological and genetic stability of UV-tolerant baculoviruses for improved control of FCM**

Project 1298 (2021/22 – 2023/24) by P Iita, K Lufhondo, M Jukes, C Knox, M Hill (RU), S Moore (CRI)

#### **Summary**

The false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) is native to southern Africa and it is a key pest of citrus. The control strategy for FCM involves an Integrated Pest Management (IPM) programme, incorporating the baculovirus, *Cryptophlebia leucotreta* granulovirus (CrleGV-SA), which has been successful in citrus orchards for almost two decades. Nonetheless, there remain various challenges when using baculoviruses, one of which is the high level of sensitivity to UV irradiation, with direct exposure to sunlight shown to decrease pathogenicity. To mitigate this, isolates of CrleGV with increased UV tolerance (UVT) have been developed via successive UV exposure. However, the successful implementation of these novel isolates as biopesticides will require mass production in suitable hosts. In doing so, this process could lead to the contamination of these isolates with covert wild type (WT) isolates, which have previously been shown to be present within host populations. Alternatively, repeated infection in the host population without any form of UV selective pressure could lead to reversion and a loss of UV tolerance. A series of preliminary biological assays have been conducted to determine the activity of the WT and UVT isolates against neonate FCM larvae. The results from these assays will be used to determine the concentration of virus that will be applied during the passage assay, while also providing a point of comparison for future biological assays. A trial run of the passage assay has been completed using the UVT isolate. Several important methodological challenges were identified which are now being addressed. Recovered samples will be re-evaluated with and without exposure to UV to determine whether the increased tolerance remains stable following passage in FCM larvae. These results will assist in developing procedures for the mass production of the CrleGV-UVT isolate.

#### **Opsomming**

Die valskodlingmot (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) is inheems aan Suider-Afrika en dit is 'n sleutelplaag van sitrus. Die beheerstrategie vir VKM behels 'n Geïntegreerde Plaagbestuur (IPM)-program, wat die bakulovirus, *Cryptophlebia leucotreta* granulovirus (CrleGV-SA) insluit, wat al byna twee dekades lank suksesvol in sitrusboorde gebruik word. Nietemin bly daar verskeie uitdagings met die gebruik van bakulovirusse, waarvan een die hoë vlak van sensitiwiteit vir UV-bestraling is, met direkte blootstelling aan sonlig wat gewys is om patogenisiteit te verminder. Om dit te versag, is isolate van CrleGV met verhoogde UV-toleransie (UVT) ontwikkel deur opeenvolgende UV-blootstelling. Die suksesvolle implementering van hierdie nuwe isolate as bioplaagdoders sal egter massaproduksie in geskikte gashere vereis. Sodoende kan hierdie proses lei tot die kontaminasie van hierdie isolate met kovertes wilde tipe (WT) isolate wat voorheen getoon is dat dit in gasheerpopulasies voorkom. Alternatiewelik kan herhaalde infeksie in die gasheerpopulasie sonder enige vorm van UV-selektiewe druk lei tot terugkeer en 'n verlies aan UV-toleransie. 'n Reeks voorlopige biologiese toetse is uitgevoer om die aktiwiteit van die WT- en UVT-isolate teen pasuitgeborede VKM-larwes te bepaal. Die resultate van hierdie toetse sal gebruik word om die konsentrasie virus te bepaal wat tydens die in vivo produksie toets toegedien moet word, terwyl dit ook 'n vergelykingspunt vir toekomstige biologiese toetse verskaf. 'n Proeflopie van die produksie toets is voltooi met die UVT-isolaat. Verskeie belangrike metodologiese uitdagings is geïdentifiseer wat nou aangespreek word. Herwonne monsters sal herevalueer word met en sonder blootstelling aan UV bestraling om te bepaal of die verhoogde toleransie stabiel bly na produksie in VKM-larwes. Hierdie resultate sal help met die ontwikkeling van prosedures vir die massaproduksie van die CrleGV-UVT-isolaat.

### 3.2.19 PROGRESS REPORT: Accurate monitoring of FCM fruit infestation

Project 1308 (April 2021 to March 2023) by Sean Moore, Mellissa Peyper, Tammy Marsberg, Luke Cousins, Marcel van der Merwe, Wayne Kirkman (CRI)

#### Summary

Included in the false codling moth risk management system (FMS) is obligatory preharvest monitoring of FCM infestation, which is done in fallen fruit underneath sets of five data trees for the last 12 weeks before harvest. If any fruit infestation is recorded at any stage, intervention with an appropriate control measure is mandatory. If any infested fruit are recorded during the last 4 weeks before harvest, fruit from the orchard may only be exported under the most stringent shipping temperature option available. Despite this risk mitigation measure, there have been a number of cases reported where no infestation is recorded in an orchard, but postharvest infestation thresholds are surpassed or infested fruit is even intercepted in the market. This project aims to determine how accurately representative the current monitoring method can be of infestation in the orchard and to determine if we can devise a more reliable method of monitoring fruit infestation. FCM infestation was monitored in seven Navel orange orchards over a 10-week period. This was done by evaluating infestation under four sets of five data trees in each orchard and in all of the sanitised fruit from each orchard. These data will be used to develop a more accurate and sensitive system for monitoring FCM infestation preharvest. Although the project will continue into a second season, we can already draw several reliable conclusions. The 5-data tree protocol for preharvest monitoring of FCM, as currently used in the FMS, is adequately effective and generally provides an over-estimation of FCM infestation in the orchard. Therefore, industry dissatisfaction with the protocol is most likely a reflection of the difficulty to implement the protocol reliably. Despite this, an assessment of infestation of all fallen fruit in an orchard, indicates that an approximately 100-fruit sample of fallen fruit per orchard is likely to provide a sufficient level of accuracy in representing the infestation in the orchard as a whole.

#### Opsomming

Ingesluit in die Valskodlingmot risiko bestuur stelsel (FMS) is die verpligtende vooroes monitering van VKM besmetting, wat in gevalde vrugte onder stelle van vyf databome gedurende die laaste 12 weke voor oes gedoen word. As enige vrugbesmetting op enige stadium aangeteken word, is intervensie met 'n geskikte bestrydings maatreel verpligtend. As enige besmette vrugte gedurende die laaste vier weke voor oes aangeteken word, mag vrugte van die boord net onder die strengste verskepings temperatuur opsie uitgevoer word. Ondanks hierdie risiko verminderings maatreel, was daar verskeie gevalle waar geen besmetting in 'n boord aangeteken is nie, maar na-oes besmettings drempelwaardes is oorskry of besmette vrugte is selfs in die mark onderskep. Die doel van hierdie projek is om te bepaal hoe akuraat die huidige moniterings metode die werklike besmettingsvlak in die boord kan verteenwoordig en om te bepaal of ons 'n meer betroubare metode vir die monitering van vrugbesmetting kan ontwikkel. VKM besmetting is in sewe Nawellemoen boorde oor 'n 10-weke tydperk gemonitor. Hierdie is gedoen deur ontleding van besmetting onder vier stelle van vyf databome in elke boord en in al die gesaniteerde vrugte van elke boord. Hierdie data sal gebruik word om 'n meer akkurate en sensitiewe stelsel te ontwikkel vir die monitering van VKM besmetting vooroes. Alhoewel die projek in 'n tweede seisoen sal voortgaan, kan ons reeds verskeie betroubare gevolgtrekkings maak. Die 5-data boomprotokol vir voor-oes monitering van VKM, soos tans in die FMS gebruik word, is voldoende doeltreffend en verskaf oor die algemeen 'n oorskatting van VKM besmetting in die boord. Daarom is die bedryfsontevredenheid met die protokol heel waarskynlik 'n weerspieëling van die moeilikheid om die protokol betroubaar te implementeer. Ten spyte hiervan, dui 'n beoordeling van besmetting van alle gevalle vrugte in 'n boord aan dat 'n ongeveer 100-vrugtemonster van gesaniteerde vrugte per boord waarskynlik 'n voldoende vlak van akkuraatheid sal verskaf om die besmetting in die boord as geheel voor te stel.

### 3.2.20 **PROGRESS REPORT: Evaluating hot air treatments for postharvest FCM control** Project 1060 (2013/4, 2015/6-2022/3) by T G Grout, P R Stephen and K C Stoltz (CRI)

#### **Summary**

Although the USDA-APHIS has treatment schedules for vapour heat on citrus and several papers were published in the 1980s and 90s that said it could be done safely, only Thailand is risking this treatment on a commercial basis. Their treatment is at 43°C and 50-65% relative humidity (RH) and the fruit is held at 5-10°C afterwards. Previous research showed that the third instar of false codling moth (FCM) was more tolerant to heat than younger instars or the egg stage, and that when fruit was placed in 46°C air for 6 h, 100% mortality of 681 larvae was obtained. At 44°C for 6 h, there were survivors so this temperature and period was used in 2016/7 to compare the susceptibility of third, fourth and fifth instars. Third instars were more susceptible than the other two instars, which were similar in their susceptibility, although mortalities of the latter instars ranged from 37 to 58%. Susceptibility to heat did seem to vary between egg batches. In 2020/1, further treatments were conducted with fifth instar FCM in oranges reaching pulp temperatures of 45°C and 47°C. Some replicates were discarded due to faults with the equipment but nine replicates at 45°C caused a mean corrected mortality of 83.9% in 4 572 larvae. Ten replicates at 47°C using 6 049 larvae gave a corrected mean mortality of 88.0%. Extending the dwell time at the maximum temperature for 30 min or 1 h before cooling did not increase the mortality significantly. Some cultivar susceptibility tests at 47°C with no dwell time showed a 16% increase in pitting compared to untreated fruit. Further research will focus on the detrimental effect on various cultivars of hot air at 47°C to see whether this treatment will be safe to use.

#### **Opsomming**

Alhoewel die USDA-APHIS behandelingskedules vir damphitte op sitrus het, en verskeie referate in die 1980's en 90's gepubliseer is wat gesê het dit kan veilig gedoen word, is dit net Thailand wat hierdie behandeling op 'n kommersiële basis waag. Hul behandeling is by 43°C en 50-65% relatiewe humiditeit (RH) en die vrugte word daarna by 5-10°C gehou. Vorige navorsing het getoon dat die derde instar van valskodlingmot (VKM) meer bestand was teen hitte as jonger instars of die eierstadium, en dat wanneer vrugte vir 6 uur in 46°C lug geplaas is, 100% mortaliteit van 681 larwes verkry is. By 44°C vir 6 uur was daar oorlewendes, so hierdie temperatuur en tydsduur is in 2016/7 gebruik om die vatbaarheid van derde, vierde en vyfde instars te vergelyk. Derde instars was meer vatbaar as die ander twee instars, wat soortgelyk was in hul vatbaarheid, hoewel mortaliteite van laasgenoemde instars van 37 tot 58% gewissel het. Die vatbaarheid vir hitte blyk tussen eiergroepe te varieer. In 2020/1 is verdere behandelings uitgevoer met vyfde instar VKM in lemoene wat pulptemperature van 45°C en 47°C bereik het. Sommige herhalings is weggegooi as gevolg van foute met die toerusting, maar nege herhalings by 45°C het 'n gemiddelde gekorrigeerde mortaliteit van 83.9% in 4 572 larwes veroorsaak. Tien herhalings by 47°C met 6 049 larwes het 'n gemiddelde gekorrigeerde mortaliteit van 88.0% gegee. Verlenging van die verblyftyd by die maksimum temperatuur vir 30 min of 1 uur vóór afkoeling, het nie die mortaliteit betekenisvol verhoog nie. Sommige kultivar vatbaarheidstoetse by 47°C sonder verblyftyd, het 'n 16% toename in gepokteskil getoon, in vergelyking met onbehandelde vrugte. Verdere navorsing sal fokus op die nadelige effek van warm lug by 47°C op verskeie kultivars, om te sien of hierdie behandeling veilig sal wees om te gebruik.

### 3.3 **PROGRAMME: FRUIT FLY** Programme coordinator: Aruna Manrakhan (CRI)

#### 3.3.1 **Programme summary**

Fruit flies are pests of phytosanitary concern in export citrus from southern Africa. Three fruit fly species are listed as pests of citrus in southern Africa: *Ceratitis capitata*, *Ceratitis rosa* and *Bactrocera dorsalis*. There have however been records of other fruit fly species on citrus in the region. *Ceratitis cosyra* was recorded in citrus from South Africa in Europe and *Ceratitis quilicii* was reared from citrus in Reunion Island. Not all citrus types are however susceptible to fruit flies. Commercial export grade lemons and limes are non-hosts for fruit flies.

The aim of the fruit fly research programme is to develop a more effective management strategy for fruit flies of concern on citrus from southern Africa. This can be done through a better understanding of the biology, ecology, monitoring and control of these fruit fly species.

A total of 12 projects was carried out under the fruit fly research programme between March 2021 and April 2022. Two of these projects: on post-harvest cold treatment and on efficiency of traps for monitoring, were completed. Two new time temperature treatments for fruit fly disinfestation: at or below 3.5°C for 24 days and at or below 5°C for 27 days were developed in Project 1171 (3.3.2). In Project 1229 (3.3.3), trap efficiency was found to vary with changing weather parameters, more specifically temperature and relative humidity, and this has important implications in decision making with regard to trap captures.

Ten other projects are still ongoing from previous years. The fruit fly rearing project (3.3.6) continues and supports other projects within the programme. Fruit fly colonies were successfully refreshed to provide quality materials for research. The effect of cold treatment interruptions on fruit fly larval survival is being finalised (3.3.4). Preliminary results indicate that interruptions of 2°C and 4°C above target pulp temperature did not affect cold treatment efficacy. A project on the distribution, host ranges and demography of *C. quilicii* (3.3.7) was in its final year. *Ceratitis quilicii* was confirmed to be more widely distributed than *C. rosa* in South Africa. Neither *Ceratitis quilicii* nor *C. rosa* were reared from citrus collected in orchards. *Ceratitis quilicii* and *C. rosa* developed poorly on citrus in laboratory studies and both did better on other fruit types tested: peach, guava and mango. Under a project funded by the European Union Horizon 2020 programme (3.3.8), new technologies such as E-traps and detection system models for *B. dorsalis* were investigated. In a low *B. dorsalis* prevalence area, captures in electronic traps placed under a risk driven detection tactic was comparable to those in conventional traps that were evenly distributed in an area, despite electronic traps being used at half the rate of conventional traps. Work is under way to import a biological control agent for *B. dorsalis*: *Fopius arisanus* (3.3.13), from Kenya where it is currently being cultured. Trials will be conducted to determine the quality of these parasitoids and their non-target effects. In a project at Stellenbosch University (Project 1318), methods of rearing *C. quilicii* are being investigated. Culturing *C. quilicii* proved difficult at CRI and other universities in South Africa since 2014. Following findings that *C. quilicii* is more widely spread than *C. rosa* in South Africa, research on equivalence of *C. capitata* cold treatment for *C. quilicii* has become a priority. For this type of research, a good culture of the fruit fly species needs to be available. Three new projects were initiated in 2021: (1) evaluation of the fruit fly systems approach (3.3.10), (2) host status of citrus for *C. cosyra* (3.3.11) and (3) fruit fly free areas (3.3.12), which is funded by the Standards Trade and Development Facility, focussing on defining areas of low fruit fly prevalence. In the project on fruit fly systems approach, fruit fly good agricultural practices and sorting practices during harvest and at packhouse were effective in either limiting or preventing fruit fly infestation. Fruit fly infestation was only recorded on mandarins and not on oranges at the packhouse. *Ceratitis capitata* was the dominant fruit fly species in orchards and was the only species reared from infested citrus. In the study on host status of citrus for *C. cosyra*, no natural infestation of *C. cosyra* was recorded. There was however infestation by *C. cosyra* recorded in forced infestation experiments. In the study on the determination of areas of low fruit fly prevalence, historical trap records and historical fruit fly interception data at packhouses were analysed. In the majority of the cases with zero fruit fly interceptions, *C. capitata* catches in Capilure traps stayed below 7 flies per trap per week at two weeks before harvest. This indicates that the trap thresholds currently in place are conservative. New data collected will be analysed to determine a more effective trap threshold for fruit flies.

## Program-opsomming

Vrugtevlieë is plaë van fitosanitêre belang in uitvoersitrus vanaf Suider-Afrika. Drie vrugtevliespesies word as plaë van sitrus in Suider-Afrika gelys: *Ceratitis capitata*, *Ceratitis rosa* en *Bactrocera dorsalis*. Daar was egter rekords van ander vrugtevliespesies op sitrus in die streek. *Ceratitis cosyra* is aangeteken op sitrus vanaf Suid-Afrika in Europa, en *Ceratitis quilicii* is vanaf sitrus in Reunion-eiland geteel. Nie alle sitrustipes is egter vatbaar vir vrugtevlieë nie. Kommersiële uitvoergraad suurlemoene en lemmetjies is nie-gashere vir vrugtevlieë nie.

Die doel van die vrugtevlieg-navorsingsprogram is om 'n meer doeltreffende bestuurstrategie vir vrugtevlieë van belang op sitrus uit Suid-Afrika, te ontwikkel. Dit kan gedoen word deur 'n beter begrip van die biologie, ekologie, monitering en beheer van hierdie vrugtevliegspesies.

Altesaam 12 projekte is onder die vrugtevlieg-navorsingsprogram tussen Maart 2021 en April 2022 uitgevoer. Twee van hierdie projekte: oor na-oes koue-behandeling en oor doeltreffendheid van lokvalle vir monitering, is voltooi. Twee nuwe tydtemperatuurbehandelings vir vrugtevlieg-ontsmetting: by of onder 3.5°C vir 24 dae, en by of onder 5°C vir 27 dae, is in Projek 1171 (3.3.2) ontwikkel. In Projek 1229 (3.3.3) is gevind dat lokvaldoeltreffendheid met veranderende weerparameters verskil, meer spesifiek temperatuur en relatiewe humiditeit, en dit het belangrike implikasies in besluitneming met betrekking tot lokvalvangste.

Tien ander projekte is nog van vorige jare aan die gang. Die vrugtevliegdeelprojek (3.3.6) gaan voort en ondersteun ander projekte binne die program. Vrugtevliegkolonies is suksesvol verfris om kwaliteit materiaal vir navorsing te verskaf. Die effek van koue-behandeling onderbrekings op vrugtevliegglarwes se oorlewing, word tans gefinaliseer (3.3.4). Voorlopige resultate dui daarop dat onderbrekings van 2°C en 4°C bó teikenpulptemperatuur nie koue-behandeling doeltreffendheid beïnvloed het nie. 'n Projek oor die verspreiding, gasheerreeks en demografie van *C. quilicii* (3.3.7) was in sy laaste jaar. Daar is bevestig dat *Ceratitidis quilicii* meer wydverspreid as *C. rosa* in Suid-Afrika is. Nóg *Ceratitidis quilicii* nóg *C. rosa* is vanaf sitrus wat in boorde versamel is, geteel. *Ceratitidis quilicii* en *C. rosa* het swak op sitrus in laboratoriumstudies ontwikkel en albei het beter op ander vrugtipies wat getoets is, gevaar: perske, koejawel en mango. Onder 'n projek wat deur die *European Union Horizon 2020*-program (3.3.8) gefinansier is, is nuwe tegnologieë soos E-lokvalle en opsporingstelselmodelle vir *B. dorsalis* ondersoek. In 'n gebied met 'n lae voorkoms van *B. dorsalis*, was vangste in elektroniese lokvalle wat onder 'n risiko-gedrewe opsporingstaktiek geplaas is, vergelykbaar met dié in konvensionele lokvalle wat eweredig in 'n gebied versprei is, ten spyte van elektroniese lokvalle wat teen die helfte van die hoeveelheid van konvensionele lokvalle gebruik word. Werk is onderweg om 'n biologiese beheermiddel vir *B. dorsalis*: *Fopius arisanus* (3.3.13), vanaf Kenia in te voer waar dit tans opgegroeï word. Proewe sal uitgevoer word om die kwaliteit van hierdie parasitoïede en hul nie-teiken-effekte te bepaal. In 'n projek aan die Universiteit van Stellenbosch word teelmetodes van *C. quilicii* ondersoek. Die teel van *C. quilicii* was sedert 2014 moeilik by CRI en ander universiteite in Suid-Afrika. Nà bevindinge dat *C. quilicii* wyer as *C. rosa* in Suid-Afrika versprei is, word navorsing oor ekwivalensie van *C. capitata* koue-behandeling vir *C. quilicii* het 'n prioriteit geword. Vir hierdie tipe navorsing moet 'n goeie kultuur van die vrugtevliegspesies beskikbaar wees. Drie nuwe projekte is in 2021 begin: (1) evaluering van die vrugtevliegstelselbenadering (3.3.10), (2) gasheerstatus van sitrus vir *C. cosyra* (3.3.11) en (3) vrugtevlieg-vrye projek (3.3.12) wat befonds word deur die *Standards Trade and Development Facility* wat daarop fokus om gebiede met lae vrugtevliegvoorkoms te definieer. In die projek oor vrugtevliegstelselbenadering was vrugtevlieg-goeie landboupraktyke en sorteerpraktyke tydens oes en by pakhuis effektief om vrugtevliegbesmetting óf te beperk óf te voorkom. Vrugtevliegbesmetting is slegs op mandaryne en nie op lemoene by die pakhuis aangeteken nie. *Ceratitidis capitata* was die dominante vrugtevliegspesie in boorde en was die enigste spesie wat uit besmette sitrus geteel is. In die studie oor gasheerstatus van sitrus vir *C. cosyra*, is geen natuurlike besmetting van *C. cosyra* aangeteken nie. Daar was egter besmetting deur *C. cosyra* in gedwonge besmettings-eksperimente aangeteken. In die studie oor die bepaling van gebiede met lae vrugtevliegvoorkoms, is geskiedkundige lokvalrekords en geskiedkundige vrugtevlieg-onderskeppingsdata by pakhuis ontleed. In die meerderheid van die gevalle met geen vrugtevlieg-onderskeppings nie, het *C. capitata*-vangste in Capilure-lokvalle twee weke voor oes onder 7 vlieë per lokval per week gebly. Dit dui daarop dat die lokvaldrempelwaarde wat tans in plek is, konserwatief is. Nuwe data wat ingesamel word, sal ontleed word om 'n meer effektiewe lokvaldrempelwaarde vir vrugtevlieë te bepaal.

### 3.3.2 FINAL REPORT: Efficacy of FCM partial cold treatments for fruit fly pests of citrus

Project 1171 (April 2017- March 2022) by Aruna Manrakhan, John-Henry Daneel, Evans Mauda, Leani Serfontein, Sean Moore, Vaughan Hattingh (CRI)

#### Summary

Cold shipping forms part of the risk mitigation measures under systems approaches for management of fruit fly pests and false codling moth (FCM) in citrus other than lemons and limes from South Africa. Temperatures

used are at set points that vary between -1°C and 4°C with estimated pulp temperatures between 0°C and 5°C. Temperatures above 3°C for durations shorter than 24 days provide partial treatment for FCM. There are existing disinfestation treatments for fruit flies at or below 3.2°C for up to 24 days. This study aims at determining the efficacy of treatments at 3.5°C and above on fruit flies. The cold tolerances of fruit fly pests of citrus in southern Africa: *Ceratitis capitata*, *Ceratitis rosa* and *Bactrocera dorsalis* were first determined at 3.5°C. The treatment durations for the most cold tolerant species were then determined at 3.5°C and 5°C. The efficacy of these treatments were confirmed in large scale tests. *Ceratitis capitata* was the most cold tolerant species. No survivor of *C. capitata* in Valencia oranges was recorded beyond 16 continuous days of treatment at both 3.5°C and 5°C in small scale tests. In the first replicate of the large scale tests at a mean of 4.1°C for 24 days, there was one survivor of *C. capitata* from a total of 25297 treated individuals. In four further replicates of the tests at a mean of 3.5°C for 24 days, no survivor of *C. capitata* was recorded from a total of 50560 treated individuals. No survivor of *C. capitata* was recorded from a total of 76902 treated individuals in tests conducted at 5°C for 27 days. These results indicate that shipping journeys should be at least 24 and 27 days when containers are set at 2°C and above 2°C respectively to mitigate the risk of fruit flies in citrus.

## Opsomming

Koue-verskeping vorm deel van die risiko-versagtende maatreëls onder stelselbenaderings vir die bestuur van vrugtevliegplae en valskodlingmot (VKM) in ander sitrus as suurlemoene en lemmetjies vanaf Suid-Afrika. Temperature wat gebruik word is by vasgestelde punte wat tussen -1°C en 4°C wissel, met geskatte pulptemperature tussen 0°C en 5°C. Temperature b6 3°C vir tydperke korter as 24 dae, verskaf gedeeltelike behandeling vir VKM. Daar is bestaande ontsmettingsbehandelings vir vrugtevlieë by of onder 3.2°C vir tot 24 dae. Hierdie studie het ten doel om die doeltreffendheid van behandelings by 3.5°C en hoër op vrugtevlieë te bepaal. Die koue-verdraagsaamheid van vrugtevliegplae van sitrus in Suider-Afrika: *Ceratitis capitata*, *Ceratitis rosa* en *Bactrocera dorsalis* is eers by 3.5°C bepaal. Die behandelingsduurte vir die mees koue-verdraagsame spesies is dan by 3.5°C en 5°C bepaal. Die doeltreffendheid van hierdie behandelings is in grootskaalse toetse bevestig. *Ceratitis capitata* was die mees koue-verdraagsame spesie. Geen oorlewende van *C. capitata* in Valencia-lemoene is aangeteken ná 16 aaneenlopende dae van behandeling by beide 3.5°C en 5°C in kleinskaalse toetse nie. In die eerste herhaling van die grootskaalse toetse by 'n gemiddelde van 4.1°C vir 24 dae, was daar een oorlewende van *C. capitata* uit 'n totaal van 25297 behandelde individue. In vier verdere herhalings van die toetse by 'n gemiddelde van 3.5°C vir 24 dae, is geen oorlewende van *C. capitata* aangeteken van 'n totaal van 50560 behandelde individue nie. Geen oorlewende van *C. capitata* is aangeteken van 'n totaal van 76902 behandelde individue in toetse wat by 5°C vir 27 dae uitgevoer is nie. Hierdie resultate dui aan dat verskeppingsritte minstens 24 en 27 dae moet duur wanneer houters onderskeidelik op 2°C en bo 2°C gestel is, om die risiko van vrugtevlieë in sitrus te verminder.

## Introduction

Fruit fly (Diptera: Tephritidae) pests and False Codling Moth (FCM), *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) are of phytosanitary concern to most export markets of citrus produced in South Africa. Mitigation of the risks of these pests in citrus from South Africa can be done using either standalone post-harvest disinfestation methods (cold or irradiation) with efficacy of at least Probit 8.7 (no survivors in 30 000 individuals tested) or systems approach which can include both pre and post-harvest treatments.

Partial cold disinfestation post-harvest treatments for FCM were developed for inclusion in a systems approach for the pest (Moore *et al.* 2016, Hattingh *et al.* 2020). Partial cold treatments would have efficacy of less than Probit 8.7 (Moore *et al.* 2016) but when combined with other pre and post-harvest measures in a systems approach can effectively mitigate the risk of the pest with a level equivalent to Probit 9 (Hattingh *et al.* 2020). A systems approach for mitigation of risk of FCM in citrus exported from South Africa to Europe was developed, coined the False Codling Moth Risk Management System (FMS) and implemented at the end of 2017 (Hattingh *et al.* 2017).

In 2019, the regulation of non-European fruit fly pests on specified fruit types including citrus produced from third countries was amended in the European Union. Fruit originating from these countries and destined to the European Union would either have to come from fruit fly free areas or be subjected to an effective fruit fly

treatment. A systems approach for fruit flies was then proposed as a treatment for management of fruit fly pest risk on South African citrus exported to Europe. The systems approach coined the Fruit Fly Risk Management System (FFMS) was subsequently adopted (Manrakhan *et al.* 2019). The FFMS includes both pre-harvest and post-harvest measures to mitigate the risk of fruit flies in export citrus. For citrus other than commercial export grade lemons and limes, cold shipping as required by the FMS at set points varying from -1°C to 4°C (pulp temperatures estimated to vary from 0°C to 5°C) for durations of up to 24 days is one of the post-harvest measures required. There are internationally approved cold treatments for fruit flies in citrus at temperatures at or below 3°C for up to 22 days (FAO 2016, USDA 2016, FAO 2017a, b, c, d, e, f). A schedule of 3.2°C for 24 days for disinfestation of *C. capitata* is being used for some citrus types exported from Australia to Japan (Hallman *et al.* 2018). However, efficacy of treatments at temperatures higher than 3.2°C for at least 24 days for fruit fly pests of citrus in southern Africa has not yet been quantified. There are three recorded fruit fly pest species of citrus in southern Africa: *Ceratitis capitata* (Wiedemann), *Ceratitis rosa* Karsch and *Bactrocera dorsalis* (Hendel). Studies on the efficacy of cold treatment for fruit flies in citrus would be time consuming and costly if it would have to be conducted on each of the three species.

In this study, the cold tolerances of the three fruit fly species were compared in artificial larval diet and in citrus at a target temperature of 3.5°C. Prior to this, the development of the three species in artificial larval diet was studied to determine developmental rates and the incubation times for the most cold tolerant life stage of these species. The durations required for treatments at 3.5°C and 5°C were then determined for the most cold tolerant fruit fly species. Finally the efficacy of treatments at 3.5°C and 5°C was quantified in large scale tests.

### Stated objectives

The stated objectives were as follows:

- A. Determine the larval development time of three fruit fly pests of citrus in artificial rearing medium
- B. Compare the *in vitro* and *in vivo* cold tolerance of third larval instars of three fruit fly pests of citrus at 3.5°C
- C. Compare the *in vitro* (in diet) and *in vivo* (in fruit) cold tolerance of third larval instar of the most cold-tolerant fruit fly pest at 3.5°C
- D. Efficacy of a 3.5°C treatment for the most cold-tolerant fruit fly pest in citrus at 4, 8, 12, 16, 20, 24 and 27 days
- E. Efficacy of a 3.5°C treatment for the most cold-tolerant fruit fly pest in citrus for 24 consecutive days (Confirmatory trial).
- F. Determination of duration of treatment at 5°C for the most cold-tolerant fruit fly pest in citrus
- G. Efficacy of a 5°C treatment for the most cold-tolerant fruit fly pest in citrus

Work under Objective C was not carried out since results obtained under Objective B clearly demonstrated that mortality rates of all fruit fly species were higher *in vitro* (in diet) compared to *in vivo* (in fruit) particularly after four days after exposure.

### Materials and methods

- A. Determine the larval development time of three fruit fly pests of citrus in artificial larval diet

Studies on the larval developmental times for the three fruit fly pests of citrus were carried out in 2017.

#### Test insects

*Ceratitis capitata*, *C. rosa* and *B. dorsalis* were all reared at the Citrus Research Centre (CRC), Citrus Research International (CRI), Nelspruit, Mpumalanga, South Africa. Eggs of the three species were collected over 20-21 hours.

#### Artificial larval diet

The larval diet used in the studies on development and cold tolerance of immature stages was a carrot based diet with carrot powder (Amidor (Pty) Ltd., Johannesburg, South Africa) and brewer's yeast (Organic World,

Randburg, South Africa) in the ratio of 2:1. The carrot powder and brewer's yeast mixture constituted 29.3% of the diet. The remaining ingredients were water (70.4%) and preservatives, methyl 4-hydroxybenzoate and sorbic acid (both from Sigma-Aldrich Pty. Ltd., Kempton Park, South Africa) at 0.2% and 0.1% of the diet mixture respectively.

#### Development and survival of fruit fly immature stages

For each fruit fly species, 100 g of the larval diet was placed in a plastic dish (12.5 cm diameter and 2.5 cm height). In each plastic dish, a total of 100 eggs of each species were placed on 4-5 moist blue blotting paper squares (1 cm x 1 cm) on the surface of the larval diet. Diet dishes with eggs of the three species were placed concurrently in the same environmental chamber Conviron CMP3023 (Controlled Environments, Manitoba, Canada) in order to compare development between species. Two similar environmental chambers were used for tests and were alternated between replicates.

Within one environmental chamber, 30 dishes of each species were incubated for daily larval observation over 15 days. All diet dishes with eggs were covered with a lid for 3 days following which the lid was removed and the diet container was transferred to a similarly labelled transparent flip-top container (12.5 cm x 12.5 cm x 6 cm) with about 50 holes (< 2 mm in diameter) pierced on the top. Control dishes (inoculated with 100 eggs each) for each species were also incubated to determine development and survival of larval and pupal stages in the larval diet. For the control dishes for each species, a fine layer of moist sand was additionally placed at the bottom of the plastic container for pupariation.

Two diet dishes for each species were removed from the environmental chamber and examined daily. Egg hatch on the paper squares on top of the diet was first determined in each dish. Thereafter, larvae found in the diet dish were removed and counted as dead or alive. Live larvae were placed in boiling water and thereafter in absolute alcohol. The body length (from mandible to end of last body segment) of each larva from each dish was measured using a Vernier calliper. Mouthparts of each larva were examined to determine changes in instars. The mouthparts of the first instar of all species were characterised by the presence of preapical teeth and non-sclerotized mandibles (White and Elson-Harris 1994). The mouthparts of the second instar of the three species were characterised by the presence of preapical teeth and sclerotized mandibles (White and Elson-Harris 1994). The mouthparts of the third instar of *C. capitata* and *B. dorsalis* were characterised by the absence of preapical teeth and presence of sclerotized mandibles (Pieterse *et al.* 2017).

Control dishes for each species were checked on a daily basis for larval jumping and presence of pupae. Pupae found were counted and transferred onto a Petri dish (9 cm diameter) lined with a moist blotting paper. The Petri dishes were checked daily for adult emergence. The number of emerged adults found in each Petri Dish were counted.

There were three replicates of these tests with three different egg batches. There were three control dishes per species per replicate (total of 300 inoculated eggs per species).

Air temperature inside the chamber and temperature inside one dish containing 100 g of larval diet with no eggs was recorded every 5 minutes using a logger (Grant Squirrel, Monitoring and control Laboratories, Johannesburg, South Africa) with temperature probes of the type-T thermocouple system. The mean ( $\pm$ SE) air temperatures in the environmental chambers during replicates one, two and three were 26.73°C  $\pm$  0.02°C, 25.70°C  $\pm$  0.01°C and 26.54°C  $\pm$  0.01°C respectively. The mean ( $\pm$  SE) temperatures reached inside the larval diet during replicates one, two and three were 25.87°C  $\pm$  0.01°C, 25.86°C  $\pm$  0.00°C, and 26.05°C  $\pm$  0.00°C respectively. Lights were switched off in both chambers throughout the study. A humidifier was placed in each environmental chamber. A relative humidity logger was placed inside each chamber to measure hourly relative humidity. The mean relative humidity ( $\pm$  SE) inside the two chambers during the study was 57.38%  $\pm$  0.22%.

B. Compare the in vitro and in vivo cold tolerance of third larval instars of three fruit fly pests of citrus at 3.5°C

In studies of in vitro (in diet) and in vivo (in fruit) cold tolerances, third instar larvae of *C. capitata*, *C. rosa* and *B. dorsalis* were used since they were found to be the most cold tolerant immature stage in a number of

previous studies (Back and Pemberton 1915, Grout *et al.* 2011, Ware and du Toit 2017). Studies were carried out in 2019. For development to third instar larvae in either diet or fruit, eggs of the three species were collected from laboratory reared colonies at CRC, CRI Nelspruit. At the time of collection, eggs were at or less than 24 hours old.

#### In vitro treatments

The larval diet used in the in vitro study was the same as the one described under Objective A. One hundred eggs of each species were inoculated onto 100 g of diet in a plastic dish as described under Objective A. For development of each species to the third larval stage, diet containers with eggs were incubated for six consecutive days in a temperature controlled room at a target temperature of 26°C. Air temperature inside the room and temperature inside three dishes containing 100 g of larval diet with no eggs was recorded every hour using a logger (Grant Squirrel, Monitoring and control Laboratories, Johannesburg, South Africa) with temperature probes of the type-T thermocouple system. For each species, five diet containers with eggs (500 eggs in total) were prepared for use in each of ten cold treatment exposures and an untreated control. Additionally for each species, there were two diet containers with eggs (200 eggs in total) which were prepared to verify the composition of the immature stages on the day of the exposure to the cold treatment.

#### In vivo treatments

Navel oranges, *Citrus sinensis* (L.) Osbeck cv. Fukomoto, sourced in April 2019 directly from a commercial citrus orchard (Crocodile Valley, Golden Frontiers Citrus) in Ehlanzeni District, Mpumalanga Province, South Africa were used for the tests. Soon after harvest in the orchard, fruit were dipped in a Sporekill solution for 1 minute (1ml of Sporekill in 1 L of water) in 100 L plastic tubs. Sporekill dips were refreshed for every 500 fruit. After the sporekill dips, fruit were stored in a cold room set at 4°C prior to preparation for inoculations. Fruit were prepared a day before the tests by first removing calyces and second dipping in an Imazalil and Guazatine mixture of Imazalil as sulphate and Guazatine (0.67g of Imazalil and 4.8 ml of Guazatine in 1 L of water) for 1 minute in 100 L plastic tubs. The temperature of the fungicide mixture was adjusted to be between 35°C and 40°C. Eggs were inoculated into each fruit by (1) boring a 6mm-diameter hole at about 30 mm deep in the fruit beneath the calyx, (2) adding a brewer's yeast:water mixture (1:2) into the hole, (3) preparing an egg-water mixture using deionized filtered water, and (4) adding a 0.025 ml aliquot of the mixture using an automatic pipette. In order to obtain the mean number of eggs per aliquot, 10 0.025 ml aliquots were placed onto a black cloth inside one Petri dish. The number of eggs in each aliquot was counted. This was repeated two times (30 aliquots in total). The number of eggs used for each species and replicate was then averaged from the 30 aliquot samples. After addition of eggs, the hole was closed using a thin piece of cotton wool. Molten wax was then smeared on top of the cotton wool. Following inoculation and sealing of hole, fruit were dipped in a fungicide mixture as described above for 1 minute in a plastic tub. Each inoculated fruit was placed in a brown paper bag. For larval development, fruit with eggs of each species were incubated for nine consecutive days in a temperature-controlled room at a target temperature of 26°C. Air temperature inside the room and temperature inside three dishes containing 100 g of larval diet with no eggs was recorded every hour using a logger (Grant Squirrel, Monitoring and control Laboratories, Johannesburg, South Africa) with temperature probes of the type-T thermocouple system. For each species, 30 fruit with eggs were prepared for use in each of ten cold treatment exposures and an untreated control. Additionally for each species, there were 15 fruit prepared to verify the composition of the larval stage on the day of the exposure to the cold treatment.

#### Exposure to cold

Two cold rooms each equipped with a refrigeration unit and located at CRC, CRI, in Nelspruit were used for both in vitro and in vivo tests. The target temperature in both tests was 3.5°C for a total period of 18 days. The susceptibilities of third instar larvae of the three fruit fly species to the cold treatment were compared concurrently at ten exposure periods: 1, 2, 4, 6, 8, 10, 12, 14, 16 and 18 days after the start of the cold treatment. Diet containers or fruit with larvae of the three species were placed simultaneously in the cold room. Treatments (fruit or diet containers) were divided in a cold room by exposure day so that they could be removed at the end of each exposure period.

Inside the cold room, air temperatures at two points: one at air delivery and one at the air return and temperatures inside either five diet containers or five fruit, with no eggs, were recorded every 5 minutes using a logger (Grant Squirrel, Monitoring and control Laboratories, Johannesburg, South Africa) with temperature

probes of the type-T thermocouple system. Temperature probes inside either diet containers or fruit were distributed in representative sections of the stacks of treatment in the cold chamber. Prior to the start of temperature measurement in each replicate, temperature probes were calibrated by immersion in ice-water. There were 3 replicate readings of each probe at 3-5 minute intervals. The readings of the probe were averaged. A calibration factor was derived for each probe (Calibration factor = True temperature of ice/water: 0°C- average probe reading). The calibration factors of the probes used were all within +/- 0.3°C.

The start point of the treatment was considered to be when three of the probes in the diet container or fruit reached 3.5°C. At the end of each exposure period, treatments (five diet containers or 30 fruit) and two diet containers or fruit with no eggs were removed from the cold room. The treatments were placed in the same temperature-controlled room as for the incubation which was set at a target temperature of 26°C. Air temperature inside the temperature-controlled room was recorded every hour using the same logger as the one used for the incubation with separate temperature probes of the type-T thermocouple system. Temperatures inside either two cold treated fruit or two diet containers with no eggs were recorded for a period of 2 days after removal from the cold room.

The cold treated diet containers and fruit were held for two days before being dissected to determine mortality. The untreated diet containers and fruit for each species were dissected on the day of the cold treatment (for diet- six days after egg inoculation; for fruit- nine days after egg inoculation) to determine mortality in the control. In the determination of mortality, dead and live larvae were counted. A larva was considered dead if there was no visible movement even when prodded. There were four replicates of these tests using four fly cohorts. Two replicates were carried out in each cold room. Each replicate was conducted at a time such that the treatment from precooling through to the final exposure period would be separately repeated.

In tests under Objective B, *C. capitata* was found to be the most cold tolerant fruit fly species. *Ceratitidis capitata* reared at CRC, CRI, in Nelspruit was therefore used for further tests under the subsequent objectives.

D. Efficacy of a 3.5°C treatment for the most cold-tolerant fruit fly pest in citrus at 4, 8, 12, 16, 20, 24 and 27 days (Exploratory testing)

Tests were carried out in 2020 in *Citrus sinensis* L. (Osbeck) cv Midnight Valencia sourced directly in the same year from commercial citrus orchards (Crocodile Valley, Golden Frontiers Citrus and Letaba Estates) in Mpumalanga and Limpopo provinces, South Africa. Fruit obtained from these orchards were treated with fungicides and stored in the same way as described under objective B (in vivo treatment). Fruit were inoculated and incubated in the same way as described under objective B (in vivo treatment). There were three replicates of these tests using three fly cohorts. In two of the replicates, 150 fruit with eggs were prepared for use in each cold exposure period tested and an untreated control. In the last replicate, 300 fruit with eggs were prepared for use in each cold exposure period tested and an untreated control.

Before each test, the weight, diameter and internal characteristics (sugar, acidity and pH) of 12 randomly selected fruit were determined.

#### Cold treatment duration tested

A cold room equipped with a refrigeration unit and located at CRC, CRI, in Nelspruit was used for the tests. The target temperature tested was 3.5°C. The susceptibility of third instar larvae of *C. capitata* to the cold treatment was determined at the following exposure periods after the start: 4, 8, 12, 16, 20, 24 and 27 days. After the first replicate, tests at the exposure period up to 27 days were discontinued. Fruit with larvae for the different cold exposure days were placed simultaneously in the cold room. Fruit were divided in a cold room by exposure day so that they could be removed at the end of each exposure period.

Temperatures before, during and after treatment were recorded as described above under Objective B. Mortality was determined in the same way as described above.

E. Efficacy of a 3.5°C treatment for the most cold-tolerant fruit fly pest in citrus (large scale testing)

Large scale tests were carried out between 2020 and 2021 in *Citrus sinensis* L. (Osbeck) cv Late Valencia sourced in (1) August 2020 from a commercial citrus packhouse (Karino) before processing and a commercial citrus orchard, Crocodile Valley, Golden Frontiers Citrus, both near Nelspruit and (2) August 2021 from a commercial citrus orchard, Mahela in Letsitele, Limpopo Province. Fruit obtained from these orchards were treated with fungicides and stored in the same way as described under objective B (in vivo treatment) except in the last replicate where the fungicide Imazalil used during fruit preparation and after inoculation was replaced with Evolve (containing azoxystrobin and fludioxinil) in the mixture with Guazatine. The new fungicide mixture consisted of 2.5 ml of Evolve and 4.8 ml of Guazatine in 1 L of water. Another change in the same replicate with regard to fungicidal treatment was the dipping of cotton wool pieces in a fungicide mixture of Imazalil as sulphate and Guazatine (0.67g of Imazalil and 4.8 ml of Guazatine in 1 L of water) before closure of holes in fruit. The changes in fungicidal treatment were implemented due to high prevalence of green mould on fruit in previous trials despite the use of imazalil. The presence of green mould on fruit led to high natural larval mortality. Fruit were inoculated and incubated in the same way as described under objective B (in vivo treatment). There were five replicates of the large scale tests. Replicates 1 to 4 were carried out using fruit sourced in 2020 and replicate 5 was carried out using fruit sourced in 2021. Between 119 and 1124 fruit were used in the different replicates. The fruit which were untreated in all replicates were more than 1/5 of the treated fruit.

Before each test, the weight, diameter and internal characteristics (sugar, acidity and pH) of 12 randomly selected fruit were determined.

#### Cold treatment tested

Tests were carried out in one of the cold rooms equipped with a refrigeration unit and located at CRC, CRI Nelspruit. The target temperature tested was 3.5°C for 24 days. In the first replicate, 3.5°C was the target long term minimum temperature while in the other replicates 3.5°C was the target long term mean temperature. Temperatures before and after treatment were recorded as described under objective B. Temperatures during the cold treatment were this time recorded using a logger (Grant Squirrel, Monitoring and control Laboratories, Johannesburg, South Africa) with PT 100 probes. Recordings were done every 5 minutes. Air temperatures were measured at two points: one at air delivery and one at the air return. Fruit pulp temperatures were recorded inside six fruit, with no eggs. Mortality in fruit was determined as described above.

#### Effect of cold treatment on quality

The effect of the cold treatment on fruit quality was determined in the last replicate of the large-scale test. Twelve fruit were evaluated for external and sensory quality before cold treatment, immediately after cold treatment and a week after cold treatment and storage at 26°C. Different batches of fruit were used at each evaluation period. For external quality, the weight, diameter and chilling injury were determined. The chilling injury was determined using a grading method developed by CRI (grading from 0-4). The sensory quality of the fruit was determined by tasting of juice extracted from 12 fruit. The tasting was evaluated by a consumer panel consisting of twelve people. The following criteria were used to evaluate sensory quality: flavour (poor, moderate, excellent), sweetness (not sweet, sweet), sourness (sour, not sour), flavour intensity (weak, moderate and intense) and cleanliness of flavour (off flavour, fresh flavour).

#### F. Determination of duration of treatment at 5°C for the most cold-tolerant fruit fly pest in citrus

Tests were carried out in 2021 in *Citrus sinensis* L. (Osbeck) cv Midnight Valencia sourced directly in the same year from a commercial citrus orchard in Crocodile Valley farm, Golden Frontiers Citrus, in Mpumalanga province, South Africa. Fruit obtained were treated with fungicides and stored in the same way as described under objective B (in vivo treatment) except in the last replicate where changes in fungicidal treatment as described under Objective E were implemented. Fruit were inoculated and incubated in the same way as described under objective B (in vivo treatment). Fruit, between 149 and 333 fruit, with eggs were prepared for use in each cold exposure period tested and an untreated control.

Before each test, the weight, diameter and internal characteristics (sugar, acidity and pH) of 12 randomly selected fruit were determined.

### Cold treatment duration tested

A cold room equipped with a refrigeration unit and located at CRC, CRI, in Nelspruit was used for the tests. The target temperature tested was 5°C. The susceptibility of third instar larvae of *C. capitata* to the cold treatment was determined at the following exposure periods after the start: 4, 8, 12, 16, 20, 24 and 27 days. Fruit with larvae for the different cold exposure days were placed simultaneously in the cold room. Fruit were divided in a cold room by exposure day so that they could be removed at the end of each exposure period. Temperatures before, during and after treatment were recorded as described above under Objective B. Mortality was determined in the same way as described above. There were four replicates of these tests using four fly cohorts.

### G. Efficacy of a 5°C treatment for the most cold-tolerant fruit fly pest in citrus (large scale testing)

Large scale tests were carried out between in 2022 in *Citrus sinensis* L. (Osbeck) cv Late Valencia sourced directly from a commercial citrus orchard, Mahela, Limpopo Province in August 2021. Fruit obtained were treated with Sporekill soon after harvest and before storage in the same way as described under objective B (in vivo treatment). Fruit were stored in the same way as described under objective B. Fruit were prepared a day before the tests by first removing calyces and second dipping in an Evolve and Guazatine mixture (2.5 ml of Evolve and 4.8 ml of Guazatine in 1 L of water) for 1 minute in 100 L plastic tubs. Fruit were inoculated and incubated in the same way as described under objective B (in vivo treatment). The only change in methodology during inoculation was the use of cotton wool pieces which were previously dipped in a fungicide mixture of Imazalil as sulphate and Guazatine (0.67g of Imazalil and 4.8 ml of Guazatine in 1 L of water) before closure of holes in fruit.

There were four replicates of the large-scale tests. For each replicate, 500 fruit were used as treatment. The fruit which were untreated in each replicate were more than 1/5 of the treated fruit.

Before each test, the weight, diameter and internal characteristics (sugar, acidity and % juice) of 12 randomly selected fruit were determined.

### Cold treatment tested

Tests were carried out in two cold rooms equipped with a refrigeration unit and located at CRC, CRI Nelspruit. The target temperature tested was 5°C for 27 days.

Temperatures before and after treatment were recorded as described under objective B. Temperatures during the cold treatment were recorded using a logger (Eltek, Grant Squirrel, Monitoring and control Laboratories, Johannesburg, South Africa) with PT 100 probes. Recordings were done every 5 minutes. Air temperatures were measured at two points: one at air delivery and one at the air return. Fruit pulp temperatures were recorded inside ten fruit, with no eggs.

Mortality in fruit was determined as described above.

### Effect of cold treatment on quality

The effect of the cold treatment on fruit quality was determined for each replicate as described under Objective E.

### Statistical analysis

For the development study, differences between species in their survival, development rates and sizes were determined using the non-parametric Kruskal-Wallis test since the assumptions of the parametric test could not be met.

In the cold tolerance tests, data were summarized as average percentage mortality for each life stage of each species at each cold exposure period. Mortality was calculated from the number of surviving third instar larvae over the number of eggs inoculated. For each exposure period Abbot's formula (Abbott 1925) was used to correct for untreated control mortality. Corrected mortality values were arcsine square root transformed to

stabilize variances. Effects of species and exposure periods and interactions thereof on mortality were analysed using Mixed Models. Estimates of cold exposure periods to achieve 95% mortality levels for each species were derived using the Probit model.

In exploratory and large-scale tests, data was summarized as observed percentage mortality. Observed mortality was calculated from the number of surviving third instar larvae over the estimated number of treated larvae. The estimated number of treated larvae for each replicate was the product of infestation rate and the number of treated fruit. The infestation rate was calculated from the number of surviving third instar larvae over the number of fruit in the control. True mortality level (Y) at 95% CI for each replicate in large scale tests was derived from the formula:  $Y = 10^{\log(0.05)/x}$  where x is the number of estimated treated individuals in the replicate. The formula was derived from calculation of level of confidence from estimated number of larvae in Couey and Chew (1986).

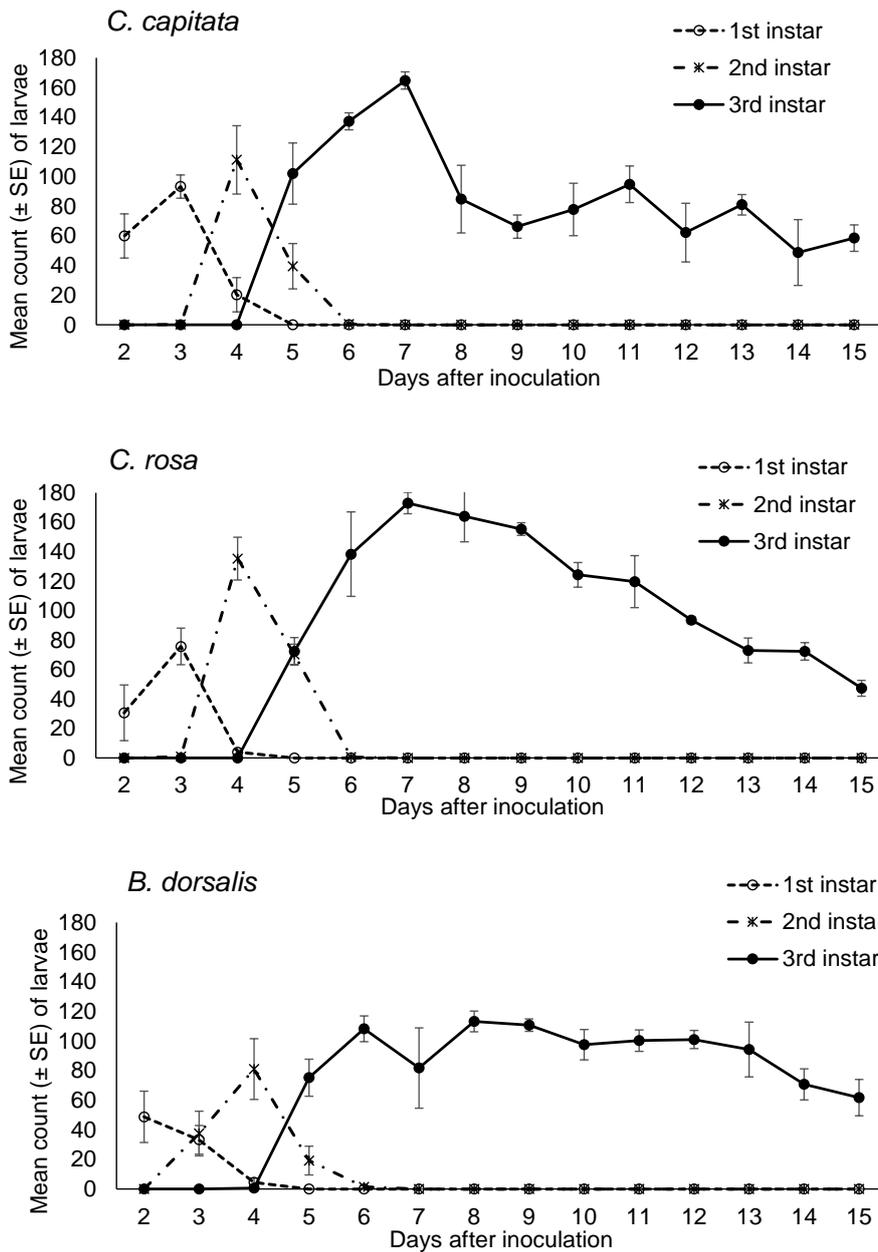
Associations between cold treatment exposure times (before cold treatment, after cold treatment and a week after cold storage) and the fruit quality variables: chilling injury and sensory criteria, were determined using Fisher's exact tests. Differences in weight and diameter at various times of fruit evaluation were determined using Analysis of Variance (ANOVA).

## Results and discussion

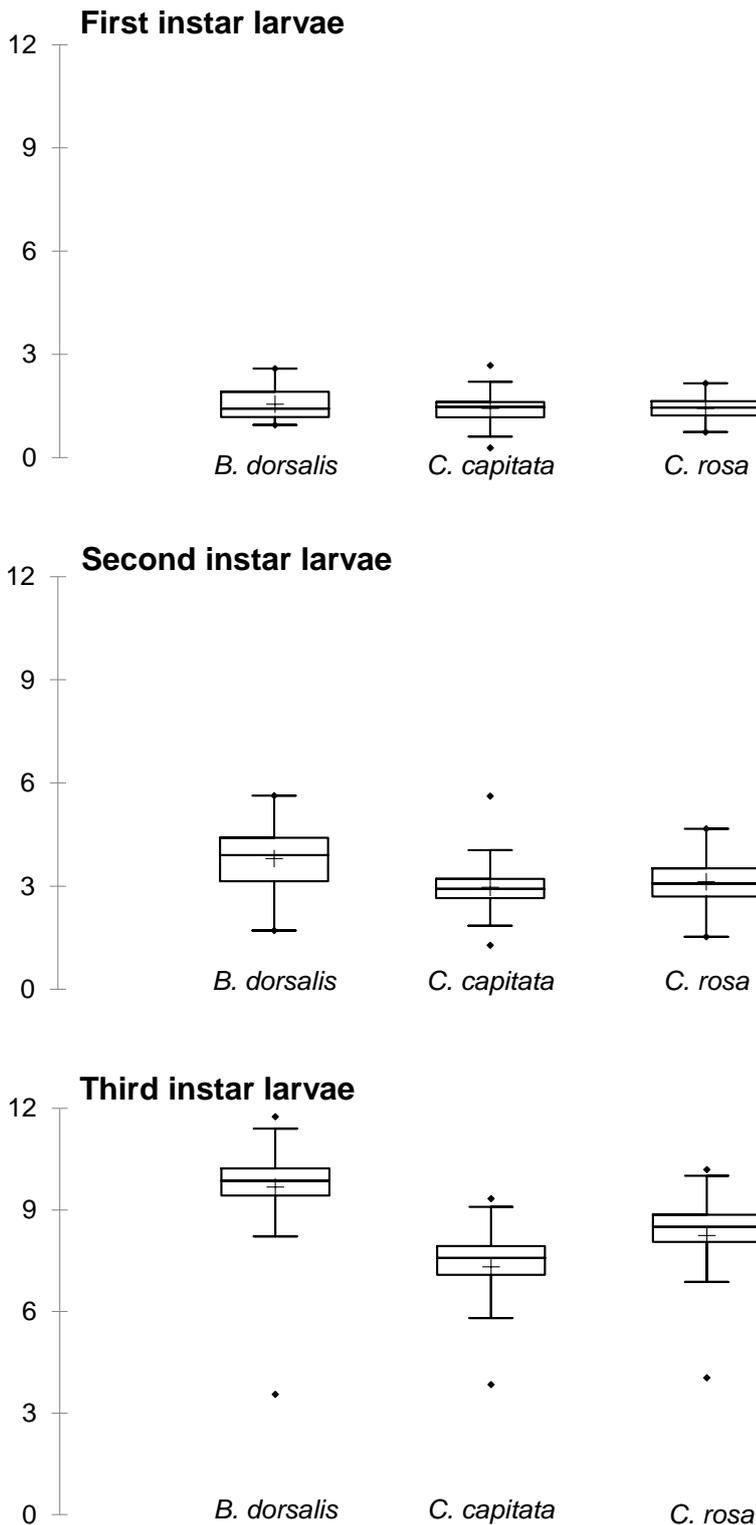
A. Determine the larval development time of three fruit fly pests of citrus in artificial diet

Eggs of *B. dorsalis* hatched earlier than those of *C. capitata* and *C. rosa*. For *B. dorsalis*, egg hatch occurred a day after inoculation in two of the replicates. For the *Ceratitidis* species, egg hatch generally occurred two days after inoculation. There were significant differences in percentage egg hatch between the three species ( $P < 0.0001$ ) with *C. capitata* ( $94.95\% \pm 0.47\%$ ) and *C. rosa* ( $95.51\% \pm 0.43\%$ ) being both higher than *B. dorsalis* ( $80.02\% \pm 1.47\%$ ).

For all species, the first instars were first recorded on day two after inoculation of eggs on diet (Fig. 3.3.2.1). The first instars were difficult to retrieve from the diet due to their small size and their translucent appearance. Consequently, the diet had to be first washed through a fine sieve. A small amount of water was then added to the washed diet in order to detect larval movement for easier retrieval of larvae. First instars were not recorded beyond day four after egg inoculation for all species (Fig. 3.3.2.1). Second instars of all species were recorded between days three and five after egg inoculation (Fig. 3.3.2.1). Third instar larvae of the *Ceratitidis* species were first recorded on day five whilst for *B. dorsalis* this occurred as from day four after egg inoculation (Fig. 3.3.2.1). On day six after egg inoculation, the larvae of all species were mostly at the third instar stage (Fig. 3.3.2.1). The larvae of *B. dorsalis* at all stages were generally longer than those of the *Ceratitidis* species (Fig. 3.3.2.2).



**Figure 3.3.2.1.** Daily composition of the different larval stages of *C. capitata*, *C. rosa* and *B. dorsalis* in the carrot based larval diet for a period of 15 days after inoculation of eggs (up to 21 hours old) at 25.92°C ± 0.00°C.



**Figure 3.3.2.2.** Box plots of measured lengths of larvae of *B. dorsalis*, *C. capitata* and *C. rosa* at different growth stages: First instar, Second Instar and Third instar, when reared on a carrot powder-based diet at a mean temperature ( $\pm$  SE) of  $25.92^{\circ}\text{C} \pm 0.00^{\circ}\text{C}$ . At all stages, larvae of *B. dorsalis* were significantly longer than those of the other two species (First instar:  $P=0.05$ , Second instar:  $P<0.0001$ , third instar:  $P<0.0001$ ).

There were no significant differences in larval developmental times between species ( $P=0.08$ ) (Table 3.3.2.1). Pupal development was however faster for *C. capitata* compared to *C. rosa* and *B. dorsalis* ( $P=0.001$ ) (Table 3.3.2.1). The two *Ceratitis* species had significantly higher larval survival rates compared to *B. dorsalis* on the carrot based diet ( $P=0.00$ ) (Table 3.3.2.1). There were however no significant differences in pupal survival rates between the three species ( $P=0.16$ ) (Table 3.3.2.1).

**Table 3.3.2.1.** Mean ( $\pm$  SE) developmental time (days) and mean ( $\pm$  SE) percentage survival of larvae and pupae of *C. capitata*, *C. rosa* and *B. dorsalis* at a mean temperature ( $\pm$  SE) of 25.92°C  $\pm$  0.00°C in control dishes. In each column, means followed by the same letters in the column are not significantly different at 0.05% probability level.

Species	Mean ( $\pm$ SE) Developmental time (days)		Mean ( $\pm$ SE) % survival	
	Larva	Pupa	Larva	Pupa
<i>Ceratitidis capitata</i>	7.22 $\pm$ 0.15 a	8.83 $\pm$ 0.17 a	83.44 $\pm$ 1.99 a	91.79 $\pm$ 2.99 a
<i>Ceratitidis rosa</i>	7.22 $\pm$ 0.15 a	9.00 $\pm$ 0.00 ab	83.22 $\pm$ 1.88 a	83.67 $\pm$ 3.36 a
<i>Bactrocera dorsalis</i>	7.78 $\pm$ 0.15 a	9.60 $\pm$ 0.25 b	61.33 $\pm$ 2.93 b	82.05 $\pm$ 4.64 a

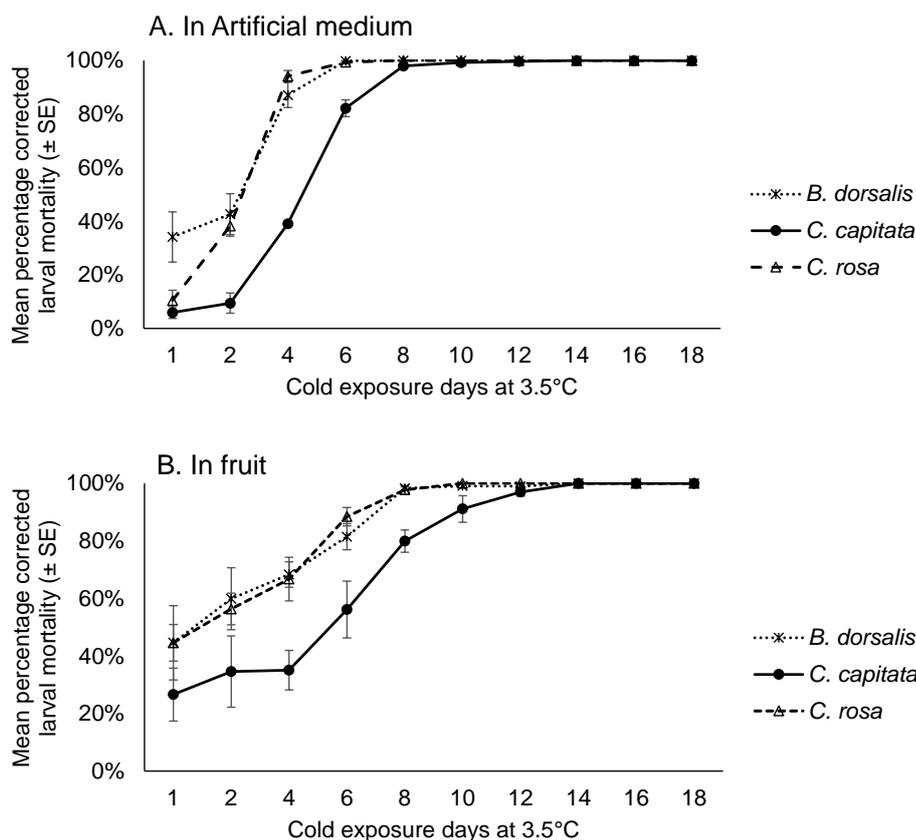
B. Compare the in vitro and in vivo cold tolerances of third larval instars of three fruit fly pests of citrus at 3.5°C

The temperatures during incubation, cold treatment and warming up after cold treatment are presented in Table 3.3.2.2. In all replicates in both tests, larvae of all species tested were mostly (over 98%) at the third larval stage.

**Table 3.3.2.2.** Temperatures before, during and after cold treatment during in vitro and in vivo cold tolerance studies.

Study	Period	Medium	Description	Temperature (°C)			
				Rep 1	Rep 2	Rep 3	Rep 4
In vitro	Incubation	Air	Mean	26.19 $\pm$ 0.06	26.02 $\pm$ 0.05	26.18 $\pm$ 0.05	26.22 $\pm$ 0.04
			Diet	Mean	25.88 $\pm$ 0.02	25.84 $\pm$ 0.02	25.95 $\pm$ 0.02
	Treatment	Diet	Mean	3.51 $\pm$ 0.00	3.52 $\pm$ 0.00	3.52 $\pm$ 0.00	3.53 $\pm$ 0.00
			Minimum	3.43 $\pm$ 0.00	3.46 $\pm$ 0.00	3.44 $\pm$ 0.00	3.44 $\pm$ 0.00
			Maximum	3.60 $\pm$ 0.00	3.61 $\pm$ 0.00	3.60 $\pm$ 0.00	3.62 $\pm$ 0.00
	Warm up	Diet	Mean	25.28 $\pm$ 0.11	25.29 $\pm$ 0.11	25.41 $\pm$ 0.09	25.48 $\pm$ 0.09
In vivo	Incubation	Air	Mean	26.16 $\pm$ 0.02	25.78 $\pm$ 0.03	26.13 $\pm$ 0.03	26.09 $\pm$ 0.06
			Fruit	Mean	25.97 $\pm$ 0.04	25.83 $\pm$ 0.03	25.85 $\pm$ 0.03
	Treatment	Fruit	Mean	3.55 $\pm$ 0.00	3.50 $\pm$ 0.00	3.51 $\pm$ 0.00	3.53 $\pm$ 0.00
			Minimum	3.46 $\pm$ 0.00	3.40 $\pm$ 0.00	3.36 $\pm$ 0.00	3.43 $\pm$ 0.00
			Maximum	3.63 $\pm$ 0.00	3.61 $\pm$ 0.00	3.67 $\pm$ 0.00	3.62 $\pm$ 0.00
	Warm up	Diet	Mean	23.95 $\pm$ 0.15	23.93 $\pm$ 0.18	23.94 $\pm$ 3.53	24.09 $\pm$ 4.11

*Ceratitidis capitata* was significantly more cold tolerant than *B. dorsalis* and *C. rosa* at 3.5°C in both in vitro ( $F_{2,90}=71.49$ ,  $P<0.0001$ ) and in vivo ( $F_{2,144}=39.13$ ,  $P<0.0001$ ) tests (Fig. 3.3.2.3). In both tests, exposure period and the interaction between species and exposure period were also significant factors in influencing mortality ( $P<0.05$ ). There were no survivors of *C. capitata* beyond 12 days of exposure at 3.5°C in both tests. After four days of cold exposure, mortality rates of all species were higher in diet compared to fruit. Based on recorded larval mortality, the estimated exposure periods (95% CI) at 3.5°C required to achieve 95% mortality of third instar larvae of *C. capitata*, *C. rosa* and *B. dorsalis* were 8.72 (8.53-8.92), 4.48 (4.35- 4.62) and 5.22 (5.05-5.41) days in artificial diet. The estimated exposure periods (95% CI) at 3.5°C required to achieve 95% mortality of third instar larvae of *C. capitata*, *C. rosa* and *B. dorsalis* were 17.31 (16.88-17.76), 7.69 (7.51- 7.88) and 8.27 (8.08- 8.48) days in fruit.



**Figure 3.3.2.2.** Mean ( $\pm$ SE) corrected mortality of late third instar larvae of *C. capitata*, *C. rosa* and *B. dorsalis* at different exposure period (days) at 3.5°C in artificial diet (A) and in Navel orange (B)

The results obtained herein concur with findings in previous studies whereby *C. capitata* was found to be as or more cold tolerant than *B. dorsalis* (Armstrong et al. 1995, Hallman et al. 2011, Hallman et al. 2013) and more cold tolerant than *C. rosa* (Ware and du Toit 2017). *Ceratitis capitata* was therefore considered to be the most cold tolerant fruit fly pest of citrus in South Africa and further objectives of this project were pursued on *C. capitata*.

**D.** Efficacy of a 3.5°C treatment for the most cold-tolerant fruit fly pest in citrus at 4, 8, 12, 16, 20, 24 and 27 days (Exploratory testing)

During the exploratory testing phase, the durations from start of cooling until at least three probes in the fruit were at  $\leq 3.5^\circ\text{C}$  were 29.08 hours, 29.33 hours and 29.92 hours in the first, second, and third replicate respectively. After the start of the cold treatment, the mean ( $\pm$  SE) fruit temperatures in the cold room were  $3.53^\circ\text{C} \pm 0.00^\circ\text{C}$ ,  $3.55^\circ\text{C} \pm 0.00^\circ\text{C}$  and  $3.57^\circ\text{C} \pm 0.00^\circ\text{C}$  for the first, second and third replicate respectively. In the first replicate, exposure to cold was evaluated for up to 27 days. In the two other replicates, exposure to cold was evaluated only for a period of up to 24 days. There were no major differences in external and internal characteristics of the fruit used in the three replicates (Table 3.3.2.3). After the cold treatment and before determination of survival from the cold treatment, the mean ( $\pm$  SE) temperatures of the fruit were recorded as  $24.13^\circ\text{C} \pm 0.17^\circ\text{C}$ ,  $24.14^\circ\text{C} \pm 0.18^\circ\text{C}$  and  $24.07^\circ\text{C} \pm 0.18^\circ\text{C}$  in the first, second and third replicate respectively. No survivors of third instar larvae of *C. capitata* were recorded beyond 16 days of cold treatment (Table 3.3.2.4).

**Table 3.3.2.3.** External and internal characteristics of Midnight Valencia oranges used in the three replicates of the exploratory phase of the cold treatment at a target temperature of 3.5°C.

Replicate	Weight/g	Diameter/mm	Acid (%)	Brix °	pH
1	249.33 ± 4.48	79.33 ± 0.73	0.9	10.3	3.55
2	217.81 ± 14.30	76.42 ± 1.50	0.85	11.6	3.61
3	255.68 ± 6.02	81.42 ± 0.61	1.15	11.2	3.8

**Table 3.3.2.4.** Mortality of third instar larvae of *C. capitata* in fruit treated at temperature of 3.5 °C for a range of exposure periods

Replicate	Exposure period	Number of fruit	Estimated number of treated larvae (based on control survivors)	Total number of survivors	Total number of dead larvae	Mortality calculated from treated larvae (%)
1	4	150	1577	473	1075	70.01%
	8	150	1577	137	1486	91.31%
	12	150	1577	5	1799	99.68%
	16	150	1577	0	1693	100.00%
	20	150	1577	0	1721	100.00%
	24	150	1577	0	1716	100.00%
	27	150	1577	0	1797	100.00%
	Control	150	-	1577	262	
2	4	150	1433	410	1218	71.39%
	8	137*	1433	19	1535	98.67%
	12	150	1433	0	1729	100.00%
	16	150	1433	0	1753	100.00%
	20	150	1433	0	1825	100.00%
	24	150	1433	0	1841	100.00%
	Control	150	-	1433	194	
	3	4	299*	4410	1182	2639
8		300	4410	302	3526	93.15%
12		300	4410	39	3946	99.12%
16		300	4410	18	3889	99.59%
20		300	4410	0	4131	100.00%
24		300	4410	0	4037	100.00%
Control		300	-	4410	136	

\*An error in the number of fruit prepared for the exposure period occurred

#### E. Efficacy of a 3.5°C treatment for the most cold-tolerant fruit fly pest in citrus (large scale testing)

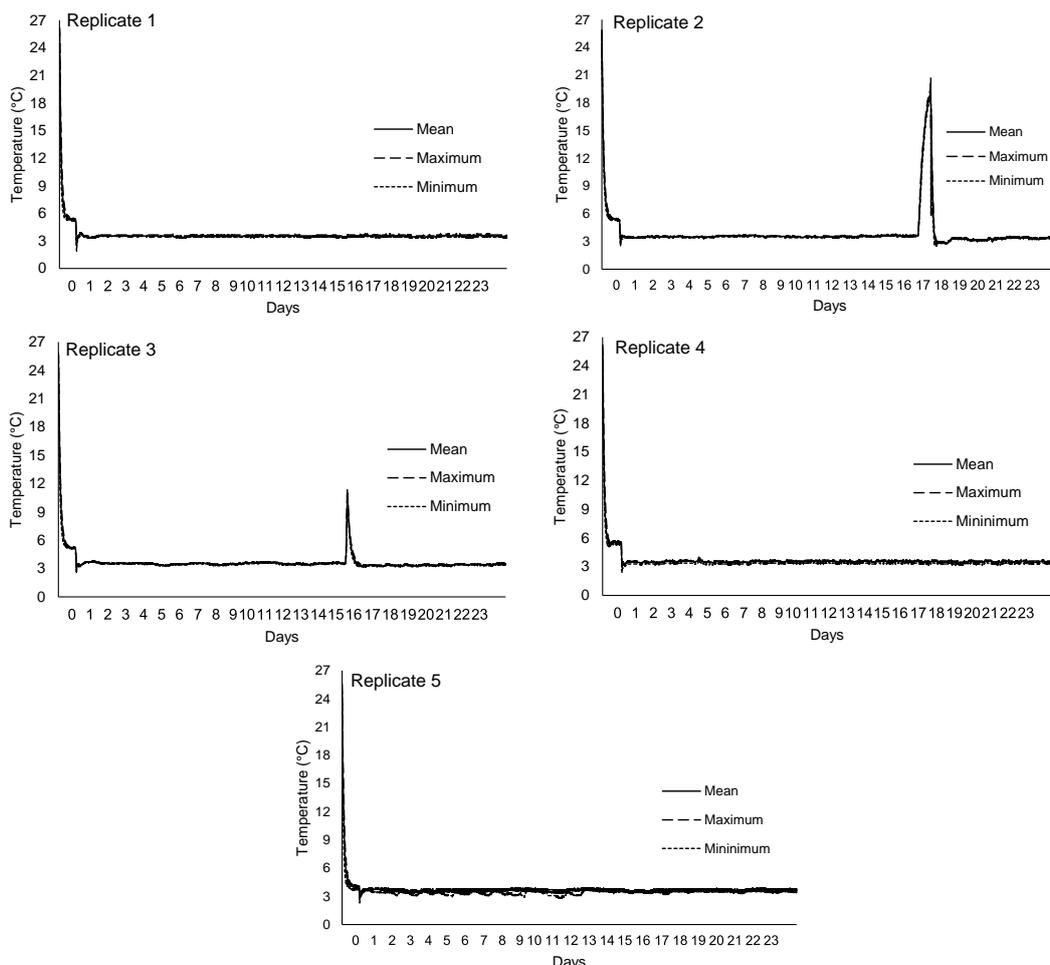
Temperature profiles and summaries of the large scale tests are shown in Figure 3.3.2.4 and Table 3.3.2.5. The external and internal characteristics of the Late Valencia oranges used are provided in Table 3.3.2.6.

In the first replicate at a mean temperature of 4°C for 24 days, there was one survivor among an estimated 25297 third instar larvae which were treated. In the four other replicates at mean temperatures varying between 3.5°C and 3.8°C for 24 days, there were no survivors among a total estimate of 50560 third instar larvae which were treated (Table 3.3.2.7). This was despite increases in fruit temperatures of up to at least two times the target temperature towards the end of the treatment (after day 15 of cold exposure) in the second and third replicates which were due to malfunctioning of the cold room (Fig. 3.3.2.4). There was no significant association of the cold treatment with sensory quality of the fruit (Fisher's exact test: Flavour: p=0.91, Flavour intensity: p=0.20; Sweetness: p=0.45; Sourness: p=1.00; Cleanliness of flavour: p=0.85) and chilling injury

(Fisher's exact test:  $p=0.50$ ) (Fig. 3.3.2.5). Differences in fruit weight and fruit diameter were found when fruit were evaluated before cold treatment, immediately after cold treatment and a week after cold treatment (Table 3.3.2.8). The weight and diameter of fruit were higher immediately after the cold treatment.

These results indicate that under the FFMS, cold shipping should be carried out for at least 24 days when set at 2°C (estimated pulp temperatures possibly varying between 3°C and 4°C) in order to ensure that there is no interception of live fruit fly larva in fruit at the port of entry.

The results also point out to a new cold disinfestation treatment schedule for fruit fly pests of citrus in southern Africa of at or below 3.5°C for 24 days. The new treatment provides a Probit 8.7 efficacy (based on table in Finney, 1952) at the 95% confidence interval.



**Figure 3.3.2.4.** Mean, maximum and minimum temperature profiles during five replicates of the large scale trial of the cold treatment at a target temperature of 3.5°C. A long term minimum temperature of 3.5°C was targeted in the first replicate. In the four other replicates a long term mean temperature of 3.5°C was targeted.

**Table 3.3.2.5.** Summary of temperatures in the cold room during large scale tests on mortality of third instar larvae of *C. capitata* at 3.5°C for 24 days

Replicate	Fruit loaded into chamber (Date and Time)	Start of treatment (Date and time)	End of treatment (Date and time)	Total Duration (days)	Average of fruit temperatures (°C)
1	05/11/2020 09:10	06/11/2020 11:10	30/11/2020 11:00	24	4.19 ± 0.00
2	28/01/2021 10:00	29/01/2021 08:15	22/02/2021 08:05	24	3.51 ± 0.00

3	25/02/2021 08:48	26/02/2021 08:58	22/03/2021 08:53	24	3.80 ± 0.02
4	08/04/2021 09:06	09/04/2021 08:51	03/05/2021 08:21	24	3.53 ± 0.01
5	26/11/2021 09:40	27/11/2021 08:25	21/12/2021 07:55	24	3.60 ± 0.00

**Table 3.3.2.6.** External and internal characteristics of Late Valencia oranges used in the four replicates of the large-scale tests of the cold treatment at a target temperature of 3.5°C.

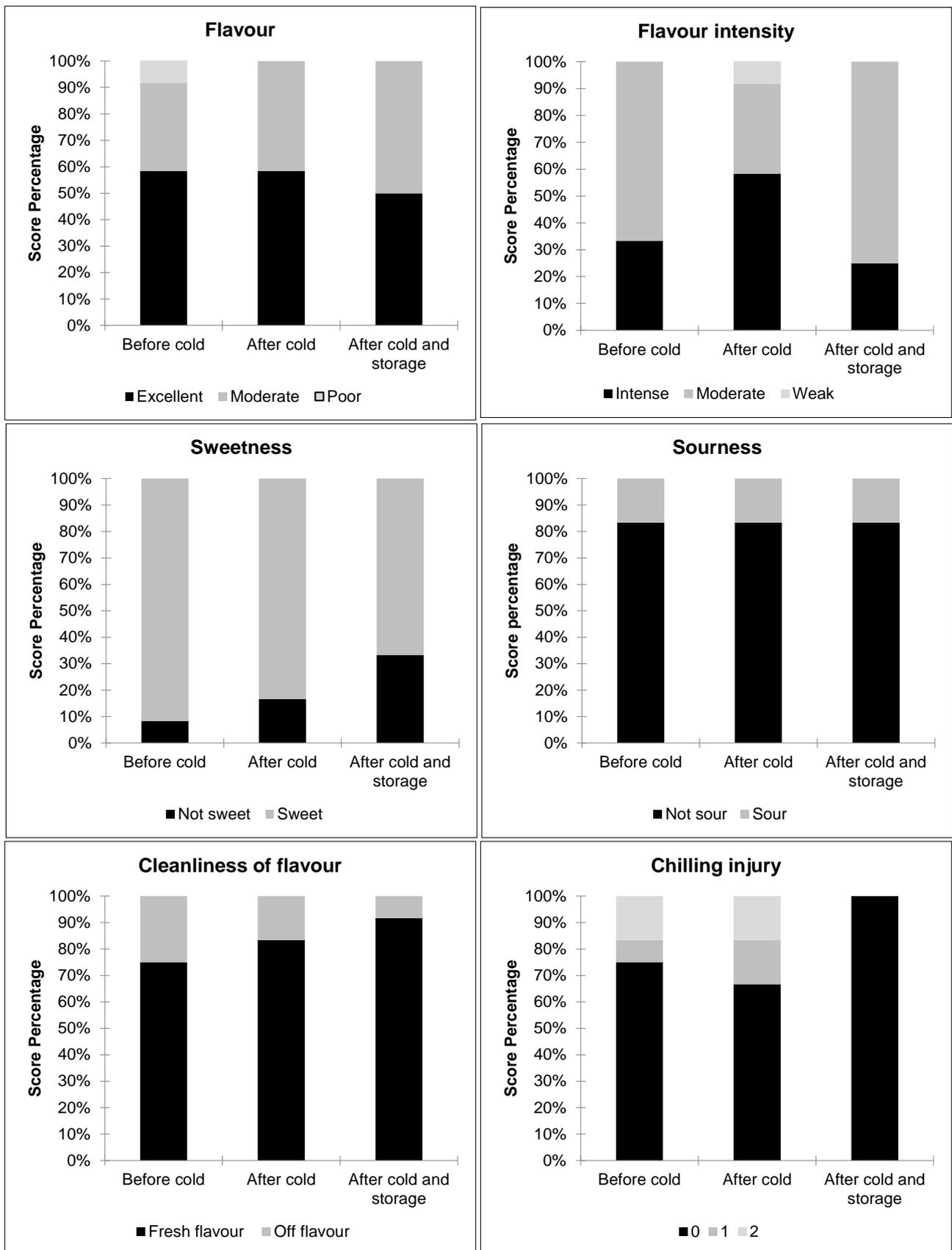
Replicate	Weight/g	Diameter/mm	Acid (%)	Brix °	pH
1	101.67 ± 1.64	58.08 ± 0.43	0.75	13.3	3.25
2	79.64 ± 3.06	53.40 ± 0.78	1.35	15.2	3.38
3	92.27 ± 3.54	57.40 ± 0.41	1.00	13.6	3.59
4	127.03 ± 9.14	63.80 ± 1.63	1.10	11.5	3.56
5	153.67 ± 6.97	66.42 ± 1.35	0.90	13.6	3.53

**Table 3.3.2.7.** Mortality of third instar larvae of *C. capitata* in large scale tests of fruit treated at between 3.5°C and 4°C for 24 days.

Replicate	Date	Control		Treated			Total No. of survivors	Mortality (from estimated treated insects)	True Mortality (95% CI)
		No. of Fruit	No. of live insects	No. of Fruit	Estimated No. of treated insects*				
1	May 11, 2020	375	8440	1124	25297	1	99.996%	99.980%	
2	January 28, 2021	125	3894	500	15576	0	100.000%	99.981%	
3	February 02, 2021	80	1374	300	5153	0	100.000%	99.942%	
4	April 08, 2021	75	2008	225	6024	0	100.000%	99.950%	
5	November 26, 2021	125	5208	500	20832	0	100.00%	99.986%	
Total**					50560			99.994%	

\* Estimated number of treated larvae = Infestation rate (No. of live insects in control/No. of fruit) X No. of treated fruits

\*\* Total calculated from replicates 2-5 which were at a mean temperature of between 3.5°C and 3.8°C over 24 days



**Figure 3.3.2.5.** Score percentages of sensory quality and chilling injury of Late Valencia oranges before cold treatment, immediately after cold treatment at 3.5°C for 24 days and a week after cold treatment and storage at 26°C.

**Table 3.3.2.8.** External characteristics of Late Valencia oranges before cold treatment, immediately after cold treatment at 3.5°C for 24 days and a week after cold treatment and storage at 26°C.

Fruit Characteristics	Before treatment	After treatment	cold	After treatment and storage	cold	ANOVA results (F and statistic and P values)
Weight (g) (mean ± SE)	153.67 ± 6.97	200.75 ± 8.93		168.67 ± 10.65		F <sub>2,32</sub> =8.23, P=0.001
Diameter (mm) (mean ± SE)	66.42 ± 1.35	75.92 ± 1.26		71.56 ± 1.57		F <sub>2,32</sub> =12.96, P<0.0001

F. Determination of duration of treatment at 5°C for the most cold-tolerant fruit fly pest in citrus

In this trial, the durations from start of cooling until at least three probes in the fruit were at ≤ 5°C were 24.75 hours in the first three replicates and 23.83 hours in the last replicate. After the start of the cold treatment, the mean (± SE) fruit temperatures in the cold room were 5.05°C ± 0.00°C, 5.07°C ± 0.00°C, 5.10°C ± 0.00°C and 5.08°C ± 0.00°C for the first, second, third and fourth replicate respectively. In all replicates, exposure to cold was evaluated for up to 27 days. There were no major differences in external and internal characteristics of the fruit used in the four replicates (Table 3.3.2.9). After the cold treatment and before determination of survival from the cold treatment, the mean (± SE) temperatures of the fruit were recorded as 23.78°C ± 0.18°C, 23.08°C ± 0.30°C, 24.20°C ± 0.21°C and 24.64°C ± 0.17°C in the first, second, third replicate and fourth respectively. No survivors of third instar larvae of *C. capitata* were recorded beyond 16 days of cold treatment (Table 3.3.2.10).

**Table 3.3.2.9.** External and internal characteristics of Midnight Valencia oranges used in the four replicates of the exploratory phase of the cold treatment at a target temperature of 5°C.

Replicate	Weight/g	Diameter/mm	Acid (%)	Brix °	pH
1	254.75 ± 10.51	80.50 ± 1.33	0.95	12.5	3.46
2	205.67 ± 7.62	74.75 ± 1.03	1.10	13.1	2.79
3	214.83 ± 7.90	76.00 ± 0.88	1.15	13.4	2.78
4	206.92 ± 9.35	74.25 ± 1.36	0.75	11.4	*na

\* na for not available. Measurement was not taken.

**Table 3.3.2.10.** Mortality of third instar larvae of *C. capitata* in fruit treated at temperature of 5 °C for a range of exposure periods

Replicate	Exposure period	Number of fruit	Estimated number of treated larvae (based on control survivors)	Total number of survivors	Total number of dead larvae	Mortality calculated from survivors (%)
1	4	298	19222	12003	5990	62.44%
	8	300	19351	3614	14750	18.68%
	12	298	19222	984	17424	5.12%
	16	300	19351	56	18825	0.29%
	20	301	19416	0	19316	0.00%
	24	301	19416	0	19022	0.00%
	27	302	19480	0	18658	0.00%
	Control	300		19351	81	
2	4	149	252*	126	2449	49.94%
	8	149	252*	59	2458	23.38%
	12	150	254*	0	2540	0.00%
	16	150	254*	0	2729	0.00%
	20	150	254*	0	2753	0.00%

	24	150	254*	0	2597	0.00%
	27	149	252*	0	2638	0.00%
	Control	150		254	2512	
3	4	276	375*	105	4576	28.02%
	8	275	373*	70	4900	18.75%
	12	300	407*	0	5557	0.00%
	16	276	375*	0	5010	0.00%
	20	275	373*	0	5060	0.00%
	24	275	373*	0	4953	0.00%
	27	278	377*	0	5122	0.00%
	Control	274		352	4265	
4	4	333	9140	6354	1263	69.52%
	8	334	9167	2546	4891	27.77%
	12	333	9140	230	6916	2.52%
	16	336	9222	7	7205	0.08%
	20	333	9140	0	7263	0.00%
	24	333	9140	0	7498	0.00%
	27	332	9113	0	7348	0.00%
	Control	333		9140	38	

\* The estimated number of larvae is not accurate due to high level of natural mortality as shown by the high number of dead larvae in control.

#### G. Efficacy of a 5°C treatment for the most cold-tolerant fruit fly pest in citrus (large scale testing)

Temperature profiles and summaries of the large scale tests are shown in Figure 3.3.2.6 and Table 3.3.2.11.

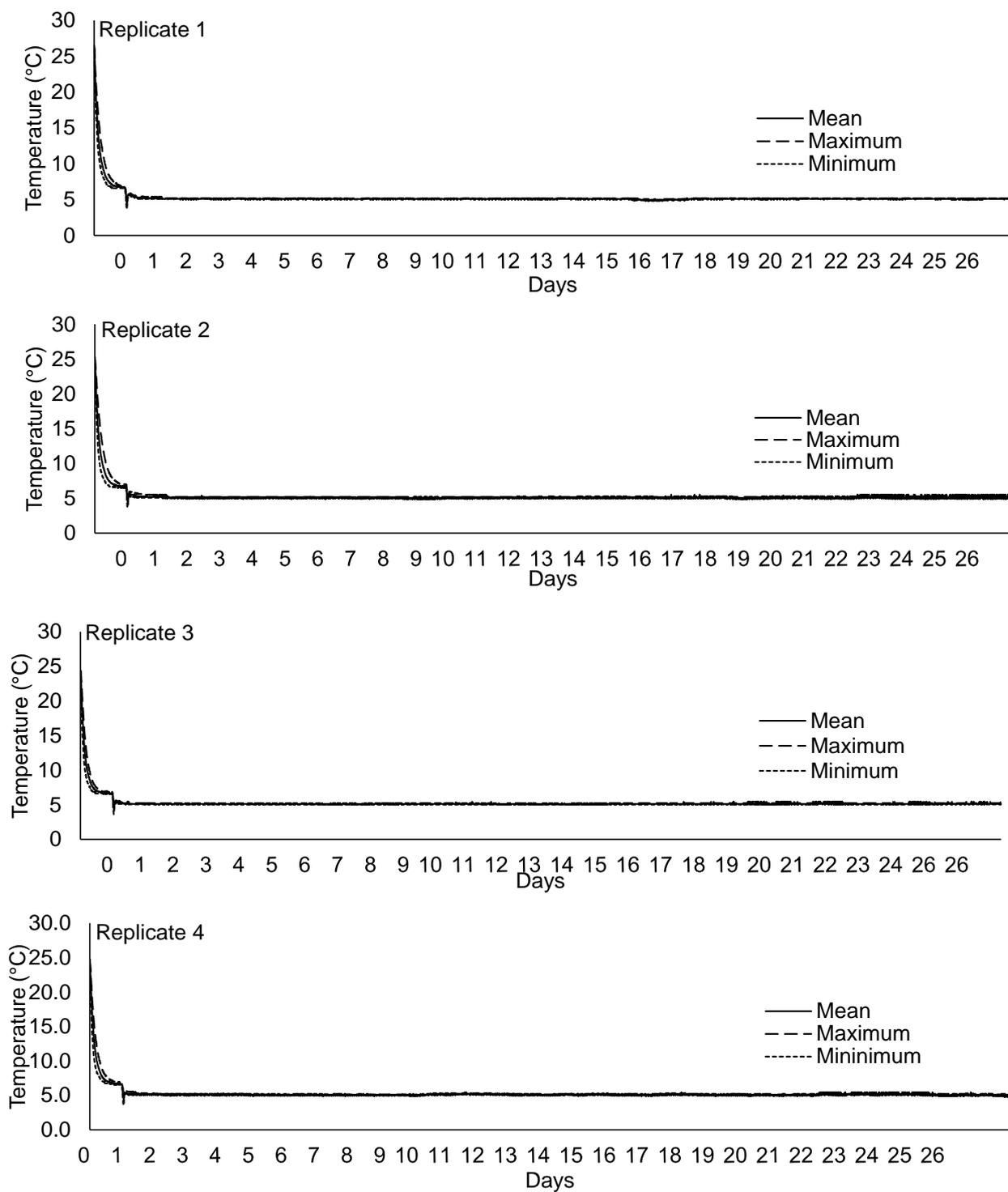
In the four replicates of the large-scale trials at mean temperatures of 5.1°C for 27 days, there were no survivors among a total estimate of 76902 third instar larvae which were treated (Table 3.3.2.12). There was a significant association of the cold treatment with some of the sensory quality parameters of the fruit, more specifically on the flavour and cleanliness of flavour (Fisher's exact test: Flavour:  $p=0.003$ , Cleanliness of flavour:  $p<0.0001$ ) (Fig. 3.3.2.7). These two parameters were significantly poorer after cold treatment and storage for one week at 26°C (Fig. 3.3.2.7). There was a significant association of the cold treatment with chilling injury with an increase in area of the fruit affected (Fisher's exact test:  $p=0.002$ ). The fruit used for cold treatment however had some chilling damage prior to the treatment since they had been stored for at least 5 months at 4°C before the trial. For the other parameters on the sensory quality of the fruit: sweetness, sourness, intensity of flavour, there were no significant associations with the cold treatment (Fisher's exact test:  $p>0.05$  for all parameters).

Differences in fruit weight and fruit diameter were found when fruit were evaluated before cold treatment, immediately after cold treatment and after cold treatment plus storage for a week with significant loss of weight and diameter particularly after cold treatment and storage for a week (Table 3.3.2.12). There was also a change in the acid of the fruit juice with a lower acidity being recorded after cold treatment (Table 3.3.2.12). There were no differences in Brix and pH as a result of the cold treatment.

These results indicate that under the FFMS, cold shipping should be carried out for at least 27 days when set at set points above 2°C (estimated pulp temperatures reaching around 5°C) in order to ensure that there is no interception of live fruit fly larva in fruit at the port of entry.

The results also point out to a new cold disinfestation treatment schedule for fruit fly pests of citrus in southern Africa of at or below 5°C for 27 days. The treatment provides a Probit 8.7 efficacy at the 95% confidence interval.

Given that some damage to external and internal characteristics of the fruit were recorded, albeit on fruit that were stored for at least 5 months before start of trial, it will be important to re-evaluate the effects of the cold treatment on fruit quality in a future trial.



**Figure 3.3.2.6.** Mean, maximum and minimum temperature profiles during five replicates of the large scale trial of the cold treatment at a target temperature of 5°C.

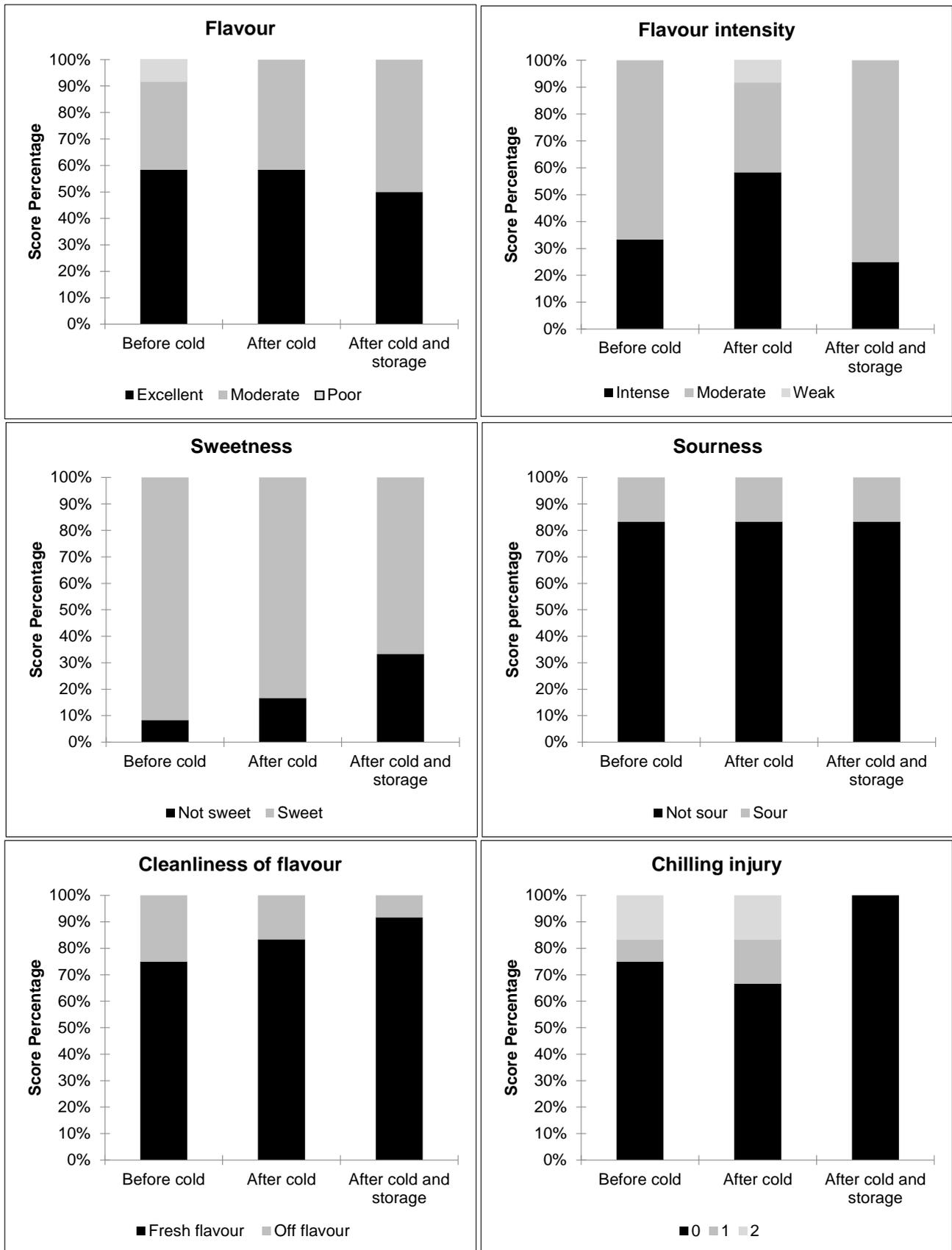
**Table 3.3.2.11.** Summary of temperatures in the cold room during large scale tests on mortality of third instar larvae of *C. capitata* at 5°C for 27 days

Replicate	Fruit loaded into chamber (Date and Time)	Start of treatment (Date and time)	End of treatment (Date and time)	Total Duration (days)	Average of fruit temperatures (°C)
1	17/02/2022 09:05	18/02/2022 08:35	17/03/2022 08:05	27	5.10 ± 0.00
2	24/02/2022 09:09	25/02/2025 08:49	24/03/2022 08:19	27	5.11 ± 0.00
3	24/03/2022 08:58	25/03/2022 08:48	21/04/2022 08:18	27	5.10 ± 0.00
4	31/03/2022 08:37	01/04/2021 08:52	28/04/2022 08:22	27	5.10 ± 0.00

**Table 3.3.2.12.** Mortality of third instar larvae of *C. capitata* in large scale tests of fruit treated at 5.1°C for 27 days.

Replicate	Date	Control		Treated			Total No. of survivors	Observed Mortality	True Mortality (95% CI)
		No. of Fruit	No. of live insects	No. of Fruit	Estimated No. of treated insects*	No. of			
1	February 17, 2022	125	5630	498	22430	0	100.0000%	99.987%	
2	February 24, 2022	125	5513	502	22140	0	100.00%	99.986%	
3	March 24, 2022	126	5200	500	20635	0	100.00%	99.985%	
4	March 31, 2022	126	8448	509	34127	0	100.00%	99.991%	
Total					76902			99.996%	

\* Estimated number of treated larvae = Infestation rate (No. of live insects in control/No. of fruit) X No. of treated fruits



**Figure 3.3.2.7.** Score percentages of sensory quality and chilling injury of Late Valencia oranges before cold treatment, immediately after cold treatment at 5°C for 27 days and a week after cold treatment and storage at 26°C.

**Table 3.3.2.13.** External characteristics of Late Valencia oranges before cold treatment, immediately after cold treatment and a week after cold treatment and storage at 26°C

Mean Characteristics (all replicates)	Fruit	Before treatment	After treatment	cold	After treatment and storage	cold	ANOVA results (F statistic, degrees of freedom (df) and P values)
Weight (g) (mean ± SE)		174.67 ± 4.12	163.19 ± 4.03		149.03 ± 3.08		F <sub>2,130</sub> = 15.45, P<0.0001
Diameter (mm) (mean ± SE)		70.21 ± 0.71	69.76 ± 0.62		67.05 ± 0.74		F <sub>2,130</sub> = 5.90, P=0.004
Acid % (mean ± SE)		0.90 ± 0.08	0.66 ± 0.01		0.61 ± 0.02		F <sub>2,11</sub> = 9.06, P=0.007
Brix ° (mean ± SE)		13.20 ± 0.07	12.95 ± 0.27		13.25 ± 0.17		F <sub>2,11</sub> = 1.15, P=0.36
Arithmetic mean pH		3.61	3.73		3.82		F <sub>2,11</sub> = 3.46, P=0.08

## Conclusion

The most cold tolerant fruit fly pest of citrus in southern Africa is *C. capitata*. Cold disinfestation treatment for *C. capitata* should be equally effective against the two other fruit fly pests: *B. dorsalis* and *C. rosa*. Two new cold treatments: at or below 3.5°C for 24 days and at or below 5°C for 27 days can be proposed for disinfestation of *C. capitata* in citrus. These new cold treatments either on their own or when included in a systems approach should effectively mitigate the risk of fruit fly pests of export citrus produced from South Africa.

## Future research

If these new time temperature treatments found in this study are also effective against FCM, it would be important to conduct further large-scale tests in order to demonstrate efficacy of these treatments at a Probit 9 level. The effects of these longer treatment times at 3.5°C and 5°C on fruit characteristics should be confirmed in future trials using freshly packed fruit.

## Technology transfer

Manrakhan et al. 2019. Integrated management of quarantine insect pests: the case of frugivorous fruit flies, 21<sup>st</sup> ESSA congress, Durban.

Manrakhan A. 2019. Citrus FF-MS and research updates on fruit fly control. 2019 CRI IPM & DM workshops.

Manrakhan et al. 2020. A systems approach for mitigation of fruit fly risk in citrus produced in South Africa. 4<sup>th</sup> TEAM meeting 2020, La Grande Motte, France.

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### 3.3.3 FINAL REPORT: Understanding fruit fly trap efficiency: the role of physical and biotic variables Project 1229 (2019/20 – 2021/22) by Chris Weldon (UP)

#### Summary

A systems approach for quarantine pests is a phytosanitary risk mitigation measure that can be used to achieve quarantine security. At least two independent risk mitigating measures should be implemented in a fruit fly systems approach. These measures can include areas of low fruit fly prevalence, fruit fly control and monitoring measures in the field, inspection, and cold storage. In the citrus industry, risk of fruit fly infestation in the field is measured by trapping. This project set out to establish the role of temperature, relative humidity and fly physiology on the efficiency of fruit fly lures. Two specific objectives were addressed, to (A) evaluate loss of lure formulation mass in relation to temperature and relative humidity under controlled laboratory and semi-field conditions, and (B) determine the effects of weather (temperature and relative humidity) and fly physiology (age, size, nutrient reserves, mating status) on performance of fruit fly attractants under semi-field conditions. Weight loss in attractant dispensers of BioLure, EGOlure and Methyl Eugenol Lure was found to increase with increases in weekly average temperature. Weekly average relative humidity affected only BioLure dispensers, which can gain weight when relative humidity is high and temperatures are low. Fly response to lures was limited during cooler morning conditions. Fly sex and age were more important than diet for determining lure response, but their importance was lure-specific. These results suggest a role for weather in the attractiveness of lures due to increased volatilisation of lure components, with lure concentration known to affect responsiveness of some fruit fly species. This may necessitate that management actions triggered by trap captures be adjusted depending on prevailing environmental conditions.

#### Opsomming

'n Stelselbenadering vir kwarantynplae is 'n fitosanitêre risiko-versagende maatreël wat gebruik kan word om kwarantynsekuriteit te bewerkstellig. Ten minste twee onafhanklike risiko-versagende maatreëls moet in 'n vrugtevliegstelselbenadering geïmplementeer word. Hierdie maatreëls kan gebiede met lae vrugtevliegvoorkoms, vrugtevliegbeheer en moniteringsmaatreëls in die veld, inspeksie en koue-opberging insluit. In die sitrusbedryf word die risiko van vrugtevliegbesmetting in die veld deur lokvalle gemeet. Hierdie projek het ten doel gehad om die rol van temperatuur, relatiewe humiditeit en vlieg fisiologie op die doeltreffendheid van vrugtevlieglokmiddels vas te stel. Twee spesifieke doelwitte is aangespreek, om (A) verlies aan lokmiddelformulasiemassa in verhouding tot temperatuur en relatiewe humiditeit onder gekontroleerde laboratorium- en semi-veldtoestande te evalueer, en (B) die uitwerking van weer (temperatuur en relatiewe humiditeit) en vlieg fisiologie (ouderdom, grootte, voedingstofreserwes, paringstatus) op prestasie van vrugtevlieglokmiddels onder semi-veldtoestande te bepaal. Daar is gevind dat gewigsverlies in lokmiddelvrystellers van BioLure, EGOlure en Metiel-eugenol lokmiddel toeneem met toenames in weeklikse gemiddelde temperatuur. Weeklikse gemiddelde relatiewe humiditeit het slegs BioLure-vrystellers beïnvloed, wat gewig kan optel wanneer relatiewe humiditeit hoog en temperatuur laag is. Vliegreaksie op lokmiddels was tydens koeler oggendtoestande beperk. Vlieggeslag en -ouderdom was belangriker as dieet vir die bepaling van lokmiddelreaksie, maar hul belang was lokmiddel-spesifiek. Hierdie resultate dui op 'n rol vir weer in die aanloklikheid van lokmiddels as gevolg van verhoogde vervlugtiging van lokmiddelkomponente, met lokmiddelkonsentrasie wat bekend is dat dit die reaksie van sommige vrugtevliegspesies beïnvloed. Dit kan noodsaak dat bestuurs-aksies wat deur lokvalvangste veroorsaak word, aangepas word na gelang van heersende omgewingstoestande.

#### Introduction

A complex of African fruit flies (Diptera: Tephritidae) places considerable pressure on the productivity, sustainability, and profitability of citrus farming interests. There is zero tolerance of fruit fly pests in export citrus from South Africa. Whilst this is mostly achieved by pre-harvest field control measures and post-harvest measures such as grading at packing houses, some markets require additional assurances of fruit fly free fruit, which are then met through bilaterally agreed disinfestation treatments. The disinfestation treatments mostly used for exported citrus are cold treatments at temperatures at 1°C or below for 16 to 22 days [1, 2]. However,

not all citrus cultivars can be exported through the existing cold treatment schedules due to detrimental effects on the quality of some citrus cultivars.

A systems approach for quarantine pests is a phytosanitary risk mitigation measure that can be used to achieve quarantine security [3, 4]. A systems approach integrates different measures and factors along the production chain, which cumulatively reduce infestation risk [3]. Recently, a systems approach was developed for another quarantine pest of citrus in South Africa, the false codling moth, *Thaumatotibia leucotreta* (FCM), and was found to be a suitable alternative to a stand-alone cold treatment [2]. The FCM systems approach includes pre harvest control and monitoring, postharvest fruit inspections, packing house grading and official inspection of a 2% fruit sample per pallet of fruit. On fruit flies, an international standard on systems approaches for risk management of fruit fly pests (ISPM 35) was also recently developed and provides a guideline for development, implementation and verification of integrated measures. At least two independent risk mitigating measures should be implemented in a fruit fly systems approach. These measures can include areas of low fruit fly prevalence, fruit fly control and monitoring measures in the field, inspection and cold storage.

In the citrus industry, risk of fruit fly infestation in the field is measured by trapping. Threshold levels have been established for certain trapping systems and form the basis for determination of fruit fly infestation risk. However, trapping levels depend not only on insect abundance but also on environmental conditions and the responsiveness of the pest to the attractant used [5]. Pest responsiveness may relate to sexual maturity, daily rhythms, feeding status and nutrient reserves [6]. There are numerous attractants available for trapping pest fruit flies, but it is often noted that trap captures are patchily distributed [7] with only a theoretical understanding of why this occurs. Developing an understanding of how the physical environment and fly biology influences trap efficiency will improve estimation of population density from trap catch. In turn, it will help determine a range of threshold values rather than single values for trapping systems in line with prevailing conditions (temperature, relative humidity, fruiting period which in turns influences composition of fruit fly populations). It will also inform the use of attractant-based suppression techniques such as male or bait-based annihilation technique (MAT and BAT, respectively), which rely on attraction of target flies to a lure (e.g., methyl eugenol for male *Bactrocera dorsalis*, protein odour for *Ceratitis* species) that is mixed with an insecticide. Further, if sterile insect technique (SIT) programmes are deployed to target pest fruit flies, knowledge of the variables that limit attraction to lures can be used to apply pre-release treatments that limit response by sterile males to MAT or BAT dispensers. This would permit the simultaneous application of MAT and/or BAT with SIT [8].

For both female and male *Bactrocera dorsalis*, we recently found that trap captures increased significantly as average minimum temperature increased. Based on the results of further laboratory experiments, it is clear that voluntary flight by *B. dorsalis* is limited by temperature, with a lower threshold of approximately 20°C. At temperatures lower than this, flies are still capable of movement within a tree canopy, but will be less likely to respond to traps. In addition, at temperatures favourable for *B. dorsalis* flight, higher temperatures may be associated with higher release rates of lures and greater fly attraction, as reported recently for trimedlure and the Mediterranean fruit fly [9]. We also found that rainfall affected female recaptures in BioLure traps and male recaptures in methyl eugenol traps differently. This raises the possibility that associated changes in humidity also affect the efficiency of each type of trap, perhaps by influencing the carrying capacity of air for volatilised lure components. Considering that other research funded by CRI [10] has found a correlation between fruit infestation and relative abundance of adult male *B. dorsalis* caught in methyl eugenol traps, improved understanding of this relationship represents an important component in calculating the risk of fruit infestation by fruit flies.

### **Stated objectives**

We set out to establish the role of temperature, relative humidity and fly physiology on the efficiency of fruit fly lures. More specifically, it sought to:

- A. Evaluate loss of lure formulation mass in relation to temperature and relative humidity under controlled laboratory and semi-field conditions
- B. Determine the effects of weather (temperature and relative humidity) and fly physiology (age, size, nutrient reserves, mating status) on performance of fruit fly attractants under semi-field conditions

## Materials and methods

### **Objective A: Loss of lure formulation mass in relation to temperature and relative humidity**

Commercial formulations of BioLure Fruit Fly and methyl eugenol (from Chempac), enhanced ginger oil (EGO) lure (from Insect Science) and Capilure capsule (from River Bioscience) were obtained from suppliers and stored until use in a refrigerator (~6°C) or freezer (for Capilure; ~-20°C). We then planned to estimate the release rate of each lure at four constant temperatures and three levels of relative humidity.

The initial weight of each freshly unpacked lure formulation was to be determined (n = 48, resulting in n = 4 for each combination of temperature and relative humidity). Thereafter, the lures were to be placed in climate rooms with controlled temperature and relative humidity in the University of Pretoria Insect Rearing and Research Facility. The conditions in the climate rooms were to be set at 18, 22, 26 or 30°C, and relative humidity within ranges of 10-30, 40-60 or 70-90%. Temperature and relative humidity were to be verified with temperature and relative humidity data loggers. The individual weight of each lure formulation was to be recorded every day over a period of five days. Linear mixed effects regression models were to be used to establish the daily rate of lure formulation weight loss at each combination of time, temperature and relative humidity, with lure dispenser identity included as a random effect (for repeated measures). We planned to use thin plate splines to present the three-dimensional relation between temperature, relative humidity and daily rate of formulation weight loss.

In addition, each lure type was weighed and placed in a separate yellow bucket trap suspended in potted citrus trees within a lemon orchard. One of each lure type was located at the northern edge, centre and southern edge of the lemon orchard. The field cages are located on the University of Pretoria Innovation Africa campus. Temperature and relative humidity at each location within the lemon orchard was recorded using temperature and relative humidity data loggers placed within Stevenson screens hung in the foliage. At daily intervals over a period of 30 days, weight of each lure was recorded to determine weight loss in relation to daily mean temperature and relative humidity. The experiment was replicated three to five times to encompass differences in temperature and natural relative humidity. Weekly weight loss from lures was related to weekly average temperature and relative humidity using linear mixed effects models. The random effects of lure dispenser identity and 'month' were included in the model. Three-dimensional plots were created to display the contribution of temperature and relative humidity to lure weight loss.

### **Objective B: Interactions between time of day and physiology on fruit fly responsiveness to lures**

The responsiveness of some fruit fly species is known to change throughout their adult life. This has been demonstrated in the Queensland fruit fly, *Bactrocera tryoni*, where the response of females and males to cue-lure varied according to age but also the diet they had been fed [9]. The same study also demonstrated that responsiveness varied across the course of an individual day, which may have been associated with patterns of resource assimilation to improve sexual performance. Similarly, recent results from CRI project 1075 on the dispersal of *B. dorsalis* indicate that rainfall affected female recaptures in BioLure traps and male recaptures in methyl eugenol traps differently. This raises the possibility that associated changes in humidity affect the efficiency of each type of trap, perhaps by influencing the rate of volatilisation of the lure components.

This study was to be conducted in shade houses on the University of Pretoria Innovation Africa campus with potted plants as a habitat for flies to rest and feed during the day. The species that we planned to test were *B. dorsalis*, *C. capitata*, and *C. quilicii*. We did not plan to test *C. rosa* due to its absence in Pretoria. Lures that were to be tested for *B. dorsalis* were Biolure and methyl eugenol. Lures that were to be tested for *C. capitata* and *C. quilicii* were BioLure, Capilure and EGOLure. We planned to place one lure in a bucket-type trap containing an insecticide block and placed in foliage in each cage. Another unbaited trap with insecticide was to also be placed in each cage. A control trap is important when recapturing flies within a restricted space to account for random entry of flies into traps.

For each species and lure combination, pupae were to be divided into two batches and dyed with two contrasting fluorescent pigment colours. On emergence, we would then sort flies by sex into 48 small cages, each containing 30 flies dyed the same colour. Cages would then be furnished with sugar, or sugar and hydrolysed yeast, plus a source of water. Fly responses to each lure were to be determined in relation to their

sex, diet, and age. At ages 2, 10, and 20 days after adult emergence, 25 females and males of each diet treatment would then be released at four times during the day (6:30, 10:00, 13:30 and 17:00) and left for 90 minutes to move around the field cage and respond to the lure. After each test period, traps were to be emptied and unresponsive flies were to be caught with an aspirator. Flies would then be placed on ice before being transferred to a freezer at -80°C. During each 90-minute period, minimum, maximum and average temperature and relative humidity were to be recorded within the shade house with a thermochron iButton, and light intensity on the base of the trap at the beginning and end of each three-hour period would also be recorded. The response of each species to the lure relative to the control trap was to be calculated for each sex, diet, age and time of day combination. For each species and lure combination, this procedure was to be repeated four times.

At a later stage, a subsample of five responsive and unresponsive flies of each treatment combination were to be weighed to determine their body mass. We then planned to freeze-dry them, weigh them again to determine their dry mass, and then assay their individual carbohydrate, lipid and protein content to establish the role of nutrient stores on responsiveness to lures.

Analysis of the relative response to lures in relation to species, sex, diet, time of day, and environmental variables (or principal components if they are autocorrelated) was to be performed using general linear models. General linear models would also be used to analyse nutrient reserves, with responsiveness, species, sex, diet, and time of day as categorical independent variables, and body mass as a covariate.

## Results and discussion

### **Objective A: Loss of lure formulation mass in relation to temperature and relative humidity**

The determination of lure formulation weight under controlled laboratory conditions could not be completed. We had persistent problems with climate rooms in the new Insect Rearing and Research Facility at the University of Pretoria. Temperatures are stable but we have had and continue to experience problems with maintaining required levels of relative humidity. A different type of humidifier was ordered from overseas by the University of Pretoria and one was only installed in April 2022. If the new humidifier performs well at controlling relative humidity in the climate room, further units will be purchased to equip the climate rooms.

Despite the problems that we experienced in the laboratory, we obtained very informative results on loss of lure formation weight in the field. From the end of June 2020 we deployed BioLure, EGOlure, Methyl Eugenol Lure and Capilure in a lemon orchard on the University of Pretoria Innovation Africa campus. The environmental conditions experienced during deployment of lures are summarised in Table 3.3.3.1.

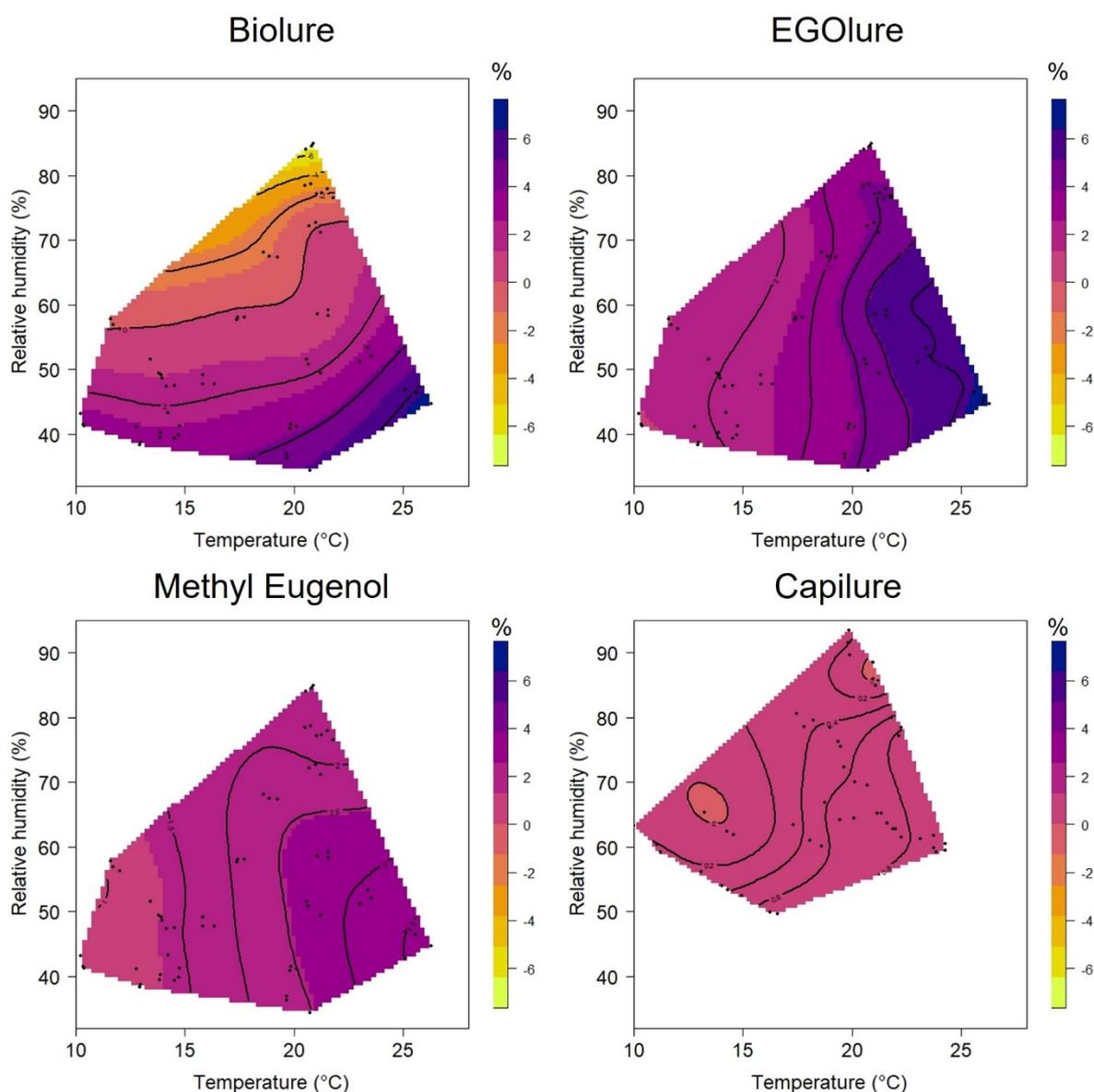
**Table 3.3.3.1.** Average weekly and maximum temperatures recorded in a lemon orchard during assessment of lure formulation loss in the field for a duration of 30 days. The lemon orchard was located on the University of Pretoria Innovation Africa campus. BioLure, EGOlure and Methyl Eugenol Lure were tested during the five periods in 2020. Capilure was tested in the four periods in 2021.

Environmental conditions	2020					2021			
	25/06	29/07	01/09	06/10	19/11	15/01	16/02	23/03	10/05
Average ( $\pm$ s.e.) weekly temperature (°C)	11.75 (0.16)	14.49 (0.15)	19.13 (0.14)	21.89 (0.15)	20.95 (0.10)	21.80 (0.09)	21.42 (0.12)	18.69 (0.13)	13.52 (0.15)
Maximum temperature (°C)	31.09	41.57	32.65	45.11	35.15	41.57	37.64	39.14	38.14
Minimum temperature (°C)	-3.48	0.59	6.12	10.08	12.08	14.08	11.64	6.56	0.04
Average ( $\pm$ s.e.) weekly relative humidity (%)	48.92 (0.52)	45.48 (0.49)	48.73 (0.52)	58.30 (0.54)	77.89 (0.42)	79.22 (0.45)	66.49 (0.49)	69.82 (0.51)	57.85 (0.50)
Maximum relative humidity (%)	100	100	100	100	100	100	100	100	99.22

Minimum relative humidity (%)	3.82	4.03	5.54	11.46	24.58	9.89	11.55	11.84	9.17
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When tested in the field, temperature significantly affected weight loss from BioLure, EGOlure, Methyl Eugenol Lure and Capilure (Figure 3.3.3.1; yellow to purple shading). In each case, weekly weight loss increased with temperature, with the effect being strongest in EGOlure, with approximately 1% more lure formulation weight lost per week for each 1°C increase in temperature (log-transformed data, linear mixed effects: coefficient = 0.016;  $\chi^2 = 17.737$ , df = 1,  $P < 0.001$ ). This was followed by Capilure (coefficient = 0.004,  $\chi^2 = 15.04$ ,  $p < 0.001$ ), BioLure (coefficient = 0.295;  $\chi^2 = 11.718$ , df = 1,  $P < 0.001$ ) and Methyl Eugenol Lure (coefficient = 0.007;  $\chi^2 = 8.245$ , df = 1,  $P = 0.004$ ). Capilure lost a small amount of weight as relative humidity decreased (coefficient = - 0.001,  $\chi^2 = 8.74$ ,  $p = 0.003$ ). In addition, relative humidity interacted significantly with temperature to affect BioLure weight loss (coefficient = -0.006;  $\chi^2 = 9.247$ , df = 1,  $P = 0.002$ ); at high weekly average relative humidity and low weekly average temperature BioLure gained weight. Weekly average relative humidity did not affect weight loss from EGO lure or Methyl Eugenol Lure.

These results suggest a role for weather in the attractiveness of lure dispensers due to increased volatilisation of lure components, in addition to higher levels of fly activity at higher temperatures that have been observed by us. Recent results show that the attractiveness of methyl eugenol to *B. dorsalis* is higher when the concentration of the attractant in the lure preparation is increased [11], so more lure volatiles resulting from high rates of lure loss or a stronger source can lead to higher rates of trapping. This is also likely to be the case with other species and lures. For example, EGOlure was shown to be equally or more effective for trapping *Ceratitis* species [12], and this may relate to the relatively high rates of lure formulation weight loss that we found even at cooler temperatures. However, BioLure dispensers lost little or even gained weight (Figure 3.3.3.1; turquoise to blue shading) as relative humidity increased. This is likely due to BioLure dispensers being hygroscopic. For Capilure (which also has an hygroscopic structure), results suggest that, although both temperature and relative humidity significantly affect the lure, the weight variation remains minor and in a narrower range than in BioLure (Figure 3.3.3.1).



**Figure 3.3.3.1.** Relation between weekly average temperature and relative humidity on weekly weight loss from commercially available lure formulations: BioLure Fruit Fly (from Chempac), EGOlure (from Insect Science), Methyl Eugenol Lure (from Chempac), and Capilure (from River Bioscience). The same lure formulation was measured repeatedly at daily intervals for 30 days when placed in a lemon orchard on the Innovation Africa campus of the University of Pretoria. For BioLure, EGOlure and Methyl Eugenol Lure, three lure formulations were tested in five 30-day periods commencing on the five dates in 2020 presented in Table 3.3.3.1 ( $n = 15$  lure formulations). For Capilure, four lure formulations were tested in three 30-day periods commencing on the four dates in 2021 presented in Table 3.3.3.1 ( $n = 12$  lure formulations).

**Objective B: Interactions between time of day and physiology on fruit fly responsiveness to lures**

The determination of the effect of time of day and physiology on fruit fly responsiveness to lures is yet to be completed. We have experienced problems that slowed our progress. Primary among these include ant attacks on cultures in the fruit fly climate rooms at the University of Pretoria, heavy rain on days when tests had been planned, and high levels of egg infertility in the fruit fly cultures. A decision was also made to replace *Ceratitidis quilicii* with *C. cosyra* for the tests because it proved difficult to establish a sustainable culture of the former. To date, we have collected the replicates summarised in Table 3.3.3.1. The remaining replicates will be completed in the next spring, summer and autumn season, starting in August 2022. Data for the weather variables collected during the semi-field trials are being analysed and will be included with the completion of all remaining replicates. Thankfully, we have a very competent and hard-working MSc (Entomology) student, Tania Pogue, who is funded by Citrus Academy and is making good progress to complete this objective.

When tested in semi-field conditions, the lure response of *C. capitata* differed between males and females for both BioLure and EGOlure (Figure 3.3.3.1 and Figure 3.3.3.2). In each case a significantly higher proportion of *C. capitata* males responded to the lures with this effect being the strongest with EGOlure (coefficient = 1.414;  $\chi^2 = 25.6$ , df = 98,  $P < 0.001$ ). This was followed by BioLure (coefficient = 0.064;  $\chi^2 = 14.442$ , df = 56,  $P < 0.001$ ). Sex was the only variable that significantly affected lure response for *C. capitata*, with no significant differences shown between diet or fly age. The lure response of *B. dorsalis* under semi-field conditions differed by sex and age (Figure 3.3.3.3 and Figure 3.3.3.4). A significantly higher proportion of *B. dorsalis* females responded to Biolure than males (coefficient = 0.107;  $\chi^2 = 8.042$ , df = 83,  $P = 0.018$ ). Conversely, only *B. dorsalis* males responded to methyl eugenol (coefficient = 0.890;  $\chi^2 = 14.442$ , df = 56,  $P < 0.001$ ). Fly age significantly affected lure response of *B. dorsalis* for both Biolure and methyl eugenol (Figure 3.3.3.3 and Figure 3.3.3.4), with immature flies responding to lures less than mature flies. Overall, two-day old flies had a significantly decreased response to Biolure (coefficient = 0.052;  $\chi^2 = 3.604$ , df = 83,  $P = 0.047$ ) and methyl eugenol (coefficient = 0.064;  $\chi^2 = 5.623$ , df = 56,  $P = 0.018$ ) when compared to ten-day old and twenty-day old flies. There was however no significant difference in lure response between ten-day old and twenty-day old flies.

These results suggest that fly physiology plays a role in the attractiveness of lures under semi-field conditions. Additionally, decreased trap captures have been observed during early morning release periods. This aligns with previous results that report that lure volatilisation and fly activity is decreased at lower temperatures. Biolure was shown to be more effective for trapping female *B. dorsalis*, which supports findings from previous studies [13]. However, we found that for *C. capitata* more males responded to Biolure, despite this being an effective food-based attractant for females. Temperature and the presence of ripening host fruit can significantly affect female selectivity in mass trapping systems [14]. Temperature differences and the absence of ripening fruit in the semi-field conditions may explain why proportionately more males were trapped by Biolure. The greater proportion of male trap captures for EGOlure and methyl eugenol lures aligns with results from similar studies [13, 15]. Differential age response to lures for male attractants are closely linked to sexual maturity and mating success of the fly [16, 17]. This relates to the lower number of immature *B. dorsalis* trapped with methyl eugenol. However, age did not significantly influence trap capture for male attractants for *C. capitata*. This could likely be due to *C. capitata* maturing at earlier ages - in comparison to *B. dorsalis* - and the difference between tested age groups being too similar to detect subtle age-related differences in lure response.

**Table 3.3.3.1.** Number of replicates completed for each combination of species, lure, nutritional status and age for field cage experiments on lure response.

Species	Lure	Diet	Age (days post emergence)	Replicates completed	Minimum remaining replicates
<i>B. dorsalis</i>	Biolure	SU	2	2	2
			10	3	1
			20	3	1
		SU+YH	2	2	2
			10	3	1
			20	3	1
	Methyl eugenol	SU	2	2	2
			10	3	1
			20	3	1
		SU+YH	2	2	2
			10	3	1
			20	3	1
<i>C. capitata</i>	Biolure	SU	2	3	1

			10	4	0
			20	3	1
		SU+YH	2	3	1
			10	4	0
			20	3	1
	EGO Pherolure	SU	2	3	1
			10	4	0
			20	3	1
		SU+YH	2	3	1
			10	4	0
			20	3	1
	Trimedlure	SU	2	1	3
			10	1	3
			20	1	3
		SU+YH	2	1	3
			10	1	3
			20	1	3
<i>C. cosyra</i>	Biolure	SU	2	0	4
			10	1	3
			20	1	3
		SU+YH	2	0	4
			10	1	3
			20	1	3
	EGO Pherolure	SU	2	0	4
			10	1	3
			20	1	3
		SU+YH	2	0	4
			10	1	3
			20	1	3

## Conclusion

Our results paint a clearer picture regarding the sensitivity of various attractants for surveillance, and even control, of several fruit fly pests. Cooler morning temperatures are associated with lower responsiveness of lures. Coupled with the earlier observation that temperatures below 20°C are associated with lower *B. dorsalis* flight in the laboratory, and our findings here that lure formulation loss, while dependent on each specific lure, also drops to low levels below the same temperature clearly indicates a more nuanced approach to the interpretation of fruit fly captures in surveillance traps. Cooler temperatures do not necessarily kill fruit flies but rather suppress flight and reproduction, and this can mean that they can survive for longer [18]. This may call for management actions triggered by trap captures, *B. dorsalis* eradication programmes, be adjusted depending on prevailing weather conditions. The delay in response by *B. dorsalis* to methyl eugenol also permits flies to move away from where they emerged before being attracted to traps and being removed from the population.

## Future research

Besides completion of our data collection, the empirical results obtained in this project need to be extrapolated using mathematical models to provide the tools to account for weather conditions for interpretation of trap captures. For application of these results, easy-to-use online calculators could be developed.

## Technology transfer

### Oral presentation

Pogue T, Malod K, and Weldon CW (2022). Interactions between time of day and physiology on fruit fly responsiveness to lures. Thursday Morning Seminar. 24 April 2022. Forestry and Agriculture Biotechnology Institute, University of Pretoria.

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### 3.3.4 **PROGRESS REPORT: The impact of interruptions on Medfly cold treatment efficacy** Project 1204 (2018/9 – 2023/4) by T G Grout, P R Stephen, K C Stoltz and V Hattingh (CRI)

#### **Summary**

Interruptions of cold treatments can occur for various reasons but apart from the recommendation by APHIS for the cold treatment for false codling moth there are few specific mitigation protocols in the event of one happening. This research is using *Ceratitis capitata* as a test subject to quantify the effect of different interruptions on the mortality of the second instar in Valencia oranges packed in export cartons. A cold treatment of 6 days at an internal pulp temperature of 0.9°C is being used to guarantee some survival of larvae. A single interruption after either 2 days of cold or 4 days of cold where the pulp temperature increased to 2°C above the upper threshold of 1.1°C did not cause consistent differences in mortality from the treatment with no interruption in four replicates conducted. Trials with double interruptions of either 2°C above the threshold or 4°C above the threshold were also similar to the treatment with no interruptions. The mean corrected mortalities of three replicates were 94.25% for two interruptions of +2°C, 95.31% for two interruptions of +4°C and 95.49% for no interruptions. When two cold rooms became available, one cold room was used for a cold control at 0.9°C for 6 days and the other cold room had an interruption where the pulp temperature was left to increase to 5.1°C before the chiller unit was restarted. Five replicates were conducted using a total of more than 34 500 second instar Medfly in each treatment. Mean corrected mortalities for each treatment did not differ significantly ( $P=0.869$ ) and logistic regression also showed little difference between treatments. One more replicate will be conducted before a decision on possible further treatments is made.

#### **Opsomming**

Onderbrekings van koue-behandelings kan om verskeie redes voorkom, maar afgesien van die aanbeveling deur APHIS vir die koue-behandeling vir valskodlingmot, is daar min spesifieke versagtingsprotokolle in die geval van een. Hierdie navorsing gebruik *Ceratitis capitata* as 'n toets-onderwerp om die effek van verskillende onderbrekings op die mortaliteit van die tweede instar in Valencia-lemoene, wat in uitvoerkartonne verpak is, te kwantifiseer. 'n Koue-behandeling van 6 dae by 'n interne pulptemperatuur van 0.9°C word gebruik om 'n mate van oorlewing van larwes te verseker. 'n Enkele onderbreking na óf 2 dae van koue óf 4 dae van koue, waar die pulptemperatuur tot 2°C bokant die boonste drempelwaarde van 1.1°C toegeneem het, het nie konsekwente verskille in mortaliteit veroorsaak in vergelyking met die behandeling met geen onderbreking, in vier herhalings wat uitgevoer is, nie. Proewe met dubbele onderbrekings van óf 2°C bo die drempelwaarde óf 4°C bo die drempelwaarde, was ook soortgelyk aan die behandeling sonder onderbrekings. Die gemiddelde gekorrigeerde mortaliteite van drie herhalings was 94.25% vir twee onderbrekings van +2°C, 95.31% vir twee onderbrekings van +4°C en 95.49% vir geen onderbrekings. Wanneer twee koelkamers beskikbaar geword het, is een koelkamer vir 'n koue-beheer by 0.9°C vir 6 dae gebruik, en die ander koelkamer het 'n onderbreking gehad waar die pulptemperatuur gelaat is om tot 5.1°C te verhoog voordat die verkoelingseenheid weer aangeskakel is. Vyf herhalings is uitgevoer deur 'n totaal van meer as 34 500 tweede instar Medvlieg in elke behandeling te gebruik. Gemiddelde gekorrigeerde mortaliteite vir elke behandeling het nie betekenisvol verskil nie ( $P=0.869$ ) en logistiese regressie het ook min verskil tussen behandelings getoon. Nog een herhaling sal uitgevoer word voordat 'n besluit oor moontlike verdere behandelings geneem word.

### 3.3.5 **PROGRESS REPORT: Alternate hosts of the Oriental Fruit Fly, *Bactrocera dorsalis*, in the Sundays River Valley**

Project 1323 (April 2021 – April 2023) by Emiel van Son, Prof. Julie Coetzee (CBC), Wayne Kirkman and Aruna Manrakhan (CRI)

#### **Summary**

In 2020 one *Bactrocera dorsalis* individual was detected in the Sundays River Valley in a trap 5km from any cultivated host, leading to speculation that *Bactrocera dorsalis* had established a low-level population on indigenous thicket species. Twenty-four Methyl Eugenol and 24 Biolure traps were set up in citrus orchards as well as in adjacent native thicket to monitor populations of fruit fly species in the region. In May and June of 2021, a total of 3 individual *B. dorsalis* were detected in the traps. Since June 2021, no other individuals have

been caught, presumably due to the declaration of establishment of *B. dorsalis* in the SRV and the accompanying management regulations. Monthly surveys of the traps continue as well as ongoing emergence experiments of indigenous fruit collected from around trap sites. Catches of Mediterranean fruit fly, *Ceratitidis Capitata*, are highest around the cultivated host during fruit season. Ten thicket species have been investigated for suitability as alternate host through the emergence experiments, based on fruit phenology. *Lycium ferocissimum*, *Azima tetracantha*, and *Capparis sepiaria* have shown to host *C. capitata*, with infestation rates ranging from 33.8 flies/kg fruit to 114.1 flies/kg fruit. Twenty-seven individuals of a *Neoceratitis* species, an indigenous Tephritid, have been reared from *Lycium ferocissimum*. Two previously confirmed hosts of Oriental Fruit fly, *Capparis sepiaria* and *Opuntia ficus-indica*, have to date not produced any *B. dorsalis*. Monthly trap monitoring and fruit collection will continue to April 2023.

## Opsomming

In 2020 is 'n *Bactrocera dorsalis* vlieg in die Sondagsriviervallei in 'n lokval gevind, 5 km weg van enige veroude gasheer. Dit het tot die gedagte gelei dat 'n klein *B. dorsalis* populasie in die inheemse bos gevestig het. Vier-en-twintig Methyl Eugenol en Biolure lokvalle elk, is in die sitrusboorde sowel as die aangrensende bos, uitgehang, om die populasievlakke van vrugtevlieë in die streek te monitor. Drie *B. dorsalis* vlieë is in Mei en Junie 2021 gevang. Sedert Junie 2021, is geen vlieë gevang nie, moontlik as gevolg van die feit dat dit verklaar is dat *B. dorsalis* in die SRV gevestig het en die gepaartgaande beheermaatreëls. Maandelikse opnames van die lokvalle, sowel as die evaluasie van inheemse vrugte wat in die bos rondom die lokvalle versamel is, gaan voort. Vangste van Mediterseense vrugtevlieg, *Ceratitidis capitata*, is die hoogste in die veroude gasheer tydens die vrugseisoen. Tien bos spesies is vir geskiktheid as alternatiewe gasheer, deur uitbroeiings-eksperimente, ondersoek. Dit is op vrug fenologie gebaseer. *Lycium ferocissimum*, *Azima tetracantha*, en *Capparis sepiaria* het getoon om gasheer vir *C. capitata* te wees, met infestasietyempo's van 33.8 tot 114.1 vlieë/kg vrugte. Sewe-en-twintig individue van 'n *Neoceratitis* spesie, 'n inheemse Tephritid, is van *Lycium ferocissimum* uitgebroei. *Capparis sepiaria* en *Opuntia ficus-indica*, wat beide voorheen as gasheer vir *B. dorsalis* bevestig is, het geen *B. dorsalis* vlieë opgelewer nie. Maandelikse lokval monitoring en versameling van vrugte sal tot April 2023 voortgaan.

### 3.3.6 PROGRESS REPORT: Fruit fly rearing

Project 407 (April 1999 – March 2023) by Aruna Manrakhan, John-Henry Daneel, Glorious Shongwe and Rooikie Beck (CRI)

## Summary

Colonies of five fruit fly species: *Ceratitidis capitata*, *Ceratitidis rosa*, *Ceratitidis quilicii*, *Ceratitidis cosyra* and *Bactrocera dorsalis* continue to be maintained at Citrus Research International (CRI), Nelspruit. Fruit flies from these colonies were used in CRI Projects 1171, 1245, 1254, 1299 and 1320. Fruit fly larval diet and fruit fly materials were supplied to University of Pretoria, Stellenbosch University, Agribiotech Research Consultancies and Nemlab for research on biology and control. Volumes of pupae produced per week for all species reared including *C. quilicii* were above 100 ml (between 3000 and 5000 pupae). For all colonies except *C. quilicii*, percentages of egg hatch and adult emergence were on average above 90 and 60 respectively. The quality of the *C. quilicii* colony was not monitored due to few flies available until September 2021. All colonies were refreshed by using flies reared from fruit. Between December 2021 and March 2022, new *C. rosa*, *C. cosyra* and *B. dorsalis* colonies were founded from a total of 921, 1028 and 749 flies respectively. For *C. rosa*, most of the flies were reared from *Syzygium jambos* collected from Nelspruit. For *C. cosyra*, most of the flies were reared from *Sclerocarya birrea* collected from Hoedspruit. Most of the wild *B. dorsalis* flies were reared from *Mangifera indica* collected in Nelspruit. In February 2022, a new *C. capitata* colony was founded, initially from flies reared from *Syzygium jambos* collected from Stellenbosch. The new *C. capitata* colony was further augmented using wild flies reared from *Coffea arabica* collected from Burgershall after March 2022. The *C. quilicii* cultures were continuously refreshed throughout the year from April 2021 to March 2022. A total of 7166 wild *C. quilicii* were added to the colony and these were mainly reared from *Acca sellowiana* collected from Ermelo.

## Opsomming

Kolonies van vyf vrugtevliespesies: *Ceratitis capitata*, *Ceratitis rosa*, *Ceratitis quilicii*, *Ceratitis cosyra* en *Bactrocera dorsalis* word steeds by Citrus Research International (CRI), Nelspruit, onderhou. Vrugtevlies van hierdie kolonies is in CRI-projekte 1171, 1245, 1254, 1299 en 1320 gebruik. Vrugtevliesglarwe-dieet en vrugtevliesmateriaal is aan die Universiteit van Pretoria, Universiteit van Stellenbosch, Agribiotech Research Consultancies en Nemlab vir navorsing oor biologie en beheer verskaf. Volumes van papier wat per week vir alle spesies wat geteel word, geproduseer is, insluitend *C. quilicii*, was meer as 100 ml (tussen 3000 en 5000 papier). Vir alle kolonies behalwe *C. quilicii*, was persentasies eiers wat uitgebroei het en die volwasse opkoms gemiddeld bó 90 en 60 onderskeidelik. Die kwaliteit van die *C. quilicii*-kolonie is nie gemonitor nie weens min vlieë beskikbaar tot September 2021. Alle kolonies is verfris deur vlieë te gebruik wat van vrugte geteel is. Tussen Desember 2021 en Maart 2022 is nuwe *C. rosa*-, *C. cosyra*- en *B. dorsalis*-kolonies uit 'n totaal van onderskeidelik 921, 1028 en 749 vlieë, gestig. Vir *C. rosa* is die meeste van die vlieë van *Syzygium jambos* geteel, wat in Nelspruit versamel is. Vir *C. cosyra* is die meeste van die vlieë van *Sclerocarya birrea* geteel wat in Hoedspruit versamel is. Die meeste van die wilde *B. dorsalis*-vlieë is van *Mangifera indica* geteel wat in Nelspruit versamel is. In Februarie 2022 is 'n nuwe *C. capitata*-kolonie gestig, aanvanklik van vlieë wat van *Syzygium jambos* geteel is, wat in Stellenbosch versamel is. Die nuwe *C. capitata*-kolonie is verder aangevul met wilde vlieë wat van *Coffea arabica* geteel is, wat ná Maart 2022 in Burgershall versamel is. Die *C. quilicii*-kulture is voortdurend deur die jaar vanaf April 2021 tot Maart 2022 verfris. Altesaam 7166 vlieë wilde *C. quilicii* is by die kolonie gevoeg en dit is hoofsaaklik van *Acca sellowiana* geteel, wat in Ermelo versamel is.

### 3.3.7 PROGRESS REPORT: Redefining dispersal potential for adequate fruit fly pest management (Diptera, Tephritidae)

Project 1254 (2019/20 – 2021/23) by A Manrakhan, L Serfontein (CRI), S K Tsatsu, M Karsten, P Addison (SU), M De Meyer and M. Virgilio (Royal Museum for Central Africa).

#### Summary

The dispersal potential of two cryptic fruit fly pest species in South Africa: *Ceratitis rosa* and *C. quilicii* can be quantified by determination of their distributions and host ranges in the country as well as by an assessment of their demographic parameters on different fruit types. The distributions and host ranges of the two species were determined by trapping and fruit sampling. Traps baited with EGO lure, an attractant known for males of the two species, were set up in selected sites in seven provinces in South Africa. Traps were set up for a period of four weeks during each of three periods: autumn-winter (May-August), spring-summer (October-December) and late summer (March) over two years between 2020 and 2021. *Ceratitis quilicii* was found in all provinces except Northern Cape. *Ceratitis rosa* was recorded only in Limpopo, Mpumalanga and KwaZulu Natal provinces. *Ceratitis quilicii* was more abundant than *C. rosa* in these three provinces. Catches of *C. quilicii* were higher in late summer. No *C. quilicii* nor *C. rosa* was reared from a total of 22.82 kg of citrus sampled across South Africa. Demographic studies of the two species were completed on five fruit types: peach, mango, guava, orange and mandarin. The five fruit types were inoculated with eggs of *C. rosa* and *C. quilicii*. Survival and development of immature and adult stages of the two species were then determined for each fruit type. Larval and pupal survival rates of both fruit fly species were poorer in the two citrus types tested compared to the other fruit types. On the other fruit types: peach, mango and guava, *C. rosa* had a higher net reproductive rate and intrinsic rate of increase than *C. quilicii*.

#### Opsomming

Die verspreidingspotensiaal van twee kriptiese vrugtevliesplaagspesies in Suid-Afrika: *Ceratitis rosa* en *C. quilicii* kan gekwantifiseer word deur die bepaling van hul verspreidings en gasheerreekse in die land, asook deur 'n assessering van hul demografiese parameters op verskillende vrugtypes. Die verspreidings en gasheerreekse van die twee spesies is deur lokvalvangste en vrugmonsters bepaal. Lokvalle met EGO-lokmiddel, 'n lokmiddel bekend vir mannetjies van die twee spesies, is op geselekteerde terreine in sewe provinsies in Suid-Afrika opgestel. Lokvalle is vir 'n tydperk van vier weke gedurende elk van drie periodes opgestel: herfs-winter (Mei-Augustus), lente-somer (Oktober-Desember) en laat somer (Maart) oor twee jaar tussen 2020 en 2021. *Ceratitis quilicii* is in alle provinsies behalwe Noord-Kaap gevind. *Ceratitis rosa* is slegs in Limpopo-, Mpumalanga- en KwaZulu Natal-provinsies aangeteken. *Ceratitis quilicii* was meer volop as *C.*

*rosa* in hierdie drie provinsies. Vangste van *C. quilicii* was hoër in die láát somer. Geen *C. quilicii* of *C. rosa* is uit 'n totaal van 22,82 kg sitrus wat regoor Suid-Afrika versamel is, geteel nie. Demografiese studies van die twee spesies is op vyf vrugtipos voltooi: perske, mango, koejawel, lemoen en mandaryn. Die vyf vrugsoorte is met eiers van *C. rosa* en *C. quilicii* geïnkuleer. Oorlewing en ontwikkeling van onvolwasse en volwasse stadiums van die twee spesies is dan vir elke vrug tipe bepaal. Larf- en papie-oorlewingsyfers van beide vrugtevliespesies was swakker in die twee sitrustipes wat getoets is in vergelyking met die ander vrugtipos. Op die ander vrugtipos: perske, mango en koejawel, het *C. rosa* 'n hoër netto reproduksietempo en intrinsieke toenametempo gehad as *C. quilicii*.

### 3.3.8 PROGRESS REPORT: In-silico boosted, pest prevention and off-season focussed IPM against new and emerging fruit flies

Project 1261 (April 2019-March 2023): A Manrakhan, L Serfontein, R Beck (CRI), D Nestel (Agricultural Research Organisation, Israel) and D Kriticos (CSIRO, Australia)

#### Summary

The aim of this study is to develop an optimal detection system for the Oriental fruit fly, *Bactrocera dorsalis*, in both time and space. A climatic model will be developed to determine optimal trapping time and sites for early pest detection. Baseline data on *B. dorsalis* populations in Mpumalanga Province continued to be collected for the construct of the model. Three grids with methyl eugenol baited traps and Biolure baited traps were set up in three sites: Crocodile Valley, Schoemanskloof and Ermelo, which were at different altitudes (low to high). Prevalence of *B. dorsalis* became lower with increasing altitude. It was clear that at the high-altitude site, detection of the pest in traps was seasonal (only between January and June). In the medium and low altitudes, detection of the pest continued throughout the year, with an increase in the summer periods. In the low *B. dorsalis* prevalence and high-altitude area, a risk-based network where e-traps were only set up in areas deemed more favourable for the pest was compared to a conventional network where traps were evenly set up across the landscape. The detection time of the pest in the risk-based network was comparable to the detection time in a conventional network which used twice the number of traps than the risk-based one. Capture rates of *B. dorsalis* in traps in the risk-based network were comparable to capture rates in the conventional network.

#### Opsomming

Die doel van hierdie studie is om 'n optimale opsporingstelsel vir die Oosterse vrugtevlies, *Bactrocera dorsalis*, in beide tyd en ruimte te ontwikkel. 'n Klimaatmodel sal ontwikkel word om optimale vangtyd en plekke vir vroeë plaag-opsporing te bepaal. Basislyndata oor *B. dorsalis* populasies in Mpumalanga-provinsie word steeds ingesamel vir die konstruksie van die model. Drie roosters met metiel-eugenol-lokvalle en Biolure-lokvalle is op drie terreine opgestel: Krokodilvallei, Schoemanskloof en Ermelo, wat op verskillende hoogtes (laag tot hoog) was. Voorkoms van *B. dorsalis* het laer geword met toenemende hoogte bo seespieël. Dit was duidelik dat die opsporing van die plaag in lokvalle op die hoë-hoogte terrein seisoenaal was (slegs tussen Januarie en Junie). In die medium en lae hoogtes het die opsporing van die plaag deur die jaar voortgeduur, met 'n toename in die somerperiodes. In die lae *B. dorsalis* voorkoms en hoë-hoogte gebied, is 'n risiko-gebaseerde netwerk waar e-lokvalle slegs opgestel is in gebiede wat as gunstiger vir die plaag beskou word, met 'n konvensionele netwerk waar lokvalle eweredig oor die landskap opgestel is, vergelyk. Die opsporingstyd van die plaag in die risiko-gebaseerde netwerk was vergelykbaar met die opsporingstyd in 'n konvensionele netwerk wat twee keer die aantal lokvalle as die risiko-gebaseerde een gebruik het. Vangtempo's van *B. dorsalis* in lokvalle in die risiko-gebaseerde netwerk was vergelykbaar met vangtempo's in die konvensionele netwerk.

### 3.3.9 PROGRESS REPORT: Rearing technology and taxonomy of the Cape fly *Ceratitis quilicii*.

Project 1318 (Apr 2021 – March 2023) by Prof P Addison, Dr W Pieterse and Nicholaas Basson (SU)

#### Summary

*Ceratitis quilicii* (Cape fly) is a newly described fruit fly pest which occurs throughout South and East Africa. *Ceratitis quilicii* was considered to be in a single taxon with *Ceratitis rosa* (Natal fly) up until 2016, at which point sufficient morphological and molecular data separated them into two taxa. In order to understand the biology of *C. quilicii* and to evaluate treatments on the species, a good laboratory colony would have to be established. So far, it has proven difficult to rear this species in the laboratory using diets suitable for other fruit fly species including *C. rosa*. Furthermore, differentiation of *C. quilicii* and *C. rosa* is problematic particularly for immature stages and females of the adult stages. This makes efficient monitoring difficult. This project seeks to develop suitable rearing and identification techniques for *C. quilicii*. For rearing of *C. quilicii*, diets varying in protein content were evaluated. The effects of these diets on pupal weight, adult emergence, adult weight, body mass, body contents, stress resistance and stress tolerance are currently being determined. Preliminary results indicate differences in stress resistance, stress tolerance, adult emergence, adult weight and wing shape between flies reared on different diets. Identification tools for *C. quilicii* continue to be developed.

#### Opsomming

*Ceratitis quilicii* (Kaapse vrugtevlieg) is 'n nuut-beskryfde vrugtevliegplaag wat oral in Suid- en Oos-Afrika voorkom. *Ceratitis quilicii* is tot 2016 as in 'n enkele takson met *Ceratitis rosa* (Natalse vrugtevlieg) beskou, op watter stadium voldoende morfologiese en molekulêre data hulle in twee taksa geskei het. Ten einde die biologie van *C. quilicii* te verstaan en behandelings op die spesie te evalueer, sal 'n goeie laboratoriumkolonie gevestig moet word. Tot dusver was dit moeilik om hierdie spesie in die laboratorium te teel deur diëte te gebruik wat vir ander vrugtevliegspesies, insluitend *C. rosa*, geskik is. Verder is differensiasie van *C. quilicii* en *C. rosa* problematies, veral vir onvolwasse stadiums en wyfies van die volwasse stadiums. Dit maak doeltreffende monitering moeilik. Hierdie projek poog om geskikte teel- en identifikasietegnieke vir *C. quilicii* te ontwikkel. Vir die teel van *C. quilicii* is diëte wat in proteïen-inhoud verskil, geëvalueer. Die uitwerking van hierdie diëte op papiegewig, volwasse-opkoms, volwasse-gewig, liggaamsmassa, liggaamsinhoud, stresweerstand en stresverdraagsaamheid word tans bepaal. Voorlopige resultate dui op verskille in stresweerstand, stresverdraagsaamheid, volwasse-opkoms, volwasse-gewig en vlerkvorm tussen vlieë wat op verskillende diëte grootgemaak word. Identifikasie-instrumente vir *C. quilicii* word steeds ontwikkel.

### 3.3.10 PROGRESS REPORT: Efficacy of fruit fly systems approach for export of citrus to EU

Project 1319 (2021- 2023) by Aruna Manrakhan, John-Henry Daneel, Rooikie Beck, Courtney Morris, Davina Saccagi (CRI)

#### Summary

A systems approach is currently being used to mitigate the risk of fruit fly pests in citrus produced in South Africa and exported to the EU. The aim of this project was to test the efficacy of the systems approach in two susceptible citrus types: mandarin and oranges in South Africa. A total of 13 mandarin and 13 Valencia orange orchards which were under the FFSA were selected from three provinces: Limpopo, Mpumalanga and Western Cape. Fruit fly trapping systems were set up to monitor fruit fly populations in these orchards. Fruit fly infestation of fruit from these orchards before and after harvest (before and after sorting and grading at packhouse) was also determined. In Western Cape Province, infestation could only be assessed for fruit at the packhouse. At each sampling point, 800 fruit were examined for fruit fly damage. In the northern regions at four weeks prior to harvest, exceedances of trap thresholds mainly for *C. capitata* were recorded in four out of 14 orchards. These exceedances were mainly recorded in mandarin orchards. In Western Cape, fruit fly trapping could only be continuously carried out as from October 2021. Only *C. capitata* was recorded in the study orchards in Western Cape. Fruit fly infestation (less than 1%) was recorded only on mandarin fruit in three out of seven orchards in the northern regions. In one of the orchards where fruit fly infestation was recorded, trap thresholds were exceeded. The fruit fly species reared out of infested fruit was *C. capitata*.

Infestation was recorded before harvest and at the packhouse, even after sorting and grading. Measures taken at harvest and at the packhouse however reduced overall infestation by 80% in mandarin. In the northern regions and the Western Cape Province, no fruit fly infestation was recorded in Valencia oranges.

## Opsomming

'n Stelselbenadering word tans gebruik om die risiko van vrugtevliegplae in sitrus wat in Suid-Afrika geproduseer en na die EU uitgevoer word, te verminder. Die doel van hierdie projek was om die doeltreffendheid van die stelselbenadering in twee vatbare sitrustipes te toets: mandaryne en lemoene in Suid-Afrika. Altesaam 13 mandaryn- en 13 Valencia-lemoenboorde wat onder die FFSA was, is uit drie provinsies gekies: Limpopo, Mpumalanga en Wes-Kaap. Vrugtevlieglokvalstelsels is opgestel om vrugtevliegpopulasies in hierdie boorde te monitor. Vrugtevliegbesmetting van vrugte van hierdie boorde vóór en ná oes (vóór en ná sortering en gradering by pakhuis) is ook bepaal. In die Wes-Kaap-provinsie kon besmetting slegs vir vrugte by die pakhuis geassesseer word. By elke monsternemingspunt is 800 vrugte vir vrugtevliegskade ondersoek. In die noordelike streke, vier weke voor oes, is oorskryding van lokvaldrempelwaardes hoofsaaklik vir *C. capitata* in vier uit 14 boorde aangeteken. Hierdie oorskrydings is hoofsaaklik in mandarynboorde aangeteken. In die Wes-Kaap kon lokvalvangste van vrugtevlieë slegs vanaf Oktober 2021 deurlopend uitgevoer word. Slegs *C. capitata* is in die studieboorde in die Wes-Kaap aangeteken. Vrugtevliegbesmetting (minder as 1%) is slegs op mandarynvrugte in drie uit sewe boorde in die noordelike streke aangeteken. In een van die boorde waar vrugtevliegbesmetting aangeteken is, is lokvaldrempelwaardes oorskry. Die vrugtevliegspesie wat uit besmette vrugte geteel is, was *C. capitata*. Besmetting is vóór oes en by die pakhuis aangeteken, selfs ná sortering en gradering. Maatreëls getref tydens oes en by die pakhuis het egter algehele besmetting in mandaryne met 80% verminder. In die noordelike streke en die Wes-Kaap-provinsie is geen vrugtevliegbesmetting in Valencia-lemoene aangeteken nie.

### 3.3.11 PROGRESS REPORT: Host status of citrus for *C. cosyra*

Project 1320 (2021/22 – 2022/23) by Aruna Manrakhan, Evans Mauda, John-Henry Daneel, Leani Serfontein, Rooikie Beck, Glorious Shongwe (CRI)

## Summary

The marula fly, *Ceratitis cosyra* (Walker), is listed as a quarantine pest in some of the current export markets. The main aim of this project is to determine the host status of export grade Valencia orange grown in South Africa for the marula fly. This was carried out by conducting field surveys to determine natural infestation and forced infestation experiments. Seven commercial Valencia orange orchards in the Mpumalanga and Limpopo Provinces were surveyed for the presence of marula fly and fruit fly infestation. Forced infestation experiments were carried out in three Valencia orange orchards (three cultivars: Midnight, Delta and Late Valencia) between July and August 2021. In each orchard, 60 cages were set up over ripe and undamaged oranges on 30 randomly selected trees. Each cage contained five oranges. On each tree, there was one control cage. Each control cage contained five detached guava fruit. In each cage, 20 females and 20 males of marula fly were released. Fruit were exposed to marula flies for four days. At the end of the four days, fruit were collected, brought back to the lab, weighed and incubated for at least six weeks in order to rear flies to adulthood. No natural infestation of marula fly was detected after examination of 10400 export grade oranges collected after harvest, this despite the presence of the marula fly in these orchards. In forced infestation experiments, however, infestation of oranges by marula fly was recorded in all orchards. The average percentage infestation (over total number of exposed fruit) by marula fly were on average  $14.7 \pm 7.0$  and  $65.5 \pm 3.9$  for oranges and guava respectively. The average levels of infestation (number of adult flies over kg of exposed fruit) of marula fly were  $2.8 \pm 1.3$  and  $245.2 \pm 23.1$  for oranges and guava respectively.

## Opsomming

Die maroelavlieg, *Ceratitis cosyra* (Walker), word in sommige van die huidige uitvoermarkte as 'n kwarantynplaag gelys. Die hoofdoel van hierdie projek is om die gasheerstatus van uitvoergraad Valencia-lemoene wat in Suid-Afrika geproduseer word, vir die maroelavlieg te bepaal. Dit is uitgevoer deur veldopnames te doen om natuurlike besmetting te bepaal, en geforseerde besmettingseksperimente. Sewe

kommersiële Valencia-lemoenboorde in die Mpumalanga- en Limpopo-provinsies is vir die teenwoordigheid van maroelavlieg- en vrugtevliegbesmetting ondersoek. Geforseerde besmettingseksperimente is in drie Valencia-lemoenboorde (drie kultivars: Midnight, Delta en Laat Valencia) tussen Julie en Augustus 2021 uitgevoer. In elke boord is 60 hokke oor ryp en onbeskadigde lemoene op 30 ewekansig geselekteerde bome opgestel. Elke hok het vyf lemoene bevat. Op elke boom was daar een kontrole hok. Elke kontrole hok het vyf losstaande koejawelvrugte bevat. In elke hok is 20 wyfies en 20 mannetjies maroelavlieë vrygelaat. Vrugte is vir vier dae aan maroelavlieë blootgestel. Aan die einde van die vier dae is vrugte versamel, teruggebring na die laboratorium, geweeg en vir ten minste ses weke geïnkubeer om vlieë tot volwassenheid te teel. Geen natuurlike besmetting van maroelavlieg is opgespoor ná ondersoek van 10400 uitvoergraad lemoene wat ná oes versamel is nie, dit ten spyte van die teenwoordigheid van die maroelavlieg in hierdie boorde. In geforseerde besmettingseksperimente is besmetting van lemoene deur maroelavlieg egter in alle boorde aangeteken. Die gemiddelde persentasie besmetting (oor totale aantal blootgestelde vrugte) deur maroelavlieg was gemiddeld  $14.7 \pm 7.0$  en  $65.5 \pm 3.9$  vir lemoene en koejawel onderskeidelik. Die gemiddelde vlakke van besmetting (aantal volwasse vlieë oor kg blootgestelde vrugte) van maroelavlieg was onderskeidelik  $2.8 \pm 1.3$  en  $245.2 \pm 23.1$  vir lemoene en koejawel.

### 3.3.12 PROGRESS REPORT: F<sup>3</sup> (Fruit fly free)

Project 1321 (2020/21-2022/23) by Aruna Manrakhan, Leani Serfontein, John-Henry Daneel and Rooikie Beck (CRI)

#### Summary

The establishment of areas of low fruit fly pest prevalence can be one of the control measures to reduce risk of fruit flies in fruit commodity in a systems approach. For citrus exported to Europe from South Africa, a fruit fly systems approach has to be implemented to effectively treat fruit flies. For citrus other than lemons and limes, prevalence of fruit fly pests in orchards are kept at low levels through implementation of fruit fly good agricultural practices. The specified low levels in the citrus industry are thresholds established for recommended fruit fly trapping systems. In this fruit fly free project, we seek to verify and determine the trap thresholds for two fruit fly pests: *Ceratitis capitata* and *Bactrocera dorsalis*. This will be carried out through analysis of historical and newly collected trapping and fruit infestation data. The analysis of historical data formed the first part of this project. Fruit fly trapping data collected between 2019 and 2020 were received from citrus farms across South Africa. Data on fruit fly infestation of citrus on those farms as recorded in packhouses were obtained from Phytclean and PPECB. There were no significant correlations between catches of males and females of the target pests and % rejections. *Ceratitis capitata* dominated in trap catches in citrus orchards. The majority of the cases with zero rejections at packhouses and with the PPECB in Eastern Cape Province was where catches of *C. capitata* males in Capilure baited traps were below 7 flies per trap per week. New trap and fruit infestation data are currently being collected under Project 1319 and will be correlated.

#### Opsomming

Die vestiging van gebiede met lae voorkoms van vrugtevliegplae, kan een van die beheermaatreëls wees om risiko van vrugtevlieë in vrugkommoditeite in 'n stelselbenadering te verminder. Vir sitrus wat vanaf Suid-Afrika na Europa uitgevoer word, moet 'n vrugtevliegstelselbenadering geïmplementeer word om vrugtevlieë doeltreffend te behandel. Vir ander sitrus as suurlemoene en lemmetjies, word die voorkoms van vrugtevliegplae in boorde op lae vlakke gehou deur die implementering van vrugtevlieg goeie landboupraktyke. Die gespesifiseerde lae vlakke in die sitrusbedryf is drempelwaardes wat vasgestel is vir aanbevole vrugtevlieg lokvalstelsels. In hierdie vrugtevliegvrue projek poog ons om die lokvaldrempelwaardes vir twee vrugtevliegplae te verifieer en te bepaal: *Ceratitis capitata* en *Bactrocera dorsalis*. Dit sal uitgevoer word deur ontleding van historiese en nuut-versamelde lokval- en vrugbesmettingsdata. Die ontleding van historiese data het die eerste deel van hierdie projek gevorm. Vrugtevlieglokvaldata wat tussen 2019 en 2020 ingesamel is, is van sitrusplase regoor Suid-Afrika ontvang. Data oor vrugtevliegbesmetting van sitrus op daardie plase, soos aangeteken in pakhuse, is vanaf Phytclean en PPECB verkry. Daar was geen betekenisvolle korrelasies tussen vangste van mannetjies en wyfies van die teikenplae, en % afkeurings nie. *Ceratitis capitata* het in lokvalvangste in sitrusboorde oorheers. Die meerderheid van die gevalle met geen afkeurings by pakhuse en met die PPECB in die Oos-Kaap-provinsie nie, was waar vangste van *C. capitata* mannetjies in Capilure-

lokvalle minder as 7 vlieë per lokval per week was. Nuwe lokval- en vrugbesmettingdata word tans onder Projek 1319 ingesamel en sal gekorreleer word.

### 3.3.13 **PROGRESS REPORT: Utilization of *Fopius arisanus* for control of Oriental fruit fly** Project 1269 (April 2020 – March 2023) by Dr Roger Price (ARC-PHP)

#### **Summary**

The CRI-1269 project 'Utilization of *Fopius arisanus* for control of Oriental fruit fly' is to try and develop a classical biological control agent for the management of *Bactrocera dorsalis* (Bd). The *F. arisanus* wasp, an exotic egg-pupal endo-parasitoid species from Asia, has already been released in East Africa against Bd. The initial focus of the project was to monitor the possible incursion of the *F. arisanus* parasitoid into South Africa from Mozambique and surveys were undertaken in mango and marula orchards in the Nkomazi municipality during Jan-Feb 2021 and again in 2022. Low numbers of Bd were found in both surveys, along with low numbers of indigenous parasitoids of fruit flies. However, no *F. arisanus* were found. ARC researchers visited CRI in May 2021 to learn how to rear Bd and were provided with a starter culture and diet mix. Adult flies emerged, but they failed to oviposit in the ARC laboratory and the culture died out in July. We undertook further training with colleagues at UP in September using their guava and parafilm techniques, along with further advice and a new Bd culture from CRI. The culture remained low until we were able to procure regular supplies of Bd larval diet formula, but since early 2022 the Bd culture has been strong. The ARC obtained a quarantine import permit for *F. arisanus*, issued by DALRRD (Permit No. PO105377). Contact was made with ICIPE, Nairobi, for them to supply a culture of *F. arisanus*. However, when our Bd culture was strong in February 2022, the ICIPE then had delays obtaining an export permit. This coincided with the expiry of our import permit, so again delays ensued. However, we now have both the export and import permits, so we will now make arrangements to travel to Kenya to hand-courier a *F. arisanus* culture back to ARC quarantine. The 2021-2022 project is behind schedule as we have not yet imported the *Fopius* parasitoid.

#### **Opsomming**

Die CRI-1269-projek 'Gebruik van *Fopius arisanus* vir beheer van Oosterse vrugtevlieg' is om 'n klassieke biologiese beheermiddel vir die bestuur van *Bactrocera dorsalis* (Bd) te probeer ontwikkel. Die *F. arisanus* wesp, 'n eksotiese eier-papie endo-parasitoïede spesie uit Asië, is reeds in Oos-Afrika teen Bd vrygelaat. Die aanvanklike fokus van die projek was om die moontlike indringing van die *F. arisanus* parasitoïed in Suid-Afrika vanaf Mosambiek te monitor, en opnames is in mango- en maroelaboorde in die Nkomazi-munisipaliteit gedurende Jan-Feb 2021 en weer in 2022 onderneem. Lae getalle Bd is in beide opnames gevind, saam met lae getalle inheemse parasitoïede van vrugtevlieë. Geen *F. arisanus* is egter gevind nie. LNR-navorsers het CRI in Mei 2021 besoek om te leer hoe om Bd te teel, en is van 'n beginkultuur en dieetmengsel voorsien. Volwasse vlieë het te voorskyn gekom, maar hulle kon nie in die LNR-laboratorium eiers lê nie, en die kultuur het in Julie uitgesterf. Ons het in September verdere opleiding saam met kollegas by UP onderneem deur hul koejawel- en parafilmtegnieke te gebruik, tesame met verdere advies en 'n nuwe Bd-kultuur van CRI. Die kultuur het laag gebly totdat ons gereelde voorrade van Bd-larwe-dieetformule kon bekom, maar sedert vroeg 2022 is die Bd-kultuur sterk. Die LNR het 'n kwarantyn-invoerpermit vir *F. arisanus* verkry, uitgereik deur DALRRD (Permit No. PO105377). Kontak is met ICIPE, Nairobi, gemaak sodat hulle 'n kultuur van *F. arisanus* kan verskaf. Toe ons Bd-kultuur egter in Februarie 2022 sterk was, het die ICIPE verdragings met die verkryging van 'n uitvoerpermit gehad. Dit het saamgeval met die verstryking van ons invoerpermit, so verdragings het weer ontstaan. Ons het egter nou beide die uitvoer- en invoerpermitte, so ons sal nou reëlings tref om na Kenia te reis om 'n *F. arisanus*-kultuur terug te stuur na LNR-kwarantyn. Die 2021-2022-projek is agter skedule aangesien ons nog nie die *Fopius*-parasitoïed ingevoer het nie.

### 3.4 PROGRAMME: OTHER PESTS

Programme coordinator: Tim G Grout (CRI)

#### 3.4.1 Programme summary

Some pests that are not concealed within exported fruit can still be considered to be phytosanitary pests for some markets who have no records of that pest. Other pests like psyllids are vectors of greening disease so their pest status is higher than pests that just cause cosmetic damage to the fruit rind. With the increasing trend to place 20% shade net over orchards, the pest status of mealybug species has increased in these environments. An investigation into possible causes for this showed that it was not due to the antifeedant effects of phenolic compounds being less under the net, and natural enemies could still move through the net. It was most likely due to an improved microclimate for mealybugs and mites on the leaf surface (3.4.2). A more general study of IPM on citrus under nets in the Lowveld also showed an increase in the levels of mealybug infestation with time (3.4.5) but a study in the Western Cape had more variable results with fewer obvious trends (3.4.6). Another comparison between conventional pest management and more bio-intensive IPM is also being conducted under net in the Western Cape (3.4.9). After the loss of buprofezin as a corrective control option for mealybug infestations late in the season a trial was conducted to evaluate some alternatives (3.4.7). The results showed that preventive control options were much more effective than corrective control options but that Tivoli was probably the most effective corrective control option in the place of buprofezin.

Citrus thrips can seriously downgrade export quality fruit and there have been questions about the efficacy of Delegate in the Sundays River Valley. A project over three seasons was conducted to determine whether there was any evidence of resistance, but none was found (3.4.3). Alternative treatments to control mites on imported budwood are required because the methyl bromide fumigation that is usually used often kills the buds. Suitable orchards with trees that are evenly infested with bud mite have not been found so little further progress on this project has been possible (3.4.4). In the past, augmentative releases of *Aphytis lingnanensis* against red scale infestations could not be shown to be more effective than conserving *A. africanus* that was already present in the orchards. Now some insectaries can provide *A. melinus* so augmentative releases with this parasitoid are being evaluated (3.4.8). The efficacy of microbial control products can be greatly influenced by the formulation. A patented additive for these formulations is being evaluated and giving promising results (3.4.10). With many citrus orchards being adjacent to indigenous vegetation it is possible for Lepidoptera that are not recognised as citrus pests to oviposit on citrus fruit and their larvae to survive for a while. This can cause confusion when these insects are mistaken for citrus pests. Currently a project is being conducted to identify unusual lepidopteran larvae on fruit (3.4.11). Along similar lines, there are some natural enemies and pests that are extremely similar morphologically and difficult to identify correctly unless the correct life stage is found. Molecular techniques are being developed to separate these (3.4.12). With the increasing numbers of mandarin hybrids being planted it has been found that the status of some pests is different on these compared to other types of citrus. One example is Australian bug that is normally under biological control but reaches significant levels of infestation on mandarin hybrids. This is under investigation in Mpumalanga (3.4.13). The parasitoid *Anagyrus vladimiri* is available for augmentative releases for the control of mealybug but levels of hyperparasitism are variable and can negate its impact (3.4.14).

Attempts are being made to rear large enough numbers of *Diaphorina citri* on potted citrus plants in Kenya to be able to screen some new systemic pesticides (3.4.15). However, numbers of *D. citri* and levels of infestation continue to be inadequate for such trials. In the meantime, personnel from ICIPE in Kenya have surveyed for *D. citri* in the warmer citrus production regions and only found it present at Lungalunga near the Tanzanian border. Due to the presence of many *Diaphorina* species in southern Africa, including some undescribed ones that are extremely similar to *D. citri*, research is being conducted to identify all psyllids likely to be found on sticky traps hung in citrus orchards near indigenous bush and adults collected from growth flush on citrus (3.4.16). Both morphological and molecular identification techniques are being used. In another project conducted with University of Pretoria, odour-based monitoring tools are being evaluated for psyllids and it is hoped that the use of AI to identify *Trioza erytreae* and *D. citri* from photographs of sticky traps will reduce the burden of scanning hundreds of traps manually (3.4.17). It is hoped that the results of these research projects will assist the biosecurity officers in identifying *D. citri* when it arrives in the region.

## Program-opsomming

Sommige plaë wat nie in uitvoervrugte versteek is nie, kan steeds as fitosanitêre plaë beskou word vir sommige markte wat geen rekords van daardie plaag het nie. Ander plaë soos bladvlooië is vektore van vergroeningsiekte, dus is hul plaagstatus hoër as plaë wat net kosmetiese skade aan die vrugskil veroorsaak. Met die toenemende neiging om 20% skadunet oor boorde te plaas, het die plaagstatus van witluisspesies in hierdie omgewings toegeneem. 'n Ondersoek na moontlike oorsake hiervoor het getoon dat dit nie te wyte was aan die teenvoedingseffekte van fenoliese verbindings wat minder onder die net was nie, en natuurlike vyande kon steeds deur die net beweeg. Dit was heel waarskynlik as gevolg van 'n verbeterde mikroklimaat vir witluise en myte op die blaaroppervlak (3.4.2). 'n Meer algemene studie van IPM op sitrus onder nette in die Laeveld het ook 'n toename in die vlakke van witluisbesmetting met tyd getoon (3.4.5), maar 'n studie in die Wes-Kaap het meer veranderlike resultate gehad met minder ooglopende neigings (3.4.6). Nog 'n vergelyking tussen konvensionele plaagbestuur en meer bio-intensiewe IPM word ook onder net in die Wes-Kaap gedoen (3.4.9). Ná die verlies van buprofezin as 'n korrektiewe beheer-opsie vir witluisbesmetting láát in die seisoen, is 'n proef uitgevoer om 'n paar alternatiewe te evalueer (3.4.7). Die resultate het getoon dat voorkomende beheer-opsies baie meer doeltreffend as korrektiewe beheer-opsies was, maar dat Tivoli waarskynlik die mees effektiewe korrektiewe beheer-opsie in die plek van buprofezin was.

Sitrusblaaspootjies kan uitvoerkwaliteit vrugte ernstig afgradeer en daar was vrae oor die doeltreffendheid van Delegate in die Sondagsriviervallei. 'n Projek is oor drie seisoene uitgevoer om te bepaal of daar enige bewyse van weerstand was, maar niks is gevind nie (3.4.3). Alternatiewe behandelings om myte op ingevoerde okuleerhout te beheer, word vereis omdat die metielbromiedberoking wat gewoonlik gebruik word, dikwels die ogies doodmaak. Geskikte boorde met bome wat eweredig met knopmyt besmet is, is nie gevind nie so min verdere vordering met hierdie projek was moontlik (3.4.4). In die verlede kon daar nie getoon word dat aanvullende vrystellings van *Aphytis lingnanensis* teen rooidopluisbesmettings meer effektief was as die bewaring van *A. africanus* wat reeds in die boorde voorkom nie. Nou kan sommige insektariums *A. melinus* voorsien, dus word aanvullende vrystellings met hierdie parasitoïed geëvalueer (3.4.8). Die doeltreffendheid van mikrobiële beheerprodukte kan grootliks deur die formulering beïnvloed word. 'n Gepatenteerde bymiddel vir hierdie formulering word geëvalueer en lewer belowende resultate (3.4.10). Aangesien baie sitrusboorde aangrensend aan inheemse plantegroei is, is dit moontlik vir Lepidoptera wat nie as sitrusplaë erken word nie, om op sitrusvrugte hul eiers te lê en vir hul larwes om vir 'n rukkie te oorleef. Dit kan verwarring veroorsaak wanneer hierdie insekte verkeerdelik as sitrusplaë geïdentifiseer word. Tans word 'n projek uitgevoer om ongewone Lepidoptera-larwes op vrugte te identifiseer (3.4.11). Soortgelyk is daar 'n paar natuurlike vyande en plaë wat morfologies uiters soortgelyk is en moeilik is om korrek te identifiseer, tensy die korrekte lewensfase gevind word. Molekulêre tegnieke word ontwikkel om hierdie te skei (3.4.12). Met die toenemende aantal mandaryn-hibriede wat geplant word, is gevind dat die status van sommige plaë anders is op sommige van hierdie hibriede, in vergelyking met ander tipes sitrus. Een voorbeeld is Australiese wolluis wat normaalweg onder biologiese beheer is, maar beduidende vlakke van besmetting op mandaryn-hibriede bereik. Dit word in Mpumalanga ondersoek (3.4.13). Die parasitoïed *Anagyrus vladimiri* is beskikbaar vir aanvullende vrylatings vir die beheer van witluis, maar vlakke van hiperparasitisme varieer en kan die impak daarvan negeer (3.4.14).

Daar word gepoog om groot genoeg getalle *Diaphorina citri* op gepotte sitrusplante in Kenia te kweek om nuwe sistemiese plaagdoders te kan evalueer (3.4.15). Getalle van *D. citri* en vlakke van besmetting is egter steeds onvoldoende vir sulke proewe. Intussen het personeel van ICIPE in Kenia 'n opname vir *D. citri* in die warmer sitrusproduksiestreke gedoen, en dit net by Lungalunga naby die Tanzaniese grens gevind. As gevolg van die teenwoordigheid van baie *Diaphorina*-spesies in Suider-Afrika, insluitend 'n paar onbeskryfde spesies wat uiters soortgelyk aan *D. citri* is, word navorsing gedoen om alle psilloïede te identifiseer wat waarskynlik op kleeflokvalle, wat in sitrusboorde naby inheemse bosse gehang word, gevind kan word, en volwassenes wat vanaf groeistuwings op sitrus versamel word (3.4.16). Beide morfologiese en molekulêre identifikasietegnieke word gebruik. In 'n ander projek wat saam met die Universiteit van Pretoria uitgevoer is, word reuk-gebaseerde moniteringsinstrumente vir bladvlooië geëvalueer, en daar word gehoop dat die gebruik van AI om *Trioza erythrae* en *D. citri* vanaf foto's van kleeflokvalle te identifiseer, die las van die skandering van honderde lokvalle handmatig, sal verminder (3.4.17). Daar word gehoop dat die resultate van hierdie navorsingsprojekte die biosekuriteitsbeamptes sal help om *D. citri* te identifiseer wanneer dit in die streek aankom.

### 3.4.2 FINAL REPORT: Determine the primary cause for mealybug repercussions under netting

Project 1195 (RCE-2-23) (2018/9 – 2021/2) by T Grout, P Stephen, L Serfontein and E Mauda (CRI)

#### Summary

In citrus orchards under 20% shade net in both Australia and South Africa, mealybug has become a primary pest. This research was conducted to determine the main reasons for this. Shade net structures at the Citrus Research Centre in Nelspruit were built, potted Midnight Valencia trees placed under the shade net and in the open adjacent to the structures, and citrus mealybug cultures initiated on butternuts. Plans to compare the growth rate of mealybug in parasitoid-proof containers outside the structures and under 20% white shade net were not possible because the cultures heated up to lethal temperatures in the sun. Difficulty was also experienced in preventing contamination of mealybug cultures with the parasitoid *Coccidoxenoides perminutus*, but this was finally resolved. However, placing 130 mealybug crawlers per plant did not result in infestation, neither did the careful transfer of adults to citrus plants, finally the attachment of a container to each plant with egg sacs was successful. After being infested for 65 days, plants under 20% net had significantly more citrus mealybug nymphs per leaf than those in the open. After 112 days, no citrus mealybug could be found on plants in the open whereas plants under net had approximately four nymphs per leaf in the middle of the plant and at a lower position. After 151 days, numbers per leaf under the net had declined but nymphs could still be found. Pest infestation of potted Midnight Valencia trees in the open adjacent to potted Midnight Valencia trees of the same age and source under 20% net at CRC, Nelspruit showed that under the net citrus red mite infestation was 75% higher, silver mite was 14% higher, red scale 33% higher and mealybug 6% higher. A photo-radiometer was used to measure PAR and UV-B radiation in adjacent commercial orchards in the open and under 20% netting at various locations in Mpumalanga and Limpopo during summer and autumn 2018/19. Results were variable on different occasions but in general the nets reduced PAR by approximately 20% and UV-B by 26%. Leaf samples at the same commercial sites were taken on two occasions in the open and under 20% net to test for levels of total phenolics. These results showed more difference between the northern and southern sides of trees than between net and no net. Tests of chemical residues on fruit under nets and in the open during February and March showed little difference, except for a trend for some strobilurin residues to be slightly higher under net. None of these differences would have been problematic at harvest time. Insect-proof cages were constructed at CRI to be used for testing the effects of 20% shade net in preventing the natural enemy *Anagyrus vladimiri* from finding citrus mealybug when released outside containers in these cages. Open containers and those closed with 20% white shade net were placed inside the insect-proof cage where *A. vladimiri* was released. The rate at which third instar citrus mealybugs on butternuts in the containers were parasitized was recorded in both types of containers. Approximately 73 *A. vladimiri* were released inside each insect-proof cage. Mealybugs were examined for parasitism weekly for thirty days in both control (open containers) and covered containers (20% shade net), recording the number of parasitised mealybug in each replicate. This objective was repeated using the natural enemy *Cryptolaemus montrouzieri* to determine the ability of the beetle to find mealybugs from outside 20% shade net and in open containers, both with mealybug-infested butternuts. Approximately 71 *C. montrouzieri* were released in each large cage. The two beneficial insects released inside the insect proof cages showed that the 20% white shade net did not affect their ability to either parasitize or prey on mealybug populations when released outside these structures. We found that the efficacy of both insects was not affected by the net and they reduced the number of mealybugs infesting the butternut to a level of complete control. It is therefore clear that the 20% net does not physically exclude the natural enemies but from the comparison with potted plants in the open and under 20% net it was clear that the mealybug and other citrus pests became more abundant under the net so perhaps the searching abilities of the natural enemies are being compromised by the net, leading to more sustained population growth.

#### Opsomming

Witluis het 'n primêre plaag in sitrusboorde onder 20% skadunet, in beide Australië en Suid-Afrika, geword. Hierdie navorsing is uitgevoer om die hoofredes hiervoor vas te stel. Skadunetstrukture is by die Sitrusnavorsingsentrum (CRC) in Nelspruit gebou. Gepotte Midnight Valencia bome is onder die skadunet en in die oopte langsaan die strukture geplaas, en sitrus witluis kulture is op botterskorsies geïnisieer. Planne

om die groeitempo van witluis in parasitoïed-bestande houers buite die strukture en onder 20% wit skadunet met mekaar te vergelyk, was onmoontlik aangesien die kulture tot dodelike temperature in die son verhit het. Probleme is ook ondervind in die voorkoming van kontaminasie van witluis kulture met die parasitoïed, *Coccidoxenoides perminutus*, maar dit is uiteindelik opgelos. Die plasing van 130 witluis kruipers per plant het egter nie tot besmetting gelei nie, ook nie die versigtige oordrag van volwasse witluis na sitrusplante nie. Die heg van 'n houer met eiersakke aan elke plant was uiteindelik suksesvol. Nadat hulle vir 65 dae besmet is, het plante onder 20% net betekenisvol meer sitrus witluis nimfe per blaar gehad, in vergelyking met dié in die oopte. Geen sitrus witluis kon ná 112 dae op plante in die oopte gevind word nie, terwyl plante onder net ongeveer vier nimfe per blaar in die middel van die plant en by 'n laer posisie gehad het. Getalle per blaar onder net het ná 151 dae afgeneem maar nimfe kon steeds gevind word. Plaagbesmetting van gepotte Midnight Valencia bome in die oopte langsaan gepotte Midnight Valencia bome van dieselfde ouderdom en bron onder 20% net by CRC, Nelspruit, het getoon dat onder net, sitrus rooimyt besmetting 75% hoër was, silwermyt was 14% hoër, rooi dopluis was 33% hoër en witluis was 6% hoër. 'n Foto-radiometer is gebruik om PAR en UV-B bestraling in aangrensende kommersiële boorde in die oopte en onder 20% net by verskeie plekke in Mpumalanga en Limpopo gedurende somer en herfs 2018/19 te meet. Resultate was by verskillende geleenthede veranderlik, maar oor die algemeen het die nette PAR met ongeveer 20% verminder en UV-B met 26%. Blaarmonsters by dieselfde kommersiële terreine is by twee geleenthede in die oopte en onder 20% net geneem ten einde vir vlakke van totale fenole te toets. Hierdie resultate het groter verskil tussen die noordelike en suidelike kante van bome getoon, as tussen net en geen net. Hierdie rTests van chemiese residue op vrugte onder nette en in die oopte gedurende Februarie en Maart, het min verskille getoon, behalwe vir die neiging vir sommige strobilurien residue om effens hoër onder net te wees. Geen van hierdie verskille sou tydens oestyd problematies gewees het nie. Insek-bestande hokke is by CRI gebou om gebruik te word om die uitwerking van 20% skadunet te toets om te verhoed dat die natuurlike vyand, *Anagyrus vladimiri*, sitrus witluis vind wanneer dit buite houers in hierdie hokke vrygelaat word. Oop houers en dié wat met 20% wit skadunet toegemaak is, is binne die insek-bestande hok geplaas waar *A. vladimiri* vrygelaat is. Die tempo waarteen derde instar sitrus witluis op botterskorsies in die houers geparasiteer is, is in beide tipes houers aangeteken. Ongeveer 73 *A. vladimiri* is binne elke insek-bestande hok vrygelaat. Witluis is weekliks vir dertig dae vir parasitisme in beide kontrole (oop houers) en bedekte houers (20% skadunet) ondersoek, en die aantal geparasiteerde witluis is in elke herhaling aangeteken. Hierdie doelwit is herhaal deur die natuurlike vyand, *Cryptolaemus montrouzieri*, te gebruik om die vermoë van die kewer te bepaal om witluis van buite 20% skadunet en in oop houers te vind, beide met witluis-besmette botterskorsies. Ongeveer 71 *C. montrouzieri* is in elke groot hok vrygelaat. Die twee voordelige insekte wat binne die insek-bestande hokke vrygestel is, het getoon dat die 20% wit skadunet nie hul vermoë beïnvloed het om óf te parasiteer óf op witluis populasies te voed wanneer dit buite hierdie strukture vrygelaat word nie. Ons het gevind dat die doeltreffendheid van beide insekte nie deur die net beïnvloed is nie, en hulle het die aantal witluis wat die botterskorsie besmet het tot 'n vlak van volledige beheer verminder. Dit is dus duidelik dat die 20% net nie fisies die natuurlike vyande uitsluit nie, maar uit die vergelyking met gepotte plante in die oopte en onder 20% net, was dit duidelik dat die witluis en ander sitrusplae meer volop onder die net geword het. Die soekvermoë van die natuurlike vyande word dus miskien deur die net in gedrang gebring, wat tot meer volgehoue populasiegroei lei.

## Introduction

In citrus orchards under 20% shade net in both Australia and South Africa, mealybug has become the primary pest. There are a number of possible reasons for this but only some of these are manageable. Research is therefore required to determine whether the likelihood of mealybug repercussions can be reduced, because many citrus growers are going ahead with erecting nets over citrus for horticultural reasons.

Possible reasons for an increase in mealybug populations include:

1. Reduced detrimental direct effects of solar radiation, resulting in increased dispersion on sun-exposed surfaces and less intraspecific competition.
2. Reduced UV-B irradiation resulting in more palatable food (less phenolics or more starch from more photosynthesis) and more rapid insect growth with shorter life cycles.
3. Increased vegetative growth resulting in poor spray coverage of mealybug on the tree framework.
4. Compromised natural enemy impact due to: a) visual confusion or repellency, b) physical obstruction, c) altered chemical cues that compromise the ability to find the host.

Depending on the colour of the net, the reduction in visible light or photosynthetically active radiation (PAR) under a 20% shade net may only be in the order of 10-15%, whereas the reduction of UV-B radiation, irrespective of the colour of the net is at least 20% on a new net according to both manufacturers in South Africa. An old, dirty net may therefore block as much as 30% of UV-B radiation. Plants produce phenolics and flavonoids in response to UV-B radiation that act as sunscreens for the plant but also act as antifeedants and toxins (Treutter 2006; Kuhlmann and Müller 2010). Under reduced UV-B and PAR, lower levels of phenolics in leaves have resulted in increased insect feeding (Dudt and Shure 1994; Zavala *et al.* 2001; Warren *et al.* 2002; Rousseaux *et al.* 2004; Ohtsuka and Osakabe 2009).

Volatile organic compounds such as beta-caryophyllene are released from citrus more rapidly under conditions of high temperature and high light (Hansen and Seufert 2003). These chemicals can be used by natural enemies to find feeding hosts, so under netting with lower surface temperatures and less solar radiation it may be more difficult for natural enemies to find their prey (Foggo *et al.* 2007).

UV-B radiation can be directly damaging to insects and many species will try to reduce exposure by feeding under the calyx or under leaves, between fruit etc. (Mazza *et al.* 2002). With lower levels of UV-B, insects may disperse more and therefore be less influenced by intraspecific competition and this may make host finding by natural enemies more difficult too.

### **Stated objectives**

1. Determine the direct effects of reduced solar radiation on citrus mealybug's rate of increase ( $r$ ) and dispersion
2. Determine citrus mealybug's rate of increase when reared on plants grown under net versus plants grown in the open.
3. Quantify UV-B levels under different grower net structures at different times of the year relative to the open and quantify total levels of phenolics in leaves from covered and open trees. Fruit will be tested for differences in pesticide residue levels too.
4. Determine the efficiency of a parasitoid (*Anagyrus vladimiri*) and predator (*Cryptolaemus montrouzieri*) against citrus mealybug reared on potted Valencia plants grown in the sun versus those grown under netting.
5. Determine the ability of *C. montrouzieri* and *A. vladimiri* to attack citrus mealybug on butternuts on the other side of a white 20% shade net barrier in an insect-proof cage.

### **Materials and methods**

#### Preparation

A structure was built at CRC in Nelspruit that was covered with 20% white net and could accommodate at least 100 potted Midnight Valencia plants. These were grown alongside other potted plants in the open, exposed to full sun. All plants had individual pot irrigation emitters.

A mother culture of citrus mealybug (*Planococcus citri*) was established and maintained on butternuts in a controlled environment room at CRC, Nelspruit. Delays were caused by contamination with the parasitoid *Coccidoxenoides perminutus* until large cake boxes were used with adequate sealing and fine gauze lids.

#### Objective 1

Butternuts seeded with equal numbers of second instar citrus mealybug were placed in natural-enemy-proof boxes with gauze sides and Perspex tops under the white 20% net and in the open to determine the mean rate of increase for each situation. However, boxes in the open heated up significantly in the direct sun resulting in temperatures much higher than the ambient temperature. Attempts were made to move air through the boxes with a small fan but this effect was inadequate. After trying different cage designs this comparison had to be abandoned because the fine mesh required to exclude *C. perminutus* hindered air flow.

#### Objective 2

While Objective 1 was being addressed, 60 potted Midnight Valencia plants were maintained under the 20% shade net and another 60 in the open with occasional acephate stem treatments to control aphids and citrus psylla. After several months of new growth in those environments the leaves were tested for total phenolic content to see whether there were differences between the two environments that were similar to differences found between commercial trees grown under netting and in the open (Objective 3). If the differences in levels of phenolics in the potted plants were at significant levels, acephate treatments would be stopped and 30 plants from each environment labelled and separated from one another, then infested with equal numbers of second instar citrus mealybug. Sticky polybutene bands would prevent the movement of mealybug between trees. After approximately two months, infestation levels on all the potted plants would be determined.

While trying to obtain adequate mealybug cultures on butternuts, the Midnight Valencia plants in pots became infested with various other pests. Fifty-two plants under the 20% net and another 52 plants in the open were inspected on 12 April 2019 for the presence or absence of various pests, and damage from citrus thrips.

Several attempts to infest potted plants with citrus mealybug failed until the following technique was used. Ten female citrus mealybugs with egg sacs were collected from the culture maintained on butternuts at CRI. These were placed in a small plastic container with a punctured lid (Fig. 3.4.2.1). Each plastic tub had a small piece of tissue paper inside as a substrate. Fifty-two of these containers were prepared and each attached to a potted Midnight Valencia plant on 20 Jun 2020 approximately 5-7 cm from the top of the tree using an elastic band. Twenty-six of these plants were left in the open, exposed to the sun and the other 26 plants that had been kept under white 20% shade cloth for several months, were retained under the net. All plants were irrigated in the same way with a drip emitter in each bag.

After 29 days a second release was made on the same trees as for the first release and following the same procedure as the first release, but with five egg sacs per container instead of ten.

The number of citrus mealybug nymphs on leaves was determined 65 days after the first release on 24 Aug 2020. Three types of leaves: upper leaf (from the top of the tree), middle leaf (found just above the release container) and lower leaf (found below the release container), were collected from each tree. The leaves were examined for the presence of mealybug nymphs using a dissecting microscope.

There was a second evaluation of the number of citrus mealybugs on leaves at 112 days after starting the trial (after the first release), on 15 October 2020. The same method as described before for the collection and evaluation was used.

A third evaluation of the number of citrus mealybugs on leaves from trees under the net was made at 151 days after the start of the trial, on 20 November 2020. Once again, the same method as described before was used for the collection and evaluation.

Two ibuttons were placed in plastic containers suspended from the trees under the net and in the open. Both ibuttons had an average temperature of 18°C (Std Dev 7.2°C under net and Std Dev 6.6°C in the open).



**Figure 3.4.2.1.** Release container containing egg sacs on tissue paper.

### Objective 3

Measurements of Photosynthetically Active Radiation (PAR) and UV-B radiation were taken under existing commercial net structures and alongside in the open on two occasions at various commercial locations in Mpumalanga and Limpopo and using the potted plants at Nelspruit. Where possible, different types and colours of net were included in these measurements. Reductions in PAR and UV-B radiation caused by the netting were expressed as percentages. Fruit were picked in February and March 2019 at the same sites where radiation measurements were taken in order to test for residues of certain pesticides that the grower may have used to determine if breakdown of residues under nets was significantly prolonged. Leaf samples were also taken from the commercial sites in adjacent orchards of the same citrus type and cultivar where one orchard was under net and the other uncovered. Sampling was done in November and December 2018 and again in April 2019. The leaf samples were sent to Stellenbosch University where they were analysed for total phenolics by Dr Leandra Moller under supervision by Prof Paul Cronjé.

### Objective 4

Fifteen Midnight Valencia plants grown in the sun and another 15 Midnight Valencia plants grown under 20% netting for long enough to show differences in total phenolics will be infested with equal numbers of second instar citrus mealybug and placed in separate halves of an insect-proof tunnel, separated by an insect-proof curtain. Releases of a recommended number of *Anagyrus vladimiri* will be made in each half of the tunnel when the mealybugs have reached the optimal life stage. Infestation of the plants will then be determined one month after the release. If adequate numbers of adult mealybug remain on the plants, this experiment will be repeated with the release of *Cryptolaemus montrouzieri* beetles. If the numbers of adults are too few, more plants will have to be infested with mealybug and kept until adults are obtained before introducing *Cryptolaemus*.

### Objective 5

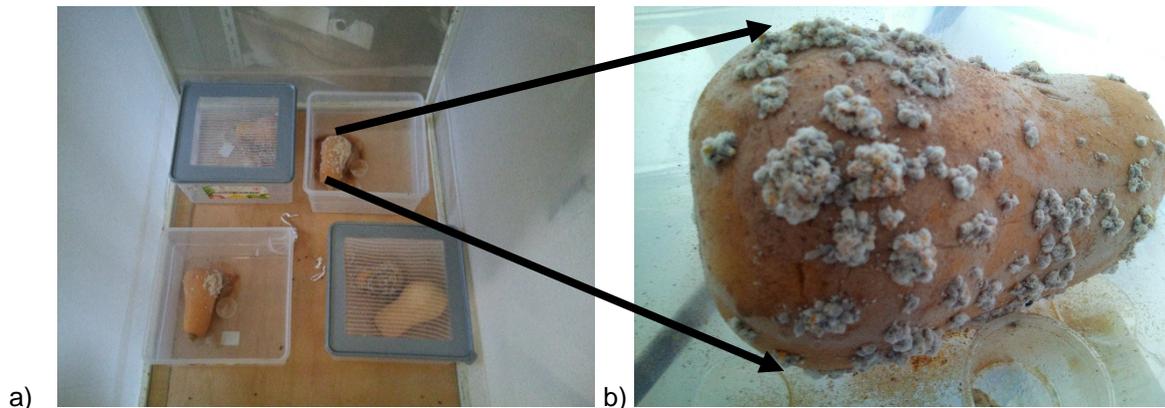
Objective 5 was conducted in two parts, repeating the experiment with two different beneficial insects that either parasitize or predate on citrus mealybug. The two beneficial insects for evaluation were *Anagyrus vladimiri* and *Cryptolaemus montrouzieri* and were purchased from BIOBEE, Letsitele, South Africa. *Anagyrus vladimiri* were purchased in mealybug mummies and *C. montrouzieri* were purchased as adult beetles inside insect proof containers.

Using insect-proof cages in the insectary at an average temperature of 26°C, citrus mealybug on a butternut at the correct life stage for *A. vladimiri* parasitism, were placed within a cake box container covered with 20% white shade netting. Simultaneously, citrus mealybug at the same life stage on a butternut were placed in a cake box but fully exposed and not covered by 20% white shade net. Seven insect-proof cages were used of variable sizes such that a total of 20 boxes with 20% net covers and 20 open boxes were used with an equal number of each treatment (replicates) per large cage. The cages ranged from 1.86 m (height) x 1.10 m (depth) x 0.60 m (width) and 1.50 m (height) x 1.31 m (depth) x 0.65 m (width). Some were with shelves and some were without shelves inside. The number of replicates in the cage was determined by the cage dimensions, starting with four replicates per cage to nine replicates per cage (Fig. 3.4.2.2). Seventy-three *A. vladimiri* parasitoids were released within each large cage and left for 48 h to parasitize the mealybug. Mealybugs were examined for parasitism weekly for thirty days in both control (open containers) and covered containers (20% shade net), recording the number of parasitized mealybug in each replicate.

A similar comparison was conducted with *Cryptolaemus montrouzieri* to see whether beetles released outside 20% shade net are able to find adult citrus mealybug on butternuts on the other side of the net. Seven insect-proof cages were used in the insectary with an optimum temperature of 26°C and an equal number of cake box containers were placed inside these cages. In each cage four to nine cake box containers with 20% white shade net and without shade net were placed with a butternut infested with mealybug in each (Fig. 3.4.2.2). A total of five hundred adult beetles were released over seven insect proof cages, giving approximately seventy-one *C. montrouzieri* in each insect-proof cage. A total of forty cake container boxes were used with 20 closed with 20% white shade net and 20 control containers left open.

The total number of *C. montrouzieri* was recorded in each replicate one week after release. Monitoring and counting of beetles inside replicates was done weekly for a period of six weeks and butternuts were replaced

in week 2 of the experiment. Results were assessed as the beetles' ability to find citrus mealybug in open and closed (20% shade net) treatments mimicking an orchard situation with shade net and without shade net. Containers inside the cages were not allowed to touch and had spaces in between to avoid any spill-over effect or assistance to beetles in entering containers (Fig. 3.4.2.2a).



**Figure 3.4.2.2.** a) Four cake containers inside an insect proof cage with two covered with 20% white shade net (closed) and two open. Each container held one mealybug-infested butternut. b) Close-up view of mealybug-infested butternut inside an open replicate.

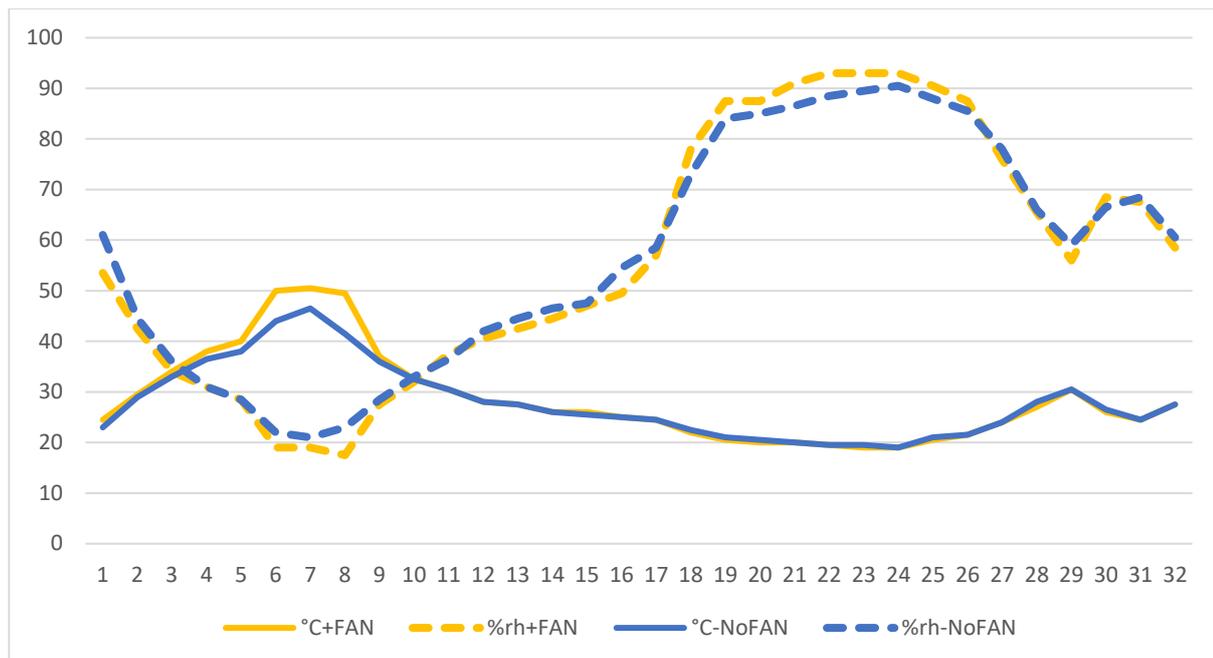
**Extra funding**

This project received some funding from DSI via the RCE-2 programme.

**Results and discussion**

Objective 1

We found that it was impossible to place mealybug cultures on butternuts in boxes with screen sides in the sun compared with under the 20% net because the cultures rapidly heated up to around 50°C in the sun (Fig. 3.4.2.3). The objective was to compare direct sun with sun through the netting so this meant that the trial design did not work. Even with the use of a small fan to move air through the cultures the temperature still increased to lethal levels in the sun. This objective was therefore abandoned.

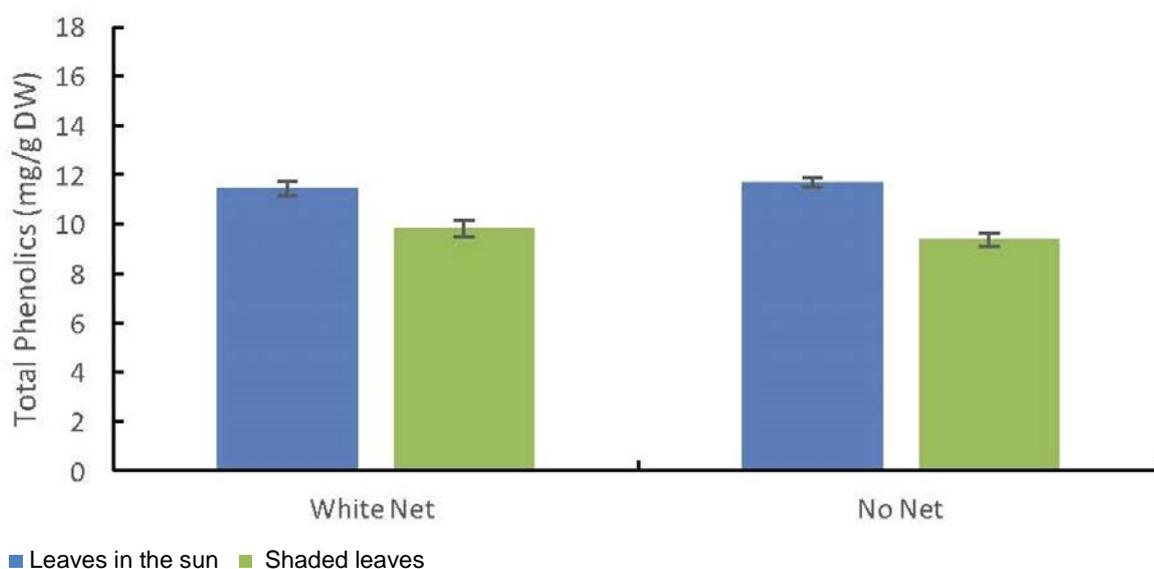


**Figure 3.4.2.3.** Air temperature (°C) and relative humidity (%) test 5-6 Nov 2018 over 32 h.

## Objective 2

Potted Midnight Valencia plants were kept under the net and in the open for several months before total phenolics were determined in leaf samples taken in January 2019 from leaves in the sun near the top of the plants and from leaves that were shaded by the upper canopy, lower down on the plants. The quantity of phenolics was higher in the sun-exposed leaves than the shaded leaves in both environments but there was no difference between the plants in the sun and those under the 20% net (Fig. 3.4.2.4). This meant that any difference in survival of citrus mealybug on potted plants in the open or under 20% net was not due to different anti-feedant effects caused by higher levels of phenolics induced by exposure to the sun.

Comparisons between plants in the open and those under the 20% white net showed that infestations by citrus red mite, silver mite and red scale were much higher under net than in the open (Table 3.4.2.1). Orange dog butterflies were excluded by the net.



**Figure 3.4.2.4.** Comparison of total phenolics for leaves exposed to the sun versus those in the shade in potted Midnight Valencia plants under 20% white net or no net at Nelspruit.

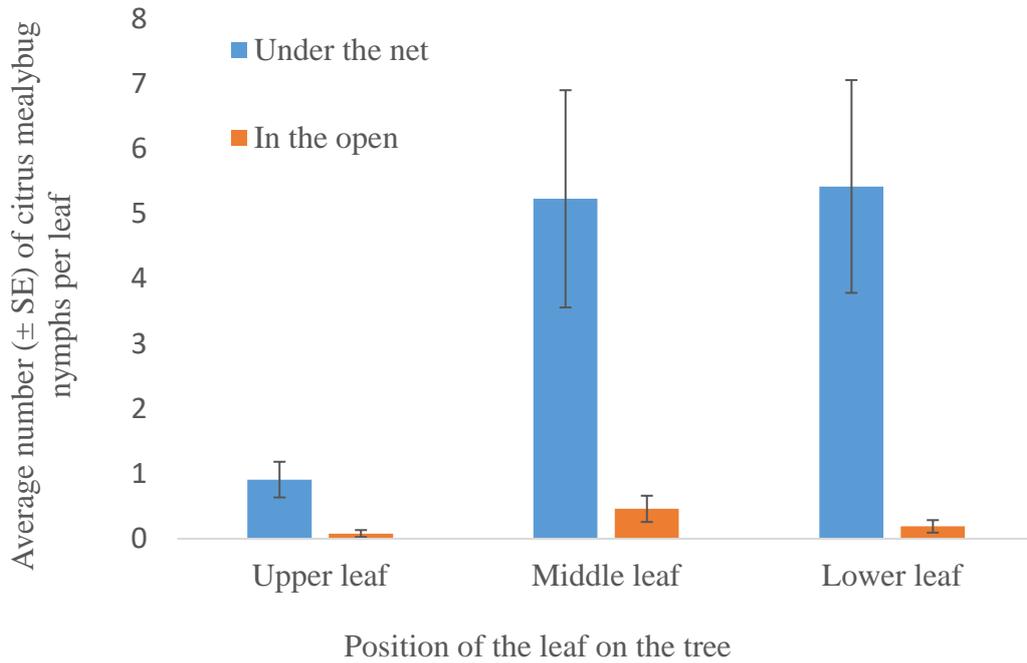
**Table 3.4.2.1.** Trees infested (%) with pests on 12 April 2019 (visual examination of 52 trees in each area)

Area	Red mite	Rust mite	Silver mite	Red scale	Mealy-bug	Thrips damage	Orange dog	Soft scale	Woolly whitefly	Leaf-miner
Outside tunnel	0.0	0.0	0.0	0.0	0.0	23.1	5.8	1.9	0.0	0.0
Inside 20% net	75.0	0.0	13.5	32.7	5.8	19.2	0.0	9.6	7.7	1.9

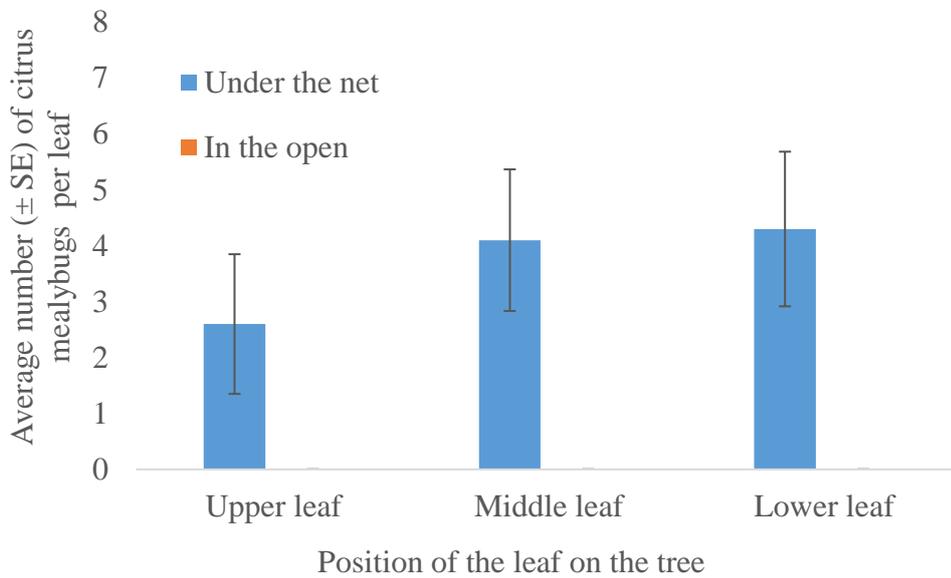
At 65 days after the start of the trial, the middle and lower leaves showed a greater infestation of citrus mealybug nymphs compared to the upper leaves (Fig. 3.4.2.5). The leaves from trees under the net had a greater infestation of nymphs than the leaves from trees in the open (Fig. 3.4.2.5).

No citrus mealybugs were found in the open at 112 days (Fig. 3.4.2.6). As with the earlier observations for trees under the net, the middle and lower leaves showed a greater infestation of citrus mealybug compared to the upper leaves. The infestation rates of the middle and lower leaves under the net remained more or less constant over time. There was an increase in infestation rate of the upper leaves with time for trees under net. The majority of the citrus mealybugs found under the net at 112 days after the trial started were nymphs (Fig. 3.4.2.7).

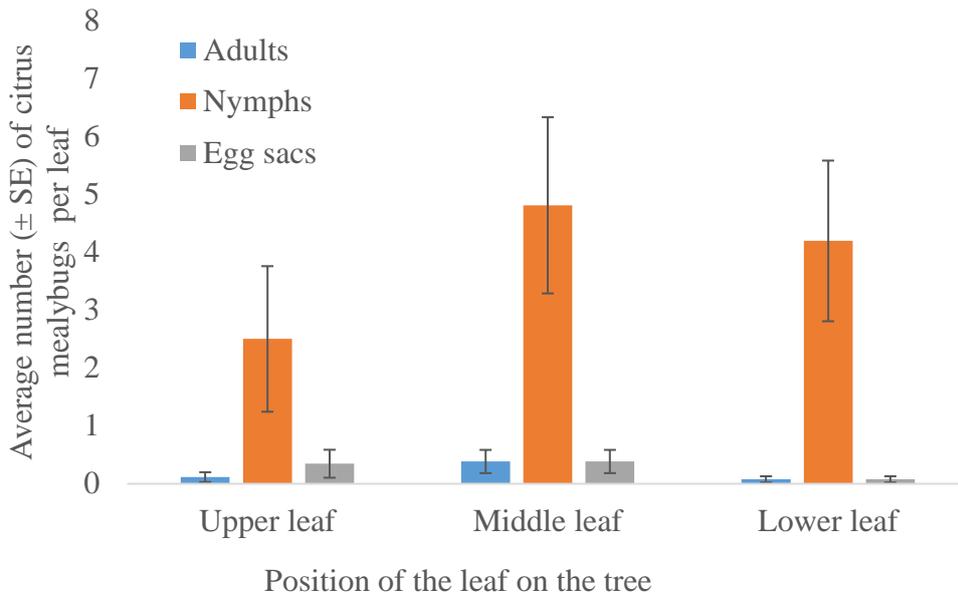
At 151 days, low numbers of citrus mealybug adults and nymphs were found on the leaves of plants under the net. No citrus mealybug egg sacs were found (Fig. 3.4.2.8).



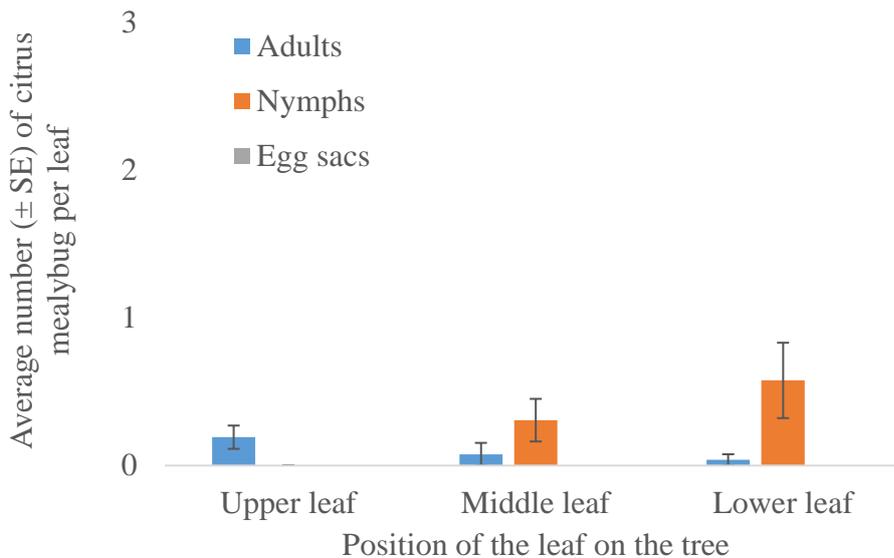
**Figure 3.4.2.5.** Average number ( $\pm$  SE) of citrus mealybug nymphs per leaf for trees under the net and in the open at 65 days.



**Figure 3.4.2.6.** Average number ( $\pm$  SE) of citrus mealybugs (adults and nymphs) per leaf for trees under the net and in the open at 112 days.



**Figure 3.4.2.7.** Average number ( $\pm$  SE) of the different citrus mealybug life stages (adults, nymphs and egg sacs) per leaf for trees under the net at 112 days.



**Figure 3.4.2.8.** Average number ( $\pm$  SE) of the different citrus mealybug life stages (adults and nymphs) per leaf for trees under the net at 151 days.

### Objective 3

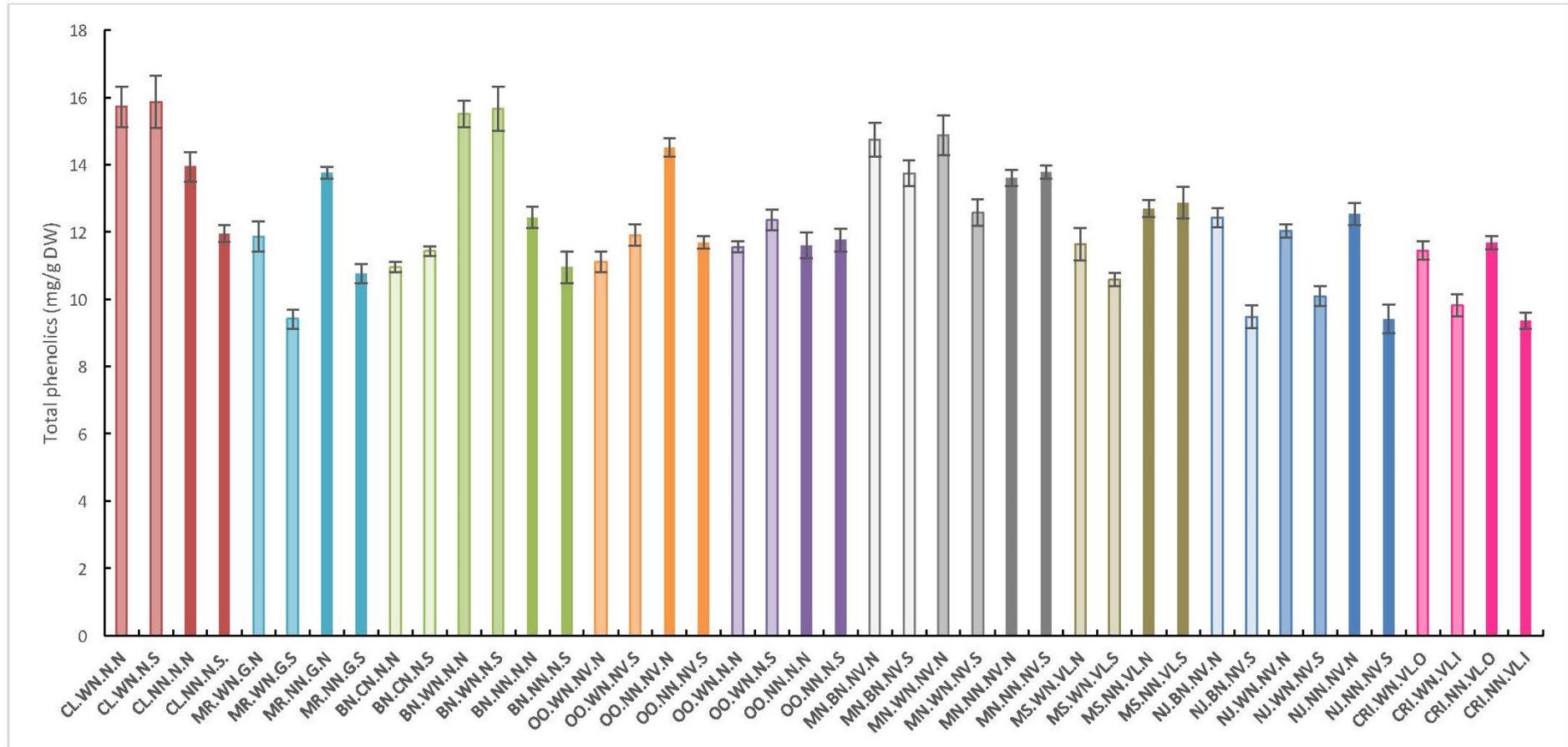
Measuring of total phenolics in November and December 2018 and April 2019 in leaves from commercial farms generally showed higher levels on the northern sides of the trees compared with the southern sides and not much difference between plants under net and in the open (Figs. 3.4.2.9 and 3.4.2.10). However, two sites with Nadorcotts had unusual results that gave higher phenolics under white net than in the open. Reduction in PAR and UV-B radiation due to 20% nets was variable but the mean PAR reduction was by 20.3% when there was little cloud cover and the UV-B mean reduction was 26.4% (Table 3.4.2.2). It therefore does not appear that differences in UV-B radiation under 20% net can be responsible for the increased pest status of citrus mealybug through production of variable amounts of total phenolics that act as antifeedants.

**Table 3.4.2.2.** Reduction in PAR and UV-B radiation under 20% net at different commercial farms

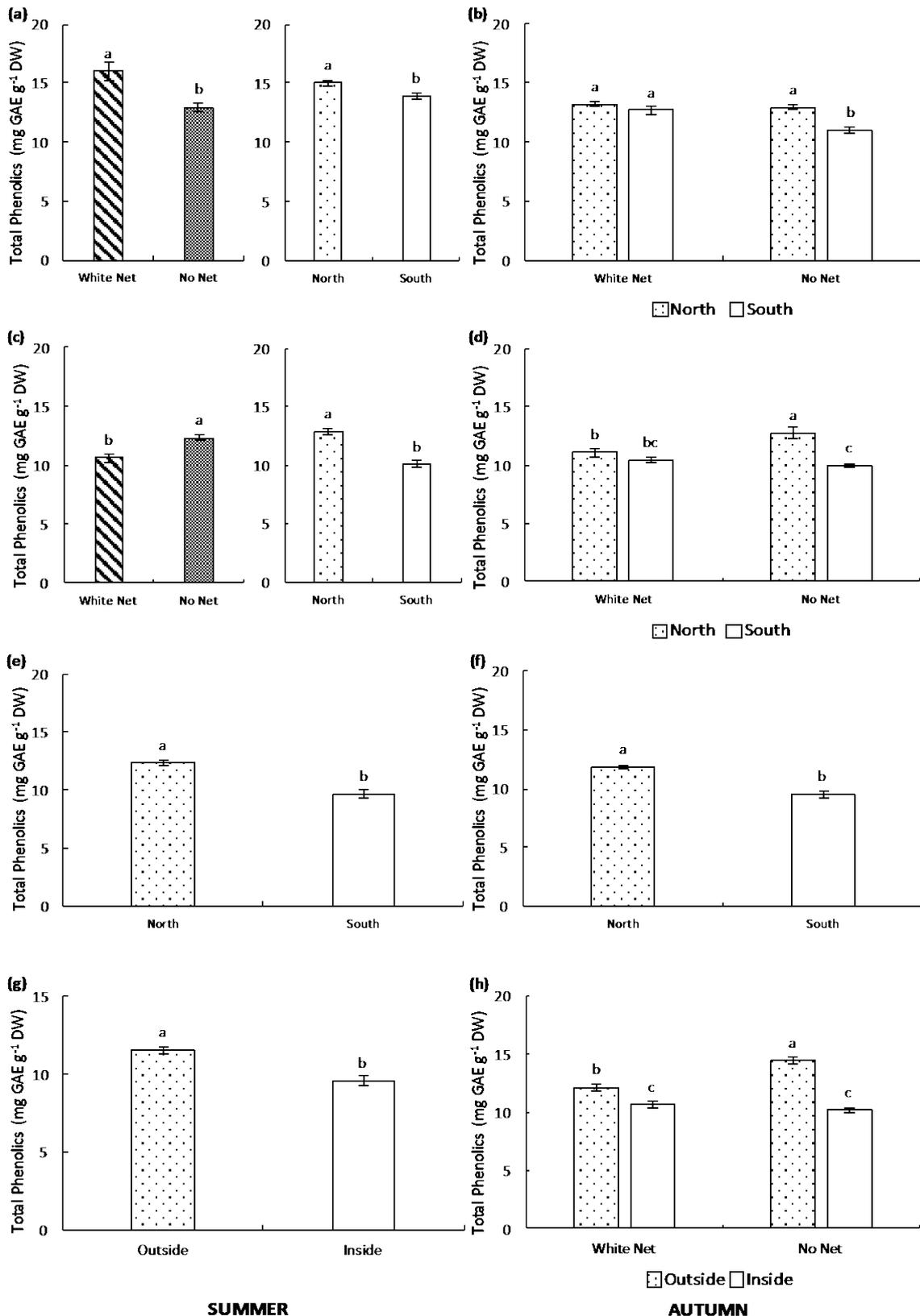
Site	Net colour	Date	Cloud %	Reps	% Reduction Net vs No net	
					PAR	UV-B
CRI, Nelspruit	White	13 11 2018	?	1	41.9	34.7
Indigo	White	06 03 2019	10	2	17.2	28.3
Indigo	White	11 12 2018	30 - 50	2	51.9	43.7
Indigo	Crystal	04 12 2018	80 - 100	2	1.8	-0.2
Indigo	Crystal	06 03 2019	10	2	17.9	18.5
Indigo	Crystal	11 12 2018	80 - 100	2	8.4	16.7
Joubert & Sons	Blue	14 11 2018	100	1	33.4	26.6
Joubert & Sons	White/blue	14 11 2018	100	1	25.7	22.3
Joubert & Sons	Blue	14 11 2018	0 -10	1	24.5	32.5
Joubert & Sons	White/blue	14 11 2018	0 -10	1	22.2	29.3
Joubert & Sons	Blue	28 02 2019	5	2	24.2	36.5
Joubert & Sons	White/blue	28 02 2019	5	2	17.7	23.1
Larten	White	21 11 2018	0	1	18.9	17.1
Nyawa	Blue	14 03 2019	5	2	21.1	29.6
Nyawa	White	14 03 2019	5	2	17.3	25.6
Nyawa	Blue	20 11 2018	100	1	14.4	22.5
Nyawa	White	20 11 2018	100	1	13.5	23.4
Nyawa	Blue	26 11 2018	100	1	28.3	15.5
Nyawa	White	26 11 2018	100	1	22.9	19.2
Ohrpack-Nadorcott	White	04 12 2018	5 - 10	2	23.1	16.1
Ohrpack-Nadorcott	White	06 03 2019	2	2	12.3	21.6
Ohrpack-Navels	White	04 12 2018	5 - 10	2	40.0	41.5
Ohrpack-Navels	White	06 03 2019	5	2	15.5	28.3
Riverside	White	22 11 2018	5 - 10	2	16.1	18.9
Riverside	White	27 02 2019	5	1	21.6	25.9
Schoeman Boerdery	White	14 03 2019	5	2	18.3	36.8
Schoeman Boerdery	White	20 11 2018	100	1	-1.9	28.8
Schoeman Boerdery	White	26 11 2018	10	1	16.7	18.5

Average reduction - when cloud cover 10% or less		20.3	26.4
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Average reduction - when cloud cover 100%		21.0	27.9
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**Figure 3.4.2.9.** Total phenolics in different samples from the first round of samples in summer 2018 where one colour represents a cultivar at a site, WN = white net, CN = crystal net, BN = blue net, NN = no net. The last letter N or S represents north or south facing.



**Figure 3.4.2.10.** Total phenolic content in *Citrus* spp. leaves sampled from trees located at Larten (area: Karino, cv. Nadorcott) (a & b); Riverside (area: Malelane, cv. Ruby star) (c & d), Joubert and Sons (area: Nelspruit, cv. Glen Ora) (e & f) and at Citrus Research International (area: Nelspruit, cv. Midnight) (g & h). Leaves were sampled in the summer of 2018 (a, c, e & g) and in the autumn of 2019 (b, d, f & h) from trees that were covered (White Net) or uncovered (No Net) with shade netting. The leaves were collected from the northern (North) and southern (South) side of the trees (a – f) and the outside and inside of potted trees at CRI (g & h). Bars represent the treatment means (n = 8), and whiskers indicate 1 standard error. Significant

differences at  $P < 0.05$  are shown by lettering above the whiskers and when no interaction was detected between the factors (i.e. sampling side and cover type) significant main effects are presented for each location.

Fruit were picked from three sites in February 2019 and another site in March 2019 to compare chemical residues on fruit under net and in the open. There were very few discernible differences (Table 3.4.2.3) and only a trend for a slight increase in strobilurin residues under net, but none of the residues would have been problematic at harvest a few months later.

**Table 3.4.2.3.** Chemical residues in ppm found in whole fruit collected from commercial farms under 20% net and in the open.

Samples collected: 27 Feb 2019

<b>Riverside - Star Ruby</b>	<b>White net</b>	<b>No net</b>
	Result (mg/kg)	Result (mg/kg)
Dithiocarbamates	1.1	1.2
Pyraclostrobin	0.035	0.025

<b>Larten - Nadorcott</b>	<b>White net</b>	<b>No net</b>
	Result (mg/kg)	Result (mg/kg)
Dithiocarbamates	5.4	3.9
Azoxystrobin	0.019	0.019
Imidacloprid	0.066	0.067

<b>Joubert &amp; Sons - Navels</b>	<b>White net</b>	<b>No net</b>
	Result (mg/kg)	Result (mg/kg)
Dithiocarbamates	0.22	0.19
Carbendazim	0.11	0.53
Pyraclostrobin	0.0131	0.0086
Tebuconazole	0.017	0.0098
Trifloxystrobin	0.058	0.04
Mercaptothion/Malathion	-	0.022

Samples collected: 14 March 2019

<b>Nyawa (Neels Kok Citrus)</b>	<b>Blue net</b>	<b>White net</b>	<b>No net</b>
	Result (mg/kg)	Result (mg/kg)	Result (mg/kg)
Dithiocarbamates	1.6	0.65	1.3
Carbendazim	0.014	0.017	0.013
Imidacloprid	None	None	0.020 <sup>1</sup>
Pyriproxyfen	0.022	0.023	0.030
Trifloxystrobin	0.058	0.035	0.033

<sup>1</sup> Farm management stated that no Imidacloprid applied this season.

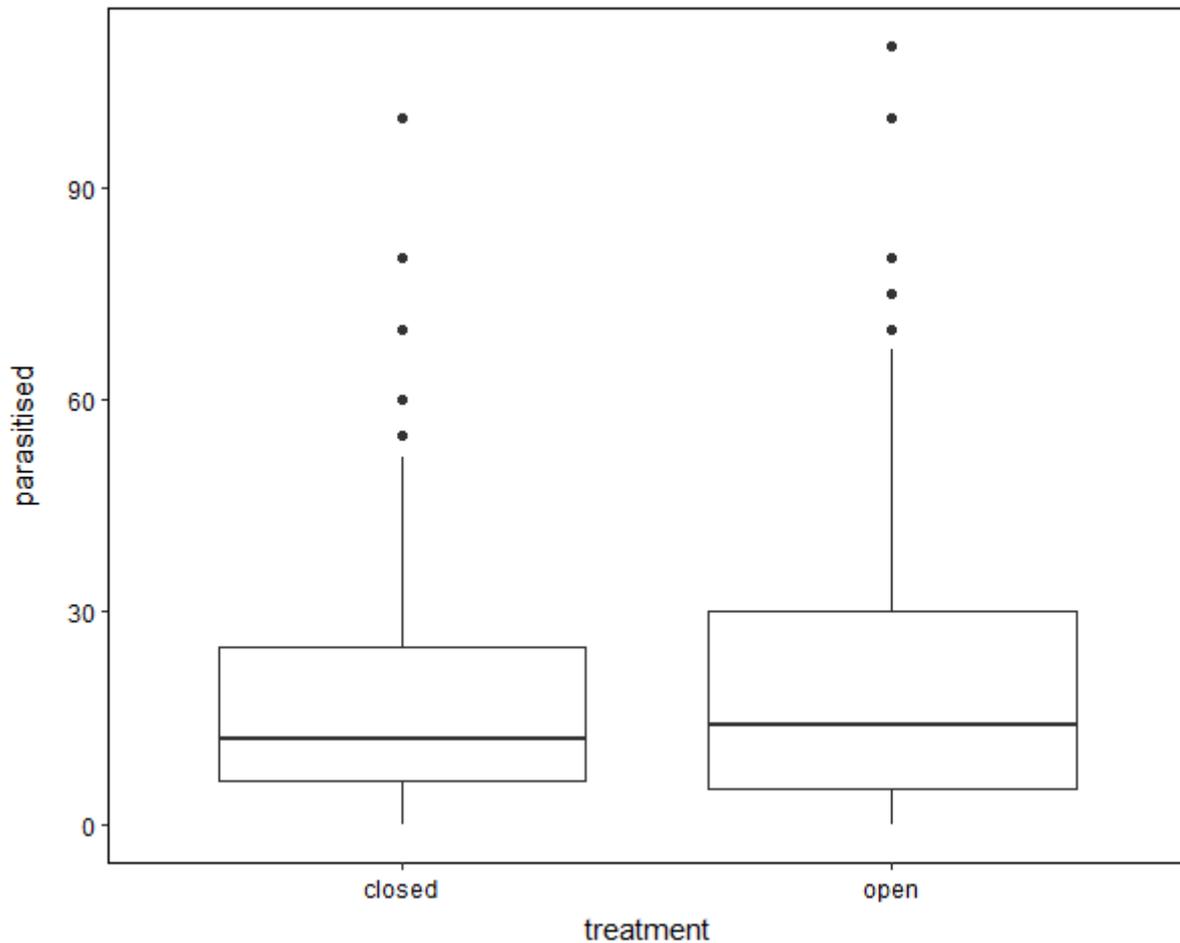
#### Objective 4

Due to there being no differences in total phenolics between potted plants kept in the sun and those under 20% white net for several months at Nelspruit, this objective to compare rates of infestation in the two different environments was not addressed.

#### Objective 5

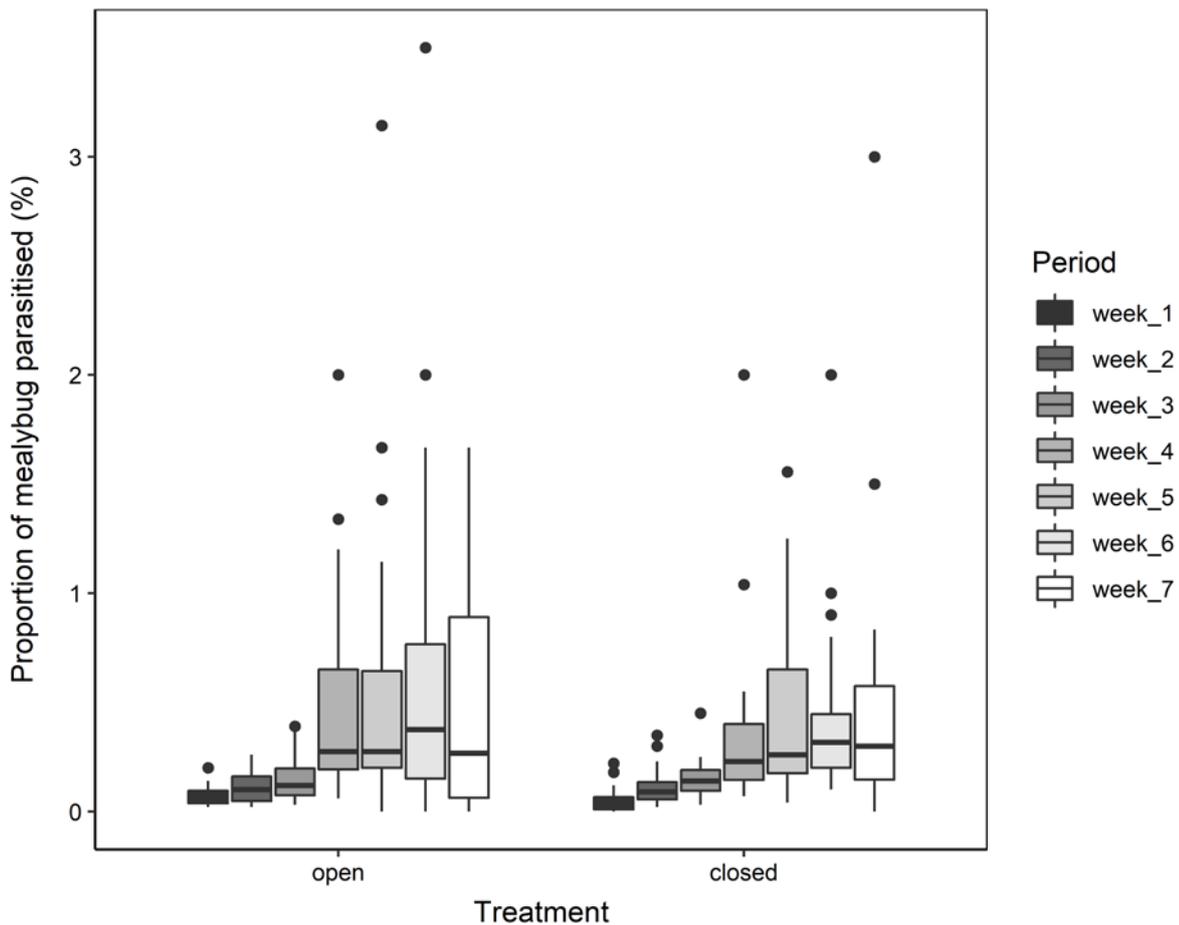
##### Part one: *Anagyrus vladimiri* experiment

Over the observation period, numbers of citrus mealybug parasitized by *A. vladimiri* in open containers and those covered with 20% net did not differ significantly (Fig. 3.4.2.11).



**Figure 3.4.2.11.** Numbers of parasitized mealybug in closed (20% shade net) and open containers.

Comparing treatment and time had shown that there was little to no difference in the parasitized mealybugs under 20% shade net and in the open. However, 20% shade net had shown an increase in parasitism during weeks 4, 5 and 6 (Fig. 3.4.2.12). The open treatment showed a constant rate of parasitism throughout the period of the experiment with the least number of outliers. The open treatment did show a decrease in parasitism in week 7 perhaps due to the mealybug being under complete control from the parasitoid (Fig. 3.4.2.12).

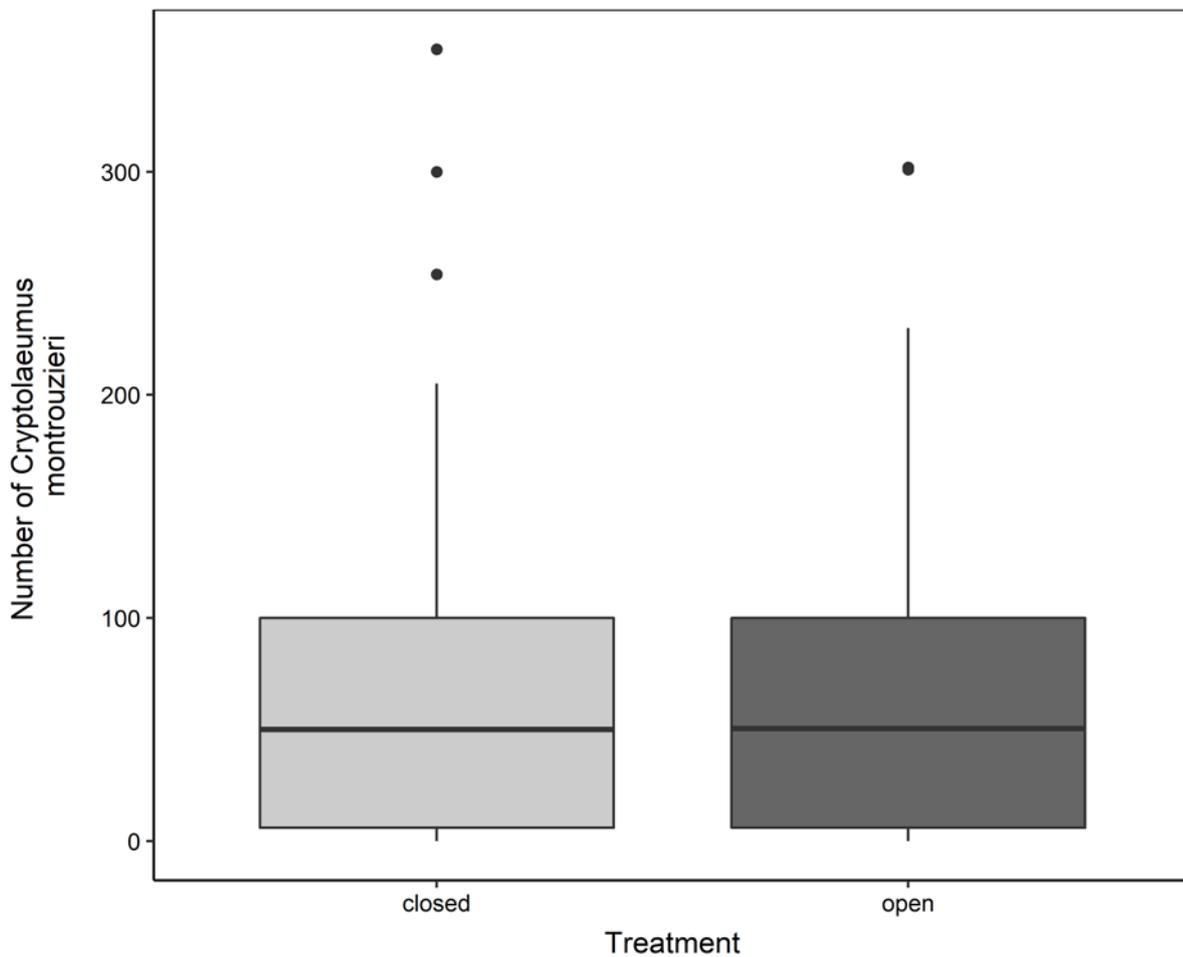


**Figure 3.4.2.12.** Percentage of mealybug parasitised by *Anagyrus vladimiri* for different treatments and weeks, averaged across treatment.

Part two: *Cryptolaemus montrouzieri* experiment

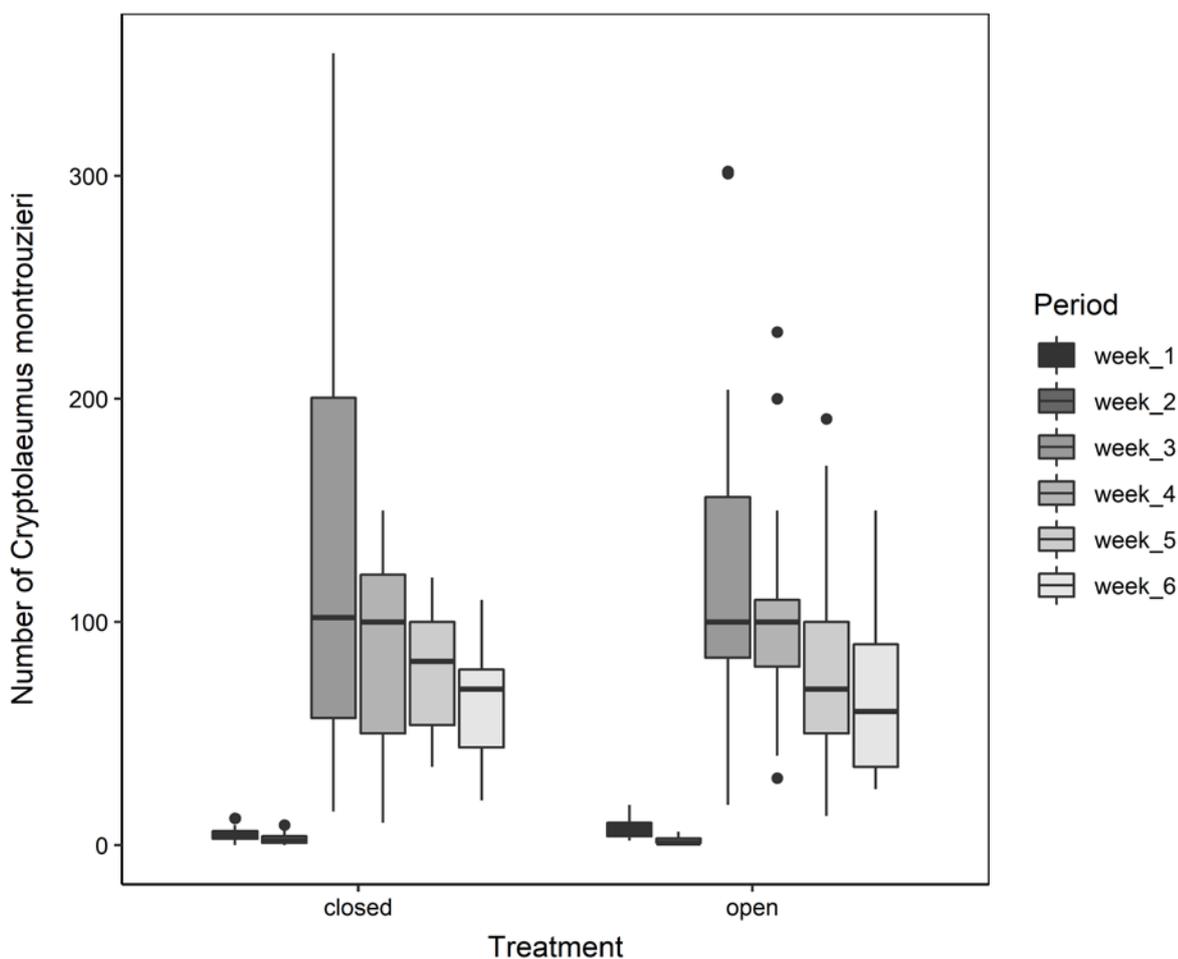
The experiment was repeated using the natural enemy *Cryptolaemus montrouzieri* to determine the ability of the beetle to find citrus mealybugs on a butternut from outside 20% shade net and in open containers.

There was no difference in the ability of the beetle to find citrus mealybugs in the experimental containers with 20% shade net or open containers. The closed treatment showed the highest number of outliers with high beetle numbers inside. This might be due to beetles having completely controlled mealybugs in the open containers and then moving into the 20% shade net containers in search of food (Fig. 3.4.2.13).



**Figure 3.4.2.13.** Boxplot showing two treatments (closed and open) versus the total number of *Cryptolaemus montrouzieri* inside the replicates during the experiment.

Furthermore, there was no difference in the numbers of beetles per week per treatment with an average of 64.07 beetles in the closed treatment and an average of 64.13 beetles in the open treatment, further indicating the ability of the beetles to be able to move in and out of 20% white shade net (Fig. 3.4.2.14). The beetles did not find the net as an obstruction to finding mealybug. The open treatment had more outliers than the closed 20% white shade net treatment.



**Figure 3.4.2.14.** Boxplot showing total numbers of *Cryptolaemus montrouzieri* beetles per week in the two treatments (closed with 20% white shade net and open).

### Conclusions

1. Infestation of citrus mealybug on Midnight Valencia plants in pots increased on plants under 20% white net before slowly declining, whereas infestations on plants in the open declined rapidly to zero. It is assumed that this is due to variable impact of natural enemies in the two environments.
2. UV-B radiation was reduced by 26% on average under shade net but any resultant effect on levels of total phenolics in foliage that could act as antifeedants was not consistent. It is therefore unlikely that phenolics are directly responsible for the increase in pest status of mealybugs under nets.
3. Nets did not cause significant increases in chemical residues of whole fruit on the commercial farms tested.
4. Infestations by mealybug, mites and red scale increased significantly under 20% white net, although the reason for this could not be determined.
5. There was no difference in efficacy of the parasitoid *Anagyrus vladimiri* against citrus mealybug under 20% white shade net or in open containers, so its effect does not appear to be compromised.
6. Furthermore, there was no difference in the ability of the beetle *Cryptolaemus montrouzieri* to find and predate on the mealybugs under 20% shade net, bringing them to complete control. In both treatments, citrus mealybug was controlled by week 4 or week 5 and the beetles started to disperse in search of food. This indicated that 20% white shade net should not compromise the beetle's ability to provide control under net or in an open orchard.

Growers should be warned that the pest status of mealybug, red scale, citrus red mite and silver mite are likely to increase in orchards under net.

## Technology transfer

Results will be presented at the 2022 Citrus Research Symposium in the Drakensberg.

## Acknowledgements

We thank Dr Leandra Moller at Stellenbosch University for her work in quantifying the total phenolics in leaf samples from trees under nets and in the open.

## Future research

No further research on this topic is planned. Growers will need to adapt to growing citrus under net as they do to a different climatic region or soil type.

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- 3.4.3 **FINAL REPORT: Determining the LC<sub>50</sub> and LC<sub>90</sub> values for spinetoram on *Scirtothrips aurantii***  
Project 1284 by S Moore, M Peyper, T Marsberg, L Cousins, W Kirkman (CRI) and M van der Merwe (RU)

## Summary

Due to queries about the efficacy of spinetoram against citrus thrips in the Sundays River Valley, Corteva Agriscience contracted CRI to conduct some baseline efficacy studies with spinetoram against field collected citrus thrips in dose-response bioassays. These were conducted on three occasions in Munger cells. However, the comprehensiveness of the bioassays differed, based on how many thrips could be collected. Ultimately, we were able to collect sufficient thrips to calculate an LC<sub>50</sub> and estimate an LC<sub>90</sub> for spinetoram against citrus thrips in the Sundays River Valley. Furthermore, a comparison was conducted between thrips from a site where resistance to spinetoram was suspected and a site where spinetoram had not been frequently used and was reported to be working well. Bioassays indicated no sign of resistance. Although we now have a benchmark for thrips susceptibility to spinetoram in the Sundays River Valley, it is recommended that similar bioassays be conducted with citrus thrips collected from a site where there has never been any exposure to

spinetoram or spinosad, or if this is not possible, as was the case in Sundays River Valley, where last exposure occurred at least 12 months previously.

## Opsomming

As gevolg van navrae oor die doeltreffendheid van spinetoram teen sitrusblaaspootjies in die Sondagsriviervallei, het Corteva Agriscience CRI gekontrakteer om 'n paar basislyn doeltreffendheidstudies met spinetoram teen veldversamelde sitrusblaaspootjies in dosis-respons biotoetse uit te voer. Dit is by drie geleenthede in Munger-selle uitgevoer. Die omvattendheid van die biotoetse het egter verskil, gebaseer op hoeveel blaaspootjies versamel kon word. Uiteindelik kon ons genoegsame blaaspootjies insamel om 'n LC<sub>50</sub> te bereken en 'n LC<sub>90</sub> te skat vir spinetoram teen sitrusblaaspootjies in die Sondagsriviervallei. Verder is 'n vergelyking gedoen tussen blaaspootjies vanaf 'n perseel waar weerstandbiedendheid teen spinetoram vermoed is en 'n perseel waar spinetoram nie gereeld gebruik is nie en blykbaar nog goed gewerk het. Biotoetse het geen teken van weerstandbiedendheid getoon nie. Alhoewel ons nou 'n maatstaf het vir blaaspootjie vatbaarheid vir spinetoram in die Sondagsriviervallei, word dit aanbeveel dat soortgelyke biotoetse uitgevoer word met sitrus blaaspootjies wat versamel is vanaf 'n perseel waar daar nog nooit enige blootstelling aan spinetoram of spinosad was nie. Indien dit nie moontlik is nie, soos wat die geval in Sondagsriviervallei was, dan waar die laaste blootstelling minstens 12 maande tevore plaasgevind het.

## Introduction

For the last few seasons, there have been reports of sub-optimal efficacy with spinetoram (Delegate 250 WG) against citrus thrips, *Scirtothrips aurantii*, on citrus in the Sundays River Valley, Eastern Cape, and elsewhere in South Africa. Consequently, Corteva Agriscience contracted CRI to try to clarify the existence and status of thrips resistance to Delegate 250 WG in the Sundays River Valley, through laboratory bioassays with field collected thrips populations. Thrips resistance to spinetoram has previously been recorded in other thrips species elsewhere (e.g. Fu *et al.* 2018; Shi *et al.* 2020; Gao *et al.* 2021).

## Stated objectives

- A. To obtain baseline dose-response values for spinetoram against citrus thrips in the Sundays River Valley.
- B. To establish if there are any signs of resistance by citrus thrips to spinetoram in the Sundays River Valley.

## Materials and methods

One trial was conducted during each of 2019, 2020 and 2021. The protocol used in 2019 and in the subsequent two years differed slightly, as Corteva Agriscience only provided the protocol (EA19T6P021E – Appendix 1) during 2020, based on the IRAC method, being similar to that reported by Doria *et al.* (2017).

### General collection and bioassay protocols

Several young twigs, each with several fully unfurled leaves, were collected from a citrus orchard that had not recently received any insecticidal sprays. For the 2019 trial, twigs were hung from tree branches in the garden outside the CRI research laboratories in Gqeberha. One twig of leaves was sprayed with water and one twig of leaves was sprayed with each of the Delegate 250WG treatments (concentrations) used in the trial. Treatments were applied with a 500 ml capacity hand-held pressurised spray bottle, as diffuse sprays, emulating the coverage recommended in commercial field application. As soon as leaves were completely dry, shortly before 17h00 on the same date, twigs were recovered and kept in the laboratory at room temperature overnight. For the 2020 and 2021 trials, the leaves were individually dipped in the treatments for 15 s and hung to air dry in the same manner as described above.

In the first trial in 2019, only the registered rate of Delegate 250 WG and half the registered rate of Delegate 250 WG were used (25 and 12.5 ppm), as well as a water-treated control, whereas in the subsequent two trials, a four-fold serial dilution was prepared (Table 3.4.3.1).

**Table 3.4.3.1.** A four-fold dilution series of Delegate 250 WG treatments prepared for bioassays with citrus thrips (based on protocol EA19T6P021E).

Treatments	Concentration	
	Mass (g) per 100 L water	Parts per million (ppm) active ingredient (ai)
1 (control)	0	0
2	0.625	1.56
3	2.500	6.25
4 <sup>1</sup>	10.000	25.00
5	40.000	100.00
6	160.000	400.00

<sup>1</sup>This is the rate of Delegate 250 WG registered against citrus thrips on citrus in South Africa.

The following morning, three (2019) or four (2020 and 2021) modified Munger cells were loaded with treated leaves, for each of the treatments. Munger cells were designed as per Morse *et al.* (1986) and Grout *et al.* (1996). For each cell, a thin layer of polyurethane foam was saturated with water and placed on a rectangle of glass 65 x 100 mm. The air-dried, treated leaf was placed on the foam with the abaxial surface uppermost. The centre of the cell comprised a slab of Perspex with a 33 mm diameter hole. Two additional holes were drilled through opposite edges of the slab to provide ventilation via screened openings into the central hole. Plasticine (Giotto, Pero, Italy) was applied in a ring around the large central hole in the Perspex to form a waterproof gasket between the leaf and Perspex. A second rectangle of glass with the same dimensions as the first formed the top of the cell. This top section of glass had a hole through the middle, approximately one third of its length from one end. Once the cells had been perfectly manipulated into position, they were held together with clamps. Munger cells were transported to the field in a cooler box with frozen ice-bricks.

Thrips were collected from the sites (citrus orchards) identified as suitable for the trial. This was done by beating new growth over a black plastic tray using a flexible length of irrigation tubing (Fig. 3.4.3.1). Thrips were transferred from the tray to the cells with a fine paintbrush (size 000). In 2019 we attempted to transfer 30-40 thrips into each cell, whereas in 2020 and 2021, we aimed at loading 20-30 thrips per cell. In 2019, both adults and second instars were collected, whereas in the subsequent two years' trials, only adults (of both sexes) were collected. The cells were returned to the cooler box as soon as the requisite number of thrips had been placed into them.



**Figure 3.4.3.1.** Collecting thrips from new flush on citrus trees, using a beating tray.

Immediately on return to the laboratory from the field, each cell was examined under a stereomicroscope and the numbers of citrus thrips that had died from injury or some cause other than that of the toxicant, were noted. The cells were then maintained in the laboratory at approximately 26°C for 24 or 48 h, using an i-button to log temperature and humidity readings at 5 min intervals. All cells were connected in parallel, by polyethylene piping, to an air pump, set at an air flow rate of 5 L/min (Fig. 3.4.3.2). After 24 h, and again after 48 h in the 2020 and 2021 trials, the numbers of dead thrips in each cell were determined before immobilizing the survivors by placing the Munger cells in a freezer at approximately -20°C for at least 30 min. The number of dead thrips recorded upon returning to the laboratory was subtracted from the number of dead thrips at the end of the 24-h and 48-h-periods and the total numbers of thrips per cell were also adjusted accordingly. Additionally, life stages of all thrips were recorded, in order to confirm that we had indeed collected what we had intended to.



**Figure 3.4.3.2.** An assembled Munger cell connected in parallel to a plenum for air flow.

In all three trials, mean values of treatments (mortality) were compared in Statistica (TIBCO Software Inc. (2020), Data Science Workbench, version 14. <http://tibco.com>), using a Generalised Linear Model (GLM) to conduct an ANOVA, followed by a post-hoc test (either Fisher or Bonferroni). In 2019, values between sites were compared in the same way, but treatment mortalities were first corrected for the control mortality (Abbott, 1925). A probit analysis was conducted on the 2021 data, using RStudio 2021.09.0+351 and R 3.6.1., after which the LC<sub>50</sub> (concentration required to kill 50% of individuals in a sample) was determined.

### 2019

A citrus orchard was identified where there was suspected citrus thrips resistance to Delegate 250 WG and where Delegate 250 WG had been used recently. A second citrus orchard was identified where Delegate 250 WG had historically been used a maximum of once per year, where it had not yet been used in the current season and where there was no report of inadequate efficacy with Delegate 250 WG. Both sites were in the Sundays River Valley, Eastern Cape (Table 3.4.3.2).

**Table 3.4.3.2.** Details of sites where thrips were collected, suspected to be resistant/tolerant<sup>1</sup> to Delegate 250 WG and suspected to be susceptible to Delegate 250 WG.

	“Resistant” site	“Susceptible” site
<b>Farm</b>	Vleiview, Dunbrody	Tweeling, Kirkwood
<b>Orchard number</b>	34E	7
<b>Coordinates</b>	33.452350 25.557597	33.404258 25.396372
<b>Cultivar</b>	Genoa lemon	Washington Navel
<b>Rootstock</b>	Carrizo	Carrizo
<b>Year planted</b>	2015	2014
<b>Tree spacing (row x tree)</b>	7 x 4 m	5.4 x 3 m

<sup>1</sup>In the remainder of the report, we will simply refer to “resistance”.

On 18 November 2019, fresh unfurled leaves on twigs were collected from Orchard 7 at Tweeling. The last spray applied to that orchard was tau-fluvalinate, which was applied on 1 October for thrips control. No residues would have been in the collected leaves, both because this insecticide was applied 48 days previously and

because the flush collected was new and had therefore not been exposed to the treatment, and tau-fluvalinate is not systemic.

Twigs were treated at 15h00 on 18 November 2019, as previously described. At 07h00, the following morning (19 November 2019), Munger cells were loaded with the treated leaves as described above. Thrips were collected from both sites on 19 November 2019: from 09h15 to 10h45 at Vleiview and from 11h15 to 12h45 at Tweeling.

## 2020

A citrus nursery was identified on Tweeling Farm (-33.402975, 25.397831), the previously used Delegate-susceptible site, where limited use of Delegate 250 WG had been made and satisfactory efficacy against citrus thrips was reported. Several sites where inadequate efficacy of Delegate 250 WG was reported were identified and followed up, but very low levels or no thrips were found at all sites, making collection of suspected Delegate-resistant thrips impossible.

On 25 November 2020, young leaves on twigs were collected from the same Palmer Navel orchard as previously used (Orchard 7 at Tweeling), again ensuring no recent insecticide applications, and dipped in the treatments in Table 3.4.3.1 at 15h00 on the same day, as previously described.

At 06h45 the following morning (26 November 2020), Munger cells were loaded with the treatments. Thrips were collected from small potted trees of a range of different cultivars in the nursery from 09h00 to 14h00 on 26 November 2020. Only sufficient thrips for the following treatments could be collected: water control, 25 ppm and 100 ppm. Immediately on return to the laboratory, at 15h00, Munger cells were checked for mortality, before attaching them to the plenum in the insectary.

## 2021

In March 2021, a suspected Delegate-susceptible orchard on Hermiston Farm in the Sundays River Valley was identified (Table 3.4.3.3), with sufficient thrips to conduct bioassays with the full suite of intended Delegate 250 WG concentrations. Unfortunately, once again, all sites that reported dissatisfaction with the efficacy of Delegate 250 WG, had insufficient thrips to collect for bioassays. Furthermore, not a single farm in the Sundays River Valley could be identified where no spinetoram or spinosad had been used and consequently no site could with absolute certainty be declared as fully Delegate-susceptible.

**Table 3.4.3.3.** Details of the site where thrips were collected in 2021, suspected to be susceptible to Delegate 250 WG.

<b>Farm</b>	<b>Hermiston, Heritage, Addo</b>
Orchard number	18
Coordinates	-33.538678 25.673869
Cultivar	Eureka Lemons
Year planted	2018
Tree spacing (row x tree)	6 x 3 m

Several young lemon leaves were collected and treated on the day preceding the thrips collection, on 17 March 2021. The following morning (18 March 2021) the Munger cells were prepared for each treatment. Thrips were collected from Hermiston on 18 March 2021, from 09h00 to 13h00.

## **Results**

### 2019

Precisely the same number of citrus thrips were collected from each site, i.e. a total of 264 thrips. These were the totals once the number of initially dead thrips had been deducted. Mean numbers of thrips per life stage and per treatment per site are provided in Table 3.4.3.4.

**Table 3.4.3.4.** Mean ( $\pm$  SE) numbers of thrips larvae (second instars) and adults used per replicate and treatment from each site used in 2019.

Treatment (ppm ai <sup>1</sup> )	Vleiview (R <sup>3</sup> )			Tweeling (S <sup>4</sup> )		
	Larvae	Adults	Combined	Larvae	Adults	Combined
0 (control)	15.33 $\pm$ 4.18	15.00 $\pm$ 6.00	30.33 $\pm$ 9.02	27.67 $\pm$ 3.28	8.67 $\pm$ 3.71	36.33 $\pm$ 6.84
6.25	3.33 $\pm$ 0.33	21.00 $\pm$ 4.16	24.33 $\pm$ 3.93	18.67 $\pm$ 3.28	13.00 $\pm$ 4.73	31.67 $\pm$ 8.01
25.00 <sup>2</sup>	4.00 $\pm$ 0.58	29.33 $\pm$ 1.20	33.33 $\pm$ 0.88	9.00 $\pm$ 4.04	11.00 $\pm$ 3.51	20.00 $\pm$ 4.51

<sup>1</sup>ppm ai=parts per million active ingredient

<sup>2</sup>This is the registered rate for citrus thrips control

<sup>3</sup>R=site where resistance was suspected

<sup>4</sup>S=site where susceptibility was suspected

Mortality for citrus thrips larvae and adults varied, with neither life stage appearing to be consistently more susceptible or tolerant to Delegate 250 WG (raw data in Appendix 2). Consequently, mortality of the two life stages was combined. This was justifiable, as Grout *et al.* (1996) also determined no difference in insecticidal susceptibility between second instar and adult citrus thrips in similar bioassays, using tartar emetic. Control mortality at Vleiview was 27.0% and at Tweeling was 10.2%. Mortality for both Delegate 250 WG treatments at both sites was significantly higher than mortality for both untreated controls. There was no significant difference in mortality between any of the four Delegate 250 WG treatments, ranging from 59.1% to 69.8% (Table 3.4.3.5). There was also no significant difference in mortality between the two untreated controls. However, in the case of Vleiview, control mortality for both adults and adults and larvae combined was above 20%. This would have compromised the reliability of the treatment related mortality and would thus have made the data unsuitable for a probit analysis, if there were indeed sufficient treatments in order to calculate this. This relatively high level of control mortality was most likely due to handling. Thrips are small fragile insects and great care is required in order not to damage them during collection.

**Table 3.4.3.5.** Mortality (%) ( $\pm$  SE) of citrus thrips larvae and adults used per replicate and treatment from each site in 2019.

Treatment (ppm ai) <sup>1</sup>	Vleiview (R <sup>3</sup> )			Tweeling (S <sup>4</sup> )		
	2 <sup>nd</sup> instar	Adults	Combined <sup>5</sup>	2 <sup>nd</sup> instar	Adults	Combined <sup>5</sup>
0 (control)	9.26 $\pm$ 4.90	52.59 $\pm$ 9.42	26.96 $\pm$ 3.09a	6.78 $\pm$ 4.14	15.56 $\pm$ 15.56	10.25 $\pm$ 5.48a
6.25	77.78 $\pm$ 22.22	66.34 $\pm$ 16.51	69.79 $\pm$ 11.36b	46.11 $\pm$ 12.03	88.53 $\pm$ 8.17	63.07 $\pm$ 7.87b
25.00 <sup>2</sup>	50.00 $\pm$ 28.87	64.11 $\pm$ 12.29	64.02 $\pm$ 10.74b	66.67 $\pm$ 33.33	58.73 $\pm$ 7.94	59.12 $\pm$ 12.20b

<sup>1</sup>ppm ai=parts per million active ingredient

<sup>2</sup>This is the registered rate for citrus thrips control

<sup>3</sup>R=site where resistance was suspected

<sup>4</sup>S=site where susceptibility was suspected

<sup>5</sup>Values followed by a different letter in the two "Combined" columns were significantly different ( $P < 0.05$ ; Fisher multiple range test; GLM).

When mortality levels for the two Delegate 250 WG treatments at the two sites were corrected for control mortality, there was again little difference in mortality between any of the four treatments, ranging from 41.3% to 49.6% (Table 3.4.3.6). There were no significant differences between any of the treatments, both within and between sites ( $P > 0.05$ ), indicating that Delegate 250 WG concentration did not make a difference and that there was no difference in thrips susceptibility to Delegate 250 WG between the two sites. As previously mentioned, the accuracy of the data from Vleiview may be compromised, due to the relatively high level of control mortality (Table 3.4.3.5). However, on the other hand, despite Abbot's (1925) correction being greater for the Vleiview mortality (i.e. reducing the Vleiview treatment mortality to a greater extent than was the case for the Tweeling treatment mortality), there was still no significant difference between the levels of mortality.

**Table 3.4.3.6.** Mortality (%) of thrips (larvae and adults combined), corrected for control mortality, for two concentrations of Delegate 250 WG at each of two sites used in 2019. (None of the values differed significantly from one another ( $P > 0.05$ ; Bonferroni post-hoc test, GLM).

Treatment (ppm ai) <sup>1</sup>	Vleiview (R <sup>3</sup> )	Tweeling (S <sup>4</sup> )
6.25	43.96	47.27
25.00 <sup>2</sup>	41.30	49.58

<sup>1</sup>ppm ai=parts per million active ingredient

<sup>2</sup>This is the registered rate for citrus thrips control

<sup>3</sup>R=site where resistance was suspected

<sup>4</sup>S=site where susceptibility was suspected

## 2020

The mean numbers of adult thrips collected per Munger cell (after deducting the initial number of dead thrips at the onset of the trial) for each treatment is provided in Table 3.4.3.7.

**Table 3.4.3.7.** Mean ( $\pm$  SE) number of adult thrips used per replicate and treatment duration.

Treatment (ppm ai) <sup>1</sup>	Mean number thrips
0 (control)	25.33 $\pm$ 1.86
25.00 <sup>2</sup>	29.33 $\pm$ 4.06
100.00	21.67 $\pm$ 0.89

<sup>1</sup>ppm ai=parts per million active ingredient

<sup>2</sup>This is the registered rate for citrus thrips control

Control mortality after 24 h was 17.95% (Table 3.4.3.8). Mortality for both 25 ppm and 100 ppm after this duration were significantly greater than for the control. However, control mortality after 48 h was very high – 48.14%. Consequently, there was no longer any significant difference between control and treatment mortalities at this time (raw data in Appendix 2).

**Table 3.4.3.8.** Mortality (%) of thrips, for two concentrations of Delegate 250 WG collected from Tweeling Farm for two durations (24 and 48 h).

Treatment (ppm ai) <sup>1</sup>	Mortality (%) after duration <sup>3</sup>	
	24 h	48 h
0 (control)	17.95 $\pm$ 16.06a	46.14 $\pm$ 7.78a
25.00 <sup>2</sup>	56.63 $\pm$ 10.92b	60.97 $\pm$ 9.33a
100.00	64.27 $\pm$ 17.87b	73.60 $\pm$ 13.94a

<sup>1</sup>ppm ai=parts per million active ingredient

<sup>2</sup>This is the registered rate for citrus thrips control

<sup>3</sup>Values in the same column followed by different letters denote significant differences (Bonferroni post-hoc test; GLM;  $P < 0.05$ ).

## 2021

The mean numbers of adult thrips collected per Munger cell (after deducting the initial number of dead thrips at the onset of the trial) for each treatment is provided in Table 3.4.3.9.

**Table 3.4.3.9.** Mean ( $\pm$  SE) number of adult thrips used per replicate and treatment duration.

Treatment (ppm ai)	Mean number thrips
0 (control)	22.25 $\pm$ 3.71
1.56	24.75 $\pm$ 3.57

6.25	28.50 ± 2.10
25.00	27.50 ± 1.32
100.00	27.50 ± 2.90
400.00	24.00 ± 3.85

**Table 3.4.3.10.** Mortality (%) of adult thrips for five concentrations of Delegate 250 WG after each of two durations.

Treatment (ppm ai) <sup>1</sup>	Mortality (%) after duration <sup>3</sup>	
	24 h	48 h
0 (control)	4.17 ± 2.5a	36.01 ± 11.74a
1.56	31.94 ± 7.23ab	79.29 ± 6.97ab
6.25	20.09 ± 8.38a	61.68 ± 8.07ab
25.00 <sup>2</sup>	28.72 ± 8.49ab	67.86 ± 10.22ab
100.00	56.25 ± 5.87bc	83.80 ± 5.98ab
400.00	83.27 ± 8.91c	98.15 ± 1.85b

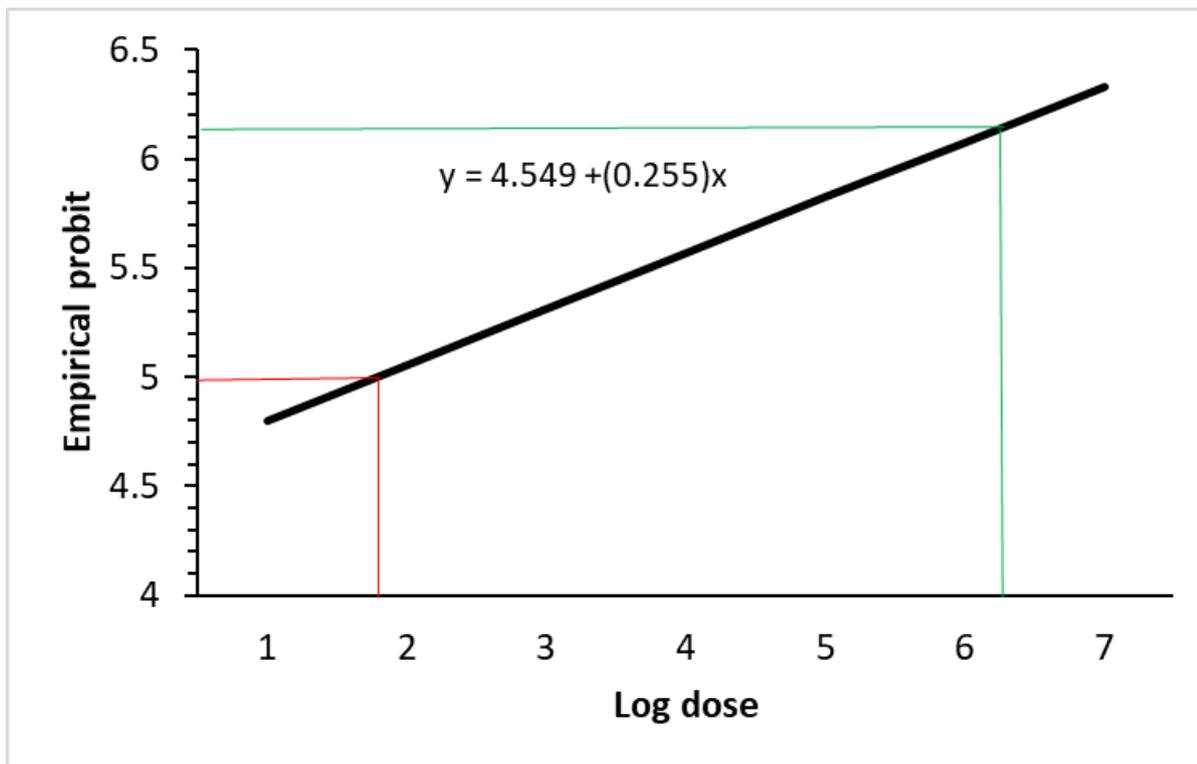
<sup>1</sup>ppm ai=parts per million active ingredient

<sup>2</sup>This is the registered rate for citrus thrips control

<sup>3</sup>Values in the same column followed by different letters denote significant differences, P < 0.05 (Bonferroni post-hoc test; GLM).

Control mortality after 24 h and 48 h was 2.70% and 36.49%, respectively. The treatment mortalities after 24 h ranged from 19.1% to 78.67%. Mortality from the 1.56 ppm, 6.25 ppm and 25 ppm treatments did not differ significantly from the control mortality. Mortality after 48 h ranged from 60.67% to 97.33%. Only the 400-ppm mortality was significantly different from control mortality (raw data in Appendix 2). However, results after 48 h cannot be considered as reliable, as control mortality was too high.

A dose-response curve for adults was generated for the 24 h exposure data from the trial conducted in 2021, using a probit analysis, with the regression equation of  $y = a + bx$ , where y is the empirical probit (mortality), x is the log dose, a is the intercept and b is the regression coefficient (R Core Team, 2020) (Fig. 3.4.3.3). From the analysis the LC<sub>50</sub> was calculated to be 58.83 ppm and the LC<sub>90</sub> was calculated to be 9033.91 ppm. However, as 90% mortality was not achieved (Table 3.4.3.10), determination of the latter was inaccurate, as one cannot reliably estimate an LC value outside of the range measured. (See Appendix 3 for workings in R).



**Figure 3.4.3.3.** Dosage-mortality probit line for Delegate 250 WG against citrus thrips adults, including the regression equation for the probit analysis. The red lines indicate the LC<sub>50</sub> and the green lines indicate the LC<sub>90</sub>.

### Discussion and conclusion

Three Munger cell bioassays were conducted with Delegate 250 WG against citrus thrips over two seasons (2019, 2020 and 2021). In the first season, thrips were collected from a site at which there were assertions of resistance to Delegate 250 WG and from a site where Delegate 250 WG was claimed to be working well. However, no significant difference in citrus thrips susceptibility to Delegate 250 WG was recorded between the two sites. Only sufficient thrips for two treatments and an untreated control could be collected from each site. Consequently, it was not possible to also compare the dose-response between sites.

On the following two occasions (2020 and 2021), it was only possible to collect thrips from sites at which thrips was considered to be fully susceptible to Delegate 250 WG. Regular inspections were conducted at several sites where there were claims of thrips resistance to Delegate 250 WG. However, it appears that vigilance had increased due to uncertainty about product efficacy and thus thrips were generally controlled well and expeditiously. In these two trials, the protocol was changed slightly at the request of Corteva. Now only adult thrips were used in bioassays and a range of specific concentrations in a four-fold dilution were used in order to more accurately determine a dose-response relationship.

In 2020, it was not possible to collect sufficient thrips in order to use all five concentrations. However, this was indeed possible in 2021 and consequently, a dose-response was calculated and an LC<sub>50</sub> for Delegate 250 WG against adult thrips in the Sundays River Valley was calculated. This can thus be used as a benchmark for any further studies.

Unfortunately, there were a number of hurdles, preventing conclusive findings in this study:

- As it is not possible to successfully rear citrus thrips (Tim Grout, pers. comm.), all thrips used in bioassays had to be field collected. Establishment of a laboratory culture of thrips would enable one to permanently have a susceptible population against which any suspected field-collected resistant population could be tested.
- Not a single orchard in the entire Sundays River Valley could be found where spinetoram or spinosad had never been used. Consequently, it was not possible to test any thrips population from the region

that had not been exposed to these active ingredients and it was therefore also not possible to claim with absolute certainty that there was any population of thrips in the area that was fully susceptible to Delegate 250 WG.

- Control mortality of thrips after 48 h was too high in order for these data to be useful. Consequently, it is recommended that such trials only be conducted for 24 h. This is not ideal, as according to the Delegate 250 WG label, although exposed target pests stop feeding immediately, they can take up to 3 days to die.

Despite these challenges, we can conclude as follows:

- From the one trial in which we were able to compare the toxicity of Delegate 250 WG to a suspected resistant and suspected susceptible population of citrus thrips, there was no indication of any difference in susceptibility of the populations and therefore no indication of the existence of thrips resistance to Delegate 250 WG.
- The concentration determined to kill 50% of adult thrips in a sample (LC<sub>50</sub>) after 24 h exposure was 59 ppm. We were unable to determine this accurately after 48 h exposure, as control mortality was too high. This was nonetheless surprising, as the registered concentration for citrus thrips is 25 ppm.
- 100% mortality was never recorded, even with the highest concentration – 400 ppm, which is 16x the registered concentration – and even after 48 h exposure.

Although we now have a benchmark for thrips susceptibility to Delegate 250 WG in the Sundays River Valley, it is recommended that similar bioassays be conducted with citrus thrips collected from a site where there has never been any exposure to spinetoram or spinosad, or if this is not possible, where last exposure occurred at least 12 months previously. Thrips resistance to insecticides is generally unstable and where exposure is restricted for a few generations, partial reversion to susceptibility usually occurs (Grout *et al.* 1996).

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### 3.4.4 PROGRESS REPORT: Controlling mites on budwood

Project 1203 (2018/9, 2022/3) by T G Grout, P R Stephen, K C Stoltz and E Mauda (CRI)

## Summary

Citrus budwood often requires fumigation with methyl bromide on arrival in South Africa and this often kills many of the buds. In the search for a more benign means of control, preliminary work with Vapormate (ethyl formate) and carbon dioxide showed that the Vapormate was more efficacious but that it was also more

phytotoxic. A heat treatment of bud sticks over a water bath at 45°C for 4 h was not very detrimental to navel orange buds with 93% taking, but it was very detrimental to mandarin buds with only 18% alive three weeks after budding. This is therefore not an option for treating mites. Dips of bud sticks infested with citrus bud mite for different periods of time (30 seconds, 2 minutes and 8 minutes) in fenpyroximate at the registered rate of 150 ml/hl water gave promising results. The solution was constantly agitated when dips were made providing overall cover of the buds. Dips for 2 min and 8 min had no live bud mite in the leaf axil buds compared to high levels of infestation in the control. Further dip treatments in acaricides will be conducted before the better treatments are tested for bud viability.

## Opsomming

Sitrus okuleerhout vereis dikwels beroking met metielbromied met aankoms in Suid-Afrika en dit maak dikwels baie van die ogies dood. In die soeke na 'n meer sagte wyse van beheer, het voorlopige werk met Vapormate (etielformaat) en koolstofdioksied getoon dat Vapormate meer doeltreffend was, maar dat dit ook meer fitotoksies was. 'n Hitte-behandeling van ogiestokkies oor 'n waterbad by 45°C vir 4 h was nie baie nadelig vir nawel lemoen ogies nie, met 93% wat gevat het, maar dit was baie nadelig vir mandaryn ogies met slegs 18% wat drie weke ná okulering lewendig was. Dit is dus nie 'n opsie vir die behandeling van myte nie. Doop van ogiestokkies wat met sitrus knopmyt besmet is, vir verskillende tydspannes (30 sekondes, 2 minute en 8 minute) in fenpiroksimaat teen die geregistreerde dosis van 150 ml/hl water, het belowende resultate gelewer. Die oplossing is konstant geroer wanneer die doopbehandeling uitgevoer is, wat algehele bedekking van die ogies verskaf het. Doopbehandelings vir 2 min en 8 min het geen lewendige knopmyt in die blaar aksiale knop gehad, in vergelyking met hoër vlakke van besmetting in die kontrole nie. Verdere doopbehandelings in mytdoders sal uitgevoer word voordat die beter behandelings vir ogie lewensvatbaarheid getoets word.

### 3.4.5 PROGRESS REPORT: IPM under nets in Mpumalanga Province

Project 1205 (April 2018 – December 2020) by K Muller, M Hill (RU) and S Moore (CRI)

## Summary

The incidence and severity of citrus thrips throughout the three citrus growing seasons (August 2018 – April 2021) were found to be less under the 20% enclosed shade net in comparison to the open citrus orchard. The severity of thrips damage in comparison, confirmed the scouting results. Mealybug populations were higher and more concentrated during the 2018/2019 season in the open orchard. During the following two seasons, the mealybug populations were more under netting than in the open orchard. FCM total trap catches were higher in the first two seasons under netting, but in the last season the FCM moths were found to be lower under net than in the open orchard. Fruit fly species were caught in higher quantities in the open orchard for three consecutive seasons in comparison to the netted orchard. Red scale (crawlers, white caps, male scales and adult females) were found in higher populations under the netted orchard when compared to the open orchard. Citrus fruit sampled for residue tests resulted in higher concentrations of active ingredients for three consecutive seasons under the enclosed netting in comparison to the open orchard. Seychelles scale and pink wax scale were present at higher populations under litchi shade net in comparison to the control, for three growing seasons. Mango scale seemed to be similar in population densities at both litchi sites. More FCM was trapped under netted orchards for two consecutive seasons, with the last season being lower under the netted orchard in comparison to the open orchard. Higher trap catches of litchi moth were recorded in the open orchards for two seasons, while higher counts were recorded under nets during the last season. Fruit fly species were trapped in higher numbers in the open orchard for three consecutive seasons. There was a higher percentage of class 1 fruit under the netted orchard in comparison to the open orchard.

## Opsomming

Die intensiteit en populasie druk van sitrus blaaspootjie deur die drie seisoene (Augustus 2018 – April 2021) was laer op vrugte onder 20% skadunet teenoor die oop boord. Die persentasie van skade ondervind tydens oes evaluasies bevestig die moniterings verslae. Witluis populasies was meer opgemerk en in hoër populasies tydens die 2018/2019 seisoen in die oop boord. Die opvolgende twee seisoene was die witluis intensiteit hoër onder die nette as teenoor die oop kontrole boord. VKM se totale mot vangste was vir die eerste twee seisoene

meer onder die net teenoor die oop boord, waar die opvolgende jaar die resultate omgekeerd was. Meer vrugtevlieë was in die oop boord gelok en gevang vir drie seisoene teenoor die boord onder net. 'n Hoër intensiteit van rooidopluis kruipers, 1ste tot finale stadiums, was gevind op vrug monsters onder die net teenoor die oop boord se vrugte. Vrug residu toetse het hoër konsentrasies van aktiewe bestandele getoon onder die net teenoor vrugte van die kontrole boord. Seychelles dopluis en pienk was-dopluis was in hoër populasies gevind onder die lietjie net teenoor die kontrole boord vir drie seisoene. Die net het nie 'n effek getoon op mangodopluis se voorkoms nie aangesien vlakke soortgelyk opgemerk was tydens al drie seisoene. VKM getalle was hoër onder net vir twee seisoene, met die derde seisoen laer getalle as buite die net. Meer lietjiemot was buite die net gevang vir twee seisoene, waar die laaste seisoen meer motte gevang was binne die net. Vrugtevlieg getalle was vir al drie seisoene meer buite die net as binne die net. 'n Hoër persentasie klas 1 vrugte was aangeteken aan bome onder net teenoor bome buite die net.

#### 3.4.6 **PROGRESS REPORT: Integrated pest management under nets in the Western Cape** Project 1228 (2020/21 – 2021/22) by D Saccaggi and C Morris (CRI)

##### **Summary**

The use of netting in the citrus orchard environment is becoming increasingly common. In order to examine the influence of netting on pest populations, two sites in the Western Cape with netted and open orchards of the same cultivar were surveyed for pests. At each site FCM, fruit fly (Medfly), citrus thrips and leafhoppers were monitored. Traps were monitored fortnightly in April and May 2021, and again from October 2021 to March 2022. At the first site in Porterville, Medfly numbers were three times higher in open than in netted orchards, while citrus thrips were higher in netted orchards. Green citrus leafhopper and citrus (brown) leafhopper were caught in low numbers in both netted and open orchards. Only one FCM was caught. At the second site in Breede River Valley, FCM numbers were high at the start of monitoring, with more caught in the open than in the netted orchard, after which they quickly decreased to very few in both orchards for the remainder of the monitoring period. Medfly numbers were similar in both netted and open orchards, while citrus thrips were more prevalent in open orchards. Similar to Porterville, leafhopper numbers were very low and did not differ noticeably between netted and open orchards. In conclusion, although differences were found in pest numbers between open and netted orchards at the same site, most of these differences were small, and the trends differed at the two sites. The use of netting in citrus orchards may have different effects at different sites, even for the same pest, and should thus be considered on a site-by-site basis.

##### **Opsomming**

Die gebruik van nette in die sitrusboordomgewing word al hoe meer algemeen. Om die invloed van nette op plaagbevolkings te ondersoek, is twee persele in die Wes-Kaap met genette en oop boorde van dieselfde kultivar vir plaë ondersoek. By elke perseel is VKM, vrugtevlieg (Medvlieg), blaaspootjies en bladspringers gemonitor. Lokvalle is tweeweekliks in April en Mei 2021 gemonitor, en weer van Oktober 2021 tot Maart 2022. By die eerste perseel in Porterville was Medvlieg-getalle drie keer hoër in oop as in genette boorde, terwyl sitrusblaaspootjies hoër was in genette boorde. Groensitrus bladspringer en sitrus (bruin) bladspringer is in lae getalle gevang in beide genette en oop boorde. Slegs een VKM is gevang. By die tweede perseel in Breederiviervallei was VKM-getalle hoog aan die begin van monitering, met meer in die ope as in die genette boord gevang, waarna dit vinnig afgeneem het tot baie min in beide, boorde vir die res van die moniteringsperiode. Medvlieggetalle was soortgelyk in beide net- en oop boorde, terwyl sitrusblaaspootjies meer algemeen in oop boorde was. Soortgelyk aan Porterville was bladspringergetalle baie laag en het nie merkbaar tussen genette en oop boorde nie verskil nie. Ten slotte, alhoewel verskille in plaaggetalle tussen oop en genette boorde op dieselfde perseel gevind is, was die meeste van hierdie verskille klein, en die tendense het by die twee persele verskil. Die gebruik van nette in sitrusboorde kan verskillende effekte op verskillende persele hê, selfs vir dieselfde plaag, en moet dus op 'n geval tot geval-basis oorweeg word.

### 3.4.7 PROGRESS REPORT: Chemical control of mealybug on citrus

Project 1257 (Apr 2021 – Mar 2022) by S Moore, W Kirkman, M Peyper, T Marsberg and L Cousins (CRI)

#### Summary

The EU has placed various MRL restrictions on commonly used chemicals for mealybug control. Thus there was a need to identify alternative treatments that could be added to the mealybug control programme, both for preventative and corrective control. Newly registered products include: Closer (sulfoxaflor), Lesson (fenpyroximate) and Tivoli (spirotetramat). Additional options, either for preventative or corrective treatment or both, include methomyl, Tokuthion, buprofezin and dichlorvos (not registered against mealybug on citrus). These products were used in a preventative and corrective spray trial in the Sundays River Valley. Tokuthion (applied in spring), Closer, Tivoli and buprofezin were the most effective as preventative treatments for mealybug. Tivoli and buprofezin were the most persistent of these treatments. None of the products used in the corrective trial worked satisfactorily or at all. This was due to an extremely high mealybug infestation. A second trial will be initiated next season to compare the efficacy of different mealybug control programmes.

#### Opsomming

Die EU het verskeie MRL-bepelings op algemeen gebruikte chemikalieë vir witluis beheer geplaas. Daar was dus 'n behoefte om alternatiewe behandelings te identifiseer wat by die witluis beheer program gevoeg kan word, wat vir beide voorkomende en korrektiewe beheer gebruik kan word. Onlangs geregistreerde produkte sluit in: Closer (sulfoxaflor), Lesson (fenpyroximate) en Tivoli (spirotetramat). Bykomende opsies, hetsy vir voorkomende of korrektiewe behandeling of albei, sluit in methomyl, Tokuthion, buprofezin en dichlorvos (nie geregistreer teen witluis op sitrus nie). Hierdie produkte is in 'n voorkomende en korrektiewe spuitproef in die Sondagsriviervallei gebruik. Tokuthion (toegedien in die lente), Closer, Tivoli en buprofezin was die mees doeltreffende van hierdie behandelings. Geen van die produkte wat in die korrektiewe proef gebruik is het bevredigend gewerk, of geensins gewerk nie. Dit was as gevolg van die ongelooflike hoë witluisbesmetting. 'n Tweede proef sal volgende seisoen begin word om die doeltreffendheid van verskillende witluis beheer programme te vergelyk.

### 3.4.8 PROGRESS REPORT: Augmentation of *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) for the control of California red scale (Hemiptera: Diaspididae) in citrus.

Project 1259 (Apr 2020-Mar 2023) by E de Beer, M Hill (RU), T Marsberg, M Peyper and S Moore (CRI)

#### Summary

*Aphytis lingnanensis* Compere was reared and tested in South Africa for augmentation against red scale on citrus. It was concluded that there is little evidence that augmentation of *A. lingnanensis* influenced the population of red scale. *A. melinus* is now available for augmentation in South Africa. It is important that the efficacy of augmentation of this species on red scale is determined locally. Six orchards were selected in the Eastern and Western Cape. *A. melinus* was released in three orchards in each region and the remaining three orchards were studied as untreated controls. Releases were 240 000 parasitoids/ha, released over a five-week period. Releases started in November in organic orchards and in January in conventional orchards. Four yellow sticky traps were hung in each orchard to monitor adult *Aphytis* spp. activity and were replaced weekly. Fortnightly, levels of red scale infestation were determined by scouting 10 fruit on each of 10 trees per orchard. Once a level of 5% red scale fruit infestation was reached, a sample of 20 infested fruit from each orchard was collected randomly every 4 weeks. Red scale on collected fruit was inspected and classified as either live, dead, or parasitized, using 20 scales per fruit. Parasitoid species responsible for parasitism were identified and percentage parasitism recorded. *Aphytis* spp. were identified morphologically and are to be confirmed by sequencing the COI gene from representative samples. Preliminary results of this study during three years of field trials suggest that the augmentation of *A. melinus* did not significantly control red scale infestation and did not increase the level of parasitism above that of the untreated control. The natural presence of *A. africanus*

dominated parasitism in the control and treatment orchards. It has been found that hyperparasitoids of *Aphytis* spp. occur in higher numbers at certain sites than what has been found during previous studies.

## Opsomming

*Aphytis lingnanensis* Compere was geteel en getoets in Suid-Afrika vir die aanvulling in sitrus boorde teen rooidopluis. Dit is egter bevind dat daar te min bewyse is dat aanvullings van *A. lingnanensis* 'n effek op rooidopluis populasies gehad het. *A. melinus* is nou beskikbaar in Suid-Afrika vir vrylatings. Dit is belangrik dat die effektiwiteit van die aanvulling met hierdie spesie teen rooidopluis bepaal word. Ses boorde is gekies in die Oos- en Wes-Kaap. Vrylatings van *A. melinus* is gedoen in drie boorde in elke streek terwyl die oorblywende drie boorde in elke streek as kontrole gemonitor was. 240 000 *A. melinus* wespes/ ha was vrygelaat oor 'n vyf week periode. In organiese produksie boorde het vrylatings in November begin en in Januarie in konvensionele produksie stelsels. Vier geel kleef kaartjies, wat weekliks vervang was, is in elke boord gehang om die aktiwiteit van volwasse *Aphytis* spp. wespes te monitor. Boord verkenning is elke twee weke gedoen om rooidopluis infestasiëvlakke te monitor deur 10 vrugte op elk van die 10 bome te verken. Sodra 'n infestasië vlak van meer as 5% waargeneem was, is 20 geïnfesteerde vrugte vanuit elke boord ewekansig gemonster. Rooidopluis op gemonsterde vrugte is as lewendig, dood of geparasiteer geklassifiseer op 20 dopluise per vrug. Parasitoïede spesies wat verantwoordelik was vir die parasitisme van rooidopluis was geïdentifiseer en die aandeel in parasitisme was bepaal. *Aphytis* spp. was morfologies geïdentifiseer en moet bevestig word deur die opstelling van die COI geen volgorde. Voorlopige resultate van die veld proewe tydens die drie jaar van proewe dui aan dat die aanvulling van *A. melinus* nie rooidopluis beheer verbeter het in die boorde waar hierdie vrylatings gemaak is nie. Parasitisme het ook nie verhoog bokant die van die kontrole boorde nie. Die natuurlike teenwoordigheid *A. africanus* het gevolglik die parasitisme gedomineer in die behandeling en kontrole boorde. Dit bevind by sekere lokaliteite dat hiperparasitoïedes van *Aphytis* spp. in groter getalle voorkom teenoor vorige navorsing.

### 3.4.9 PROGRESS REPORT: A comparison of control of key citrus pests in orchards under nets, in a biointensive IPM programme and a conventional programme

Project 1289 (2020/21 – 2021/22) by D Grabe, M Hill (RU) and S Moore (CRI)

## Summary

Around the world, commodity markets are demanding that citrus should be produced with less chemical intervention to reduce risks to humans and the environment. The aim of this study was to determine if it was possible to obtain the same yield and export percentage, in citrus orchards where an integrated management programme, heavily based on biological control, was applied, in comparison to orchards that relied solely on chemical pest control. We focussed particularly on the following pests and their natural enemies: citrus mealybug, *Planococcus citri*, citrus thrips, *Scirtothrips aurantii*, red scale, *Aonidiella aurantii*, and citrus red mite, *Panonychus citri*.

During the first season different control strategies were applied. Scouting initiated intervention, both spraying and natural enemy augmentation. However, in December we had an outbreak of citrus mealybug and different interventions had to be applied in order to protect the crop. More chemicals were used within the 16 ha, commercial chemical treatment block and the 10 ha, Integrated Pest Management trial site, than within the 4 ha, strong biological trial site. The biological management relied strongly on more beneficial insects, with predators such as *Cryptolaemus montrouzieri* and *Nephus bipunctatus* being released.

Thrips, red scale, red mite, and even the outbreak we had of citrus mealybug were controlled by harvest time and most of the sooty mould had been washed off by the winter rain. All four key species were managed in such a way as to protect the crop and to protect the natural habitat of beneficial insects that started to bloom. Certain fruit export problems included cosmetic damage from mealybug rind damage, and cosmetic damage from dead and alive red scale on rind. Due to the mealybug outbreak, interventions were made with specifically chosen chemical sprays and or increased releases of beneficial parasitoids and predators. This increased the cost/ha considerably. To farm sustainably, it should also be economical. It was found after the first season that switching from chemical treatment to biological in one season, can be done, but it is too expensive.

The lessons learnt in the first season of 2020/21 led to certain changes in the control strategy within the integrated management programme. Certain chemicals were replaced for others and increased numbers of beneficial insects were added early in the season. During the second season of 2021/22 considerable focus was placed on controlling mealybug. To date mealybug is under good control due to the presence of beneficial insects. Red mite remained low throughout the season. Some thrips damage has occurred on fruit and this can be related to the infestation levels recorded during the season. It was never zero, but always low – very low. Late season red scale is present on some outside fruit, although some parasitism is evident. Final damage and infestation analyses will be conducted at harvest. Production costs were reduced substantially for both the chemical and biological programmes, from the first to the second season. However, these will have to be weighed up against losses to thrips and red scale damage.

Through this study we hope to find a commercially viable option to farm sustainably and economically using an IPM programme.

## Opsomming

Markte reg oor die wêreld is besig om produksie van sitrus te verander. Die druk om sitrus te produseer met minder chemiese beheer word al hoe meer. Sodoende, dat mense die gesondheids en omgewings voordele daarvan sal benut.

Die doel van die studie was om te toets of dieselfde opbrengste asook uitvoer uitpakkie, volhoubaar geproduseer kon word tussen 'n boord waar 'n geïntegreerde plaag bestuur (GPB) program wat swaar gesteun het op biologiese beheer toegepas was en 'n boord waar streng chemiese beheer toegepas was. Die interaksies tussen die volgende peste asook hulle natuurlike vyande was geondersoek: sitrus witluis, *Planococcus citri*, sitrus blaaspootjie, *Scirtothrips aurantii*, rooidopluis, *Aonidiella aurantii*, en sitrus rooimyt, *Panonychus citri*.

Gedurende die eerste seisoen was daar verskillende beheer strategieë toegepas. Plaag verkenning het aksie geïnisieer op beide aspekte van chemiese spuite en biologiese vrylatings. In Desember van 2020 het ons 'n uitbreek van sitrus witluis gehad en dit het gelei tot ingryping deur middel van verskillende spuite en aangepaste vrylatings om die oes te beskerm. Meer chemie was gebruik op die 16 ha, kommersiële chemiese boord en die 10 ha, geïntegreerde plaagbestuur boord as in die 4 ha, streng biologiese boord. Hier was daar sterk gesteun op meer voordelige insekte, insluitende predatore soos; *Cryptolaemus montrouzieri* en *Nephus bipunctatus*.

Blaaspootjie, rooidopluis, rooimyt en selfs die uitbraak van witluis was onder beheer voor oestyd begin het. Selfs die roetskimmel het genoeg tyd gehad om uit te droog en die winter reën kon meeste van dit af was. Al vier sleutel plaagspesies was beheer op so 'n manier om die oes te beskerm asook om die natuurlike omgewing waarin die voordelige insekte begin het om vanself te begin ontstaan. Daar was sekere uitvoer probleme wat kosmetiese skade van witluis op die skouers van die vrug en kosmetiese skade van lewendige en dooie rooidopluis op die vrug ingesluit het. As gevolg van die witluis uitbraak was daar sekere drastiese stappe geneem om die oes te beskerm; spesifieke chemie was gekies en/of meer voordelige insekvrylatings van parasitoïde en predatore was gedoen. Dit het wel die produksie koste/ha aansienlik opgestoot. Om volhoubaar te boer, moet dit ook ekonomies uitvoerbaar wees. Ons het na die eerste seisoen gevind dat dit moontlik is om oor te slaan van 'n volle chemiese program na 'n biologiese program, maar dit was duurder.

Die lesse wat geleer was in die eerste seisoen van 2020/21 het gelei tot veranderinge in die beheer strategieë binne die geïntegreerde bestuursprogram. Sekere chemie was veruil vir ander en hoër getalle van voordelige insekte vroeër in die seisoen was ingestel. In die tweede seisoen van 2021/22, was daar baie fokus geplaas op die beheer van witluis. Tot op hede is die witluis goed onder beheer gehou deur hulle natuurlike vyande. Rooimyt getalle was laag deur die seisoen. Sekere vrugte het wel blaaspootjie skade opgedoen en dit kan moontlik wees as gevolg van die konstante lae getalle blaaspootjie deur die seisoen. Laat seisoen rooidopluis is sigbaar op van die buite vrugte, alhoewel daar goeie parasitisme gesien kan word. Die finale skade analise sal gedoen word na die oes. Produksie kostes vanaf die eerste tot die tweede seisoen was aansienlik

verminder vir beide die chemie en biologiese programme. Dit sal wel opgeweeg moet word teen die moontlike verliese van blaaspootjie en dopluis skade.

Deur die studie, hoop ons om 'n kommersiële en lewensvatbare opsie te vind om volhoubaar en ekonomies te boer deur middel van 'n GPB program.

#### 3.4.10 **PROGRESS REPORT: Investigating delivery systems for formulation and application of microbial control agents**

Project 1290 (2021/2 – 2022/3) by Anne Grobler, Johnny van den Berg (NWU), Sean Moore and M. van der Merwe (CRI).

##### **Summary**

Various environmental factors constrain the efficacy of entomopathogens as microbial control agents against agricultural pests, such as sensitivity to UV-irradiation and moisture dependence. The effective formulation of these agents could potentially make a significant positive difference to their efficacy, mainly when applied against arboreal pests where they are exposed to these harmful environmental conditions. The first possibility that will be investigated is a patented delivery system called Pheroid®. It has been shown to enhance the absorption of various pharmacological compounds and biological molecules, including those in agriculture. The efficacy of Pheroid®-based formulations has been established with agricultural chemicals. For example, the efficacy and phytotoxicity of a chlorpyrifos-Pheroid® formulation were determined via trial plot studies on bollworm and aphids. Increased efficacy was obtained with the formulation without any signs of phytotoxicity. We are currently conducting a study with Pheroid® formulated microbial control agents to determine if a similar positive effect can be obtained. Desiccated CrleGV-SA was formulated with Pheroid® at three concentrations, namely 7, 14 and 28 mg of virus per ml of Pheroid®. Dose-response bioassays against FCM neonates were conducted with the aforementioned formulations and desiccated unformulated CrleGV-SA. A total of 5 replicates were conducted. CrleGV-SA with all three Pheroid® concentrations were significantly more virulent than desiccated unformulated CrleGV-SA, when comparing LC<sub>50</sub>. The 28 mg of virus per ml of Pheroid® concentration was also more virulent than the two lower concentrations. The effectiveness of formulating CrleGV-SA with Pheroid® has been demonstrated, and now, the entomopathogenic fungus *Metarhizium anisopliae* will be formulated with Pheroid® and its efficacy tested against FCM fifth instars or prepupae.

##### **Opsomming**

Verskeie omgewingsfaktore beperk die doeltreffendheid van entomopatogene as mikrobiële beheermiddels teen landbouplae, soos sensitiviteit vir UV-bestraling en vogafhanklikheid. Die doeltreffende formulering van hierdie middels kan moontlik 'n beduidende positiewe verskil aan hul doeltreffendheid maak, hoofsaaklik wanneer dit toegedien word teen boomplae waar hulle aan hierdie skadelike omgewingstoestande blootgestel word. Die eerste moontlikheid wat ondersoek sal word, is 'n gepatenteerde afleweringstelsel genaamd Pheroid®. Daar is getoon dat dit die absorpsie van verskeie farmakologiese verbindinge en biologiese molekules verbeter, insluitend dié in die landbou. Die doeltreffendheid van Pheroid®-gebaseerde formuleringe is vasgestel met landbouchemikalieë. Byvoorbeeld, die doeltreffendheid en fitotoksiteit van 'n chlorpyrifos-Pheroid® formulering is bepaal deur middel van proefperseelstudies op bolwurm en plantluise. Verhoogde doeltreffendheid is verkry met die formulering sonder enige tekens van fitotoksiteit. Ons is tans besig met 'n studie met Pheroid®-geformuleerde mikrobiële beheermiddels om te bepaal of 'n soortgelyke positiewe effek verkry kan word. Uitgedroogde CrleGV-SA is geformuleer met Pheroid® teen drie konsentrasies, naamlik 7, 14 en 28 mg virus per ml Pheroid®. Dosis-respons biotoetse teen VKM pasuitgeborede larwes is uitgevoer met die voorgenoemde formuleringe en uitgedroogde ongeformuleerde CrleGV-SA. Altesaam is 5 herhalings uitgevoer. CrleGV-SA met al drie Pheroid® konsentrasies was aansienlik meer virulent as uitgedroogde ongeformuleerde CrleGV-SA, wanneer LC<sub>50</sub> vergelyk word. Die 28 mg virus per ml Pheroid® konsentrasie was ook meer virulent as die twee laer konsentrasies. Die doeltreffendheid van die formulering van CrleGV-SA met Pheroid® is gedemonstreer, en nou sal die entomopatogeniese swam *Metarhizium anisopliae* geformuleer word met Pheroid® en die doeltreffendheid daarvan getoets word teen VKM vyfde instars of voorpapië.

### 3.4.11 PROGRESS REPORT: Identification and management of new lepidopteran pests on citrus

Project 1292: (April 2021 – March 2022) by T Marsberg, M Peyper, W Kirkman, L Cousins (CRI), A Timm, T Gilligan (USDA APHIS) and B van de Vossen (DNPPPO), (Once novel viruses are identified, Rhodes University will also be included)

#### Summary

Currently only two lepidopteran species are listed that can cryptically infest citrus fruit in South Africa. These are false codling moth, *Thaumatotibia leucotreta*, and carob moth, *Ectomyelois ceratoniae*. However, more incidents of lepidopteran larvae that are not considered citrus pests, are being recorded on citrus, sometimes at disconcertingly high levels. Consequently, we need to be proactive in correctly and swiftly identifying these species and provide feedback of control options to the growers. Thus, the aim of the study, is to potentially collect all lepidopterans occurring on citrus, attempt to rear these lepidopterans in the laboratory and identify/isolate alternative control options. A genetic 'fingerprint' will also be created during this process to allow for rapid and correct identification of these species.

Several lepidopteran species were collected from the field and attempts have been made to establish these species in the laboratory. Species collected thus far, include; *Thaumatotibia batrachopa* (macadamia nut borer), *Ectomyelois ceratoniae* (carob moth), *Lobesia vanillana* (grape moth), *Cryptoblabes gnidiella* (honeydew moth), Prays *citri* (citrus blossom moth) and a leaf roller species. The *T. batrachopa* culture was established but crashed after the second generation. Multiple field collections have been completed since then to try and re-establish the laboratory culture. However, no success has yet been achieved. The main problem with the establishment of a *T. batrachopa* laboratory culture is the lack of mating and oviposition. Multiple attempts have been made to induce mating and oviposition. However, nothing has proven effective thus far. The *E. ceratoniae* culture also crashed due to eggs being non-viable. A second attempt is currently in progress and viable eggs have been collected and larvae are developing on FCM artificial diet. An *L. vanillana* laboratory culture has been established. However, the last three generations have not provided numerous viable eggs, thus the culture is small and needs to be bulked up. The leaf roller culture was established with a single couple, which produced three generations before it collapsed. The culture has been re-established but is still in its first generation. Attempts were made to rear *P. citri* but no oviposition occurred. The *C. gnidiella* culture also crashed. During the attempts to establish these species in the laboratory, larvae showing symptoms of microbial infection were collected. The majority of symptomatic larvae collected exhibited signs of viral infection. Viral DNA was extracted from these larvae and the lef-8, lef-9 and granulin/polyhedrin genes were amplified. *Thaumatotibia batrachopa* and *E. ceratoniae* viruses were ±97% similar to CrleGV. The leaf roller species, *L. vanillana* and *C. gnidiella* all showed a low percentage match to various other GVs, which may indicate new viruses for each of these lepidopteran species. Further genetic analysis is required to determine if these isolates are novel GVs.

Samples of each of these Lepidoptera species were sent to the USDA to develop a suitable technique to create a genetic 'fingerprint'. The USDA investigated the feasibility of developing such a molecular assay based on analysis of ~650bp of the cytochrome oxidase I (COI) gene. This gene is often useful for discriminating species based on sequence analysis and forms the basis of DNA barcoding. Recently, Yokomi et al. (2022) published a molecular assay using COI to develop primers to distinguish between two species of *Cydia* and three species of *Grapholita* that feed on fresh cherries in the U.S. The same methods were examined to determine whether such an approach was feasible for this study. Several COI sequences from each relevant species were obtained from public databases (NCBI and BOLD) or generated from our own samples. For each species, alignments were used to generate a consensus sequence. Consensus sequences for each species were aligned to each other and used in an in-silico analysis to identify species-specific regions that could be used to develop amplification primers. Although nucleotide base differences were evident among species, and sufficient to allow species identification based on Sanger sequencing, these differences were either inconsistent within a species or not long enough to be useful for designing species-specific primers. Therefore it was concluded that COI was not ideal for genetic 'fingerprint' purposes. Further analyses will focus on examining differences in the internal transcribed spacer regions (ITS) to determine whether this gene is more suited to developing diagnostic assays. Previous studies have shown that ITS evolves at a faster rate than COI and may therefore show more species-specific differences. Since few ITS sequences are available for

relevant species in public databases, both the USDA and the CRI (Gqeberha) laboratories will concentrate on generating Sanger sequences using universal primers for each of the species.

Delta traps with PheroLure designed for monitoring *T. batrachopa* have been placed in 20 selected citrus orchards on seven farms around Mbombela. These selected orchards have macadamia orchards within 10 m to 2 km from the citrus orchard. Traps are monitored monthly in all these selected orchards, recording moths trapped and replacing the pheromone dispenser every eight weeks. In total six citrus cultivars (Nadorcott, Cambria Navels, Orri mandarins, Valencia, Midnight Valencia and Leanri mandarin) are being monitored for *T. batrachopa* using delta traps, with drop fruit stations being established later in the season. No *T. batrachopa* were caught, however, a total of 28 *T. leucotreta* were recorded in *T. batrachopa* delta traps in seven orchards on 3 farms.

## Opsomming

Tans word slegs twee Lepidoptera spesies gelys wat kripties sitrusvrugte in Suid-Afrika kan besmet. Dit is valskodlingmot, *Thaumatotibia leucotreta*, en karobmot, *Ectomyelois ceratoniae*. Al hoe meer voorvalle van lepidoptera-larwes wat nie as sitrusplae beskou word nie, word egter op sitrus aangeteken, soms op onstellende hoë vlakke. Gevolglik moet ons proaktief wees om hierdie spesies korrek en vinnig te identifiseer en terugvoer van beheeropsies aan die produsente te gee. Die doel van die studie is dus om potensieel alle Lepidoptera wat op sitrus voorkom te versamel, te probeer om hierdie Lepidoptera in die laboratorium te teel en alternatiewe beheeropsies te identifiseer/isoleer. 'n Genetiese 'vingerafdruk' sal ook tydens hierdie proses geskep word om vinnige en korrekte identifikasie van hierdie spesies moontlik te maak.

Verskeie Lepidoptera spesies is van die veld af versamel en pogings is aangewend om culture van hierdie spesies in die laboratorium te vestig. Spesies wat tot dusver versamel is, sluit in: *Thaumatotibia batrachopa* (makadamia-neutboorder), *Ectomyelois ceratoniae* (karobmot), *Lobesia vanillana* (druiwemot), *Cryptoblabes gnidiella* (heuningdoutmot), *Prays citri* (sitrusblommot) en 'n bladrollerspesie. Die *T. batrachopa* kultuur is gevestig, maar het na die tweede generasie neergestort. Verskeie veldversamelings is sedertdien voltooi om die laboratoriumkultuur te probeer herstel. Geen sukses is egter nog behaal nie. Die hoofprobleem met die vestiging van 'n *T. batrachopa* laboratoriumkultuur is die gebrek aan paring en eierlegging. Veelvuldige pogings is aangewend om paring en eierlegging te veroorsaak. Niks is egter tot dusver effektief bewys nie. Die *E. ceratoniae* kultuur het ook neergestort omdat eiers nie lewensvatbaar was nie. 'n Tweede poging is tans aan die gang en lewensvatbare eiers is versamel en larwes ontwikkel op VKM kunsmatige dieet. 'n *L. vanillana* laboratoriumkultuur is gevestig. Die laaste drie generasies het egter nie talle lewensvatbare eiers verskaf nie, dus is die kultuur klein en moet aangevul word. Die bladrollerspesie kultuur is gevestig met 'n enkele paartjie, wat drie generasies voortbring het voordat dit in duie gestort het. Die kultuur is hervestig, maar is steeds in sy eerste generasie. Pogings is aangewend om *P. citri* te teel, maar geen eierlegging het plaasgevind nie. Die *C. gnidiella*-kultuur het ook ineengestort. Tydens die pogings om hierdie spesies in die laboratorium te vestig, is larwes wat simptome van mikrobiële infeksie getoon het, versamel. Die meerderheid simptomatiese larwes wat versamel is, het tekens van virus infeksie getoon. Virus DNA is uit hierdie larwes onttrek en die lef-8, lef-9 en granulien/polihedrien gene is geamplifiseer. *Thaumatotibia batrachopa* en *E. ceratoniae* virusse was ±97% verwant aan CrleGV. Die bladrollerspesie, *L. vanillana* en *C. gnidiella* het almal 'n lae persentasie verwantskap met verskeie ander GVs getoon, wat aandui dat hierdie dalk nuwe virusse is vir hierdie Lepidoptera spesies. Verdere genetiese analise is nodig om te bepaal of hierdie isolate nuwe GVs is.

Monsters van elk van hierdie Lepidoptera spesies is na die USDA toe gestuur om 'n geskikte tegniek te ontwikkel om 'n genetiese 'vingerafdruk' te skep. Die USDA het die haalbaarheid ondersoek om so 'n molekulêre toets te ontwikkel gebaseer op ontleding van ~650bp van die sitochroomoksidase I (COI) geen. Hierdie geen is dikwels nuttig om spesies te onderskei op grond van volgorde-analise en vorm die basis van DNA strepieskodering. Onlangs het Yokomi *et al.* (2022) 'n molekulêre toets gepubliseer wat COI gebruik om 'primers' te ontwikkel om te onderskei tussen twee spesies *Cydia* en drie spesies *Grapholita* wat op vars kersies in die VSA voed. Dieselfde metode is ondersoek om te bepaal of so 'n benadering haalbaar was vir hierdie studie. Verskeie COI sekwensies van elke relevante spesie is verkry uit openbare databasisse (NCBI en BOLD) of gegenereer uit ons eie monsters. Vir elke spesie is belynings gebruik om 'n konsensus-sekwensie

te genereer. Konsensus-sekwensies vir elke spesie is in lyn gebring met mekaar en gebruik in 'n in-siliko analise om spesie-spesifieke streke te identifiseer wat gebruik kan word om amplifikasie-primers te ontwikkel. Alhoewel nukleotiedbasisverskille tussen spesies sigbaar was en voldoende was om spesie-identifikasie gebaseer op Sanger-sekwensiebepaling toe te laat, was hierdie verskille of inkonsekwent binne 'n spesie of nie lank genoeg om bruikbaar te wees vir die ontwerp van spesie-spesifieke 'primers' nie. Daarom het ons tot die gevolgtrekking gekom dat COI nie ideaal was vir genetiese 'vingerafdruk' doeleindes nie. Verdere ontledings sal daarop fokus om verskille in die interne transkriberende spasieerstreke (ITS) te ondersoek om te bepaal of hierdie geen meer geskik is om diagnostiese toetse te ontwikkel. Vorige studies het getoon dat ITS teen 'n vinniger tempo as COI ontwikkel en dus meer spesie-spesifieke verskille kan toon. Aangesien min ITS-volgorde vir relevante spesies in openbare databasisse beskikbaar is, sal beide die USDA en CRI (Gqeberha) laboratorium konsentreer op die generering van Sanger-sekwensies deur gebruik te maak van universele primers vir elk van die spesies.

Deltalokvalle met PheroLure wat ontwerp is vir die monitering van *T. batrachopa* is in 20 geselekteerde sitrusboorde op sewe plase rondom Mbombela geplaas. Hierdie geselekteerde boorde het makadamia boorde binne 10 m tot 2 km vanaf die sitrusboord. Lokvalle word maandeliks in al hierdie geselekteerde boorde gemonitor, motvangste is aangeteken en die feromoonvrysteller is elke agt weke vervang. In totaal word ses sitruskultivars (Nadorcott, Cambria Navels, Orri mandaryne, Valencia, Midnight Valencia en Leanri mandaryn) gemonitor vir *T. batrachopa* met behulp van delta lokvalle, met gevaldevrugstasies wat later in die seisoen gebruik sal word. Geen *T. batrachopa* is tot dusver gevang nie, maar 'n totaal van 28 *T. leucotreta* is aangeteken in *T. batrachopa* delta lokvalle in sewe boorde op 3 plase.

#### 3.4.12 **PROGRESS REPORT: Development of molecular detection tools for the identification of citrus pests and natural enemies.**

Project 1299 (2020/21 – 2022/23) by R Bester, G Cook, T Marsberg, A Manrakhan, S Moore, H Maree (CRI) and E De Beer (RU)

##### **Summary**

The aim of this project is firstly to construct genetic databases for mealybug, fruit fly, *Aphytis* and false codling moth (FCM) species and then secondly to use these resources to develop validated detection assays for specific species of each insect.

There are seven mealybug species known to be pests on citrus in South Africa namely: *Planococcus citri*; *Paracoccus burnerae*; *Nipaecoccus viridis*; *Delottococcus aberiae*; *Pseudococcus longispinus*; *Pseudococcus calceolariae*; *Ferrisia virgata*. All but one (*P. citri*) of these may be considered as phytosanitary pests by one or more of South Africa's export markets, the most problematic market being South Korea. A rapid molecular identification tool is needed that can differentiate between these mealybugs to prevent unnecessary rejections.

In this project three multiplex assays were designed and validated to differentiate between the abovementioned mealybug species.

Three fruit fly species: *Ceratitis capitata* (Mediterranean fruit fly/Medfly), *Ceratitis rosa* (Natal fly) and *Bactrocera dorsalis* (Oriental fruit fly) are present in South Africa and are considered pests of citrus and some of these species are considered as quarantine pests in some of our export markets. There are other fruit fly species present in South Africa that are not considered as citrus pests but are regulated by some export markets of citrus from South Africa. *C. rosa* and *C. quilicii* which are members of the same species complex (*C. rosa* is considered a citrus pest while *C. quilicii* is not). These two species are morphologically very similar and only the males of the species can be differentiated. A more reliable genetic method was developed, to enable identification of existing and potential fruit fly pests of citrus in South Africa. In this project a multiplex assay for the identification of 5 fruit fly species, (*Ceratitis capitata*, *Ceratitis rosa*, *Ceratitis quilicii*, *Ceratitis cosyra* and *Bactrocera dorsalis*) was developed.

With the pressure from the markets to reduce the amount of chemicals used in the citrus industry, there is a growing need to improve biological control of key citrus pests. California red scale, *Aonidiella aurantii* is

primarily controlled by the use of chemicals such as imidacloprid, pyriproxyfen and spirotetramat. Two *Aphytis* species, *A. melinus* and *A. lingnanensis* have the potential to be used as biological control agents against red scale. It is important to morphologically and genetically identify various species of *Aphytis* and update the barcoding database to ensure correct identification of these species and that the correct species are used in augmentation programmes. To date, a method to extract DNA from *Aphytis* species was validated and a potential assay identified that can be used to differentiate between species.

A genetic resource for FCM will also be built to attempt differentiation between larvae that originate from the sterile moths released or from wild moth populations.

## Opsomming

Die doel van hierdie projek is eerstens om genetiese databasisse vir witluis, vrugtevlieg, *Aphytis* en valskodlingmot (VKM) spesies op te stel en dan tweedens om hierdie hulpbronne te gebruik om opsporingstoetse vir spesifieke spesies van elke insek te ontwikkel.

Daar is sewe witluisspesies wat bekend is as plae op sitrus in Suid-Afrika, naamlik: *Planococcus citri*; *Paracoccus burnerae*; *Nipaecoccus viridis*; *Delottococcus aberiae*; *Pseudococcus longispinus*; *Pseudococcus calceolariae*; *Ferrisia virgata*. Almal behalwe een (*P. citri*) kan deur een of meer van Suid-Afrika se uitvoermarkte as fitosanitêre plae beskou word, die mees problematiese mark is Suid-Korea. 'n Vinnige molekulêre identifikasie-toets is nodig wat tussen hierdie witluise kan onderskei om onnodige verwerpings te voorkom. In hierdie projek is drie multiplekstoetse ontwerp en gevalideer om tussen die bogenoemde witluisspesies te onderskei.

Drie vrugtevliespesies: *Ceratitis capitata* (Mediterreense vrugtevlieg/medvlieg), *Ceratitis rosa* (Nataalse vlieg) en *Bactrocera dorsalis* (Oosterse vrugtevlieg) kom in Suid-Afrika voor en word as plae van sitrus beskou en van hierdie spesies word ook beskou as kwarantynplae in sommige van die uitvoermarkte. Daar is ander vrugtevliespesies in Suid-Afrika wat nie as sitrusplae beskou word nie, maar gereguleer word deur uitvoermarkte van sitrus uit Suid-Afrika. *C. rosa* en *C. quilicii* wat lede van dieselfde spesiekompleks is (*C. rosa* word as 'n sitrusplaag beskou terwyl *C. quilicii* nie). Hierdie twee spesies stem morfologies baie ooreen en slegs die mannetjies van die spesie kan onderskei word. 'n Meer betroubare genetiese metode was ontwikkel om die identifikasie van bestaande en potensiële vrugtevliesplae van sitrus in Suid-Afrika moontlik te maak. In hierdie projek is 'n multiplekstoets vir die identifikasie van 5 vrugtevliespesies (*Ceratitis capitata*, *Ceratitis rosa*, *Ceratitis quilicii*, *Ceratitis cosyra* en *Bactrocera dorsalis*) ontwikkel.

Met die druk van die markte om die hoeveelheid chemikalieë wat in die sitrusbedryf gebruik word te verminder, is daar 'n groeiende behoefte om biologiese beheer van sleutelsitrusplae te verbeter. Kalifornië rooidopluis, *Aonidiella aurantii* word hoofsaaklik beheer deur die gebruik van chemikalieë soos imidacloprid, pyriproxyfen en spirotetramat. Twee *Aphytis* spesies, *A. melinus* en *A. lingnanensis* het die potensiaal om as 'n biologiese beheermiddel teen rooidopluis gebruik te word. Dit is belangrik om verskeie spesies *Aphytis* morfologies en geneties te identifiseer en die kodedatabasis by te werk om die korrekte identifikasie van hierdie spesies te verseker en dat die korrekte spesies in aanvullingsprogramme gebruik word. Tot op datum is 'n metode om DNS uit *Aphytis* spesies te onttrek, gevalideer en 'n potensiële toets is geïdentifiseer wat gebruik kan word om tussen spesies te onderskei.

'n Genetiese hulpbron vir FCM sal ook in die volgende jaar gebou word om te probeer onderskei tussen larwes wat afkomstig is van die steriele motte wat vrygestel is of van wilde motpopulasies.

### 3.4.13 PROGRESS REPORT: Monitoring and control techniques for Australian bug

Project 1314 (April 2021 – April 2023) by L Serfontein, A Manrakhan and E Mauda (CRI)

## Summary

Populations of the Australian bug, *Icerya purchasi*, and one of its important predators: the Vedalia beetle, *Novius cardinalis*, were monitored monthly in four mandarin orchards in Mpumalanga Province for one year

from May 2021. The orchards selected for the study were those known to have heavy Australian Bug infestation. Spray programmes of the orchards where the studies were carried out were obtained. In the first year of the study, populations of the Australian bug and Vedalia beetle were found to increase in the study orchards up until after harvest (June 2021). Peak abundances of Australian bug and Vedalia beetle occurred in July 2021 with average percentage of trees infested with Australian bug being 67.5 % ( $\pm$  3.3%) and average percentage of trees with Vedalia beetle being 12.0% ( $\pm$  2.3%) in a mandarin orchard. At the same time the mean number of Australian bug and Vedalia beetle individuals per branch were 16.7 ( $\pm$  0.003) and 0.008 ( $\pm$ 0.003) respectively. Just after harvest, crop protection products such as pyriproxyfen, imidacloprid and buprofezin were applied in all study orchards. Declines in the populations of both the pest and predator were recorded and coincided with the timing of application of these products. All study orchards were devoid of Australian bug and Vedalia beetle populations thereafter. Low populations of Australian bug were detected in the orchards in May 2022. Monitoring of the Australian bug and its natural enemy will be carried out in the same four mandarin orchards for another year. It is possible that applications of pyriproxyfen, imidacloprid and buprofezin suppressed Australian bug populations. But these sprays might also have impacted negatively on the populations of the Vedalia beetle.

## Opsomming

Populasies van die Australiese wolluis, *Icerya purchasi*, en een van sy belangrike predatore: die Vedalia-kewer, *Novius cardinalis*, is maandeliks in vier mandarynboorde in Mpumalanga-provinsie vir een jaar, vanaf Mei 2021, gemonitor. Die boorde wat vir die studie gekies is, was dié wat bekend is vir swaar Australiese wolluis besmetting. Spuitprogramme van die boorde waar die studies uitgevoer is, is verkry. In die eerste jaar van die studie is gevind dat populasies van die Australiese wolluis en Vedalia-kewer in die studieboorde tot ná oes (Junie 2021) toeneem. Piek voorkoms van die Australiese wolluis en Vedalia-kewer het in Julie 2021 voorgekom, met die gemiddelde persentasie bome wat met Australiese wolluis besmet was, op 67.5 % ( $\pm$  3.3%), en gemiddelde persentasie bome met Vedalia-kewer op 12.0% ( $\pm$  2.3%) in 'n mandarynboord. Terselfdertyd was die gemiddelde aantal Australiese wolluis en Vedalia-kewer individue per tak onderskeidelik 16.7 ( $\pm$  0.003) en 0.008 ( $\pm$ 0.003). Net ná oes, is gewasbeskermingsprodukte soos pyriproxyfen, imidacloprid en buprofezin in alle studieboorde toegedien. Afnames in die populasies van beide die plaag en predator is aangeteken en het saamgeval met die tydsberekening van toediening van hierdie produkte. Alle studieboorde was daarna vry van die Australiese wolluis en Vedalia-kewer-populasies. Lae populasies van die Australiese wolluis is in Mei 2022 in die boorde waargeneem. Monitering van die Australiese wolluis en sy natuurlike vyand sal vir nog 'n jaar in dieselfde vier mandarynboorde uitgevoer word. Dit is moontlik dat bespuitings van pyriproxyfen, imidacloprid en buprofezin Australiese wolluis populasies onderdruk het, maar hierdie bespuitings het moontlik ook 'n negatiewe impak op die populasies van die Vedalia-kewer gehad.

### 3.4.14 PROGRESS REPORT: The influence of timing, insecticide residues and hyperparasitism on the efficacy of *Anagyrus vladimiri* augmentation for mealybug control

Project 1258 (April 2021 – March 2022) by W Mommsen, S Moore (CRI) and M Hill (RU)

## Summary

Mealybug has escalated as a pest of phytosanitary concern for various export markets. Agricultural insecticides alone are ineffective in suppressing mealybug populations and thus much research has been dedicated to investigate various biocontrol options. Releases of parasitic wasps for control of citrus mealybug, *Plannococcus citri*, has taken place commercially for over two decades in South Africa. *Anagyrus vladimiri*, previously known as *Anagyrus sp. near pseudococci*, has recently been described as a separate species. The aim of this study was to determine efficacy of *A. vladimiri* augmentations influenced by different timing of releases. Mealybug infestation and the percentage parasitism of third instars was recorded every two weeks in trial orchards in Burgersfort and Hoedspruit over two consecutive seasons. We compared early (October) vs. late (January) releases of *A. vladimiri* in open and netted orchards. In the 2020 season, mealybug infestation was lowest in trial plots where early releases of *A. vladimiri* took place. Later releases were still effective in reducing mealybug infestation through harvest. However, in 2021, in Burgersfort orchards, mealybug infestation was very high and no efficacy from releases was recorded, despite following programmes with low impact on beneficial insects. In semi-field non-target bioassays we tested various insecticides on *A.*

*vladimiri* which showed that *A. vladimiri* was less susceptible to insecticide residues compared to other parasitoids that had been tested previously and this confirmed that our IPM strategy was sound. However, high levels of hyperparasitism of *A. vladimiri* and *Coccidoxenoides perminutus* were observed, which was not the case in the previous season. This could perhaps explain the uncontrollable mealybug levels. Because of the many factors that can influence our dataset, we will be running a multivariable data analysis using the statistics programme “R” so that we can determine the influence of these factors on the efficacy of *A. vladimiri* augmentative releases.

## Opsomming

Witluis bly belangrike plaë van sitrus en is 'n fitosanitêre bekommernis vir sekere uitvoermarkte. Landbou-insekdoders alleen is onvoldoende om witluis populasies te onderdruk en daarom is baie navorsing, om verskeie bio-beheeropsies te ondersoek, gedoen. Vrylaatings van parasitiese wespes vir die beheer van sitrus witluis, *Plannococcus citri*, word vir die laast twee dekades kommersieel versprei in Suid Afrika. *Anagyrus vladimiri*, voorheen bekend as *Anagyrus* sp. near *pseudococci* was onlangs geklassifiseer as 'n aparte spesie. Die doel van hierdie studie is om doeltreffendheid van *A. vladimiri* aanvullings en die invloed van tydsberekening van die vrylaatings te bepaal. Witluis besmetting en persentasie parasitisme van derde instars het elke twee weke in Burgersfort en Hoedspruit proefboorde plaasgevind oor twee opeenvolgende seisoene. Ons het Vroeë (Oktober) en laat (Januarie) vrystellings van *A. vladimiri* in oop en sitrusboorde onder net vergelyk. In die 2020-seisoen was witluisbesmetting die laagste in boorde aangevul met vroeë vrystellings. Latere vrystellings was egter ook effektief deur oes. In 2021 was witluisbesmetting in Burgersfort boorde baie hoog en geen doeltreffendheid van vrystellings is aangeteken nie, ten spyte van die gevolg van programme met 'n lae impak op voordelige insekte. In semi-veld, nie-teiken effek biotoetse het ons 'n reeks insekdoders getoets wat toon dat *A. vladimiri* oor die algemeen minder vatbaar vir residue is, as ander voorheen getoetste parasitoïede, wat dus 'n goeie IPM strategie bevestig. Hoë vlakke van hiperparasitisme van *A. vladimiri* en *Coccidoxenoides perminutus* deur drie hiperparasietespesies, is egter in 2021 aangeteken, wat nie die geval in die vorige seisoen was nie en kan dus die onbeheerbare witluisvlakke verklaar. As gevolg van faktore wat ons datastel kan beïnvloed, moet ons 'n multifaktor analiese doen met die wiskundige program “R” sodat ons die invloed van hierdie faktore op doeltreffendheid van *A. vladimiri* aanvullings kan bepaal.

### 3.4.15 PROGRESS REPORT: New systemic insecticides for citrus

Project 1148 (2016/7-2022/3) by T G Grout, P R Stephen (CRI), S M Faris and P Nderitu (*icipe*)

## Summary

In order to prepare for the arrival of *Diaphorina citri* in South Africa, we need to find more systemic insecticides that can be used frequently in nurseries and for non-bearing trees. The brown citrus aphid *Aphis citricidus* was previously used as an indicator pest for screening systemic insecticides on potted lemon trees. The registered imidacloprid drench and recently registered acephate (Spectra Stem) stem treatment were used as standards and resulted in all aphids dropping off the leaves within 7 days. Two dosages of sulfoxaflor as a drench gave the same result after 7 days, although the mortality rate was slower. Results from an unregistered product, used in some countries as a soil drench for vegetables, were disappointing and a higher dosage will be required. Another unregistered systemic used on citrus in the USA gave promising results as a soil drench. A field trial against cotton aphid on young citrus was attempted but populations were sporadic and results very variable. Focus then switched to developing a culture of *D. citri* on potted citrus plants in an insect-proof structure in SE Kenya in collaboration with *icipe* so that the same chemicals could be evaluated against *D. citri* on potted plants. However, populations of *D. citri* on citrus in the region have remained low and the numbers in the culture have been inadequate for trials, despite the addition of *Murraya koenigii* plants to the culture. P. Nderitu did conduct a survey of the citrus production areas in Kenya in search of *D. citri* and possible HLB infections in hot regions. Of the 18 sites visited, *D. citri* was only recovered from Lungalunga in SE Kenya close to the Tanzania border.

## Opsomming

Om voor te berei vir die koms van *Diaphorina citri* in Suid-Afrika, moet ons meer sistemiese insekdoders vind wat gereeld in kwekerye en vir nie-draende bome gebruik kan word. Die bruin sitrus plantluis, *Aphis citricidus*, is voorheen as 'n indikatorplaag gebruik vir die sifting van sistemiese insekdoders op gepotte suurlemoenbome. Die geregistreerde imidacloprid deurdrenkbehandeling, en onlangs geregistreerde asefaat (Spectra Stem) stambehandeling, is as standarde gebruik en het daartoe gelei dat alle plantluis binne 7 dae van die blare afgeval het. Twee dosisse sulfoxaflor as 'n deurdrenkbehandeling het dieselfde resultaat na 7 dae gegee, alhoewel die mortaliteitsyfer stadiger was. Resultate van 'n ongeregisteerde produk, wat in sommige lande as 'n gronddeurdrenking vir groente gebruik word, was teleurstellend en 'n hoër dosis sal vereis word. Nog 'n ongeregisteerde sistemiese produk wat op sitrus in die VSA gebruik is, het belowende resultate as 'n gronddeurdrenking gegee. 'n Veldproef teen katoen plantluis op jong sitrus is probeer, maar populasies was sporadies en resultate baie wisselvallig. Fokus het toe oorgeskakel na die teel van 'n populasie van *D. citri* op gepotte sitrusplante in 'n insekbestande struktuur in SO Kenia, in samewerking met *icipes* sodat dieselfde chemikalieë teen *D. citri* op potplante geëvalueer kan word. Populasies van *D. citri* op sitrus in die streek het egter laag gebly en die getalle in die teelpopulasie was onvoldoende vir proewe, ten spyte van die toevoeging van *Murraya koenigii*-plante tot die teelpopulasie. P. Nderitu het 'n opname van die sitrusproduksiegebiede in Kenia gedoen, op soek na *D. citri* en moontlike HLB-infeksies in warm streke. Van die 18 terreine wat besoek is, is *D. citri* net vanaf Lungalunga in SO Kenia, naby die Tanzanië-grens, gevind.

#### 3.4.16 PROGRESS REPORT: Distinguishing *Diaphorina* spp. and *Trioza* spp. from other psylloids likely to be caught on yellow traps

Project 1255 (2021/2 – 2022/3) by Evans Mauda, Glynnis Cook, Aruna Manrakhan, Hano Maree, Tim Grout, Rachelle Bester, Rochelle de Bruyn, Leani Serfontein (CRI) and Daniel Burckhardt (Naturhistorisches Museum, Switzerland)

#### Summary

The identification of *Diaphorina* species in South Africa and other parts of the world is difficult. Two species (*Diaphorina punctulata* and *D. zebrana*) have been found to feed on citrus as adults, however, are not considered vectors of African greening (Catling and Atkinson 1974). Taxonomy of psylloids in the Afrotropical region has been largely ignored. There are currently no morphological keys for identification of these species. Collected specimens from the field surveys are being sorted, recorded and identified to the closest possible genus level and where possible to species level using an unpublished taxonomic key prepared by Daniel Burckhardt. Specimen collection is continuing in and around citrus environments in Limpopo, Mpumalanga, and Eastern Cape provinces. Indigenous plants were confirmed as hosts of psylloids with the presence of eggs, immature and adult *Diaphorina* species collected. Indigenous *Trioza* spp. and *Diaphorina* spp. including a *D. citri* lookalike have been sent for DNA barcoding and sequencing.

*Diaphorina* specimens collected from 2020 to 2021 were mostly obtained from sticky traps placed for biosecurity surveys. Specimens were removed from traps and DNA was extracted. PCR amplification for DNA barcoding and identification purposes using universal primers was unsuccessful. However, primers designed for specific amplification of *D. citri*, but targeting a different region of the mitochondrial Cytochrome C oxidase sub unit I (COI) to the previous primers, were able to amplify most unknown *Diaphorina* specimens and nucleotide sequences were generated for 36 specimens. These *Diaphorina* specimens all showed closer sequence identity to *D. lycii* rather than to *D. citri* or *D. communis* sequences available on GenBank. A conserved region was found in a sequence alignment allowing the design of a forward primer, better suited to *Diaphorina* and triozids, to be used with the universal reverse primer allowing for the generation of sequence data in the general COI bar-code region. Using the new assay, sequences for *D. virgata* (6) and morphologically different looking *D. punctulata* (15) specimens were obtained. A bar-code sequence for a *Diaphorina* specimen, collected in Colchester, Eastern Cape and morphologically a lookalike to *D. citri*, showed that the specimen was not *D. citri*, but an indigenous species occurring on the indigenous tree *Harpephyllum caffrum*, commonly known as African Plum tree.

Sixteen trioqid specimens, mostly collected from sticky traps were morphologically identified as either *Trioza erytrae* or *T. litseae* or *Trioza* sp. (*litseae*-group) and were bar coded using Sanger sequencing. Nucleotide sequence diversity was observed between these specimens and sequences broadly grouped into 5 clusters.

Thus far, '*Candidatus Liberibacter africanus*' (CLaf) was detected in specimens in two clusters. A unique *Liberibacter* sp. was identified in a trioza sample from a trap in Knysna and further characterisation and plant host identification of this *Liberibacter* is required. The trioza barcode of this sample grouped in a cluster with a specimen from East London.

## Opsomming

Die identifisering van *Diaphorina*-spesies in Suid-Afrika en ander dele van die wêreld is moeilik. Daar is gevind dat twee spesies (*Diaphorina punctulata* en *D. zebrana*) as volwassenes op sitrus voed, maar word egter nie as vektore van Afrika-vergroening beskou nie (Catling en Atkinson 1974). Taksonomie van psilloïede in die Afro-tropiese streek is grootliks geïgnoreer. Daar is tans geen morfologiese sleutels vir die identifikasie van hierdie spesies nie. Versamelde monsters van die veld-opnames word gesorteer, aangeteken en tot die naaste moontlike genusvlak, en waar moontlik tot spesievlak, geïdentifiseer met behulp van 'n ongepubliseerde taksonomiese sleutel wat deur Daniel Burckhardt opgestel is. Monsterversameling gaan voort in en om sitrus-omgewings in Limpopo, Mpumalanga en Oos-Kaap provinsies. Inheemse plante is as gashere van psilloïede bevestig, met die teenwoordigheid van eiers, onvolwasse en volwasse *Diaphorina* spesies wat versamel is. Inheemse *Trioza* spp. en *Diaphorina* spp., insluitend 'n monster wat soos *D. citri* lyk, is vir DNS-strepieskodering en -volgordebepaling gestuur.

*Diaphorina*-monsters wat vanaf 2020 tot 2021 versamel is, is meestal vanaf kleeflokvalle verkry wat vir biosekuriteitsopnames geplaas is. Monsters is uit lokvalle verwyder en DNS is geëkstraheer. PCR-amplifikasie vir DNS-strepieskodering en identifikasiedoeleindes met behulp van universele inleiers, was onsuksesvol. Inleiers wat vir spesifieke amplifikasie van *D. citri* ontwerp is, maar wat 'n ander area van die mitokondriale Sitokroom C-oksidasie sub-eenheid I (COI) as die vorige inleiers geteiken het, was egter in staat om die meeste onbekende *Diaphorina*-monsters te amplifiseer, en nukleotiedvolgordes is vir 36 monsters gegeneer. Hierdie *Diaphorina*-monsters het almal 'n nader volgorde-identiteit aan *D. lycii* getoon, as aan *D. citri*- of *D. communis*-volgordes, beskikbaar op GenBank. 'n Gekonserveerde area is in 'n volgorde-belyning gevind wat die ontwerp van 'n voorwaartse inleier moontlik maak, beter geskik vir *Diaphorina* en triosiede, wat gebruik kan word met die universele omgekeerde inleier, wat die generering van volgorde-data in die algemene COI-strepieskode-gebied moontlik maak. Deur die nuwe toets te gebruik, is volgordes vir *D. virgata* (6) en *D. punctulata* (15) monsters wat morfologies anders lyk, verkry. 'n Strepieskode-volgorde vir 'n *Diaphorina*-monster, versamel in Colchester, Oos-Kaap, en morfologies soortgelyk aan *D. citri*, het getoon dat die monster nie *D. citri* was nie, maar 'n inheemse spesie wat op die inheemse boom *Harpephyllum caffrum*, algemeen bekend as Afrika Pruimboom, voorkom.

Sestien triosied-monsters, meestal vanaf kleeflokvalle versamel, is morfologies as óf *Trioza erytrae* óf *T. litseae* óf *Trioza* sp. (*litseae*-groep) geïdentifiseer, en is strepieskodes met behulp van Sanger-volgordebepaling gegee. Nukleotiedvolgorde diversiteit is tussen hierdie monsters waargeneem en volgordes is breedweg in 5 groepe gegroepeer. Tot dusver is '*Candidatus Liberibacter africanus*' (CLaf) in monsters in twee groepe opgespoor. 'n Unieke *Liberibacter* sp. is in 'n triosiedmonster vanaf 'n lokval in Knysna geïdentifiseer en verdere karakterisering en plantgasheer-identifikasie van hierdie *Liberibacter* word vereis. Die triosied strepieskode van hierdie monster het in 'n groep met 'n monster vanaf Oos-Londen gegroepeer.

### 3.4.17 PROGRESS REPORT: Development of novel monitoring and control tools for citrus psyllids Project 1315 (2021/22 – 2022/23) by C W Weldon, K Krüger (UP) and A Manrakhan (CRI)

#### Summary

The South African citrus industry is currently faced with the potential introduction of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), and *Candidatus Liberibacter asiaticus* (Las) which it vectors. Las can also be vectored by the indigenous citrus psyllid pest, *Trioza erytrae* (Del Guercio) (Hemiptera: Triozidae). Techniques used for detection and surveillance of these pests are ineffective at low pest density, require skilled labour and are time consuming, so better methods are needed. Another approach to protect South African citrus from Las is to investigate alternative approaches for suppression of the vectors. One approach for controlling citrus psyllids is to use a gene silencing method called RNA interference which

has been developed in CRI project 1160. The aim of this project is to develop novel monitoring and control tools for citrus psyllids in South Africa. Our objectives are to (A) evaluate odour-based monitoring tools for psyllids, (B) develop and evaluate a vision-based system for identification of *T. erytrae* and *D. citri* on traps, and (C) test the efficacy of CTV RNAi constructs to suppress psyllids. Not much progress has been made regarding Objective A due to delays in delivery of the needed consumables and heavy rainfall when fieldwork was planned. In contrast, we have made considerable progress on Objective B. To date, 64 yellow sticky traps have been inspected for psyllids, photographed, and the images annotated and sent to our collaborators in Florida to develop and train artificial intelligence algorithms that identify psyllids. The system is producing promising results for *T. erytrae* identification but more sticky trap samples are being collected to improve accuracy and the ability to detect *D. citri* and *Diaphorina* species native to South Africa. Objective C has not yet started because we are negotiating access to facilities with biosecurity accreditation.

## Opsomming

Die Suid-Afrikaanse sitrusbedryf word tans gekonfronteer met die potensiële inkomings van die Asiatiese sitrusbladvlooi, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), en *Candidatus Liberibacter asiaticus* (Las) waarvoor dit die vektor is. Las kan ook deur die inheemse sitrusbladvlooiplaag, *Trioza erytrae* (Del Guercio) (Hemiptera: Triozidae) oorgedra word. Tegnieke wat gebruik word vir die opspoor en waarneem van hierdie plaag, is ondoeltreffend teen lae plaagdigtheid, vereis geskoolde arbeid, en is tydrowend, dus word beter metodes benodig. Nog 'n benadering om Suid-Afrikaanse sitrus teen Las te beskerm, is om alternatiewe benaderings vir die onderdrukking van die vektore te ondersoek. Een benadering vir die beheer van sitrusbladvlooi, is om 'n geen "silencing" metode, genaamd RNS-inmenging te gebruik wat in CRI-projek 1160 ontwikkel is. Die doel van hierdie projek is om nuwe moniterings- en beheerhulpmiddele vir sitrusbladvlooi in Suid-Afrika te ontwikkel. Ons doelwitte is om (A) reuk-gebaseerde moniteringshulpmiddele vir bladvlooi te evalueer, (B) 'n visie-gebaseerde stelsel te ontwikkel en te evalueer vir identifikasie van *T. erytrae* en *D. citri* op lokvalle, en (C) die doeltreffendheid van CTV RNAi-modelle te toets om bladvlooi te onderdruk. Daar is nie veel vordering gemaak met betrekking tot Doelwit A nie, as gevolg van vertraging in die aflewering van die benodigde verbruiksgoedere, en swaar reënval toe veldwerk beplan is. Daarteenoor het ons aansienlike vordering gemaak met Doelwit B. Tot op hede is 64 geel kleeflokvalle vir bladvlooi geïnspekteer, gefotografeer en die beelde is geannoteer en aan ons medewerkers in Florida gestuur om kunsmatige intelligensie-algoritmes te ontwikkel en op te lei wat bladvlooi identifiseer. Die stelsel lewer belowende resultate vir *T. erytrae*-identifikasie, maar meer kleeflokvalmonsters word ingesamel om akkuraatheid en die vermoë om *D. citri* en *Diaphorina* spesies, inheems aan Suid-Afrika, op te spoor, te verbeter. Doelwit C het nog nie begin nie omdat ons toegang tot fasiliteite met biosekuriteit akkreditasie onderhandel.

## 4 PORTFOLIO: DISEASE MANAGEMENT

### 4.1 PORTFOLIO SUMMARY

By Jan van Niekerk (Portfolio Manager: DM, CRI)

Sustainable production of high-quality citrus fruit is dependent on successful management of various pre and postharvest plant pathogens. These include fungal, viral, viroid and bacterial pathogens. In order to focus on all of these, the Disease Management portfolio of CRI is divided into different research programmes. These are preharvest (Fruit and Foliar with CBS and Soilborne diseases), Graft Transmissible Diseases (GTD) and postharvest diseases programmes (PHD). In order to focus the research aimed at preparing the South African citrus industry for the arrival of Asian Citrus Greening (HLB), the HLB Research Programme was furthermore established in 2021. Within each programme, specific industry research needs are addressed through research projects with various objectives.

Within the GTD programme the focus is aimed at preventing the damaging effects of these pathogens, such as citrus tristeza virus (CTV) and citrus viroids. These pathogens can easily spread through plant material. Therefore, research in this programme focusses on ensuring that pathogen free plant material is supplied to the industry. With this in mind, cross-protection mitigating the effect of citrus tristeza virus (CTV) is a big focus area. Field trials evaluating single-strain CTV sources for cross protection in different citrus types are ongoing

in project 1173. Viroids, due to their mechanical transmissibility, remain a problem in orchards where susceptible rootstocks are used. In project 1155 various new rootstocks are being evaluated for their sensitivity to viroids. However, no clear results are available yet. The horticultural performance of field-cut propagation material versus Improvement Scheme material has been under debate for some time. However, field trials in project 1074 clearly show that trees made from scheme material outperform trees made from field-cut material. Shoot-tip grafting is the technique used to render all new citrus accessions entering the CIS pathogen free. Confirmation of the successful elimination of pathogens is done using both molecular and biological screening. Project 1241 is aimed to investigate the use of high throughput sequencing (HTS) to potentially fast-track diagnostic processes in the STG pipeline and enable quicker release of cultivars to the industry. Results have indicated that this method indeed has the potential to be successfully employed in the CIS. Due to 'Candidatus' *Liberibacter asiaticus* (Las) and its vector's presence in Africa being confirmed, accurate diagnostics are critical for early detection and management interventions. In project 1200 a real-time diagnostic assay along with an inexpensive, reliable DNA extraction method was developed.

As indicated above, the HLB-RP was established in 2021 to coordinate and facilitate the interdisciplinary research, across research portfolios, needed to prepare the industry for the arrival of this disease. Consequently, some research projects within the IPM and Citriculture portfolios are also reported in this programme summary. However, only the projects included in the Disease Management (DM) portfolio are included in the DM annual report. One of the critical factors in management of HLB is rapid and accurate diagnostics of the pathogen. To this end, a Loop-mediated isothermal amplification (LAMP) detection assays were developed in Project 1265. This assay will be validated in field assays within a new project. Within the South African industry, we have a situation where trees are often co-infected with African Greening (CLaf) and CTV. The interaction of these two pathogens is being studied in project 1346, especially in reference to the possible effect on CTV cross-protection. Apart from early detection, control methods aimed at the HLB pathogen and vector is focused on. Within project 1160, CTV infectious clones with different "payloads," that are aimed at the insect vectors, were constructed and show that in principle they can be used to target the vector insects.

The preharvest diseases programme focusses on soilborne diseases, fruit and foliar diseases as well as Citrus Black Spot (CBS). Strong emphasis is being placed on understanding the different pathogens better through epidemiological studies as well as finding alternative control strategies or improving the current strategies used.

Sustainable management of fruit, foliar and soilborne diseases of citrus remains the primary focus of projects in the preharvest disease programme. This entails searching for and screening alternative, softer management options, improving chemical control programmes to prevent resistance development and spread, improving spray technology, and studying the epidemiology of the different pathogens.

Citrus Black Spot (CBS) remains the most important disease in the South African citrus industry, and to this end, a number of projects are aimed at improving management and detection of *Phyllosticta citricarpa*, the causal pathogen. Complementary to these projects are studies focused on understanding the population structure, epidemiology and fungicide resistance development of the pathogen in South Africa. Research on CBS encompasses all these aspects. In project 970 improvement of chemical control is a specific focus with some new chemicals showing promise. In the vein of fungicide resistance, Project 1316 focused on developing a molecular detection assay to detect benomyl resistance in *Phyllosticta citricarpa*. An assay was developed and can be employed to better manage benomyl in the control of CBS. However, chemical control alone cannot address this disease satisfactorily. Therefore, a number of projects, 1235, 1223 1238 and 1244 were conducted to improve the CRI PhytRisk CBS prediction models, better understand the population structures of *Phyllosticta citricarpa* and the role of pruning debris as ascospore source in disease epidemiology. *Alternaria* disease of citrus is gaining more importance due to increased occurrence in different citrus producing areas. To improve chemical control of *Alternaria* Brown Spot, project 750 has been ongoing for a number of years. Unfortunately, to date no fungicides have been identified that result in better control compared to existing fruit protection programmes. In certain Western Cape production areas and in certain seasons, Anthracnose, caused by *Colletotrichum* spp. and Botrytis flower blight, caused large pre-and postharvest losses due to fruit

drop and lesions or warts on export fruit. To address these two diseases, projects 1306 and 1236 were initiated. Project 1306 started in April 2021, while 1236 concluded in April 2022, having obtained some valuable results.

Also included in this programme are soilborne diseases and nematodes. Project 762 is specifically aimed at evaluating preplant soil fumigation for the management of soilborne pathogens and nematodes. Within project 1030 the focus is specifically to identify and evaluate new chemicals, non-chemical products or biocontrol agents for the management of soilborne pathogens and pests. Some encouraging results have been obtained in this project. Project 1068 has also been ongoing for some years and is investigating the decline and death of citrus trees in the Gamtoos and Sunday's River Valley. At this stage it is clear that a number of biotic and abiotic factors are playing a role in this syndrome. These will be further studied in a new project. Root and fruit rot, caused by *Phytophthora* spp., remain a serious limitation in citrus production. Chemical control of these diseases is still the cornerstone of management. To this end, project 1302 investigates any reduced phosphonate sensitivity within South African *P. nicotianae* populations, while project 1305 focusses on identifying fungicides that can be used in cases where mefenoxam resistance is present. Infested plant material is also an important method of spread of *Phytophthora* spp. Therefore, management of these pathogens in certified nurseries is critical. Project 1304 is investigating any non-traditional sources of nursery contamination by these pathogens. Parallel to this, project 1337 investigated the different factors that could influence the results obtained from routine soilborne pathogen testing that nurseries have to do as part of the CIS requirements.

Within the postharvest disease management environment, pressure on postharvest fungicides and chemical, residue causing sanitisers is increasing. In project 123 some new products that are either GRAS chemicals or non-residue-leaving products, were evaluated and some promising results, especially in the control of sour rot were obtained. One of the active ingredients that is under severe pressure in the EU, is imazalil. To prepare the industry for the banning of this active, large amounts of work have been done in project 1250. In this project alternative actives were tested alone and in combination to determine their suitability to replace imazalil. Their application parameters were also optimised, specifically in terms of application temperatures. From these numerous postharvest trials, it became evident that postharvest fungicides give variable disease control results on mandarins. To investigate this phenomenon, project 1325 was started and it focusses on the possible role that rind phytochemistry and morphology play in causing the variable efficacy of postharvest fungicides.

An increasing problem relating to increased shipping times and shipping delays, is saprophytic fungal growth on the stem-end of export fruit. The exact causes and remedial actions that can be taken are studied in project 1326. Results already indicate that mouldy pallet bases are not the source of these infections.

Pressure on postharvest fungicides has also led to greater focus on managing postharvest pathogens already in the near-harvest period. Some practices are already employed in the industry and the efficacy of these and other methods are the focus of project 1327. Along with pre-harvest management, specific investigation of alternative, non-chemical control methods are a big focus within postharvest research. Project 1366 focuses on determining whether essential oil volatiles can be used for the control of all major postharvest pathogens in degreening rooms and also possibly in cartons through novel application techniques. To date, some interesting and promising results were obtained.

## **PORTEFEULJE-OPSOMMING**

Volhoubare produksie van hoë kwaliteit sitrusvrugte is afhanklik van suksesvolle bestuur van verskeie voor- en na-oesplantpatogene. Dit sluit swam-, virus-, viroïede en bakteriese patogene in. Om op al hierdie te fokus, is die Siektebestuursportefeulje van CRI in verskillende navorsingsprogramme verdeel. Dit is voor-oes (Vrugte en Blaar met CBS en grondgedraagde siektes), ent-oordraagbare siektes (GTD) en na-oesiektprogramme (PHD). Ten einde die navorsing, wat daarop gemik is om die Suid-Afrikaanse sitrusbedryf vir die koms van Asiatiese Sitrusvergroening (HLB) voor te berei, te fokus, is die HLB Navorsingsprogram verder in 2021 gestig. Binne elke program word spesifieke bedryfsnavorsingsbehoefte aangespreek deur navorsingsprojekte met verskeie doelwitte.

Binne die GTD-program, is die fokus daarop gemik om die skadelike effekte van hierdie patogene, soos sitrus tristeza virus (CTV) en sitrusviroïede, te voorkom. Hierdie patogene kan maklik deur plantmateriaal versprei. Navorsing in hierdie program fokus dus daarop om te verseker dat patogeenvrye plantmateriaal aan die bedryf verskaf word. Met dit in gedagte, is kruisbeskerming, wat die effek van sitrus tristeza virus (CTV) versag, 'n groot fokus-area. Veldproewe wat enkel-isolaat CTV-bronne vir kruisbeskerming in verskillende sitrustipes evalueer, is aan die gang in projek 1173. Viroïede bly, as gevolg van hul meganiese oordraagbaarheid, 'n probleem in boorde waar vatbare onderstamme gebruik word. In projek 1155 word verskeie nuwe onderstamme geëvalueer vir hul sensitiwiteit vir viroïede. Daar is egter nog geen duidelike resultate beskikbaar nie. Die tuinboukundige prestasie van veld-gesnyde voortplantingsmateriaal teenoor Verbeteringskema-materiaal is al 'n geruime tyd onder debat. Veldproewe in projek 1074 toon egter duidelik dat bome gemaak van skema-materiaal beter presteer as bome wat van veld-gesnyde materiaal gemaak is. Lootpunt-enting is die tegniek wat gebruik word om alle nuwe sitrustoevoegings tot die CIS, patogeenvry te maak. Bevestiging van die suksesvolle uitskakeling van patogene word gedoen deur beide molekulêre en biologiese sifting te gebruik. Projek 1241 is daarop gemik om die gebruik van hoë deurvloei-volgorde-bepaling (HTS) te ondersoek om moontlike diagnostiese prosesse in die STG-pyplyn te versnel en om vinniger vrystelling van kultivars aan die bedryf moontlik te maak. Resultate het aangedui dat hierdie metode inderdaad die potensiaal het om suksesvol in die CIS aangewend te word. Aangesien '*Candidatus*' *Liberibacter asiaticus* (Las) en sy vektor se teenwoordigheid in Afrika bevestig is, is akkurate diagnostiek van kritieke belang vir vroeë opsporing en bestuurs-ingrypings. In projek 1200 is 'n intydse diagnostiese toets saam met 'n goedkoop, betroubare DNS-ekstraksiemetode ontwikkel.

Soos hierbo aangedui, is die HLB-RP in 2021 gestig om die interdisiplinêre navorsing óór navorsingsportefeuljes heen te koördineer en te fasiliteer, wat nodig is om die industrie voor te berei vir die koms van hierdie siekte. Gevolglik word sommige navorsingsprojekte binne die IPM- en Citriculture-portefeuljes ook in hierdie program-opsomming gerapporteer. Slegs die projekte wat in die Siektebestuur (DM)-portefeulje ingesluit is, word egter in die DM-jaarverslag ingesluit. Een van die kritieke faktore in die hantering van HLB is vinnige en akkurate diagnostiek van die patogene. Vir hierdie doel is 'n Lus-gemedieerde isotermiese amplifikasie (LAMP) opsporingstoets in Projek 1265 ontwikkel. Hierdie toets sal in veldtoets binne 'n nuwe projek gevalideer word. Binne die Suid-Afrikaanse bedryf het ons 'n situasie waar bome dikwels gelyktydig met Afrika Vergroening (CLaf) én CTV besmet is. Die interaksie van hierdie twee patogene word in projek 1346 bestudeer, veral met verwysing na die moontlike effek op CTV-kruisbeskerming. Afgesien van vroeë opsporing, word daar op beheermetodes wat op die HLB-patogene en -vektor gemik is, gefokus. Binne projek 1160 is CTV-aansteeklike klone met verskillende "payloads", wat op die insekvektore gerig is, gekonstrueer, en het getoon dat dit in beginsel gebruik kan word om die vektor-insekte te teiken.

Die voor-oessiekteprogram fokus op grondgedraagde siektes, vrug- en blaarsiektes, asook Sitruswartvlek (CBS). Sterk klem word daarop geplaas om die verskillende patogene beter deur epidemiologiese studies te verstaan, asook om alternatiewe beheerstrategieë te vind, of die huidige strategieë wat gebruik word, te verbeter.

Volhoubare bestuur van vrug-, blaar- en grondgedraagde siektes van sitrus bly die primêre fokus van projekte in die voor-oessiekteprogram. Dit behels die soeke na, en evaluering van alternatiewe, sagter bestuurs-opsies, die verbetering van chemiese beheerprogramme om weerstandsonwikkeling en -verspreiding te voorkom, die verbetering van spuitegnologie, en die bestudering van die epidemiologie van die verskillende patogene.

Sitruswartvlek (CBS) bly die belangrikste siekte in die Suid-Afrikaanse sitrusbedryf, en vir hierdie doel is 'n aantal projekte daarop gemik om die bestuur en opsporing van *Phyllosticta citricarpa*, die veroorsakende patogene, te verbeter. Aanvullend tot hierdie projekte is studies gefokus op die begrip van die populasiestruktuur, epidemiologie en swamdoderweerstandsonwikkeling van die patogene in Suid-Afrika. Navorsing oor CBS sluit al hierdie aspekte in. In projek 970 is verbetering van chemiese beheer 'n spesifieke fokus, met 'n paar nuwe chemikalieë wat belofte toon. Met betrekking tot swamdoderweerstand, het Projek 1316 gefokus op die ontwikkeling van 'n molekulêre opsporingstoets om benomilweerstand in *Phyllosticta citricarpa* op te spoor. 'n Toets is ontwikkel en kan aangewend word om benomil beter in die beheer van CBS te bestuur. Chemiese beheer alleen kan egter nie hierdie siekte bevredigend bestuur nie. Daarom is 'n aantal projekte, 1235, 1223 1238 en 1244 uitgevoer om die CRI PhytRisk CBS voorspellingsmodelle te verbeter, die

populasiestrukture van *Phyllosticta citricarpa*, en die rol van snoei-afval as askosporbron in siekte-epidemiologie, beter te verstaan. Alternaria-siekte van sitrus word al hoe belangriker as gevolg van verhoogde voorkoms in verskillende sitrusproduserende gebiede. Om chemiese beheer van Alternaria-bruinvlek te verbeter, is projek 750 al 'n aantal jare aan die gang. Ongelukkig is daar tot op hede geen swamdoders geïdentifiseer wat beter beheer tot gevolg het in vergelyking met bestaande vrugbeskermingsprogramme nie. In sekere Wes-Kaapse produksiegebiede en in sekere seisoene, veroorsaak Antraknose, veroorsaak deur *Colletotrichum* spp. en Botrytis-blomskroei, groot voor- en na-oesverliese weens vrugval en letsels of vratte op uitvoervrugte. Om hierdie twee siektes aan te spreek, is projekte 1306 en 1236 van stapel gestuur. Projek 1306 het in April 2021 begin, terwyl 1236 in April 2022 afgehandel is, met 'n paar waardevolle resultate.

By hierdie program is ook grondgedraagde siektes en aalwurms ingesluit. Projek 762 is spesifiek gemik op die evaluering van vóórplant-grondberoking vir die bestuur van grondgedraagde patogene en aalwurms. Binne projek 1030 is die fokus spesifiek om nuwe chemikalieë, nie-chemiese produkte of biobeheermiddels vir die bestuur van grondgedraagde patogene en plaë te identifiseer en te evalueer. Sommige belowende resultate is in hierdie projek verkry. Projek 1068 is ook al vir 'n paar jaar aan die gang en ondersoek die agteruitgang en dood van sitrusbome in die Gamtoos- en Sondagsriviervallei. Op hierdie stadium is dit duidelik dat 'n aantal biotiese en abiotiese faktore 'n rol in hierdie sindroom speel. Dit sal verder in 'n nuwe projek bestudeer word. Wortel- en vrugvrot, veroorsaak deur *Phytophthora* spp., bly 'n ernstige beperking in sitrusproduksie. Chemiese beheer van hierdie siektes is steeds die hoeksteen van bestuur. Vir hierdie doel ondersoek projek 1302 enige verminderde fosfonaatsensitiwiteit binne Suid-Afrikaanse *P. nicotianae* populasies, terwyl projek 1305 fokus op die identifisering van swamdoders wat gebruik kan word in gevalle waar mafenoksamweerstand teenwoordig is. Besmette plantmateriaal is ook 'n belangrike metode van verspreiding van *Phytophthora* spp. Daarom is die bestuur van hierdie patogene in gesertifiseerde kwekerye van kritieke belang. Projek 1304 ondersoek enige nie-tradisionele bronne van kwekerye kontaminasie deur hierdie patogene. Parallel hiermee het projek 1337 die verskillende faktore ondersoek wat die resultate wat van roetine grondgedraagde patogeentoetsing verkry is, wat kwekerye as deel van die CIS-vereistes moet doen, kan beïnvloed.

Binne die na-oes siektebestuurs-omgewing neem die druk op na-oeswamdoders en chemiese, residu-veroorsakende ontsmettingsmiddels toe. In projek 123 is 'n paar nuwe produkte wat óf GRAS-chemikalieë is óf produkte is wat nie residue agterlaat nie, geëvalueer, en 'n paar belowende resultate, veral in die beheer van suurvrot, is verkry. Een van die aktiewe bestanddele wat onder erge druk in die EU is, is imazalil. Om die industrie voor te berei vir die verbod op hierdie aktiewe middel, is groot hoeveelhede werk in projek 1250 gedoen. In hierdie projek is alternatiewe aktiewe middels alleen en in kombinasie getoets om hul geskiktheid te bepaal om imazalil te vervang. Hul toedieningsparameters is ook geoptimaliseer, spesifiek in terme van toedieningstemperatuur. Uit hierdie talle na-oesproewe het dit duidelik geword dat na-oeswamdoders veranderlike siektebeheerresultate op mandaryne gee. Om hierdie verskynsel te ondersoek, is projek 1325 begin en dit fokus op die moontlike rol wat skil-fitochemie en -morfologie speel om die veranderlike doeltreffendheid van na-oeswamdoders te veroorsaak.

'n Toenemende probleem wat verband hou met verlengde verskepingstye en versendingsvertraging, is saprofitiese swamgroei aan die stingel-ent van uitvoervrugte. Die presiese oorsake en regstellende aksies wat geneem kan word, word in projek 1326 bestudeer. Resultate het reeds aangedui dat muwwerige paletbassisse nie die bron van hierdie infeksies is nie.

Druk op na-oeswamdoders het ook gelei tot groter fokus op die bestuur van na-oespatogene reeds in die naby-oesperiode. Sommige praktyke word reeds in die bedryf aangewend, en die doeltreffendheid van hierdie en ander metodes is die fokus van projek 1327. Saam met voor-oesbestuur is spesifieke ondersoek na alternatiewe, nie-chemiese beheermetodes 'n groot fokus binne na-oesnavorsing. Projek 1366 fokus op die bepaling van vlugtige essensiële olies wat gebruik kan word vir die beheer van alle groot na-oespatogene in ontgroeningskamers en ook moontlik in kartonne, deur nuwe toedieningstegnieke. Tot op datum is 'n paar interessante en belowende resultate verkry.

## 4.2 PROGRAMME: GRAFT TRANSMISSIBLE DISEASES

Programme coordinator: Glynnis Cook (CRI)

### 4.2.1 Programme summary

Vegetative propagation carries the potential to disseminate pathogens including bacteria, viruses and viroids. To safeguard the industry against pathogen spread in this manner, pathogen-free propagation material is supplied through the Citrus Improvement Scheme (CIS). Research aspects of this programme is inherently linked to the requirements of the CIS to develop and implement reliable diagnostic capabilities to detect known graft transmissible pathogens. New industry cultivars, which are either selected through bud-mutations locally or are foreign introductions, are processed through shoot-tip grafting (STG) to eliminate pathogens, followed by molecular and biological indexing to confirm pathogen-free status. Project 1241 (4.2.2) was done to investigate the application of high throughput sequencing (HTS) based detection of known citrus viruses and viroids to enable faster, more sensitive pathogen detection and to fast-track release of cultivars to industry. Reliability, sensitivity and reproducibility of HTS processes were validated and HTS was shown to be more comprehensive than currently used assays. With the necessary validations and standard operating procedures, the implementation of HTS, as part of routine pathogen screening practices is possible. Another objective of this project was to investigate diseases with unknown aetiology using HTS. Citrus virus A (CiVA) was identified and associated with citrus impietratura on grapefruit and a blotchy fruit rind symptom on sweet oranges. A detection assay for the simultaneous detection of citrus infecting coguviruses was developed. The project also yielded an HTS-based CTV genotyping tool for the detection of known and unknown CTV genotypes.

Apart from the supply of pathogen-free bud-wood, management strategies are required to limit spread and to control endemic, insect transmitted pathogens. Cross-protection is one such management strategy which is applied to mitigate the damaging effects of CTV. Research is therefore conducted to understand the impact of various CTV strains and to determine which are ultimately required for cross-protection. Field trials evaluating performance of single-strain CTV sources in various citrus types are ongoing (4.2.5). Grapefruit has been the citrus type most affected by CTV in South Africa. Field trials, conducted over many years, indicated a CTV source for grapefruit cross-protection which was associated with better tree health and production. Validation of this source as a replacement pre-immunisation source for grapefruit will be done by semi-commercial trial evaluations in Star Ruby (4.2.6).

Results of a field trial comparing the horticultural performance of field-cut propagation material to budwood supplied by the CIS show that better growth, tree health and production are achieved using CIS propagation material (4.2.3). Despite the use of certified budwood, viroids remain problematic in the industry due to their mechanical transmissibility. Newly planted orchards are especially vulnerable if susceptible rootstocks are used. A field trial is underway to better understand the viroid sensitivities of commercial and potentially important rootstock selections (4.2.4).

### Programopsomming

Vegetatiewe voortplanting dra die potensiaal om patogene te versprei, insluitend bakterieë, virusse en viroïede. Om die bedryf teen patogeenverspreiding op hierdie wyse te beskerm, word patogeenvrye voortplantingsmateriaal deur die Sitrusverbeteringskema (SVS) verskaf. Navorsingsaspekte van hierdie program is inherent gekoppel aan die vereistes van die SVS om betroubare diagnostiese vermoëns te ontwikkel en te implementeer om bekende entoordraagbare patogene te kan opspoor. Nuwe bedryfskultivars, wat óf plaaslik deur mutasies geselekteer word óf ingevoer word vanaf die buiteland, word deur groeipuntenting (GPE) verwerk om patogene uit te skakel, gevolg deur molekulêre en biologiese indeksering om patogeenvrye status te bevestig. Projek 1241 (4.2.2) is gedoen om die toepassing van hoë deurvloei-volgordebepaling (HTS)-gebaseerde opsporing van bekende sitrusvirusse en viroïede te ondersoek om vinniger, meer sensitiewe patogeenvrye opsporing moontlik te maak en om die vrystelling van kultivars na die bedryf te bespoedig. Betroubaarheid, sensitiwiteit en hehaalbaarheid van HTS-prosesse is bevestig en HTS is meer omvattend as huidige toetse getoon. Met die nodige bevestiging en standaard bedryfsprosedures is die implementering van HTS, as deel van roetine patogeenvrye toetsing, moontlik. Nog 'n doelwit van hierdie projek

was om siektes met onbekende etiologie met behulp van HTS te ondersoek. Sitrusvirus A (CiVA) is geïdentifiseer en geassosieer met sitrus impietratura op pomelo en 'n vrugskil simptoem op soet lemoene. 'n Opspringstoets vir die gelyktydige opsporing van sitrus-infekterende coguvirusse is ontwikkel. Die projek het ook 'n HTS-gebaseerde CTV genotiperings hulpmiddel vir die opsporing van bekende en onbekende CTV genotipes opgelewer.

Benewens die verskaffing van patogeenvrye enthout, word bestuurstrategieë vereis om verspreiding te beperk en endemiese, insekkoordraagbare patogene te beheer. Kruisbeskerming is een so 'n bestuurstrategie wat toegepas word om die skadelike uitwerking van CTV te verlig. Navorsing word dus gedoen om die impak van verskeie CTV-rasse te verstaan en om te bepaal watter uiteindelik nodig is vir kruisbeskerming. Veldproewe wat prestasie van enkelras CTV-bronne, in verskeie sitrustipes evalueer, is aan die gang (4.2.5). Pomelo is die sitrustipe wat die meeste deur CTV in Suid-Afrika geraak word. Veldproewe wat oor baie jare uitgevoer is, het 'n CTV-bron vir pomelo-kruisbeskerming uitgewys wat geassosieer is met beter boomgesondheid en -produksie. Bevestiging van hierdie bron as 'n vervangings pre-immunisasiebron vir pomelo's sal gedoen word deur semi-kommersiële proefevaluasies in Star Ruby boorde (4.2.6).

Resultate van 'n veldproef waar die hortologiese prestasie van veldgesnyde voortplantingsmateriaal vergelyk word met voortplantingsmateriaal, verskaf deur die SVS, toon dat beter groei en boomgesondheid verkry word van SVS-voortplantingsmateriaal (4.2.3). Ten spyte van die gebruik van gesertifiseerde enthout, is viroïede steeds problematies in die bedryf vanweë hul meganiese oordraagbaarheid. Nuut aangeplante boorde is veral kwesbaar as vatbare onderstamme gebruik word. 'n Veldproef is aan die gang om die viroïed sensitiviteite van kommersiële en potensieel belangrike onderstamseleksies beter te verstaan (4.2.4).

#### **4.2.2 FINAL REPORT: Application of high-throughput sequencing (HTS) for routine virus and viroid detection in high value accessions.**

Project 1241 (2019/20 – 2021/22) by R Bester, G Cook, JHJ Breytenbach, C Steyn, R De Bruyn, PH Fourie and HJ Maree (CRI)

#### **Summary**

The ultimate aim of this project was to validate high throughput sequencing (HTS) based detection of known viruses and viroids of citrus for routine detection and to open up the possibility to fast-track multiplication of clean material within the Citrus Improvement Scheme (CIS) without additional risk. Plant material deliberately infected with a range of viruses (positive and negative stranded viruses) and viroids was established. Total RNA was extracted from three representative samples of each plant (4 plants) using two different methods (CTAB vs Zymo Research RNA kit) and sent for HTS (Macrogen, Korea). One representative sample of each plant was also sent to the Central analytical facility (CAF) in Stellenbosch for HTS on an Ion Torrent platform. The data were evaluated for biological and technical variation focussing on RNA extraction method, platform used and bioinformatic analysis. The study evaluated the influence of different HTS protocols on the sensitivity, specificity, repeatability and reproducibility of HTS as a detection tool. Both extraction methods and sequencing platforms resulted in significant differences between the data sets. However, the complete virome profile was constructed with all the above-mentioned methodology. The differences in the data sets only highlighted the need to be aware of the level of variation to enable informed adjustments to correctly interpret the data for a reliable result. The plants were also assayed one year later using the same methodology and the results were consistent.

The project also included a direct comparison between an HTS-based detection assay and the conventional methods as applied in the CIS. Seven accessions were selected for this comparison. Total RNA was extracted from these samples before and after shoot tip grafting and sent for HTS. One of these samples were selected as source material and was used to deliberately infect healthy seedlings. The infection status of these seedlings was monitored for seven months, at five time points, using both HTS and RT-PCR to test the sensitivity of both approaches to detect viruses and viroids. Even though the limit of detection of HTS can be influenced by pathogen concentration, sample processing method and sequencing depth, HTS detection in this study was found to be either equivalent or more sensitive than RT-PCR.

Additionally, one of the objectives of this project was to investigate diseases with unknown aetiology (such as the Psorosis-like diseases) using HTS. Psorosis-like symptomatic plants were identified and subjected to HTS. Citrus virus A (CiVA) was identified in these samples. The association of CiVA with citrus impietratura on grapefruit and fruit rind symptoms similar to citrus impietratura on sweet oranges was investigated as well as determining the genetic diversity of CiVA in various citrus production regions and citrus species. A detection assay for the simultaneous detection of citrus infecting coguviruses was also developed.

## Opsomming

Die hoofdoel van hierdie projek was om hoë-deurset volgordebepaling gebaseerde opsporing (HTS) van bekende virusse en viroïede in sitrus as 'n roetine toets te valideer. In die proses mag dit die geleentheid bied om die vermeerdering van plantmateriaal in die sitrus verbeteringskema te bespoedig sonder addisionele risiko. Plant materiaal geïnfekteer met 'n verskeidenheid virusse (positiewe en negatiewe string virusse) en viroïede is gemaak. Totale RNA is uit drie verteenwoordigende monsters van elke plant (4 plante) met behulp van twee verskillende metodes (CTAB vs Zymo Research RNA kit) uitgehaal en vir HTS (MacroGen, Korea) gestuur. Een verteenwoordigende monster van elke plant is ook na die Sentrale analitiese fasiliteit (CAF) in Stellenbosch vir HTS gestuur om data met die Ion Torrent-platform te genereer. Die data is geëvalueer vir biologiese en tegniese variasie met die fokus op RNA-ekstraksie-metode, volgordebepalingsplatform en bioinformatiese analise. Die studie het die invloed van verskillende HTS-protokolle op die sensitiwiteit, spesifisiteit, herhaalbaarheid en reproduseerbaarheid van HTS as 'n opsporingsinstrument geëvalueer. Beide ekstraksiemetodes en volgordebepalingsplatforms het beduidende verskille tussen die datastelle tot gevolg gehad. Die volledige viroomprofiel is egter met al bogenoemde metodologie saamgestel en die verskille in die datastelle het slegs die behoefte beklemtoon om bewus te wees van die vlak van variasie om ingeligte aanpassings moontlik te maak om die data korrek te interpreteer vir betroubare resultate. Die plante is ook een jaar later met dieselfde metodologie getoets en die resultate was dieselfde.

Die projek het ook 'n direkte vergelyking tussen 'n HTS-gebaseerde opsporingstoets en die konvensionele metodes, soos toegepas in die sitrus verbeteringskema, ingesluit. Sewe monsters is gekies vir hierdie vergelyking. Totale RNA is voor en na enting van lootpunte uit hierdie monsters onttrek en vir HTS gestuur. Een van hierdie monsters is as bronmateriaal gekies en is gebruik om doelbewus gesonde saailinge te besmet. Die infeksiestatus van hierdie saailinge is vir sewe maande, op vyf tydpunkte gemonitor, deur beide HTS en RT-PCR te gebruik om die sensitiwiteit van beide benaderings om virusse en viroïede op te spoor te toets. Selfs al kan die limiet van opsporing van HTS deur patoëenkonsentrasie, monsterverwerkingsmetode en volgordebepalingsdiepte beïnvloed word, is gevind dat HTS-opsporing in hierdie studie óf ekwivalent óf meer sensitief is as RT-PCR.

Daarbenewens was een van die doelwitte van hierdie projek om siektes met onbekende etiologie (soos die Psorose-agtige siektes) met behulp van HTS te ondersoek. Psorose-agtige simptome is geïdentifiseer en aan HTS onderwerp. Sitrusvirus A (CiVA) is in hierdie monsters geïdentifiseer. Die assosiasie van CiVA met sitrusimpietratura op pomelo- en vrugteskil simptome soortgelyk aan sitrusimpietratura op lemoene is ondersoek asook die bepaling van die genetiese diversiteit van CiVA in verskeie sitrusproduksiestreke en sitrusspesies. 'n Opsporingstoets vir die gelyktydige opsporing van sitrus-infekterende coguvirusse is ook ontwikkel.

## Introduction

The application of High-Throughput Sequencing (HTS), also known as next-generation sequencing, has proven very successful for virus discovery to resolve disease aetiology in many agricultural crops. We have utilised this technology successfully in our research group on perennial crops discovering novel viruses, new variants of known viruses and detecting viruses previously not known to be present in SA. Completed studies have been published and additional reviews written on the topic. The next challenge as highlighted in Maree *et al.* (2018), Olmos *et al.* (2018) and Massart *et al.* (2018) is to validate the application of HTS for routine virus detection.

In this project the influence of biological variation, RNA extraction method and bioinformatic analysis was investigated. The optimal approach was repeated one year later on the same material to assess reproducibility. The application of this technology has the greatest impact in certification and two objectives tied directly to the CIS. The ultimate aim of this project was to validate HTS-based detection of known viruses and viroids of citrus for routine detection and to open up the possibility to fast-track multiplication of clean material in the CIS without additional risk.

### **Stated objectives**

1. Evaluate the influence of assay parameters such as RNA extraction method, time of sampling, and sequence depth on the sensitivity, specificity and reproducibility of an HTS-based assay.
2. Determine the accuracy of an HTS-based detection assay compared to conventional methods as applied in the CIS through a side-by-side comparison.
3. Determine the influence of a bioinformatic analysis pipeline on detection efficiency.
4. Application of HTS to determine the etiological agents in Psorosis-like diseases.

### **Materials and methods**

1. Evaluate the influence of assay parameters such as RNA extraction method, time of sampling, and sequence depth on the sensitivity, specificity and reproducibility of an HTS-based assay.

In this objective three biological replicate plants and a healthy control plant were used (4 plants). These plants contained several viruses and viroids to cover as many scenarios as possible (positive and negative sense viruses of high and low concentrations and viroids). In round 1, two RNA extraction methods (CTAB vs Zymo Research RNA kit) were evaluated on three technical replicates of the same plant material using the Illumina sequencing platform (4 plant x 3 replicates x 2 extractions = 24 HTS samples). One of the technical replicates per plant was also evaluated using a second HTS platform (Ion Torrent) (4 plant x 1 replicate x 2 extractions = 8 HTS samples). The material was allowed to regrow and 12 months later the experiment was repeated with the best RNA extraction method and platform identified in round 1 (CTAB and Illumina platform) (4 plant x 3 replicates x 1 extraction = 12 HTS samples).

2. Determine the accuracy of an HTS-based detection assay compared to conventional methods as applied in the CIS through a side-by-side comparison.

The accuracy of HTS was evaluated in a side-by-side comparison with the CIS. Field collected virus symptomatic and healthy material (nucleus material) were screened for viruses and viroids before and after shoot tip grafting (STG) with the HTS-based assay optimised in objective 1. Material was also evaluated with the routine RT-PCR assays used in the scheme and results compared. (7 samples before and after STG = 14 HTS samples)

3. Determine the influence of bioinformatic analysis pipeline on detection efficiency.

The influence of bioinformatic analysis were evaluated using an array of bioinformatic tools and methods. Both de novo assembly and read mapping were compared as strategies to identify pathogens. A commercial tool for both de novo assembly and read mapping was also compared to freely available command line programs. A bioinformatic pipeline for CTV genotype detection was also constructed and evaluated using simulated and real data sets to determine the parameters to discriminate between false positive read mappings and true genotype-specific genome coverage.

4. Application of HTS to determine the etiological agents in Psorosis-like diseases.

Field samples displaying psorosis-like symptoms were collected and processed for HTS in an attempt to determine the aetiological agent of the symptom. An RT-PCR assay was developed for the agent identified and additional samples screened to assess the correlation of the agent with the observed symptom. The sequence diversity of the pathogen was also assessed.

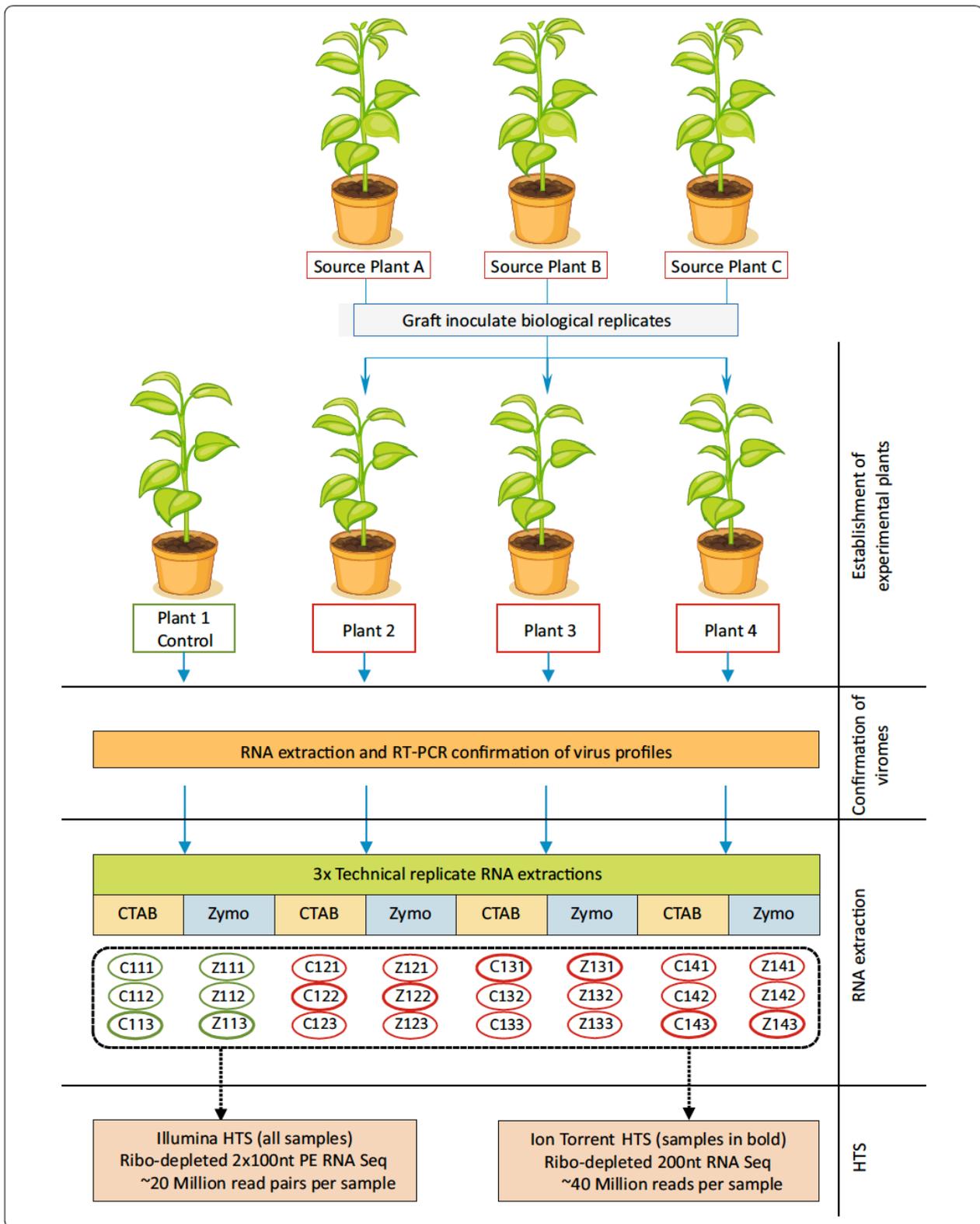
## Results and discussion

1. Evaluate the influence of assay parameters such as RNA extraction method, time of sampling, and sequence depth on the sensitivity, specificity and reproducibility of an HTS-based assay.

A set of plants (Madam Vinous / Carrizo) has been established and graft inoculated with a set of viruses and viroids (citrus tristeza virus (CTV), citrus virus A (CiVA), citrus tatter leaf virus (CTLV) and hop stunt viroid (HSVd), citrus dwarfing viroid (CDVd) and citrus exocortis viroid (CEVd). The infection status of these plants was determined with RT-PCR (see Figure 4.2.2.1 for experimental layout). One healthy plant and three plants infected with HSVd, CDVd, CEVd, CTV, CiVA and CLTV were selected and sampled. Total RNA was extracted from three representative samples of each plant using two different methods (CTAB vs Zymo Research RNA kit) and sent for Illumina HTS at Macrogen in Korea. One technical replicate of each plant was also sent for HTS on the Ion Torrent platform at CAF (Stellenbosch). The influence of the assay parameters was evaluated through bioinformatic analyses.

The experiment evaluated the efficiency of using HTS to detect two single stranded RNA viruses from different families, a negative-sense single-stranded RNA virus and three viroid species. The influence of RNA extraction protocol, sequencing platform and data analysis pipelines on the sensitivity, specificity, and repeatability of HTS as a detection tool were evaluated. Each of these parameters introduced a different bias that creates variation in the data output. Even though the different extraction methods, sequencing platform and data analysis tools resulted in variation in the present study, the result, being the virome profile of each sample, could be confirmed independent of the HTS approach. The study highlights the need to be aware of the level of variation associated with each approach in strategy, from sample collection to data interpretation and how these variables may impact on the initial objective of the HTS assay. This awareness is critical to enable informed adjustments to correctly interpret the data for a reliable result. The primary recommendation that follows from this study is that, irrespective of extraction method or sequencing platform, a combination of de novo assembly and read mapping should be used for a routine detection assay. Since the goal of this study was to evaluate HTS as a detection tool in quarantine or certification schemes, and not for discovery purposes, a list of known pathogens should be available in these settings for read mapping. The aim of a de novo assembly in the certification scheme context will be to identify unsuspected pathogens. The absence of virus/viroid related de novo assembled contigs does not automatically indicate a negative status for the respective pathogen and read mapping is required as a validation step to confirm absence. This is especially necessary for low concentration viruses and viroids. Read mapping against multiple reference genes as internal controls is also recommended to establish gene ratios for a specific assay. This allows for the evaluation of sequencing depth to accurately determine the absence of low-level infections. The inclusion of a nontarget positive and a negative control can assist significantly to evaluate cross contamination between samples. The conclusion is that sequencing depth matters and that with enough data the variation observed between extraction methods and sequencing platforms are diminished and equivalent results can be obtained.

Additionally, the two different extraction methods yielded total RNA with different rRNA profiles. No significant difference between the RIN values for the two groups was observed, however the rRNA ratio of the Zymo Research kit extracted RNA was significantly lower than for the CTAB RNA due to a higher concentration of 5S rRNA yielded by the Zymo Research kit. This indicates a potential difference in the RNA species extracted with each method and it is possible that the CTAB extraction selected against viroid sequences due to the Lithium Chloride (LiCl) precipitation step.



**Figure 4.2.2.1.** Visual representation of experimental layout. The establishment of plant material and the selection of samples subjected to high-throughput sequencing (HTS) is illustrated. Source plant A is infected with citrus tristeza virus (CTV) (genotypes RB, T3, T30, VT and S1), citrus virus A (CiVA), hop stunt viroid (HSVd), citrus dwarfing viroid (CDVd) and citrus exocortis viroid (CEVd), source plant B with only ‘Candidatus Liberibacter africanus (CLaf )’ and source plant C with citrus tatter leaf virus (CTLV)

To evaluate the reproducibility of HTS, the same plants evaluated in the first round were sampled again, one year later, and evaluated in triplicate using the same analyses to construct the virome profile. The HTS assay proved to be reproducible.

To compare the sensitivity of HTS to RT-PCR a second set of plants (5 x Mexican lime and 5 x Madam Vinous) was established using one of the plants that contained the full complement of pathogens as inoculation source. Plants were sampled before inoculation and at 30, 90, 150 and 203 days post inoculation. RNA was extracted and assayed using HTS and RT-PCR.

The time course experiment was performed to compensate for natural pathogen accumulation in plants over time. The HTS pipeline applied in this study produced reproducible and comparable results to standard RT-PCR assays for the detection of CTV and three viroid species in citrus. Even though the limit of detection of HTS can be influenced by pathogen concentration, sample processing method and sequencing depth, HTS detection in this study was found to be either equivalent or more sensitive than RT-PCR.

The selection of RNA extraction method also again proved to impact on the detection of viroids as more viroid sequences were present in the data when a LiCl free RNA extraction method was used. It is possible that some RNA extraction methods can select against viroid RNA. However, with HTS, more sequencing depth can compensate and increase detection of low concentration pathogens. The erratic detection of viroid sequences with both RT-PCR and HTS can point to either uneven viroid distribution in the plant, viroid concentration fluctuations during the different time points or selection against viroid RNA during the RNA extraction method.

2. Determine the accuracy of an HTS-based detection assay compared to conventional methods as applied in the CIS through a side-by-side comparison.

Seven accessions were selected for this comparison. Total RNA was extracted from these samples using two different methods and sent for HTS at Macrogen in Korea. The shoot tip grafting of these accessions was completed as part of the normal operations of the CIS. RNA was extracted from a successful shoot tip grafted plant per accession, RT-PCRs assays were performed and RNA was also sent for HTS. The RT-PCR and HTS assays produced identical results and HTS was proved a viable and equivalent technique compared to RT-PCR with the added benefit that unknown pathogens not assayed with RT-PCR can be detected with HTS. One of the accessions before shoot tip grafting also contained '*Ca. Liberibacter africanus*' (CLaf) and the HTS assay using total RNA as input also detected CLaf opening up the possibility to extend the HTS assay to pathogens other than RNA viruses and viroids.

3. Determine the influence of bioinformatic analysis pipeline on detection efficiency.

The bioinformatic pipeline was evaluated for efficiency and sensitivity to detect known viruses and viroids from citrus. Both de novo assembly and read mapping were compared as strategies to identify pathogens. A commercial tool for both de novo assembly and read mapping was also compared to freely available command line programs. The programs performed equivalently in producing the accurate virome profile, however user input and computer resources differed between the tools.

A bioinformatic pipeline for CTV genotype detection was also constructed and evaluated using simulated and real data sets to determine the parameters to discriminate between false positive read mappings and true genotype-specific genome coverage. Simulated and real HTS data sets were used to identify genome coverage thresholds to categorize false positive read mappings and true genotype-specific genome coverage. Using this read mapping pipeline, genome coverage below 50% was regarded as the result of non-target read mappings. A genome coverage above 90% was indicative of the presence of a specific genotype, and genome coverage between 50–90% suggested the presence of genotype variants not represented in the read mapping reference list. The abovementioned thresholds were all dependent on at least 1000 read pairs of CTV mapping to a genotype sequence. These thresholds will also be influenced by genotype concentration and sequencing depth of the library, which was not necessarily investigated to the extremes in this study. HTS with the associated bioinformatic pipeline was validated and proposed as a CTV genotyping assay.

The data generated during this project was not able to be used to evaluate the ability to differentiate species and cultivar. More representative genomes (either through database access or self-generated genomes) are needed for this objective.

#### 4. Application of HTS to determine the etiological agents in Psorosis-like diseases.

High-throughput sequencing of citrus indicator hosts, originally inoculated from field samples and showing transient chlorotic flecking or oak leaf patterns, revealed the presence of the first South African variant of citrus virus A (CiVA). Psorosis-like symptomatic plants were identified and also subjected to HTS. Citrus CiVA was identified in these samples.

The confirmed presence of CiVA in cultivars of grapefruit (*Citrus paradisi* Macf.), sweet orange (*C. sinensis* (L.) Osb.) and clementine (*C. reticulata* Blanco) in South Africa initiated a study to determine the distribution, genetic diversity, and symptom association of CiVA in three provinces and seven citrus production regions. CiVA was detected in six citrus production regions in symptomatic and asymptomatic sweet orange trees. In three citrus production regions, CiVA was detected in sweet orange trees displaying a fruit rind symptom similar to citrus impietratura. CiVA was also detected in grapefruit trees with typical citrus impietratura symptoms and in symptomless clementine trees. The three encoded gene regions of CiVA were Sanger sequenced to investigate the genetic diversity between isolates from the six citrus production regions and three citrus species. Phylogenetic analysis of the nucleotide sequences (nt) of each encoded gene region was performed through the construction of Maximum-likelihood (ML) phylogenetic trees and nucleotide identity matrices. Phylogenetic analysis and nt identity matrices indicated a higher genetic diversity within the NP than the MP and RdRp. More genetic diversity was observed between isolates from the three citrus species than between isolates from the different citrus production regions. A real-time RT-PCR detection assay targeting the RdRp was also developed to simultaneously detect CiVA and CCGaV. Two cDNA synthesis methods for reverse transcription (RT) were compared and a degenerate, dual priming oligo (DPO) reverse primer was designed to improve the specificity of the detection assay. Two PCR assays that utilised the DPO reverse primer with two different forward primers were compared. The cDNA synthesis method and choice of primer pair had an influence on the amplification efficiency, specificity, and sensitivity of the real-time detection assay. A tissue specificity assay was also performed and CiVA was detected throughout the plant in leaf midribs, leaf lamina, green bark, and roots. Lower Ct values were consistently associated with the green bark and leaf midribs. The detection assay was implemented for pathogen screening within the Citrus Improvement Scheme of South Africa, ensuring the release of CiVA free budwood to the citrus industry.

The distribution of CiVA in South Africa indicates that CiVA was introduced through planting material. CiVA was primarily detected in older orchards that were established prior to the application of shoot tip grafting for virus elimination in the South African Citrus Improvement Scheme.

## Conclusion

The aim of this project was achieved and HTS was validated as a reliable approach for the detection of citrus viruses and viroids. The application of HTS for the detection of plant viruses is commonly described as being unbiased, however this is only true within a specific context, in that it does not require any prior knowledge of the pathogens. There are, however, slight biases and variations at every step of an HTS assay, as demonstrated, which can easily be corrected for when quantified. HTS is more comprehensive than any assay previously used, and with the necessary validations and standard operating procedures, the implementation of HTS as part of routine pathogen screening practices is possible.

The project also yielded an HTS-based CTV genotyping tool for the detection of known and unknown CTV genotypes. The distribution and diversity of CiVA was also investigated and a citrus coguviruses RT-PCR assay was developed. The research outcome and outputs of this project highlighted the positive contribution HTS technology can have in the citrus industry.

## Future research

Future research in the scope of this project will include the following:

1. Evaluation of the influence of different RNA extractions on the detection of viroids.
2. The use of HTS data for differentiating species and cultivars.

## Technology transfer

1. The CiVA assay developed in this study has been incorporated in the Citrus Improvement Scheme RT-PCR assay panel.
2. The HTS Bioinformatic data analysis pipeline has been developed and is currently used at CRI for screening samples of importance.
3. A Cutting Edge on the symptomology associated with CiVA was published (Fruit rind symptoms possibly associated with citrus virus A (CiVA). G. Cook, R. Bester, H.J. Maree. April 2021, No 318).
4. Publications:

Bester R, Cook G, Breytenbach JHJ, Steyn C, De Bruyn R & Maree HJ (2021a) Towards the validation of high-throughput sequencing (HTS) for routine plant virus diagnostics: measurement of variation linked to HTS detection of citrus viruses and viroids. *Virology Journal* 18: 61. doi:10.1186/s12985-021-01523-1.

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Bester R, Karaan M, Cook G & Maree HJ (2021c) First report of citrus virus A in citrus in South Africa. *Journal of Citrus Pathology* 8. doi: Retrieved from <https://escholarship.org/uc/item/5gr6p8zh>.

de Bruyn R, Bester R, Cook G, Steyn C, Breytenbach JHJ & Maree HJ (2022) Distribution and genetic diversity of *Coguvirus eburi* in South African citrus and the development of a real-time RT-PCR assay for citrus-infecting coguviruses. *Plant Disease*. doi:10.1094/PDIS-11-21-2409-RE.

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#### **4.2.3 PROGRESS REPORT: Comparison of shoot tip grafted citrus with field-cut (old clone) material**

Project 1074 (2013 - 2023) by G Cook, J Breytenbach, R de Bruyn and C Steyn (CRI)

##### **Summary**

The objective of this study is to compare tree health, production and fruit characteristics of CIS supplied material with that of field-cut and viroid infected material. Graft transmissible pathogens, including viroids and viruses, are removed by shoot tip grafting from accessions submitted to the CIS. Thereafter a citrus tristeza virus (CTV) source is introduced to each accession within the cross-protection programme. Field trees can however acquire a range of graft transmissible pathogens over time, either by means of insect vector transmission or mechanically during routine orchard practices. Budwood was collected from original field sources of the cultivars, which contained various populations of CTV strains and citrus viroids. These were budded to 'Swingle' citrumelo, 'Carrizo' citrange and 'C35' citrange rootstocks. The same was done for budwood obtained from the CFB and for CFB budwood to which a non-cachexia causing hop stunt viroid (CVd-IIa) source was additionally inoculated. A field trial was established at Burgersfort in 2016. After five and a half years, significant differences in tree growth were observed between treatments. Significantly reduced canopy volumes were associated with field-cut material of all three cultivars. Trees made from CFB supplied budwood were consistently the largest. The 2021 season was the third harvest from the trial and also the first good harvest. Average yields of trees made with CIS budwood were correlated to tree size and were significantly higher than yields obtained from trees made with field-cut material.

##### **Opsomming**

Die doel van hierdie studie is om boomgesondheid, -produksie en vrugte-eienskappe te vergelyk van bome gemaak met veldgesnyde en viroïed-geïnfekteerde materiaal met dié wat deur die Sitrus Verbeteringskema (SVS) verskaf word. Ent-oordraagbare patogene, insluitend viroïede en virusse, word verwyder van kommersieële kultivars deur middel van groeipuntenting en word daarna geïnkuleer met 'n citrus tristeza virus (CTV) bron vir kruisbeskerming binne die SVS. Veldbome kan egter oor 'n tydperk 'n verskeidenheid ent-oordraagbare patogene optel deur middel van insekvektoroordraging of meganieseoordraging tydens roetine-boordpraktieke. Enthout is versamel uit oorspronklike veldbronne van die kultivars, wat verskeie CTV-rasse en sitrusviroïede bevat. Okuleerhout is op 'Swingle' citrumelo, 'Carrizo' citrange en 'C35' citrange onderstamme ge-okuleer. Dieselfde is gedoen met enthout verkry vanaf die SVS asook SVS enthout waarop CVd-IIa addisioneel geïnkuleer is. 'n Veldproef is in 2016 op Burgersfort geplant. Na vyf en 'n half jaar is beduidende verskille in boomgroei waargeneem. Aansienlike verminderde boom volumes is geassosieer met bome gemaak van veld-gesnyde materiaal van al drie kultivars. Bome gemaak van SVS materiaal was deurgaans die grootste. Die 2021 seisoen was die derde oes uit die proef en ook die eerste goeie oes. Die gemiddelde opbrengste van bome gemaak met SVS enthout het boomgroottes weerspieël en was statisties hoër as die opbrengste verkry van bome wat met veld-gesnyde materiaal gemaak was.

#### **4.2.4 PROGRESS REPORT: Field testing of commercial or potentially important rootstock selections for viroid sensitivity**

Project 1155 (2016/7 – 2025/6) by G Cook, C Steyn, J Breytenbach and J Joubert (CRI)

##### **Summary**

The choice of rootstock is an important consideration for the establishment of a citrus orchard. Apart from climate and soil suitability, rootstock selection should include considerations for resistance or tolerance to diseases and pests. Viroids are graft-transmissible agents which can induce a range of symptoms dependent on the sensitivity of the rootstock and scion. They are also mechanically transmitted by contaminated cutting tools and can unintentionally be introduced to, and spread in nurseries and orchards. Apart from diseases, namely Exocortis and Cachexia, viroids can also induce symptoms such as bark cracking and stunting. There is limited experience regarding the effect of viroids on hybrid rootstocks introduced in the past two decades, including new selections from the USA. A field trial is underway to test the sensitivity of these newer commercial or potentially commercial rootstocks to citrus dwarfing viroid (CDVd) and the non-cachexia variant of hop stunt

viroid (HSVd). The trial was planted in October 2019 in the Nelspruit district. Both relative and absolute quantitative PCR assays were developed and viroid distribution and titre were investigated in scions on four rootstock selections. Real-time PCR quantitative data was analysed as part of an MSc study. Viroid transmission detection was notably erratic in scions on some rootstocks, particularly MxT and C-22, indicating that rootstocks may influence viroid distribution within scions. Uneven spatial distribution of the viroids was observed and inner canopy sampling sites were more reliable for detection relative to outer canopy positions. HSVd also reached significantly higher copy numbers compared to CDVd in scions on all ten rootstocks. Growth parameters including canopy volumes, scion and rootstock circumferences were measured. Differences in tree growth between treatments was not observed two years post planting. However, growth differences were observed between rootstocks in accordance with the expected vigour of the respective rootstocks, but the influence of viroids on these growth parameters were not detected at this early stage.

## Opsomming

Die keuse van onderstam is belangrik in die vestiging van 'n sitrus boord. Benewens klimaats- en grondgeskiktheid moet hierdie oorweging weerstand of verdraagsaamheid teenoor siektes en plaë insluit. Viroïede is entoordraagbare entiteite wat verskeie simptome kan veroorsaak op sensitiewe bo- en onderstamme. Weens maklike meganiese oordraging deur snygereedskap en besmette enthout, word viroïede soms, per ongeluk, in kwekerie en boorde versprei. Afgesien van die siektetoestande, 'Exocortis' en 'Cachexia', kan viroïede ook simptome soos baskraak en verdwering veroorsaak. Daar is beperkte ervaring met betrekking tot die effek van viroïede op onderstamme wat die afgelope twee dekades bekendgestel is, insluitende nuwe onderstamme afkomsitg uit die VSA. 'n Veldproef is voorberei om die sensitiwiteit van hierdie nuwe kommersiële of potensieel kommersiële onderstamme teen 'citrus dwarfing' viroïed en die nie-patogeniese variant van 'hop stunt' viroïed, te toets. Die proef is in Oktober 2019 in die Nelspruit omgewing geplant. Beide relatiewe en absolute kwantitatiewe PKR toetse is ontwikkel en viroïed verspreiding en titer is ondersoek in bostamme op vier onderstam seleksies. PKR kwantitatiewe data is ontleed as deel van 'n MSc studie. Viroïed-opsporing was veral wisselvallig in bostamme op sommige onderstamme, veral MxT en C-22, wat aandui dat onderstamme viroïedverspreiding in die bostamme moontlik kan beïnvloed. Onegalige verspreiding van die viroïede in bome is waargeneem en die monsternemingsplekke binne die boom was meer betroubaar vir opsporing van viroïede relatief tot die buitenste takke. HSVd het ook aansienlik hoër kopiegetalle bereik in vergelyking met CDVd in bome op al tien onderstamme. Groeiparameters insluitend boomvolumes, bostam- en onderstamomtrek is gemeet. Verskille in boomgroei tussen behandelings is nie twee jaar na plant waargeneem nie. Verskille in groei is egter tussen onderstamme waargeneem in ooreenstemming met die verwagte groeikragtigheid van die onderskeie onderstamme, maar die invloed van viroïede op hierdie groeiparameters is nie op hierdie vroeë stadium waargeneem nie.

### 4.2.5 PROGRESS REPORT: Field evaluation of three single-strain CTV isolates on navel and soft citrus cultivars

Project 1173 (2017/8-2022/3) by G Cook, J Breytenbach, C Steyn and R de Bruyn (CRI)

## Summary

Single-strain citrus tristeza virus (CTV) isolates were characterized and evaluated in various industry cultivars in a glasshouse trial (project 1056). No detrimental symptoms were associated with these isolates. Selected cultivars and treatments of the trial were planted at various sites to evaluate field performance and to monitor the CTV translocation to new growth of the trees. This is done with the aim of understanding the effect of CTV single strains on various citrus types, in addition to evaluating them as potential cross-protection sources. Previous grapefruit field trials indicated that single-strain CTV sources were associated with better horticultural performance compared to the multi-strain sources (project 742). Grapefruit and Valencia trees were planted in the Northern Cape, Navels in Mpumalanga and a Clementine and a Mandarin hybrid were planted in Limpopo Province. The Northern Cape trials were terminated as numerous trial trees were lost due to a lack of water shortly after planting. Tree canopy volumes were determined for the fourth year for the navels and soft citrus. Significant tree size differences between CTV strain treatments were only noted with Bahianinha Navel, where the VT strain was associated with bigger trees relative to the T68- and RB-strains as well as the control trees. No tree size differences were observed between treatments with Palmer and Washington Navel

or the Clementine and Mandarin hybrid cultivars. The 2021 season was the third harvest for the soft citrus, the second harvest for the navels and the first good harvest from both trials. No yield differences were observed between treatments with the clementine, however the mandarin-hybrid trees containing the RB CTV strain were higher yielding compared to those containing the VT strain. Bahianinha Navel trees containing the VT strain were the highest yielding, but no yield differences were noted between treatments of the other two navel cultivars.

## Opsomming

Enkel-ras citrus tristeza virus (CTV) isolate is gekarakteriseer en geëvalueer in verskeie bedryfskultivars in 'n glashuis proef (projek 1056). Geen nadelige simptome was geassosieer met hierdie isolate nie. Geselekteerde kultivars en behandelings van hierdie proef is in verskeie proefpersele geplant om veldprestasie en CTV-translokasie in die plante te evalueer. Die doel hiermee is om die effek van enkel CTV-rasse op verskeie sitrustipes te bestudeer, benewens om hulle as potensiële kruisbeskermingsbronne te evalueer. Vorige pomelo proewe het aangedui dat enkelras CTV bronne beter presteer as CTV bronne bestaande uit ras mengsels (projek 742). Dit is dus van waarde om die enkel-ras CTV bronne as potensiële kruisbeskermingsbronne te evalueer. Die pomelo en Valencia-bome is in die Noord-Kaap geplant en Nawels in Mpumalanga. 'n Clementine- en 'n Mandaryn proef is in Limpopo geplant. Die Noord-Kaap-proef is beëindig aangesien talle proefbome gevrek het weens 'n tekort aan water kort na plant. Boomvolumes is vir die vierde jaar vir die nawels en sagte sitrus bepaal. Beduidende boomgrootte verskille tussen CTV-ras behandelings is slegs met Bahianinha Navel opgemerk, waar die VT-ras geassosieer is met groter bome relatief tot bome met T68- of die RB-ras asook die kontrolebome. Geen boomgrootte verskille is waargeneem tussen behandelings met Palmer en Washington Navel of die Clementine en Mandaryn kultivars nie. Die 2021-seisoen was die derde oes vir die sagte sitrus, die tweede oes vir die nawels en die eerste goeie oes van beide proewe. Geen opbrengsverskille is waargeneem tussen behandelings met die Clementine nie, maar die Mandarynbome wat die RB CTV-ras bevat het, het hoër opbrengste gelewer in vergelyking met dié wat die VT-stam bevat. Bahianinha Nawelbome wat die VT-ras bevat, het die hoogste opbrengste gelewer, maar geen opbrengsverskille is tussen behandelings van die ander twee nawelkultivars opgemerk nie.

### 4.2.6 PROGRESS REPORT: Field evaluation of two approved cross-protection sources for Grapefruit

Project 1329 (2021/22 – 2027/28) by G Cook, J Breytenbach, C Steyn, R de Bruyn and J Joubert (CRI)

## Summary

Historically, grapefruit has been the citrus type most affected by citrus tristeza virus (CTV) in South Africa. The Citrus Improvement Scheme (CIS) implemented a CTV cross-protection programme to mitigate the effects of field challenges by severe strains. GFMS35 is the CTV source currently used for grapefruit pre-immunisation. Comparative field trials showed that a single-strain isolate, B390-5, was associated with good tree health and production. The Citrus Improvement Scheme Advisory Committee (CISAC) approved B390-5 as a new pre-immunisation source for grapefruit pending a comparative assessment to GFMS35 in semi-commercial trials. Tree preparation for two field trials has commenced. Swingle citrumelo rootstocks were budded with Star Ruby containing the respective CTV sources at both CRI and a commercial nursery. Trials will be planted at two trial sites in the spring of 2022.

## Opsomming

Histories was pomelo die sitrustipe wat die meeste deur sitrus tristeza-virus (CTV) in Suid-Afrika aangetas is. Die Sitrusverbeteringskema (SVS) het 'n CTV-kruisbeskermingsprogram geïmplementeer om die gevolge van veldinfeksies deur strawwe rasse te verminder. GFMS35 is die CTV-bron wat tans gebruik word vir enting van pomelo's. Vergelykende veldproewe het getoon dat 'n enkelras CTV-isolaat, B390-5, goeie boomgesondheid en –produksie gelewer het. Die Advieskomitee vir Sitrusverbeteringskema (CISAC) het B390-5 goedgekeur as 'n nuwe bron vir enting vir pomelo's, afhangend van verdere vergelykende semi-kommersiele veld proewe, met GFMS35. Boomvoorbereiding vir twee veldproewe is aan die gang. Swingle-sitrumelo-onderstamme is

geokuleer met Star Ruby wat die onderskeie CTV-bronne bevat. Bome word voorberei beide by CRI en by 'n kommersiële kwekery. Proewe sal in die lente van 2022 by twee proefpersele geplant word.

#### 4.3 PROGRAMME: PREHARVEST DISEASES

Programme coordinator: Jacquie van Der Waals (CRI)

##### 4.3.1 Programme summary

Sustainable management of fruit, foliar and soilborne diseases of citrus remains the primary focus of projects in the preharvest disease programme. This entails searching for and screening alternative, softer management options, improving chemical control programmes to prevent resistance development and spread, improving spray technology and studying the epidemiology of the different pathogens.

Citrus Black Spot (CBS) remains the most important disease in the South African citrus industry, and to this end a number of projects are aimed at improving management and detection of *Phyllosticta citricarpa*, the causal pathogen. Complementary to these projects are studies focused on understanding the population structure, epidemiology and fungicide resistance development of the pathogen in South Africa.

Project 970 (4.3.10) focusses on the development of new chemical spray programmes for management of CBS. Results showed that an experimental fungicide, Product X, gave good CBS control (>99% CBS free fruit) at the highest concentration and when mixed with mineral oil. No phytotoxicity was observed on fruit treated with Product X. The season was, however, marked by low disease pressure. Nonetheless, Product X is a possible candidate for consideration for CBS registration trials and hopefully it will result in clean fruit (CBS free) suitable for the export market, even under high disease pressure.

Projects focused on understanding the epidemiology of CBS include 1235 (4.3.7), 1223 (4.3.4), 1238 (4.3.14) and 1244 (4.3.5). Correct timing of fungicide applications is critical in the management of CBS. CRI-PhytRisk is a CBS-risk management platform that provides decision support to citrus growers in CBS areas, to improve fungicide spray timing, and indicates CBS-risk on fruit destined for export, based on the past season's weather conditions and CBS infection predictions. The CBS models were again validated in a collaborative project (4.3.14) with Brazilian researchers. The CRI-PhytRisk platform has been completely upgraded to newer, more efficient technology. It is proposed that CRI-PhytRisk continue as a research service project, with funding allocated for maintenance and continuous improvement. Project 1244 investigated the epidemiology, inoculum potential and infection parameters of *P. citricarpa*. Temperatures of 21 and 27°C were found to be most suitable for infection of Troyer citrange leaves using pycnidiospores of *P. citricarpa*, and no infection was observed at 15°C. Symptom expression in the infection trials was, however, erratic (once in four repeats). Findings of these trials could be used to adjust infection parameters in the CRI-PhytRisk model, which is currently based on germination studies and not infection *per se*. Control of the disease includes management of pruning debris. Project 1223 (4.3.4) therefore aimed to determine to what extent chopped or shredded pruning debris contribute to CBS inoculum in citrus orchards. Results showed that shredding or chopping of pruning debris in the orchard can lead to reduced ascospore inoculum load and can potentially contribute to CBS management.

Besides insight into infection periods and inoculum sources of *P. citricarpa*, knowledge of the population genetic structure is important for a comprehensive understanding of CBS disease epidemiology. The study in project 1235 (4.3.7) is the first to use high throughput sequencing (HTS) to study the genetic diversity and connectivity of *P. citricarpa* populations. The results obtained confirm the clonal nature of the USA *P. citricarpa* isolates, and *in silico* detection of mating types confirmed the presence of only one mating type in the USA isolates. Three SSR marker sets were developed, and gave similar results in terms of the genetic relationship/connectivity of isolates from China, the USA and South Africa as previously published by Carstens *et al.* (2017). Isolates from the five South African provinces where citrus black spot is present, also showed a genetic overlap, corroborating the findings of Carstens *et al.* (2017). Furthermore, whole genome sequence data was used to confirm the mutations in the  $\beta$ -tubulin gene conferring benzimidazole resistance (Moyo *et al.*, 2021) in *P. citricarpa*. Highly specific PCR primers that can be used for rapid and efficient detection of benzimidazole resistance in *P. citricarpa* were designed in project 1316 (4.3.7). This testing assay will allow

growers to determine resistance frequencies in their orchards and also make better informed management decisions.

Alternaria brown spot (ABS), caused by *Alternaria alternata*, is a serious disease of tangerines (*Citrus reticulata*) and their hybrids in all citrus producing regions of South Africa. As with CBS, ABS is controlled primarily through fungicide applications. Project 750 (4.3.10) evaluated the efficacy of various spray programmes in controlling ABS on 'Nova' mandarins in the Kirkwood and Buffeljagsrivier areas in the Eastern Cape (EC) and Western Cape (WC) provinces, respectively. Unfortunately, none of the programmes provided satisfactory control of ABS, especially under high disease pressure.

Projects 762 (4.3.11) and 1030 (4.3.12) are specifically aimed at finding alternative means of control of *Phytophthora* and citrus nematode. The aim of project 762 is to identify pre-plant treatments that are effective in keeping orchard soils free from citrus nematode and *Phytophthora* spp. for as long as possible after planting. The trial has been running since January 2010. To date no treatment has stood out in terms of nematode control. However, based on tree height and trunk diameter measurements, the pre-plant fumigation treatments with 1,3 dichloropropene and metam sodium are beginning to show potential. It is therefore becoming clear that pre-plant soil fumigation in a replant situation does improve tree growth in comparison to no treatment or post-plant treatments. In project 1030, in the 2021/22 period, further monitoring of tree height, stem diameter and nematode juvenile counts in the soil was done at a preplant fumigation site in the Kirkwood area, Eastern Cape. Results indicated clearly that four years after fumigation and tree planting, trees planted in fumigated soil had significantly thicker stems and were significantly taller compared to trees planted in non-fumigated soil. It was furthermore seen that citrus nematode juvenile counts as well as *Phytophthora* levels were significantly lower in fumigated soil. The beneficial effect of preplant fumigation at this stage is again clearly evident. Two potential new, unregistered nematicides were also evaluated at two trial sites, in the Western Cape and Mpumalanga. From results obtained it was seen that the *Paecilomyces lilacinus*-based biological control product has potential to be used in an additive capacity in combination with current chemical control options. It seems that it could provide more long-term control of nematodes but that its efficacy is possibly dependent on soil type. The new chemical nematicide, A22011B, also showed great promise in comparison with currently registered chemical nematicides. It was noted that regardless of soil type, one application of 0.67 ml/tree gave consistent results in the control of nematode females and juveniles, which compared very favourably with current registered chemical nematicides.

A severe decline and subsequent death of citrus trees has been reported from the Gamtoos and Sunday's River valleys for a number of years. Surveys of diseased trees were done as part of project 1068 (4.3.13) in the Gamtoos and Sunday's River Valley production areas. Several pathogens, including four new *Neocosmospora* species, were identified in association with the internal root and trunk decay symptoms seen in declining trees. Investigations indicated that high soil pH and EC probably contributed to predisposing trees to infection by the different *Neocosmospora* spp. identified. This could also explain why orchards planted on Rough Lemon does not have any problems. To identify other rootstock options, a trial containing 10 different rootstocks was planted in March 2021. Several treatment programs with chemicals and natural plant extract products were also tested. Preliminary results showed that a benomyl drench treatment or a foliar spray treatment with specific plant extract products, were able to maintain or improve tree health to the same level as trees grown in soil fumigated preplant. These treatments will therefore be evaluated further in the rootstock trial. Comparison of bacterial communities in the roots of diseased and healthy trees showed that that certain bacterial genera are only associated with diseased, declining trees. Their significance needs to be investigated, especially their interactions with the associated pathogens.

Fibrous root rot of citrus is caused by *Phytophthora nicotianae* and to a lesser extent by *Phytophthora citrophthora* in South Africa. Infection of plants in nurseries or orchards can result in severe losses to the industry, necessitating the use of effective and sustainable control options.

Phosphonate fungicides have been effectively used for the management of this disease. Recently, reports of reduced sensitivity to these fungicides have emerged for *Phytophthora*. Project 1302 (4.3.15) aims to characterise the *in vitro* and *in planta* phosphonate sensitivity of *P. nicotianae* isolates from South African citrus production areas. To determine the sensitivity of the *P. nicotianae* isolates to potassium phosphite (Phosphite

400SL, Villa Crop Protection) and ammonium phosphite (Brilliant SL, UPL), *in vitro* phosphonate sensitivity trials were conducted by an agar dilution method. The isolates displayed a continuous range of sensitivity values, without any clear sensitivity groupings, that did not differ significantly between products. Project 1303 (4.3.16) investigated the effect of different rootstocks (Delta Valencia, Rough Lemon (RL), Carrizo Citrange (CC) and X639) on phosphite translocation and persistence in trees after phosphonate foliar applications for *Phytophthora* disease control. Results indicated that at Letaba Estates the phosphite residues in fruit from RL trees peaked sooner after spraying, and to a higher level, than in fruit from CC or X639 trees. Good control of *Phytophthora* brown rot was therefore reached much faster on RL tree fruit. It was seen that a phosphite residue of 6 mg/kg was needed for brown rot control. It therefore seems that in RL trees phosphite is translocated faster with less breakdown compared to the other rootstocks in the study. Knowledge gained from these two projects will inform industry on the current efficacy of phosphonates for the management of *Phytophthora* diseases in citrus and facilitate informed resistance management strategies.

Phenylamides have been used successfully for many years to control *Phytophthora* spp.. However, there are reports of insensitivity development against these oomycides by the pathogen. In project 1305 (4.3.18), the sensitivity of *P. nicotianae* isolates to ethaboxam, mandipropamid, zoxamide and amisulbrom was assessed *in vitro* using oomycete fungicide-amended media to determine the EC<sub>50</sub> values of each compound. The results indicate that *P. nicotianae* isolates are more sensitive to ethaboxam, mandipropamid, zoxamide than to current synthetic fungicides; however, the screened isolates showed no sensitivity to amisulbrom.

The occurrence of outbreaks of *Phytophthora* root rot in nurseries can be attributed to several factors, including the presence of *Phytophthora* propagules in growth media and/or irrigation water, over-seasoning inoculum present in asymptomatic nursery stock and wind-blown particles carrying inoculum. The main objective of project 1304 (4.3.17) is to identify novel sources of *Phytophthora* contamination in citrus nurseries. From the processed samples, 95 putative *Phytophthora* isolates have been recovered and identified microscopically, and of the 28 putative isolates whose ITS was sequenced, three isolates were confirmed to be *Phytophthora* spp. The other fungal genera recovered were *Fusarium* (12), *Phytophthium* (5), *Pythium* (6), *Neocosmospora* (1) and *Aspergillus* (1). To date, most putative *Phytophthora* isolates were recovered from potting media collected from asymptomatic potted citrus seedlings, indicating a potential contamination source in nurseries, although it is too early to state definitively. The picture may change as more samples are processed and putative *Phytophthora* isolates are identified molecularly.

Regular testing for the presence of *Phytophthora* spp. is a requirement for certified citrus nurseries. In project 1337 (4.3.20), the effects of nursery media sample storage time, storage temperature or exposure to sunlight on *Phytophthora* analysis results, were investigated. Results indicated that if coir and Klasmann peat samples were stored at 4°C, the positivity percentage declined significantly with increasing storage time. When *Phytophthora nicotianae* infested samples were stored at 25°C or 30°C, the mean percentage of positive samples remained constant at nearly 100%. Comparison of the different testing techniques showed that the traditional soil baiting gave the most accurate results and that the plating of baiting liquid and species-specific PCR gave high a percentage of false negative results. The latter two methods are therefore not suitable for use as validation methods for the soil baiting method. From this study it is evident that when samples from nurseries are collected, they can safely be stored at 25°C if long term storage is required. However, when stored at 4°C, storage should not exceed 7 days. In future studies the sun exposure experiment should be repeated during summer months. The sensitivity of soil baiting furthermore also needs to be determined.

*Colletotrichum* is one of the most important genera of plant pathogenic fungi, causing several diseases including citrus anthracnose. In recent years, postharvest anthracnose has become a serious limiting factor in various citrus production areas. Because of the potential negative impact that *Colletotrichum* infections may have on citrus production, new surveys are required to study inoculum sources (i.e. flowers, leaves, fruit and twigs) to identify which *Colletotrichum* species are associated with which citrus diseases in Southern Africa, to determine when fruit becomes infected and what the best chemical options would be to manage them. Project 1306 therefore aims to provide new insight into the epidemiology of *Colletotrichum* species associated with anthracnose-like symptoms and to establish which registered fungicides are effective in managing these species. Preliminary identifications, grouped a subset of samples into four groups, namely *C. gloeosporioides* (69% of the samples), *C. karstii* (29%), *C. cigarro* (1%) and *C. novae-zelandiae* (1%).

Project 1236 (4.3.2) is an investigation into the epidemiology of Botrytis on lemons. Work from the study indicated that lemon blossoms and broad leaf weeds are the primary sources of inoculum in lemon orchards. It was furthermore seen that some fungicides have potential to control this disease and should be pursued. Validation of the Botrytis prediction model incorporated in CRI PhytRisk is still ongoing and would be a valuable management tool.

## Programopsomming

Volhoubare bestuur van vrug-, blaar- en grondgedraagde siektes van sitrus bly die primêre fokus van projekte in die voor-oes siekteprogram. Dit behels die soeke na, en toets van alternatiewe, sagter bestuurs-opsies, die verbetering van chemiese beheerprogramme om weerstandsontwikkeling en -verspreiding te voorkom, die verbetering van spuittegnologie, en die bestudering van die epidemiologie van die verskillende patogene.

Sitruswartvlek (CBS) bly die belangrikste siekte in die Suid-Afrikaanse sitrusbedryf, en vir hierdie doel is 'n aantal projekte daarop gemik om die bestuur en opsporing van *Phyllosticta citricarpa*, die veroorsakende patogeen, te verbeter. Aanvullend tot hierdie projekte is studies wat daarop fokus om die populasiestruktuur, epidemiologie en swamdoderweerstandontwikkeling van die patogeen in Suid-Afrika te verstaan.

Projek 970 (4.3.10) fokus op die ontwikkeling van nuwe chemiese spuitprogramme vir die bestuur van CBS. Resultate het getoon dat 'n eksperimentele swamdoder, Produk X, goeie CBS-beheer gegee het (>99% CBS-vrye vrugte) by die hoogste konsentrasie, en wanneer dit met minerale olie gemeng is. Geen fitotoksiteit is waargeneem op vrugte wat met Produk X behandel is nie. Die seisoen is egter deur lae siektedruk gekenmerk. Nietemin is Produk X 'n moontlike kandidaat vir oorweging vir CBS-registrasieproewe en hopelik sal dit tot skoon vrugte (CBS-vry) lei, geskik vir die uitvoermark, selfs onder hoë siektedruk.

Projekte wat daarop gefokus is om die epidemiologie van CBS te verstaan, sluit 1235 (4.3.7), 1223 (4.3.4), 1238 (4.3.14) en 1244 (4.3.5) in. Korrekte tydsberekening van swamdodertoedienings is van kritieke belang in die bestuur van CBS. CRI-PhytRisk is 'n CBS-risikobestuursplatform wat besluitnemingsondersteuning aan sitrusprodusente in CBS-gebiede bied, ten einde swamdoder spuittyd te verbeter, en CBS-risiko op vrugte wat vir uitvoer bestem is, aan te dui, gebaseer op die afgelope seisoen se weerstoestand en CBS-infeksievoorspellings. Die CBS-modelle is weer in 'n samewerkende projek (4.3.14) met Brasiliaanse navorsers bekragtig. Die CRI-PhytRisk-platform is heeltemal na nuwer, meer doeltreffende tegnologie opgradeer. Daar word voorgestel dat CRI-PhytRisk voortgaan as 'n navorsingsdiensprojek, met befondsing wat vir instandhouding en voortdurende verbetering toegeken word. Projek 1244 (4.3.5) het die epidemiologie, inokulumpotensiaal en infeksieparameters van *P. citricarpa* ondersoek. Daar is gevind dat temperature van 21 en 27°C die geskikste is vir infeksie van Troyer citrange blare deur gebruik te maak van piknidiospore van *P. citricarpa*. Geen infeksie is by 15°C waargeneem nie. Simptoom-uitdrukking in die infeksieproewe was egter wisselvallig (een keer in vier herhalings). Bevindinge van hierdie proewe kan gebruik word om infeksieparameters in die CRI-PhytRisk-model aan te pas, wat tans op ontkiemingstudies en nie infeksie *per se* gebaseer is. Beheer van die siekte sluit die bestuur van snoei-afval in. Projek 1223 (4.3.4) het dus ten doel gehad om te bepaal tot watter mate gekapte of gesnipperde snoei-afval tot CBS-inokulum in sitrusboorde bydra. Resultate het getoon dat versnippering of kap van snoei-afval in die boord tot verminderde askospoor-inokulumlading kan lei, en moontlik tot CBS-bestuur kan bydra.

Benewens insig in infeksieperiodes en inokulumbronne van *P. citricarpa*, is kennis van die populasie-genetiese struktuur belangrik vir 'n omvattende begrip van CBS-siekte-epidemiologie. Die studie in projek 1235 (4.3.7) is die eerste wat hoë deurvloei volgorde-bepaling (HTS) gebruik om die genetiese diversiteit en verwantskap van *P. citricarpa* populasies te bestudeer. Die resultate wat verkry is bevestig die klonale aard van die VSA *P. citricarpa* isolate, en *in silico*-opsporing van paringstipes het die teenwoordigheid van slegs een paringstipe in die VSA isolate bevestig. Drie SSR merkerstelle is ontwikkel, en het soortgelyke resultate gegee in terme van die genetiese verwantskap/konnektiwiteit van isolate uit China, die VSA en Suid-Afrika, soos voorheen gepubliseer deur Carstens *et al.* (2017). Isolate van die vyf Suid-Afrikaanse provinsies waar sitruswartvlek teenwoordig is, het ook 'n genetiese oorvleueling getoon, wat die bevindinge van Carstens *et al.* (2017) bevestig. Verder is heel genoom volgordedata gebruik om die mutasies in die  $\beta$ -tubuliene te bevestig wat

bensimidazoleweerstand (Moyo *et al.*, 2021) in *P. citricarpa* verleen. Hoogs spesifieke PKR-inleiers wat vir vinnige en doeltreffende opsporing van bensimidazoleweerstand in *P. citricarpa* gebruik kan word, is in projek 1316 (4.3.7) ontwerp. Hierdie toets sal produsente in staat stel om weerstandsfrekwensies in hul boorde te bepaal en ook beter ingeligte bestuursbesluite te neem.

Alternaria bruinvlek (ABS), wat deur *Alternaria alternata* veroorsaak word, is 'n ernstige siekte van mandaryne (*Citrus reticulata*) en hul hibriede in alle sitrusproduserende streke van Suid-Afrika. Soos met CBS, word ABS hoofsaaklik deur swamdodertoedienings beheer. Projek 750 (4.3.10) het die doeltreffendheid van verskeie spuitprogramme in die beheer van ABS op 'Nova' mandaryne in die Kirkwood- en Buffeljagsrivier-gebiede in onderskeidelik die Oos- en Wes-Kaap- provinsies geëvalueer. Ongelukkig het geen van die programme bevredigende beheer van ABS verskaf nie, veral onder hoë siektedruk.

Projekte 762 (4.3.11) en 1030 (4.3.12) is spesifiek daarop gemik om alternatiewe maniere vir beheer van *Phytophthora* en sitrusaalwurm te vind. Die doel van projek 762 is om vóórplant-behandelings te identifiseer wat doeltreffend is om boordgrond vry te hou van sitrusaalwurm en *Phytophthora* spp., so lank as moontlik ná plant. Die proef duur sedert Januarie 2010. Tot op hede het geen behandeling uitgestaan in terme van aalwurmbeheer nie. Gebaseer op boomhoogte en stamdeursnee-metings, begin die vóórplant-berokingsbehandelings met 1,3 dichloorpropeen en metamnatrium egter potensiaal toon. Dit word dus duidelik dat vóórplant-grondberoking in 'n herplantsituasie wel boomgroei verbeter, in vergelyking met geen behandeling of ná-plant behandelings. In projek 1030, in die 2021/22 periode, is verdere monitering van boomhoogte, stamdeursnee en aalwurm larwe-tellings in die grond gedoen by 'n vóórplant-berokingsperseel in die Kirkwood-area, Oos-Kaap. Resultate het duidelik aangedui dat vier jaar ná beroking en boomplanting, bome wat in berookte grond geplant is, aansienlik dikker stamme gehad het en aansienlik hoër was in vergelyking met bome wat in nie-berookte grond geplant is. Daar is verder gesien dat sitrusaalwurm larwe-tellings, sowel as *Phytophthora*-vlakke, betekenisvol laer was in berookte grond. Die voordelige effek van vóórplant-beroking op hierdie stadium is weer duidelik sigbaar. Twee potensiële nuwe, ongeregisteerde aalwurmdoders is ook by twee proefpersele in die Wes-Kaap en Mpumalanga geëvalueer. Uit resultate wat verkry is, is gesien dat die *Paecilomyces lilacinus*-gebaseerde biologiese beheerproduk potensiaal het om in 'n bydraende kapasiteit, in kombinasie met huidige chemiese beheer-opsies, gebruik te word. Dit blyk dat dit meer langtermyn beheer van aalwurms kan verskaf, maar dat die doeltreffendheid daarvan moontlik afhanklik is van grondtipe. Die nuwe chemiese aalwurmdoder, A22011B, het ook groot belofte getoon in vergelyking met huidige geregisteerde chemiese aalwurmdoders. Daar is opgemerk dat, ongeag grondtipe, een toediening van 0.67 ml/boom konsekwente resultate in die beheer van aalwurmwyses en -larwes gelewer het, wat baie gunstig vergelyk het met huidige geregisteerde chemiese aalwurmdoders.

'n Ernstige agteruitgang, en daaropvolgende dood van sitrusbome, word al vir 'n aantal jare vanuit die Gamtoos- en Sondagsriviervalleie gerapporteer. Opnames van siek bome is as deel van projek 1068 (4.3.13) in die Gamtoos- en Sondagsriviervallei-produksiegebiede gedoen. Verskeie patogene, insluitend vier nuwe *Neocosmospora* spesies, is in verband met die interne wortel- en stambederf simptome wat in kwynende bome gesien word, geïdentifiseer. Ondersoek het aangedui dat hoë grond pH en EC waarskynlik daartoe bygedra het dat bome meer vatbaar vir infeksie deur die verskillende geïdentifiseerde *Neocosmospora* spp. gemaak word. Dit kan ook verklaar waarom boorde wat op 'Rough lemon' geplant is, geen probleme het nie. Ten einde ander onderstam-opsies te identifiseer, is 'n proef met 10 verskillende onderstamme in Maart 2021 geplant. Verskeie behandelingsprogramme met chemikalieë en natuurlike plant-ekstrakprodukte is ook getoets. Voorlopige resultate het getoon dat 'n benomiël-deurdrenkbehandeling of 'n blaarbespuitingsbehandeling met spesifieke plant-ekstrakprodukte in staat was om boomgesondheid te handhaaf of tot dieselfde vlak te verbeter as bome wat geplant is in grond wat vóór plant berook is. Hierdie behandelings sal dus verder in die onderstamproef geëvalueer word. Vergelyking van bakteriese populasies in die wortels van siek en gesonde bome, het getoon dat sekere bakteriese genera slegs met siek, kwynende bome geassosieer word. Die betekenis hiervan moet ondersoek word, veral hul interaksies met die geassosieerde patogene.

Sitruswortelvrot word deur *Phytophthora nicotianae* en in 'n mindere mate deur *Phytophthora citrophthora* in Suid-Afrika veroorsaak. Infeksie van plante in kwekerye of boorde kan ernstige verliese vir die bedryf tot gevolg hê, wat die gebruik van effektiewe en volhoubare beheer-opsies noodsaak.

Fosfonaat swamdoders word doeltreffend gebruik vir die bestuur van hierdie siekte. Onlangs het berigte van verminderde sensitiviteit teen hierdie swamdoders vir *Phytophthora* na vore gekom. Projek 1302 (4.3.15) het ten doel om die *in vitro* en *in planta* fosfonaat sensitiviteit van *P. nicotianae* isolate uit Suid-Afrikaanse sitrusproduksiegebiede te karakteriseer. Om die sensitiviteit van die *P. nicotianae* isolate vir kaliumfosfiet (Phosphite 400SL, Villa Crop Protection) en ammoniumfosfiet (Brilliant SL, UPL) te bepaal, is *in vitro* fosfonaat sensitiviteitsproewe met 'n agarverduunningsmetode uitgevoer. Die isolate het 'n deurlopende reeks sensitiviteitswaardes vertoon, sonder enige duidelike sensitiviteitsgroepeerings, wat nie betekenisvol tussen produkte verskil het nie. Projek 1303 (4.3.16) het die effek van verskillende onderstamme (Delta Valencia, Rough Lemon (RL), Carrizo Citrange (CC) en X639) op fosfiettranslokasie en volharding in bome ná fosfonaatblaartoedienings vir *Phytophthora*-siektebeheer ondersoek. Resultate het aangedui dat by Letaba Estates, die fosfietreste in vrugte van RL-bome vinniger 'n piek ná bespuiting bereik het, en tot 'n hoër vlak, as in vrugte van CC- of X639-bome. Goeie beheer van *Phytophthora* bruinvrot is dus baie vinniger op RL-boomvrugte bereik. Dit blyk dus dat in RL-bome fosfiet vinniger getranslokeer word met minder afbraak in vergelyking met die ander onderstamme in die studie. Kennis verkry uit hierdie twee projekte sal die industrie inlig oor die huidige doeltreffendheid van fosfonate vir die bestuur van *Phytophthora* siektes in sitrus, en ingeligte weerstandbestuurstrategieë fasiliteer.

Fenielamiede is al vir baie jare suksesvol gebruik om *Phytophthora* spp. te beheer. Daar is egter verslae van die ontwikkeling van verminderde sensitiviteit teen hierdie middels deur die patoog. In projek 1305 (4.3.18) is die sensitiviteit van *P. nicotianae* isolate vir etaboxam, mandipropamied, zoxamied en amisulbrom *in vitro* geassesseer deur gebruik te maak van oömiseet swamdoder-gewysigde media om die EC<sub>50</sub> waardes van elke verbinding te bepaal. Die resultate dui daarop dat *P. nicotianae* isolate meer sensitief is vir etaboxam, mandipropamied en zoxamied as vir huidige sintetiese swamdoders. Die getoetste isolate het egter geen sensitiviteit vir amisulbrom getoon nie.

Die voorkoms van uitbrake van *Phytophthora*-wortelvrot in kwekerye kan toegeskryf word aan verskeie faktore, insluitend die teenwoordigheid van *Phytophthora*-propagules in groeimedia en/of besproeiingswater, inokulum vanaf die vorige seisoen teenwoordig in asimptomatiese kwekeryvoorraad, en windgewaaide deeltjies wat inokulum dra. Die hoofdoel van projek 1304 (4.3.17) is om nuwe bronne van *Phytophthora*-besmetting in sitruskwekerye te identifiseer. Van die verwerkte monsters is 95 moontlike *Phytophthora*-isolate herwin en mikroskopies geïdentifiseer, en van die 28 moontlike isolate waarvan die ITS volgordes bepaal is, is drie isolate as *Phytophthora* spp. bevestig. Die ander swamgenera wat herwin is, was *Fusarium* (12), *Phytophthium* (5), *Pythium* (6), *Neocosmospora* (1) en *Aspergillus* (1). Tot op hede is die meeste moontlike *Phytophthora*-isolate uit potmedia wat van asimptomatiese sitrussaailinge versamel is, herwin, wat op 'n potensiële besmettingsbron in kwekerye dui, alhoewel dit te vroeg is om definitief te verklaar. Die prentjie kan verander namate meer monsters verwerk word en moontlike *Phytophthora*-isolate molekulêr geïdentifiseer word.

Gereelde toetsing vir die teenwoordigheid van *Phytophthora* spp. is 'n vereiste vir gesertifiseerde sitruskwekerye. In projek 1337 (4.3.20) is die uitwerking van kwekerymedia opbergingstyd, opbergingstemperatuur of blootstelling aan sonlig op *Phytophthora*-ontledingsresultate ondersoek. Resultate het aangedui dat indien kokos- en Klasmann-veenmonsters by 4°C gestoor is, die positiviteitspersentasie betekenisvol afgeneem het met toenemende opbergingstyd. Wanneer *Phytophthora nicotianae*-geïnfekteerde monsters by 25°C of 30°C gestoor is, het die gemiddelde persentasie positiewe monsters konstant op byna 100% gebly. Vergelyking van die verskillende toetstegnieke het getoon dat die tradisionele grondlokaas die mees akkurate resultate gelewer het, en dat die uitplaat van lokaasvloeistof, en spesie-spesifieke PKR 'n hoë persentasie vals negatiewe resultate gegee het. Laasgenoemde twee metodes is dus nie geskik vir gebruik as bekragtigingsmetodes vir die grondlokaasmetode nie. Uit hierdie studie is dit duidelik dat wanneer monsters vanaf kwekerye versamel word, dit veilig by 25°C gestoor kan word indien langtermyn opberging vereis word. Wanneer dit egter by 4°C gestoor word, moet opberging nie 7 dae oorskry nie. In toekomstige studies moet die sonblootstellingseksperiment gedurende somermaande herhaal word. Die sensitiviteit van grondlokaas moet verder ook bepaal word.

*Colletotrichum* is een van die belangrikste genera van plantpatogeniese swamme, wat verskeie siektes veroorsaak, insluitend sitrus antraknose. In onlangse jare het na-oes antraknose 'n ernstige beperkende faktor in verskeie sitrusproduksiegebiede geword. As gevolg van die potensiële negatiewe impak wat *Colletotrichum*

infeksies op sitrusproduksie kan hê, is nuwe opnames nodig om inokulumbronne (d.w.s. blomme, blare, vrugte en takkies) te bestudeer om te identifiseer watter *Colletotrichum*-spesies met watter sitrussiektes in Suider-Afrika geassosieer word, om te bepaal wanneer vrugte besmet raak, en wat die beste chemiese opsies sal wees om dit te bestuur. Projek 1306 het dus ten doel om nuwe insig te gee in die epidemiologie van *Colletotrichum* spesies wat met antraknose-agtige simptome geassosieer word en om vas te stel watter geregistreerde swamdoders effektief is in die bestuur van hierdie spesies. Voorlopige identifikasies het 'n substel van monsters in vier groepe gegroepeer, naamlik *C. gloeosporioides* (69% van die monsters), *C. karstii* (29%), *C. cigarro* (1%) en *C. novae-zelandiae* (1%).

Projek 1236 (4.3.2) is 'n ondersoek na die epidemiologie van *Botrytis* op suurlemoene. Werk uit die studie het aangedui dat suurlemoenbloeisels en breëblaar-onkruid die primêre bronne van inokulum in suurlemoenboorde is. Daar is verder gesien dat sommige swamdoders potensiaal het om hierdie siekte te beheer, en gevolg moet word. Bekragtiging van die *Botrytis*-voorspellingsmodel wat in CRI PhytRisk geïnkorporeer is, is steeds aan die gang en sal 'n waardevolle bestuursinstrument wees.

#### 4.3.2 FINAL REPORT: Epidemiology and management of *Botrytis cinerea* in citrus

Project 1236 (Jan. 2019 – June 2022) by Dr Cheryl Lennox and Dr Julia Meitz-Hopkins (SU)

##### Summary

When lemon blossoms are infected with *Botrytis cinerea*, rind distortion occurs on juvenile fruitlets. The cork-like scars on the surface of mature fruit result in losses due to downgraded fruit. There is currently no known prediction model for *Botrytis* infection on lemon blossoms and no registered fungicides on citrus. Thus, the aim of this study was to evaluate a risk prediction model for *B. cinerea* on citrus, developed by Citrus Research International, and to evaluate fungicide efficacy on lemons.

The fungicides azoxystrobin, fenhexamid, fludioxonil, iprodione, pyrimethanil, and benomyl are highly effective in controlling *B. cinerea* on other crops. Therefore, 20 *B. cinerea* isolates from each of three lemon orchards were tested *in vitro* against previously determined discriminatory fungicide doses to classify them as sensitive or resistant to these fungicides. An orchard trial was conducted with recommended dosages for azoxystrobin, fenhexamid, iprodione, or benomyl over two seasons. The risk prediction model was evaluated by sampling blossoms and comparing the incidence of blossom infection to the predicted risk.

*In vitro*, 70 % of the isolates were highly sensitive to azoxystrobin, 98 % of isolates were highly sensitive to fenhexamid. All 60 isolates were highly sensitive to fludioxonil and iprodione, while 70 % of isolates were highly sensitive to both pyrimethanil and benomyl. However, eight isolates from both the Jonkershoek and Franschhoek areas were highly resistant to benomyl. In the 2021 and 2022 orchard trials, benomyl had the highest efficacy and reduced the mean incidence of blossom infection by 68 % in both years, compared to the untreated control. Azoxystrobin was less effective with a mean reduction of infection incidence of 44 %.

Due to the similar incidence of *B. cinerea* infection on lemon blossoms during a predicted medium risk of infection and predicted low risk of infection, the prediction model could not be validated. During a predicted low risk in Citrusdal, an incidence of 43% was recorded, compared to 47% for a medium risk of infection. In Jonkershoek, the incidence of infection was 3 %, 39 %, and 58 %, all for predicted medium risk periods in 2019, 2020.

Fenhexamid, azoxystrobin, and benomyl were highly effective in controlling *B. cinerea* on blossoms. However, as benomyl residues are not accepted in the EU, future studies should focus on evaluating higher dosages of fenhexamid. The risk prediction model needs further development, focusing on the role of temperature on infection. The model should be evaluated over several years, as the risk of infection might be medium for the whole flowering period in one year and low or high the next. It is also recommended that the model is tested in conjunction with fungicide applications, after it has been verified. This will give a clearer indication as to whether the occurrence of rind distortion can be reduced when fungicides are applied according to the model.

## Opsomming

Wanneer suurlemoen bloeisels deur *Botrytis cinerea* geïnfekteer word, kom skilverwringing voor op jong vrugte. Dit veroorsaak 'n kosmetiese-siekte wat 'n negatiewe finansiële impak het, omdat die vrugte afgegradeer word weens die kurkagtige letsels op die volwasse vrug. Dit is tans onmoontlik om te voorspel wanneer bloeisels geïnfekteer word en daar is ook geen swamdoder geregistreer vir die beheer van *B. cinerea* op sitrus nie. Die doel van hierdie studie was dus om 'n siektevoorspellingsmodel wat deur Citrus Research International ontwikkel is vir *B. cinerea* op sitrus, te evalueer, en om die effektiwiteit van botrytisdoders te evalueer in die beheer van *B. cinerea* op suurlemoene.

Die swamdoders asoksistrobien, fenheksamied, fludioxonil, iprodioon, pirimetaniel en benomiël is hoogs effektief in die beheer *B. cinerea* op ander gewasse. Daarom is *B. cinerea* isolate van drie suurlemoen boorde wat nog nie voorheen aan swamdoders blootgestel is nie, *in vitro* getoets teen voorheen bepaalde diskriminerende dosisse van die bogenoemde swamdoders, in miselium groei-toetse. 'n Veldproef is ook uitgevoer met die aanbevole dosisse van geformuleerde swamdoders wat asoksistrobien, fenheksamied, iprodioon en benomiël as aktiewe bestanddeel bevat. Die risiko voorspellingsmodel is geëvalueer deur bloeisel monsters te neem en die voorkoms van bloeisel infeksie te vergelyk met die voorspelde risiko. Weerdata van die streke is geanaliseer om die parameters vas te stel wat tot hoër voorkoms van infeksie lei.

Die miselium groei-toetse het gewys dat 'n meerderheid van die isolate vanuit die drie streke hoogs sensitief was vir die diskriminerende dosisse. In totaal was 70 % van die isolate hoogs sensitief vir asoksistrobien, 98 % van die isolate was hoogs sensitief vir fenheksamied, en al 60 isolate was hoogs sensitief vir fludioxonil en iprodioon. Verder was 70 % van die isolate hoogs sensitief vir pirimetaniel en benomiël. Isolate van die Citrusdal area het die hoogste sensitiwiteit getoon, gevolg deur isolate van die Jonkershoek en Franschhoek areas. Agt isolate van die Jonkershoek area, asook agt isolate van die Franschhoek area was hoogs weerstandbiedend teen benomiël.

Benomiël het die hoogste effektiwiteit gehad in die veldproewe (2020 en 2021) en die voorkoms van geïnfekteerde bloeisels met 68 % verminder, in vergelyking met die onbehandelde kontrole, maar infeksie van 32 % bloedsels is steeds te hoog. Asoksistrobien, fenheksamied en iprodioon het onderskeidelik die voorkoms van skilversteuring met 44 %, 41 %, en 24 % verlaag, in vergelyking met die onbehandelde kontrole.

Aangesien die *B. cinerea* infeksie dieselfde was tydens 'n voorspelde medium risiko en 'n voorspelde lae risiko infeksieperiode, kon die model nie geverifieer word nie. Tydens 'n voorspelde lae risiko vir infeksie in die Citrusdal area was infeksie van 43 % waargeneem, in vergelyking met 47 % voorkoms van infeksie wat tydens 'n medium risiko voorspelling waargeneem is. In die Jonkershoek area was die voorkoms van infeksie 3 %, 39 %, en 58 % vir medium risiko infeksieperiodes.

Fenheksamied, asoksistrobien en benomiël het *B. cinerea* op bloeisels beheer. Benomiël residue word egter nie aanvaar in die EU nie en toekomstige studies moet daarop fokus om hoër dosisse van asoksistrobien en fenheksamied te evalueer. Dit word ook aanbeveel dat die proef in verhoogte konsentrasies van fenhexamied swamdoder herhaal word om die effektiwiteit verder te evalueer. Die risiko voorspellingsmodel benodig verdere ontwikkeling wat fokus op die rol wat temperatuur speel tydens infeksie. Die model moet ook oor 'n aantal jare geëvalueer word, omdat die risiko van infeksie medium kan wees vir die hele blomtydperk in een jaar, en laag of hoog gedurende die volgende. Daar word ook voorgestel dat die model tesame met swamdoder toedienings getoets word, nadat dit geverifieer is. Dit sal aandui of die model die voorkoms van skilverwringing verlaag wanneer swamdoders aangewend word volgens die model.

## Introduction

*Botrytis cinerea* Pers. Fr. can cause losses in over 200 crop species worldwide (Giraud et al., 1997; Williamson et al., 2007). *Botrytis cinerea* affects a wide variety of hosts, ranging from vegetables (lettuce, broccoli, beans) to small fruit (grapes and berries) and cut flowers (roses and gerbera flowers) (Droby and Lichter, 2004). This ascomycete fungus causes grey mould. Small fruit and vegetables are the most affected and Botrytis infection

leads to severe losses. In South Africa, *B. cinerea* has been reported as major pathogen on pome and stone fruit, grapevine and rooibos (Fourie and Holz, 1994; Wessels *et al.*, 2013, 2016).

Although *B. cinerea* has been studied extensively, very little is known about how this pathogen affects citrus. For many years the flower fall and premature flower fall of lemons have been associated with *B. cinerea*, as well as fruit rot occurring pre- or post-harvest (Fullerton *et al.*, 1999; Smilanick *et al.*, 2019). The first occurrence of rind distortion on lemons, associated with *B. cinerea* was noted in Florida in 1952 (Calavan *et al.*, 1952; Pelletier and Hilbron, 1954). The symptoms were described as scarring of juvenile fruit. Studies by Klotz (1973, 1978), Menge (1988) and Stevens *et al.* (1997) all referred to the symptoms, however, there is no detailed study to date about *B. cinerea* infection risk prediction on lemons.

Lemon orchards in New Zealand often report rind distortion, with a high susceptibility for rind distortion in the cultivars 'Genoa' and 'Villa Franca' (Stevens *et al.*, 1997). 'Villa Franca' has all the characteristics of 'Eureka' lemon. The cultivar, season, and climatic region can all influence the number of fruits affected in a season. Fullerton *et al.* (1999) estimates that up to 30 % of fruit can be affected by rind distortion in a season.

The symptoms associated with rind distortion can be described as conical or ridge-like outgrowths on the rind (Fullerton *et al.*, 1999). The ridges extend up to 4 mm from the surface and are irregular in shape and size. However, when the fruit mature the outgrowths on the rind disappear, but a blemish resembling a corky scar remains on the fruit. The corky appearance on the surface of the fruit makes it a cosmetic disease, that leads to the downgrading of fruit.

Although it is most commonly reported on lemons, distortions have also been noted on other citrus varieties. In 1951 malformation on limes was described similar to the description of rind distortion on lemons, caused by *B. cinerea* (Jeppson, 1951). However, it was found that the malformation on the limes was linked to the abnormal development of flowers, involving syntheses between stamens and ovaries during flower differentiation. The primary source of inoculum in an orchard is believed to be floral debris that is colonised by *Botrytis* sp. and is caught in flower clusters or directly on the surface of the fruit (Fullerton *et al.*, 1999). Rind distortion has also been reported on the mandarin cultivars Satsuma and Encore, and the tangelo cultivar Seminole. Symptoms on these cultivars resemble those caused by *B. cinerea* on lemon (Jeppson, 1951).

Fullerton *et al.* (1999) described the infection of lemon fruit by *B. cinerea* on juvenile fruit during the phenological phase after petal fall. The fungal hyphae grow over the surface of the fruit from colonised flower debris. Small necrotic pits are formed on the surface of the fruit by the compact infection cushions at the tip of hyphae. These infection cushions cause a collapse of the epidermal cells as well as of several layers of underlying cells, close to the infection cushions.

Fullerton *et al.* (1999) further reported that as the fruitlets mature, generalised hyperplasia can occur up to 20 cell layers deep in the vicinity of the necrotic pits. This results in outgrowths occurring on the surface of the fruit. As the fruit develops and becomes larger, the initial wound stretches, flattens and becomes corky. The formation of necrotic pits is distinctive of a non-pathogenic reaction between the fungus and the host, with fungal hyphae failing to manifest in the necrotic tissues of the pit. It is believed that the hyperplasia leading to rind distortion is a reaction to the initial wound and can also be observed in other injuries to the rind of lemon fruit (Fullerton *et al.*, 1999).

Due to the extended flowering period of lemons and the difficulty applying effective cover to juvenile fruit, Fullerton *et al.* (1999) found that conventional fungicides were not effective in controlling this disease. Despite the extended flowering period of lemons, the number of fungicide applications is however, limited due to cost and the possibility of exceeding maximum residue levels, as some fungicides can carry over from flowers to fruit. However, cultural management practices can lower the risk of *Botrytis* infection; these include the removal of floral debris from the orchard, lowering the density of shelter belts, pruning trees to have less dense canopies to enhance drying and to lower moisture (personal communication, J. van Niekerk, Stellenbosch University, Department of Plant Pathology, Stellenbosch, South Africa).

Producers rely on fungicides for the control of *B. cinerea*, but to date there are no registered fungicides available for the control of *B. cinerea* on citrus in South Africa. Many fungicides have, however, been registered to control *B. cinerea* on various other crops. Fungicides from the following chemical groups have showed good control of *B. cinerea*, pre- or postharvest, on other crops: phenylpyrroles, benzimidazoles, strobilurins, dicarboximides and anilinopyrimidines (Leroux, 2007; Petit *et al.*, 2011).

## Stated objectives

**1a. Determine the *in vitro* sensitivity** of South African *Botrytis* spp. isolates from Western Cape lemon orchards to fungicides (azoxystrobin, benomyl, fenhexamid, fludioxonil, iprodione, pyrimethanil) at the published discriminatory dose

**1b. Determine the most effective fungicide** (azoxystrobin, benomyl, fenhexamid, iprodione) applied as full-bloom spray on 'Eureka' lemons by testing the efficacy based on inhibition of *B. cinerea* blossom infection incidence.

**2. Primary inoculum sources:** Quantify *B. cinerea* incidence on lemon blossoms using (a) moisture chamber inoculation and stereo microscopy in comparison with (b) reverse transcription quantitative PCR (qPCR) of whole blossoms

**3. Botrytis risk model evaluation:** Evaluation of *Botrytis cinerea* infection risk model using CRI Phytrisk

## Materials and methods

### 1a. Fungicide sensitivity

To test the sensitivity of *B. cinerea* isolates from lemons, *in vitro* mycelial growth tests were conducted on 60 isolates not previously exposed to fungicides. Twenty isolates were sampled from each of the Jonkershoek, Franschoek, and Citrusdal areas. The isolates were tested against previously determined discriminatory doses of azoxystrobin (50 mg/L and 100 mg/L), benomyl (1 mg/L and 3 mg/L), fenhexamid (1 mg/L and 50 mg/L), fludioxonil (0.1 mg/L and 10 mg/L), iprodione (3 mg/L and 5 mg/L), and pyrimethanil (0.7 mg/L and 4 mg/L) (Meitz-Hopkins *et al.*, 2021). Trials were done twice on fungicide amended potato dextrose agar as described in Meitz-Hopkins *et al.* 2021 and relative mycelial growth of each isolate was compared to the isolate's growth diameter on an unamended control plate (20 isolates per orchard, three reps per isolate in each trial).

### 1b. Orchard trials - fungicide efficacy

Formulated products containing either azoxystrobin, benomyl, fenhexamid, or iprodione were applied once as full bloom spray either in September or in October in commercial lemon orchards where no fungicides were previously applied to compare their efficacy at the recommended doses (van Zyl 2017). The fungicides were evaluated in a randomised block design field trial with three replicates of each treatment under natural *B. cinerea* infection conditions. Fungicides were applied using an electric knapsack (Rovic Leers, Cape Town) fitted with an Albus ATR nozzle (Rovic Leers, Cape Town) until run-off occurred. The fungicides used were azoxystrobin (50 mg/L; Obstructo 250SC), benomyl (250 mg/L; Benomyl 500WP), fenhexamid (375 mg/L; Teldor 500SC), and iprodione (500 mg/L; Rovral). Incidence of blossom infection was evaluated by collecting 3 x 10 blossoms per treatment at each sampling time point 7 d after fungicide application from each orchard and incubating them for 14 d in a moisture chamber at 25°C. The percentage of infected blossoms was calculated after inspection of the blossoms using a stereo microscope to identify *B. cinerea* conidiophores. Isolations were conducted on semi-selective media (Edwards and Seddon, 2001) and cultures incubated for 7 d at 25°C. Culture morphology and Internal Transcribed Spacer (ITS) PCR and sequencing of a subset of isolates confirmed species identity.

## 2. Primary inoculum sources and quantification

Blossoms (N = 1200 per year), twigs (N = 10), weeds (N = 10), leaf litter (N = 10), fruit mummies (N = 10), and juvenile fruitlets (N = 100 per orchard) were sampled as possible sources of inoculum from three orchards in the Western Cape of South Africa. The prediction model developed by Citrus Research International (Phytrisk)

was evaluated by comparing the predicted risk to the percentage *B. cinerea* infection incidence on lemon blossoms and recorded weather data (rain, average temperature).

Inoculum of *B. cinerea* was evaluated for incidence in spring. Two methods were used, either a moisture chamber incubation or qPCR. Twenty blossoms were incubated in a moisture chamber for 14 d at 25°C under high relative humidity, or blossoms were surface sterilized using 70% ethanol and then incubated for 14 d at 25°C under high relative humidity in the moisture chamber. *Botrytis cinerea* infection quantification in blossoms using RNA isolations was done with a standard curve of RNA extracted from a *B. cinerea* spore suspension ( $1 \times 10^6$  spores/ml). The number of cells calculated from the qPCR reading (of each sample extracted from 100 mg blossom tissue, with RNA resuspended in 50 µl total volume) is therefore given in cells/µl, then converted to *B. cinerea* cells per mg blossom tissue.

### 3. Botrytis infection risk model evaluation

Weather data and predicted *B. cinerea* infection periods were extracted from CRI Phytrisk showing increased *B. cinerea* infection risk (values >2) or high infection risk (values >3). This data was compared to blossom infection (sterile blossoms from moisture chamber incubation) and inoculum levels (percentage infected blossoms, non-sterile).

## Results and discussion

### Task table

Objective	Achievement
<b>1. Fungicide sensitivity</b>	
<b>a. <i>In vitro</i> study at discriminatory doses</b>	Benomyl resistance was observed in some isolates from Jonkershoek and Franschhoek. Isolates from Citrusdal had highest sensitivity to all fungicides. Jonkershoek and Franschhoek isolates showed very similar sensitivity levels, only significantly different for fenhexamid at 1 mg/L and iprodione 3 mg/L (Fig. 4.3.2.1a)
<b>b. Orchard Trial</b>	Benomyl was most effective in reducing <i>B. cinerea</i> blossom infections, followed by azoxystrobin, fenhexamid and iprodione (Fig. 4.3.2.1b)
<b>2. Primary inoculum sources</b>	
a. Moisture chamber (blossoms) and ultrasound bath (twigs, weeds, mummies)	<i>Botrytis cinerea</i> was most frequently isolated from lemon blossoms. The average percentage blossoms infected over the three regions was 25 %. <i>Botrytis cinerea</i> was also detected on broadleaf weeds and some twigs.
b. qPCR of <i>B. cinerea</i> RNA in lemon blossom	Average incidence of <i>B. cinerea</i> infection ranged from 35 % in Citrusdal to 25 % in Franschhoek and 20 % in Jonkershoek based on live <i>B. cinerea</i> cells as measured by <i>B. cinerea</i> RNA present in individual lemon blossoms.
<b>3. Botrytis infection risk model</b>	Temperatures below 20°C were more favorable for <i>B. cinerea</i> infection. Exposure to high temperatures decreases viability - Gindro and Pezet (2001). No clear trend for moisture hours (leaf wetness) vs. infection.

### 1. Fungicide sensitivity and efficacy

Results from the *in vitro* trials indicated that there is a significant difference between the sensitivity of isolates from the different regions to the fungicides tested. The isolates from Citrusdal showed high sensitivity to all the fungicides tested, the exception being one isolate with moderate resistance to azoxystrobin. Approximately 50 % of the isolates from Franschhoek showed moderate resistance to azoxystrobin and pyrimethanil, and 40

% of the isolates were highly resistant to benomyl. For the Jonkershoek isolates there was moderate resistance in 35 % and 45 % of isolates to azoxystrobin and pyrimethanil, respectively. There was high resistance in 40 % of isolates from Jonkershoek to benomyl (Figure 4.3.2.1a).

Benomyl had the highest efficacy in controlling *B. cinerea* on lemon blossoms in the orchard. The *B. cinerea* blossom inoculum incidence was reduced significantly. In September, 68 % reduction of mean *B. cinerea* incidence of non-sterilised blossoms was observed compared to the *B. cinerea* incidence of the untreated control blossoms (Figure 4.3.2.1b). The trial was repeated in October (later in the same season) when incidence and inoculum levels were lower (Figure 4.3.2.1b). Azoxystrobin, hexamid, and iprodione reduced the *B. cinerea* blossom infection incidence by 44 %, 41 %, and 23 %, respectively compared to the untreated control ( $P=0.0003$ ). It was therefore shown that benomyl was significantly more effective than azoxystrobin and fenhexamid. Iprodione did not differ significantly from the untreated control and therefore it was deemed ineffective. This study determined which fungicides could effectively control *B. cinerea* on citrus, and whether there is a risk of resistance development in *B. cinerea* populations on lemons (Figure 4.3.2.1b).

## 2. Primary inoculum sources and inoculum quantification

*Botrytis cinerea* was isolated from broadleaf weeds by plating infected leaves on Botrytis – selective media from all sampled regions (Citrusdal, Franschhoek, Stellenbosch). The average percentage blossoms infected with *B. cinerea* over the three regions sampled in 2019 and 2020 was 23.90 %. *Botrytis cinerea* could only be isolated from twigs in the Citrusdal orchard, and *B. cinerea* was detected on juvenile fruitlets from all three orchards using a qPCR assay. Mostly *Penicillium* spp., *Alternaria* spp., and *Fusarium* spp. were isolated from the floral debris, and *Penicillium* from the fruit mummies sampled. *Botrytis cinerea* could not be isolated from the floral debris or fruit mummies.

On average 228 *B. cinerea* cells/ $\mu$ l, 274 cells/ $\mu$ l (137 *B. cinerea* cells/mg blossom tissue) and 198 cells/ $\mu$ l (99 *B. cinerea* cells/mg blossom tissue), were detected using RT-qPCR for the infected blossoms from Jonkershoek, Franschhoek and Citrusdal, respectively (Fig. 4.3.2.2 a,b). The qPCR assay was successful in amplifying and detecting *B. cinerea* RNA from lemon blossoms in the three orchards as well as from juvenile fruitlets. The limit of detection found in this study was 68 cells/ $\mu$ L (34 *B. cinerea* cells/ mg blossom tissue). The percentage blossoms that *B. cinerea* was detected on, using the qPCR assay, was higher compared to the incidence of infected blossoms that were sampled and incubated in a moisture chamber (Fig. 4.3.2.2). Non-sterilised blossoms that showed infection in the moisture chamber are only indicative for inoculum present in the orchard, actual blossom infection can be measured either with qPCR or by incubating surface sterilized blossoms.

## 3. Botrytis infection risk model

Results indicated that temperature and moisture (leaf wetness) had an effect on the infection of blossoms (Figs. 4.3.2.3, 4.3.2.4). The infection of lemon blossoms by *B. cinerea* in the orchard showed that if the daily average temperatures for the seven days prior to sampling were higher than 20°C, the percentage infection was very low or no infection occurred. Temperatures below 20°C were more favorable for *B. cinerea* infection since exposure to high temperatures decreased the viability of spores. No clear trend for the influence of moisture (leaf wetness) could be determined from the current dataset. The prediction model could not be validated as the same incidence of *B. cinerea* was recorded on predicted low risk days compared to predicted medium risk days. This study contributes to the understanding of the primary inoculum sources of *B. cinerea* in lemon orchards as well as to what parameters should be modified to achieve a more effective prediction of *B. cinerea* infection risk on lemons (Fig. 4.3.2.3).

It is recommended that good orchard sanitation is practiced as discussed previously in the section on *B. cinerea* inoculum sources. By controlling weeds and removing twigs and leaf litter from the orchard floor the initial amount of primary inoculum will be reduced and the chances of infection will also be lower. Although the results indicate that the blossoms from Jonkershoek had a slightly higher mean blossom infection incidence using the moisture chamber method compared to qPCR, no difference was found between the two methods for Jonkershoek (Fig. 4.3.2.2a). It could possibly be that the qPCR assay is more sensitive and specific in detection of *B. cinerea* in an orchard. It can also be that RT-qPCR will detect all viable surface spores

(conidiospores and ascospores) present on the blossom at the time of sampling, along with infections that have occurred. However, with moisture chamber incubation some of the spores at the time of sampling may not be viable enough to infect. Secondary pathogens occurred on the blossoms incubated in the moisture chambers and the growth of these pathogens could have prevented the isolation of *B. cinerea*. The qPCR methodology (measuring live cells instead of potential for infection) could also be used to determine fungicide efficacy in an orchard or monitor shifts towards fungicide resistance.

*Botrytis cinerea* overwinters in the soil or on the soil surface as sclerotia, alternatively it can overwinter as mycelium on organic matter in the orchard. For conidia and ascospore release, or mycelial growth to occur certain environmental conditions are needed. Moisture, due to high relative humidity (>95 %), rain, dew, or leaf wetness, is an important factor needed for blossom infection to occur, together with temperatures between 15°C and 20°C. However, *B. cinerea* can survive extreme temperatures for extended periods of time. According to Nair and Allen (1993) the optimum growing conditions for *B. cinerea* are 23.7°C and wetness of 4 hours. However, a minimum of 1.3 h of wetness is needed for successful infection by *B. cinerea*. A study by Ciliberti *et al.* (2014) described the optimal infection conditions for *B. cinerea* as 20°C and wetness of 16 hours are required. A year later in 2015, Ciliberti *et al.* (2015) found that a minimum relative humidity of 65 % is required for *B. cinerea* to grow.

In a future study the *B. cinerea* risk prediction model could be validated by comparing the incidence of blossom infection after fungicides were applied following a calendar-based program compared to infection incidence following fungicide applications based on the predicted risk according to the model. Thus, saving money during production if less fungicide application is required to achieve the same results making fungicide use more efficient.

The control of *B. cinerea* on lemons can have a major financial advantage for producers. Some growers have reported up to 30 % of losses due to infection of lemon blossoms, this is similar to the average incidence of 24.45 % infected blossoms observed during this study. When fruit are downgraded the value decreases significantly. The average price received by producers for fruit used for juicing is between R700-R1200 per ton of fruit. The price varies depending on the demand for lemon juice. Producers receive R7000 per ton of fruit exported to countries such as Russia, and European countries. The price for a ton of fresh lemons exported to premium markets (China, Malaysia, and Canada) can go up to R10000 (personal communication, S. Marais. Dole (Pty) Ltd, Durbanville, South Africa) compared to downgraded value of juiced fruit.

The infection of lemon blossoms in this study occurred at temperatures below 20°C and with wetness duration of less than 16 hours. In some instances, the accumulated hours of wetness in a seven-day period were less than 16 hours and blossom infection still occurred. From the results it could be concluded that the optimal conditions for *B. cinerea* to infect lemon blossoms were average temperatures (over a seven-day period) between 12°C and 18°C, and some free moisture present was required. For both the Jonkershoek and Franschoek areas the risk of infection increased with increasing average temperatures and accumulated hours of wetness. Citrusdal did not follow the same trend.

The model currently implemented by CRI on Phytrisk is therefore not accurate enough for use in industry. Although the model includes all the necessary weather parameters that should be taken into account for infection, it is important that the temperature aspect of the model should be further developed as well as extended and consecutive rain periods. It is suggested that the accumulated hours of temperature between 11°C and 18°C should be used in the model, rather than the average daily temperature, as from the results it seems that the highest percentage infection occurred in this temperature range. Results indicated that the percentage infection was higher in all orchards when the average temperature was 18°C or below. If the effect of temperature in the orchard can be understood and incorporated into the model, greater success might be achieved in predicting infection of *B. cinerea* on lemons.

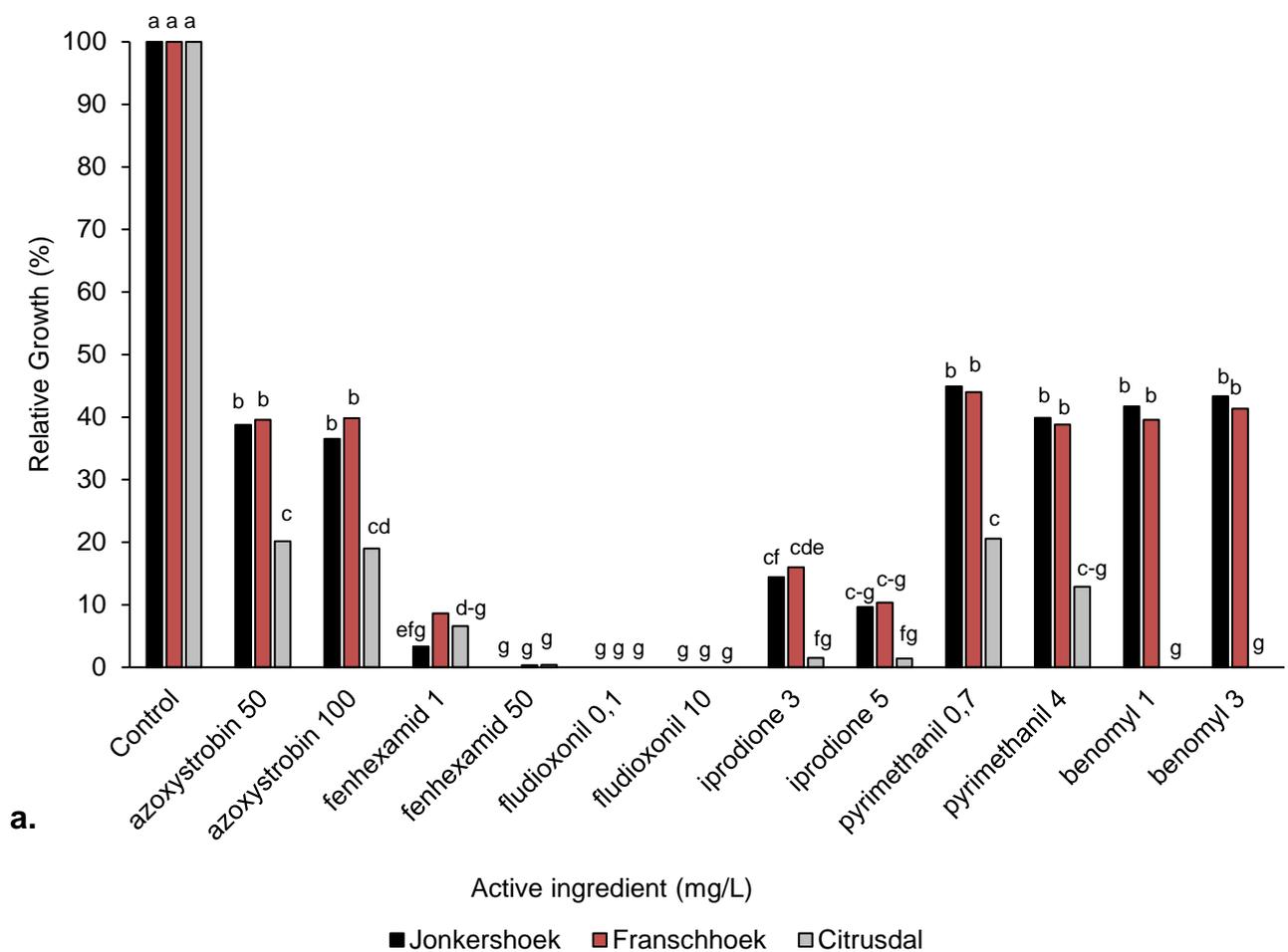
## Conclusion

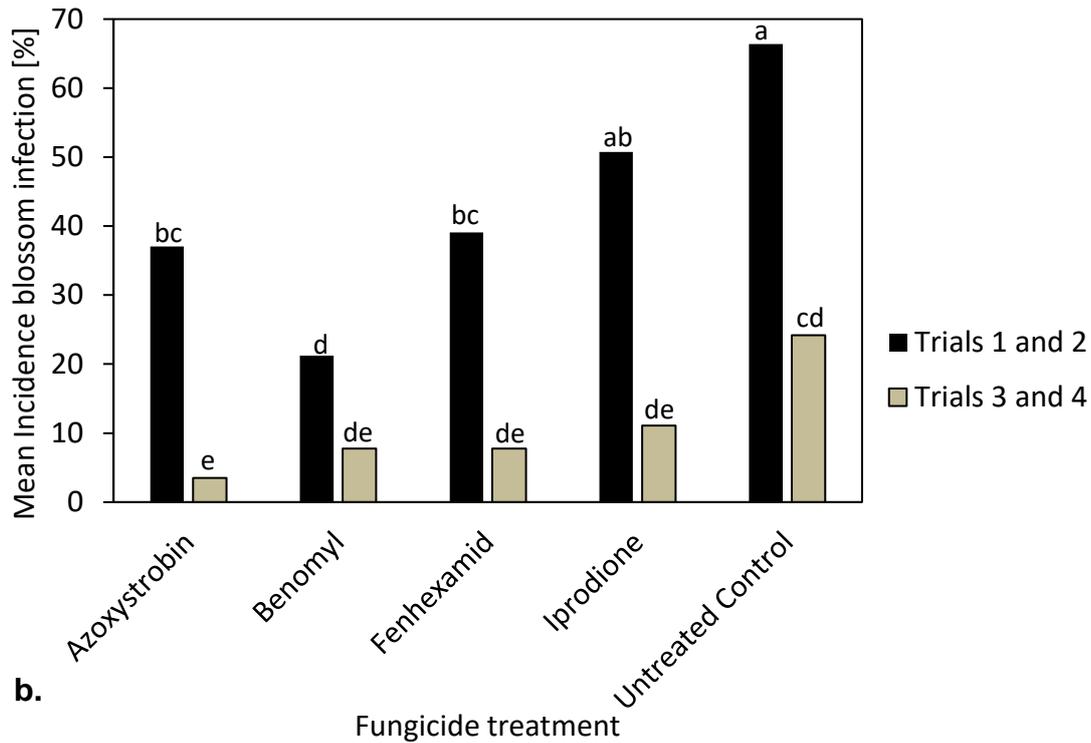
This study set out to determine the primary inoculum source of *B. cinerea* in lemon orchards, and to validate the prediction model developed by Citrus Research International. It was determined that broadleaf weeds and

lemon blossoms at full bloom are the primary sources of inoculum, as *B. cinerea* could not be isolated from flower buds, or floral debris. The model could not be validated due to the variance in infection for the same risk predicted. The risk of infection during a predicted medium risk ranged from 3 to 58 %. The study confirmed the presence of the pathogen in the Western Cape orchards with varying risk for infection depending on weather conditions. Fungicide tolerance was confirmed in the tested populations and should be considered when choosing products to prevent *B. cinerea* infection. An accurate prediction model could be useful to growers to give guidance in timing necessary fungicide spray applications.

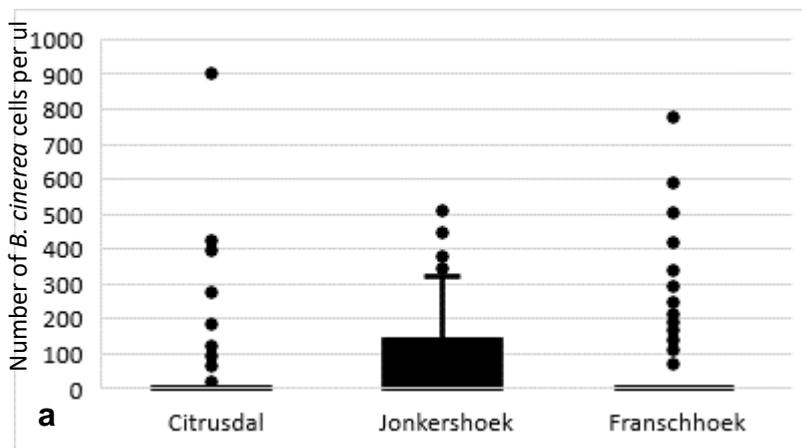
### Future research

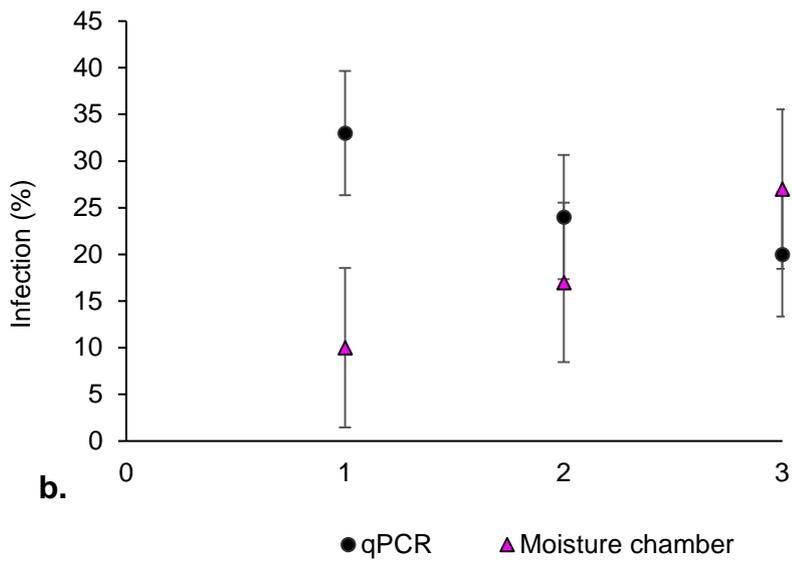
Future studies should focus on the role temperature has on infection, as well as validating the model over a number of years where different weather conditions are observed. Lastly the model should be tested in conjunction with fungicide applications, after the model has been verified. This will give a clearer indication whether the model helps reducing the occurrence of rind distortion when fungicides are applied according to the model.





**Figure 4.3.2.1.a.** *In vitro* fungicide sensitivity for Franschhoek, Jonkershoek and Citrusdal. **b.** *Botrytis cinerea* surface contamination of blossom resulting in grey mould incidence after incubation in a moisture chamber using blossom samples from four orchard fungicide trials on ‘Eureka’ lemon in two orchards in Simondium. Results from season 2020 were similar in season 2021 when fungicide was applied early in the season (September, Trials 1 and 2), while trials repeated in a second orchard later in the season were slightly different due to lower inoculum levels (Trials 3 and 4;  $P = 0.0003$ ).

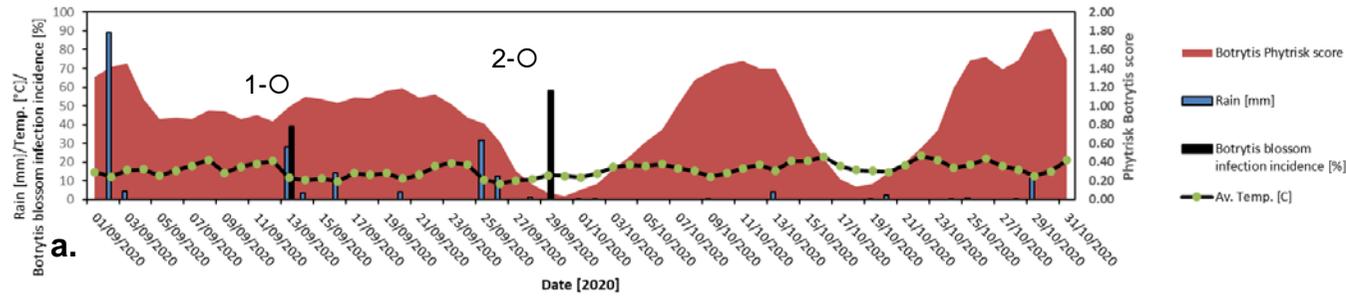




**Figure 4.3.2.2a.** Quantification of *B. cinerea* cells in samples of lemon blossoms from three orchards from Citrusdal mid-October 2019 in individual blossom tissue **b.** Comparison of incidence of blossom infection detected in moisture chamber (triangle) or qPCR (circle) Citrusdal (1), Franschhoek (2) and Jonkershoek (3) 16 Oct 2019.

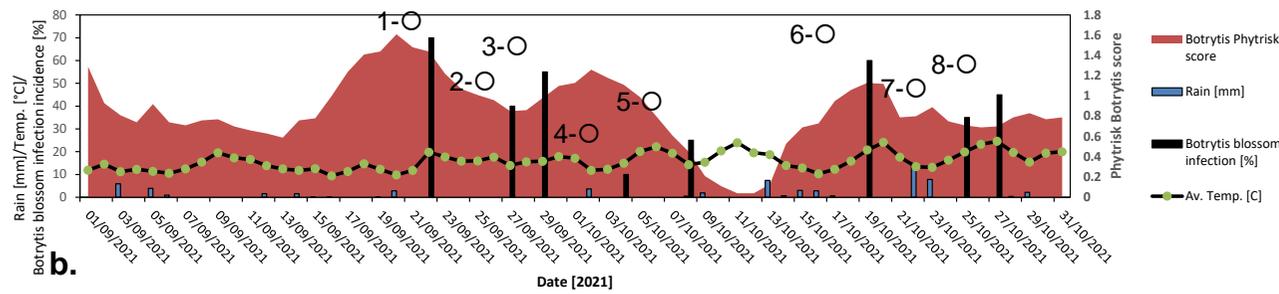
Error bars indicate standard deviation between replicates.

Franschhoek weather station (2020 Moisture chamber, non-sterile)



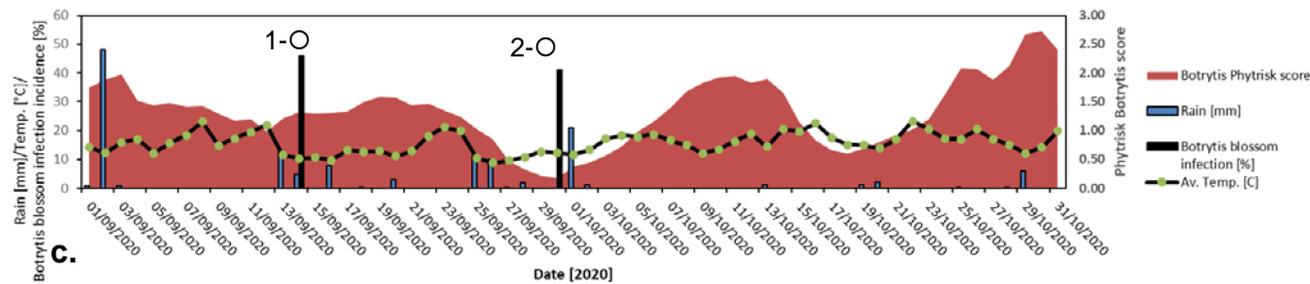
- 1- 14 September 2020 (39%)
- 2- 30 September 2020 (58%)
- 3- 14 September 2020 (39%)
- 4- 30 September 2020 (58%)

Franschhoek weather station (2021 Moisture chamber, non-sterile and sterile)

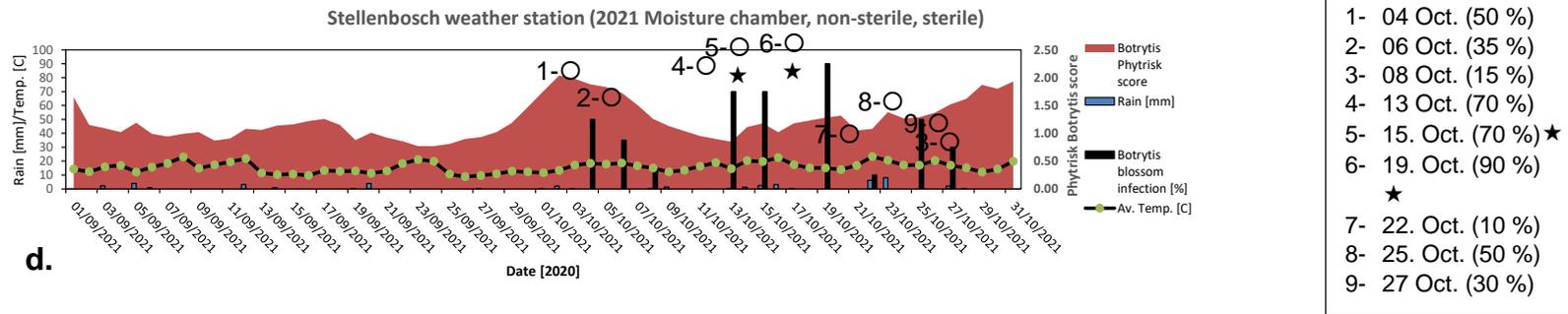


- 1- 22 Sept. (70%)
- 2- 27 Sept. (40%)
- 3- 29 Sept. (55%)
- 4- 04 Oct. (10%)
- 5- 08 Oct. (25%)
- 6- 19 Oct. (60%)
- 7- 25 Oct. (35%)
- 8- 27 Oct. (45%)

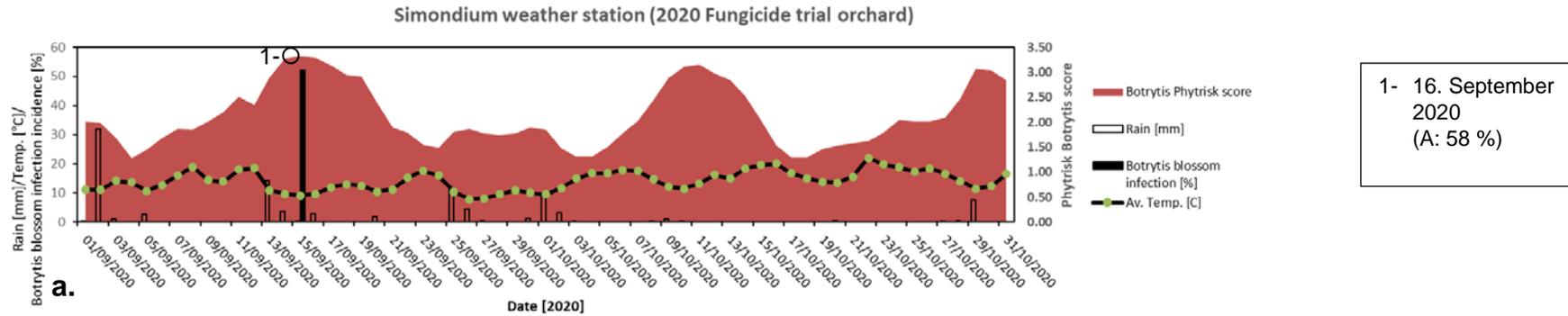
Stellenbosch weather station (2020 Moisture chamber, non-sterile)



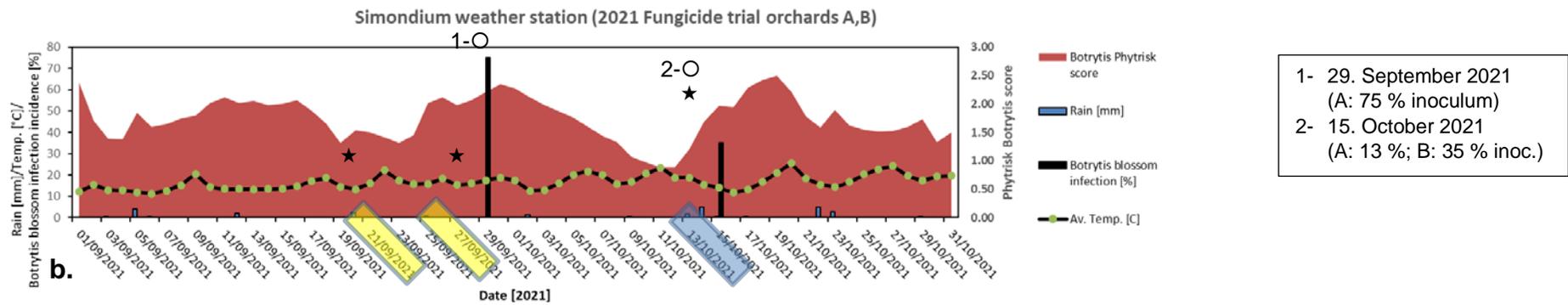
- 1- 14 September 2020 (46%)
- 2- 30 September 2020 (41%)



**Figure 4.3.2.3.** CRI PhytRisk prediction of Botrytis infection risk and indicated results of infection (sterile blossom; ★) or inoculum presence (non sterile, ○) in orchards where incidence of *B. cinerea* inoculum on blossoms was quantified. **a.** Franschhoek 2020 **b.** Franschhoek 2021 **c.** Stellenbosch 2020 **d.** Stellenbosch 2021



1- 16. September 2020 (A: 58 %)



1- 29. September 2021 (A: 75 % inoculum)  
 2- 15. October 2021 (A: 13 %; B: 35 % inoc.)

Same comment as above

**Figure 4.3.2.4.** CRI Phytrisk prediction of Botrytis infection in trial orchards used for fungicide trials (only one orchard in 2020) **a.** Simondium orchard A; **b.** Simondium orchard B (yellow highlighted date for blossom infection; sterile blossom; ★); (blue highlighted date: blossom infection in both orchards A, B; sterile blossom; ★); percentage incidence indicating inoculum presence with black bar (non sterile, ○)

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mould disease. *Molecular Plant Pathology* 8:561–580.

#### 4.3.3 **FINAL REPORT: Influence of shade nets on *Alternaria* brown spot and citrus black spot: comparing epidemiological model output for covered (under shade nets) and uncovered (normal/open) orchards' weather datasets**

Project 1187 (2017/04 - 2022/03) by Providence Moyo and Paul H. Fourie (CRI)

##### **Summary**

The use of shade/hail nets is increasing in South African orchards. These nets are mainly to mitigate the loss of yield due to detrimental climatic conditions including extreme temperatures, hailstorms and high winds. They also protect crops against insects and birds. The use of hail nets can, however, lead to the modification of the orchard microclimate, in particular humidity and temperature, which are crucial in the growth and development of pathogens such as *Phyllosticta citricarpa*, *Alternaria alternata* and *Botrytis cinerea*, which cause citrus black spot (CBS), *Alternaria* brown spot (ABS) and Botrytis mould, respectively. Thus, the use of shade nets can directly or indirectly affect the development of these diseases in citrus orchards. Disease predictions from a trial site located in the Western Cape province were analysed for the 2021-2022 season. Based on weather data collected and disease predictions, pseudothecium maturation of *P. citricarpa* was reached earlier in the orchard under the net, but no CBS risks were predicted in either orchard for the whole season. More days suitable for ABS were predicted in the orchard outside the net. These predictions coincided with high total rainfall and lower temperatures experienced in the orchard outside the net. High Botrytis infection risks were predicted inside the net, where higher humidity levels were experienced. Fungicide residue persistence in orchards was analysed from fruit collected from various orchards located in different areas. A higher number of fungicides persisted under nets in comparison to orchards outside nets.

##### **Opsomming**

Die gebruik van skadu-/haelnette neem toe in Suid-Afrikaanse boorde. Hierdie nette is hoofsaaklik om die verlies aan opbrengs as gevolg van nadelige klimaatstoestande, insluitend uiterste temperature, haelstorms en hoë winde, te verlaag. Hulle beskerm ook gewasse teen insekte en voëls. Die gebruik van haelnette kan egter tot die verandering van die boord mikroklimaat, veral humiditeit en temperatuur, lei, wat deurslaggewend is in die groei en ontwikkeling van patogene soos *Phyllosticta citricarpa*, *Alternaria alternata* en *Botrytis cinerea*, wat sitruswartvlek (CBS), *Alternaria* bruinvlek (ABS) en Botrytis skimmel, onderskeidelik, veroorsaak. Die gebruik van skadunette kan dus direk of indirek die ontwikkeling van hierdie siektes in sitrusboorde beïnvloed. Siektevoorspellings van 'n proefferrein in die Wes-Kaap-provinsie is vir die 2021-2022 seisoen ontleed. Pseudothecium-rypwording van *P. citricarpa* is vroeër in die boord onder net bereik, maar geen CBS-risiko's is in beide boorde vir die hele seisoen voorspel nie. Meer dae geskik vir ABS is in die boord buite die net voorspel. Hierdie voorspellings het met hoë totale reënval en laer temperature, wat in die boord buite die net ervaar is, saamgeval. Hoë Botrytis infeksierisiko's is binne die net voorspel, waar hoër humiditeitsvlakke ervaar is. Swamdoderresidu volharding in boorde is ontleed vanaf vrugte wat uit verskeie boorde wat in verskillende gebiede geleë is, versamel is. 'n Groot aantal swamdoders het onder die net volhard, in vergelyking met boorde buite nette.

##### **Introduction**

South African citrus producers are challenged to produce fruit of the highest quality that meet international standards, amid detrimental climatic conditions which include hail, high temperatures and light intensities. In order to produce the best export fruit and to maintain the competitiveness of the industry, citrus producers are embracing new technology that is constantly being developed. One such technology is shade (or protective) netting.

The desired effects of shade netting include the protection of orchards against birds, hail, insects, extreme radiation and temperatures, and high-speed winds (Stamps 2009). The alteration of the microclimate variables of the area under nets, particularly temperature and relative humidity, may have direct or indirect effects on the development of diseases found in citrus orchards. Humid environments, for instance, are known to favour

the growth and development of pathogens including *Alternaria alternata* (Fr.:Fr.) Keissl. pv. *citri* Solel (Timmer *et al.* 1998) and *Phyllosticta citricarpa* (McAlpine) van der Aa (Kotzé 2000), which cause Alternaria brown spot (ABS) and citrus black spot (CBS), respectively.

Alternaria brown spot and citrus black spot are two major fruit and foliar diseases that hamper fruit export to fresh markets by South African producers. The diseases cause external blemishes that reduce the value of citrus fruit for the fresh market (Timmer *et al.* 1998, 2000; Kellerman and Kotze 1977). The ABS pathogen infects young leaves, twigs and immature fruit (Whiteside 1979; Timmer *et al.* 2000) causing small black necrotic spots that appear as early as 24 hours after infection on infected tissues (Canihos *et al.* 1999; Timmer *et al.* 2000). A host specific toxin produced by the pathogen may cause lesions to expand, which often leads to leaf and fruit drop as well as twig dieback. Leaves and fruit become less susceptible to infection by the fungus with maturity (Timmer *et al.* 2000). Airborne conidia are produced primarily on lesions on mature leaves and their release is triggered by rainfall events as well as sudden changes in relative humidity (Timmer *et al.* 1998). Production of spores begins after lesions are well developed, approximately 10 days after lesions first appear, and continues for about 40 days thereafter (Reis *et al.* 2006). Optimum temperatures for infection were determined to be 22 to 27°C (Solel and Kimchi 1998; Canihos *et al.* 1999) while 8 hours of leaf wetness is the minimum period required for infection at optimum temperature (Canihos *et al.* 1999).

Similar to the ABS pathosystem, fruit and leaves are infected by the CBS pathogen during the early stages of their development (McOnie 1964). Fruits are susceptible to infection for a period of 5 months from anthesis (Kotzé 2000), whereas leaves are susceptible from development up until 10 months later (Truter *et al.* 2007). Climatic conditions also have an important influence on the successful infection of susceptible tissues by the CBS pathogen. Adequate hours of wetness and temperatures must coincide with presence of inoculum for infection to occur (Kotzé 1963). Alternate wet and dry periods and mild to warm temperatures aid in pseudothecium maturation and ascospore release (Kiely 1948) and a minimum period of 15 hours of wetness is required for successful germination of ascospores at optimal temperatures (Kotzé 1963).

In South Africa, fungicide applications are essential for effective control of ABS and CBS as well as production of blemish-free fruit suitable for the fresh market. Up to eight monthly spray applications are required to achieve acceptable control in orchards heavily infested with ABS (Schutte *et al.* 1992), whereas fungicidal sprays applied at 4- to 6-week intervals during the critical period of infection from mid-spring to mid-summer are required for the control of CBS (McOnie 1964; Schutte *et al.* 1997, 2003). Predictive models that use duration of leaf wetness and temperatures required for infection have been developed to aid in timing of fungicide applications to improve control of both CBS (Dummel *et al.* 2012; Fourie *et al.* 2013) and ABS (Timmer *et al.* 2000) as well as reduce the number of fungicide spray applications per season.

A weather-based model, called the Alter-Rater, was developed to predict the time of fungicide application for the control of ABS and is based on daily cumulative points which are assigned on the basis of rainfall, leaf wetness as well as temperature (Timmer *et al.* 2000). The Alter-Rater model has been validated and proven effective in Florida and Brazil (Bhatia *et al.* 2003; Peres and Timmer 2006). Fourie *et al.* (2013) also modelled the effect of temperature and wetness on *Phyllosticta* pseudothecium maturation and ascospore release in citrus orchards using Gompertz models and found that temperature had a major influence on pseudothecium maturation. Another predictive model was developed by Dummel *et al.* (2012) to aid in predicting ascospore release and infection risk by the CBS pathogen, based on weather parameters. Additionally, Magarey *et al.* (2015) developed a model which incorporated dispersal models of Fourie *et al.* (2013) for the prediction of *P. citricarpa* infections. Meanwhile, an ongoing project has integrated dispersal and infection models together with weather data into a web-based platform ([www.CRI-PhytRisk.co.za](http://www.CRI-PhytRisk.co.za)) for use by growers (project 1238) and in another project (1149), different CBS models were evaluated and compared for accuracy in predicting *P. citricarpa* infection. Whilst these models may provide valuable epidemiological insight and decision support, they can also be used to compare epidemiological parameters based on conditions that exist in normal (uncovered) orchards with those under shade netting, as it is expected that the changing microclimate will have some effect on the development and spread of ABS and CBS.

The overall aim of this study was to determine how shade netting could influence the development and spread of diseases within citrus orchards. Infection models will be used to predict infection periods of ABS and CBS pathogens, based on recorded climatic data.

### **Stated objectives**

1. Investigate the influence of shade nets on the risk of citrus black spot infection.
  - Measure weather data (rainfall, temperature, leaf wetness and humidity) in covered vs. uncovered orchards.
  - Use CRI-PhytRisk to compare the risk of CBS infection in covered vs. uncovered orchards.
2. Investigate the influence of shade nets on *Alternaria* brown spot severity.
  - Measure weather data (rainfall, temperature, leaf wetness and humidity) and ABS severity in covered vs. uncovered orchards.
3. Investigate the influence of shade nets on *Botrytis* infection.
4. Compare fungicide residues on fruit collected from trees growing inside and outside shade nets.

### **Materials and methods**

#### **Investigate the influence of shade nets on the risk of citrus black spot infection**

Two sites were used for trials during the 2021-2022 season. Each trial site consisted of two orchards, one orchard under a shade net and one in the open. The trial site in Ashton (Western Cape Province) consisted of Nadorcott mandarin orchards in close proximity to each other. The orchard in Nelspruit (Mpumalanga province) is a Nadorcott mandarin grafted on Carrizo and planted in January 2006. The orchard is divided into two parts, one part is under the net (Block 8) and the second is outside the net (Block 7).

The orchards in each trial site are equipped with iLeaf weather stations that are linked to CRI-PhytRisk and thus, the output of CBS infection risks in each orchard was downloaded directly from the CRI-PhytRisk website. The number of days with 3-hour periods in which pycnidiospore and ascospore infections were predicted, using CRI-PhytRisk, were compared between the orchard under the net and that outside the net, at each trial site.

#### **Investigate the influence of shade nets on *Alternaria* brown spot severity**

The same open and netted orchards used in the CBS trials at the two sites were used for the *Alternaria* brown spot trial. Since the *Alternaria* brown spot forecasting model was added to CRI-PhytRisk, the CRI-PhytRisk output of the ABS scores generated from weather data supplied by weather stations under the nets were compared with the scores generated from weather data from orchards outside the net. Thus, the number of days suitable for ABS infection in orchards under net were compared to those in open orchards.

#### **Investigate the influence of shade nets on *Botrytis* infection**

The *Botrytis* forecasting model was also programmed into CRI-PhytRisk. The number of *Botrytis* infection days corresponding to any of the three risk categories, [i.e. high infection risk (when daily infection scores are  $\geq 2.0$ ); medium risk (scores of between 0.5 and 2.0) and low risk (scores of  $<0.5$ )] were compared between the netted and open orchards for both trial sites.

#### **Compare fungicide residues on fruit collected from trees growing inside and outside shade nets**

In addition to determining the effect of shade nets on disease development, fruit from trees inside and outside the shade net were sent for multi-residue fungicide testing. Fruit were collected a few days before harvest and sample analysis was carried out using liquid chromatography tandem mass spectrometry (LC-MS/MS). This was carried out for fruit from the trial site in Nelspruit as well as from orchards in Groblersdal and Burgersfort (Limpopo Province).

### **Results and discussion**

#### **Investigate the influence of shade nets on the risk of citrus black spot infection**

CBS does not occur in the Western Cape, but weather data were analysed and CBS model output compared with those from the Mpumalanga site. Citrus fruit are susceptible to infection by the CBS pathogen between

October and March (Kotzé 1963). Thus, the number of days with suitable periods for either ascospore or pycnidiospore infection as well as the weather parameters were recorded between 1 October 2021 and 31 March 2022 (182 days in total).

The biofix date, which is the date from which ascospores will progressively be released from maturing pseudothecia, calculated from the 1<sup>st</sup> of July, was reached earlier in the orchard under the net at the Western Cape site. Pseudothecium maturity was predicted on 30 November 2021 for the orchard under the net, and 08 December 2021 for the orchard outside the net (Table 4.3.3.1). Slightly lower average minimum and maximum temperatures were experienced in the orchard under the net compared to the orchard outside the net (Table 4.3.3.1). However, higher average minimum and maximum relative humidity was experienced in the orchard under the net. No CBS infection risks were predicted by CRI-PhytRisk for either orchard in the Western Cape site, i.e. no 3-hour periods suitable for either ascospore or pycnidiospore infection risk were predicted in the orchards for the entire season.

Unfortunately, the weather station inside the net at the Nelspruit site broke and it took more than 2 months to get the station fixed and therefore, no results were obtained to compare with those of the weather station located in the orchard outside the net.

#### **Investigate the influence of shade nets on *Alternaria* brown spot severity**

The *Alternaria* Brown Spot (ABS) infection risks and weather data for the Western Cape site were analysed from 1 September 2021 to 30 April 2022 (242 days in total). No data were analysed for the Nelspruit site due to the reason mentioned above.

A higher ABS infection risk was predicted by CRI-PhytRisk in the orchard outside than inside the net in the Western Cape site. Twenty (20) orange (intermediate risk) days were predicted for the open orchard compared to two (2) orange days predicted in the orchard inside the net (Table 4.3.3.2). No days suitable for high ABS risk (zero red days) were predicted for this trial site (Table 4.3.3.2).

Disease incidence and severity was reported to be positively correlated to total rainfall, wetness duration and relative humidity (RH), but negatively correlated to average temperature when relationships between *Alternaria* brown spot (ABS) incidence or severity and environmental conditions were analysed (Timmer *et al.* 2000; Bassimba *et al.* 2014). Total rainfall was higher in the orchard outside the net than inside the net whereas average temperatures were higher inside the net (Table 4.3.3.2).

#### **Investigate the influence of shade nets on *Botrytis* infection**

Similar to the *Alternaria* brown spot trials, infection risks and weather data were measured from 1 September 2021 to 30 April 2022. More days suitable for high *Botrytis* infection risks (red days) were predicted in the orchard inside the net where high relative humidity was recorded (Table 4.3.3.2).

#### **Compare fungicide residues on fruit collected from trees growing inside and outside shade nets**

Fruit samples for multi-residue analysis were collected from orchards in the Mpumalanga and Limpopo provinces during the 2020-2021 season. Residues of several pesticides (fungicides and insecticides) were detected, with a higher number of pesticides detected in fruit samples collected from orchards inside than outside nets in Nelspruit and Groblersdal (Tables 4.3.3.3 and 4.3.3.4). Fungicides used in citrus black control spray programmes, such as azoxystrobin, pyraclostrobin and carbendazim, were detected and again higher residue levels of these fungicides were detected on fruit from orchards inside the net compared to outside, except for the trial site in Burgersfort, where residue levels of azoxystrobin were higher in the open orchard (Tables 4.3.3.3 - 4.3.3.5). Post-harvest fungicides including 2,4-D, pyrimethanil, fludioxonil, propiconazole and Ortho Phenyl Phenol were also detected. Insecticides detected included etoxazole, methoxyfenocide, imidacloprid and methoxyfenocide and these were detected at higher residue levels in fruit samples from the closed than open orchard in Nelspruit and Groblersdal (Table 4.3.3.3 and 4.3.3.4).

#### **Conclusion**

Shade nets are known to modify the microclimate conditions in orchards, i.e. they are reported to increase humidity, reduce temperature, temperature fluctuations and air movements (Wachsmann *et al.* 2014). Based on reports of high relative humidity and reduced airflow inside nets, one would expect increased risks of fungal diseases such as *Alternaria* brown spot in orchards under net. This was, however, not supported by the CRI-PhytRisk predictions of ABS infection in the Western Cape trial site during the 2021-2022 season. The high total rainfall experienced in the orchard outside the net could explain these unexpected results. *Alternaria* brown spot incidence and severity is positively correlated to total rainfall, wetness duration and relative humidity (Timmer *et al.* 2000; Bassimba *et al.* 2014).

Frequent dew and rain events, which result in prolonged high moisture conditions favour *Botrytis* infection of flowers (Ciliberti *et al.* 2015). Higher *Botrytis* risks were predicted for orchards under net compared to those outside nets in the Western Cape trial site. High humidity levels measured under the net, compared to outside the net in this site, can also prolong moisture periods thereby favouring infection.

The biofix date was reached earlier in the orchard under the net, but no CBS infection risks were predicted by CRI-PhytRisk for either orchard in the Western Cape site.

Fungicide residue persistence in orchards was analysed from fruit collected from different orchards and locations during the 2020-2021 season. Data showed that higher residues levels of pesticides persisted under the net in comparison to the orchard outside the net.

### Technology transfer

No technology transfer took place in this reporting period.

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**Table 4.3.3.1.** CRI-PhytRisk predictions of Citrus black spot in citrus orchards under net and outside nets in Ashton in the Western Cape province during the 2021-2022 growing season. Predictions reported are for the period between 1 October 2021 and 31 March 2022 (182 days)

	Orchard inside net	Orchard outside net
<b>Citrus black spot prediction</b>		
Biofix date	30-11-2021	08-12-2021
Number of days with possible ascospore infection	0	0
Number of days with possible pycnidiospore infection	0	0
Number of 3-hr periods with predicted ascospore infection	0	0
Number of 3-hr periods with predicted pycnidiospore infection	0	0
<b>Weather variables</b>		
Number of days with measurable rainfall (> 0.1 mm)	3	42
Total rainfall (mm)	1.7	96.1
Average minimum temperature (°C)	11.74	14.24
Average maximum temperature (°C)	25.88	27.74
Average minimum relative humidity (%)	48.76	31.63
Average maximum relative humidity (%)	94.35	79.14

**Table 4.3.3.2.** CRI-PhytRisk predictions of *Alternaria* brown spot and *Botrytis* infections in citrus orchards under the net and outside the nets in Ashton in the Western Cape province during the 2021-2022 growing season. Predictions reported are for the period between 1 September 2021 and 30 April 2022 (242 days)

	<b>Orchard inside net</b>	<b>Orchard outside net</b>
<b>Alternaria brown spot prediction</b>		
Number of days with daily point value (DPV) < 3 (days = green)	240	222
Number of days with $3 \leq \text{DPV} \leq 6$ (days = orange)	2	20
Number of days with DPV > 6 (days = red)	0	0
<b>Botrytis prediction</b>		
Number of days with predicted infection score of < 0.5 (days = green)	39	69
Number of days with infection score of $\geq 0.5$ and $\leq 2.0$ (days = orange)	87	151
Number of days with predicted infection score of $\geq 2.0$ (days = red)	116	22
<b>Weather variables</b>		
Number of days with measurable rainfall (> 0.1 mm)	4	51
Total rainfall (mm)	3.50	100.40
Average minimum temperature (°C)	13.13	12.74
Average maximum temperature (°C)	27.75	26.44
Average minimum relative humidity (%)	33.51	29.69
Average maximum relative humidity (%)	94.57	79.09

**Table 4.3.3.3.** Fungicides and insecticides detected in fruit samples collected during harvest in Nadorcott mandarin trees growing inside and outside shade nets in Nelspruit (Mpumalanga) in the 2020-2021 season

	Fungicides/Insecticides (mg/kg)								
	Azoxystrobin <sup>a</sup>	Etoxazole <sup>b</sup>	2.4 D <sup>c</sup>	Imidacloprid <sup>b</sup>	Propiconazole <sup>c</sup>	Methoxyfenocide <sup>b</sup>	Fludioxonil <sup>c</sup>	Pyrimethanil <sup>c</sup>	Spirotetramat <sup>b</sup>
<b>Inside shade net</b>									
Sample 1	0,011	<0,01	<0,01	0,027	<0,01	0,13	–	<0,01	–
Sample 2	0,019	<0,01	<0,01	0,036	<0,01	0,21	<0,01	<0,01	–
Sample 3	0,012	<0,01	<0,01	0,042	<0,01	0,13	–	<0,01	–
Sample 4	0,021	<0,01	0,014	0,041	<0,01	0,20	–	<0,01	<0,01
Sample 5	0,014	<0,01	<0,01	0,031	<0,01	0,15	–	<0,01	–
Sample 6	0,013	<0,01	<0,01	0,024	<0,01	0,13	–	<0,01	–
Sample 7	0,012	0,012	<0,01	0,030	<0,01	0,097	–	<0,01	–
Sample 8	0,019	<0,01	<0,01	0,024	<0,01	0,17	–	<0,01	–
Sample 9	0,012	<0,01	<0,01	0,025	–	0,14	–	<0,01	–
Sample 10	0,014	<0,01	<0,01	0,039	–	0,075	–	<0,01	–
<b>Average</b>	<b>0.0147</b>	<b>0.0102</b>	<b>0.0104</b>	<b>0.0319</b>	<b>0.008</b>	<b>0.1432</b>	<b>0.001</b>	<b>0.01</b>	<b>0.001</b>
<b>Outside a shade net</b>									
Sample 1	<0,01	–	<0,01	<0,01	–	0,21	–	<0,01	–
Sample 2	<0,01	–	0,011	0,025	–	0,21	–	<0,01	–
Sample 3	0,015	–	<0,01	0,013	–	0,31	–	<0,01	–
Sample 4	0,012	–	0,012	0,011	–	0,32	–	<0,01	–
Sample 5	0,012	–	<0,01	0,012	–	0,22	–	<0,01	–
Sample 6	<0,01	–	<0,01	0,017	–	0,19	–	<0,01	–
Sample 7	<0,01	–	0,011	0,011	–	0,19	–	<0,01	–
Sample 8	0,013	–	<0,01	0,018	–	0,27	–	<0,01	–
Sample 9	0,013	–	<0,01	0,023	–	0,21	–	0,012	–
Sample 10	<0,01	–	<0,01	0,011	–	0,21	–	<0,01	–
<b>Average</b>	<b>0.0115</b>	<b>–</b>	<b>0.0104</b>	<b>0.0151</b>	<b>–</b>	<b>0.234</b>	<b>–</b>	<b>0.0102</b>	<b>–</b>

<sup>a</sup> Fungicides used in citrus black spot programmes

<sup>b</sup> Insecticides

<sup>c</sup> Fungicides used as postharvest chemicals

**Table 4.3.3.4.** Fungicides and insecticides detected in fruit samples collected during harvest in Midnight Valencia orange trees growing inside and outside shade nets in Groblersdal (Limpopo) in the 2020-2021 season

	Fungicides/Insecticides (mg/kg)					
	Carbendazim <sup>a</sup>	Permethrin <sup>b</sup>	Pyraclostrobin <sup>a</sup>	Spirodiclofen <sup>b</sup>	Ortho Phenyl Phenol (2-Phenylphenol) <sup>c</sup>	Spirotetramat <sup>b</sup>
<b>Inside a shade net</b>						
Sample 1	<0,01	0,046	<0,01	–	<0,01	<0,01
Sample 2	<0,01	<0,01	0,013	–	0,010	0,015
Sample 3	<0,01	–	0,011	–	<0,01	<0,01
Sample 4	<0,01	–	0,012	–	0,014	–
Sample 5	<0,01	0,025	0,011	–	<0,01	0,018
Sample 6	0,013	–	0,011	–	<0,01	0,012
Sample 7	0,012	–	0,018	–	0,015	0,015
Sample 8	0,011	0,018	0,011	–	<0,01	0,017
Sample 9	0,010	–	0,012	–	<0,01	0,017
Sample 10	<0,01	–	<0,01	–	<0,01	0,017
<b>Average</b>	<b>0.0106</b>	<b>0.0099</b>	<b>0.0119</b>	<b>–</b>	<b>0.0109</b>	<b>0.0131</b>
<b>Outside shade net</b>						
Sample 1	<0,01	0,014	<0,01	–	<0,01	–
Sample 2	<0,01	–	0,013	<0,01	0,011	–
Sample 3	<0,01	–	0,011	<0,01	<0,01	–
Sample 4	<0,01	–	<0,01	–	<0,01	0,015
Sample 5	<0,01	–	0,016	0,010	0,010	<0,01
Sample 6	<0,01	–	0,014	<0,01	<0,01	–
Sample 7	<0,01	–	0,011	<0,01	<0,01	–
Sample 8	<0,01	–	<0,01	0,012	<0,01	–
Sample 9	–	–	<0,01	–	0,010	–
Sample 10	–	–	0,011	<0,01	<0,01	–
<b>Average</b>	<b>0.008</b>	<b>0.0014</b>	<b>0.0116</b>	<b>0.007</b>	<b>0.0101</b>	<b>0.0025</b>

<sup>a</sup> Fungicides used in citrus black spot programmes

<sup>b</sup> Insecticides

<sup>c</sup> Fungicide used as a postharvest chemical

**Table 4.3.3.5.** Fungicides and insecticides detected in fruit samples collected during harvest in Nadorcott mandarin trees growing inside and outside shade nets in Burgersfort (Limpopo) in the 2020-2021 season

	Fungicides/Insecticides (mg/kg)			
	Azoxystrobin <sup>a</sup>	Bromopropylate <sup>b</sup>	Propiconazole <sup>c</sup>	Spirotetramat <sup>b</sup>
<b>Inside shade net</b>				
Sample 1	0,024	<0,01	–	0,30
Sample 2	0,017	–	–	0,28
Sample 3	0,022	–	–	0,27
Sample 4	0,012	–	–	0,31
Sample 5	0,012	–	–	0,34
Sample 6	0,013	–	–	0,37
Sample 7	0,013	–	–	0,31
Sample 8	0,011	–	–	0,30
Sample 9	0,012	–	–	0,28
Sample 10	0,014	–	–	0,26
<b>Average</b>	<b>0.015</b>	<b>0.001</b>	<b>–</b>	<b>0.302</b>
<b>Outside shade net</b>				
Sample 1	0,019	–	<0,01	0,32
Sample 2	0,019	–	–	0,39
Sample 3	0,015	–	–	0,33
Sample 4	0,013	–	–	0,39
Sample 5	0,011	–	–	0,30
Sample 6	0,021	–	–	0,39
Sample 7	0,016	–	–	0,35
Sample 8	0,015	–	–	0,33
Sample 9	0,020	–	–	0,36
Sample 10	0,018	–	–	0,36
<b>Average</b>	<b>0.0167</b>	<b>–</b>	<b>0.001</b>	<b>0.352</b>

<sup>a</sup> Fungicides used in citrus black spot programmes

<sup>b</sup> Insecticides

<sup>c</sup> Fungicide used as a postharvest chemical

#### 4.3.4 FINAL REPORT: Management of pruning debris as part of the citrus black spot control strategy

Project 1223 (2019/04 – 2022/03) by P. Moyo, T. Nxumalo and P.H. Fourie (CRI)

##### Summary

This project aimed to determine to what extent chopped or shredded pruning debris contribute to CBS inoculum in citrus orchards. Chopped (shredded) and un-chopped pruning debris were collected from a Eureka lemon orchard, with a known CBS history, in Hoedspruit, placed in wire frames and left under the trees in the orchard to undergo natural decomposition and maturation of *Phyllosticta* fruiting bodies. Naturally abscised leaves were also collected. The decomposing leaves and pruning debris were sampled at monthly intervals from October to March during the 2019-2020, 2020-2021 and 2021-2022 growing seasons and sent to DALRRD in Stellenbosch, for analysis of the presence of ascospores of *Phyllosticta* using a Kotzé inoculum monitor (KIM). Generally, a higher number of ascospores were recorded using the KIM in the 2020-2021 season when compared to the other seasons, with the highest number of ascospores counted from un-chopped pruning debris. Shredding pruning debris in the Eureka lemon orchard lead to reduced ascospore inoculum load. Results of the KIM analysis were complemented by the CRI-PhytRisk prediction system which predicted more

days with possible ascospore infection in the 2020-2021 season, in comparison to other seasons. A Quest volumetric spore trap was also operated in the same lemon orchard and *Phyllosticta* ascospores were trapped from October to March of the 2020-2021 and 2021-2022 seasons. A higher number of ascospores were trapped in the 2020-2021 season compared to the 2021-2022 season.

## Opsomming

Hierdie projek het ten doel gehad om vas te stel tot watter mate gekapte of gesnipperde snoei-afval tot CBS-inokulum in sitrusboorde bydra. Gekapte (gesnipperde) en nie-gekapte snoei-afval is vanaf 'n Eureka-suurlemoenboord, met 'n CBS geskiedenis, in Hoedspruit versamel, in draadrame geplaas, en onder die bome in die boord gelaat om natuurlike ontbinding en rypwording van *Phyllosticta*-vrugliggame te ondergaan. Natuurlik-afgesnyde blare is ook versamel. Die ontbindende blare en snoei-afval is met maandelikse tussenposes vanaf Oktober tot Maart gedurende die 2019-2020, 2020-2021 en 2021-2022 groeiseisoene versamel en na DALRRD in Stellenbosch gestuur vir ontleding vir die teenwoordigheid van askospore van *Phyllosticta*, deur gebruik te maak van 'n Kotzé inokulummonitor (KIM). Oor die algemeen is 'n groter aantal askospore aangeteken dmv die KIM in die 2020-2021 seisoen, in vergelyking met die ander seisoene, met die hoogste aantal askospore wat vanaf nie-gekapte snoei-afval getel is. Die versnippering van snoei-afval in die Eureka-suurlemoenboord het tot 'n verminderde askospoor inokulumlading gelei. Resultate van die KIM-analise is deur die CRI-PhytRisk-voorspellingstelsel gekomplimenteer, wat meer dae met moontlike askospoor-infeksie in die 2020-2021 seisoen, in vergelyking met ander seisoene, voorspel het. 'n Quest volumetriese spoorlokval is ook in dieselfde suurlemoenboord gebruik, en *Phyllosticta* askospore is vanaf Oktober tot Maart van die 2020-2021 en 2021-2022 seisoene, gevang. 'n Hoër aantal askospore is in die 2020-2021-seisoen, in vergelyking met die 2021-2022-seisoen, gevang.

## Introduction

Citrus black spot (CBS), caused by *Phyllosticta citricarpa* (McAlpine) van der Aa. is the most important fungal disease of citrus in South Africa and the costliest to control. The disease does not affect the internal fruit quality but rather causes cosmetic lesions and thus influences the marketability of citrus fruit (Kiely 1948; Kotzé 2000). The importance of CBS in South Africa is largely elevated due to its quarantine status in certain markets, particularly the EU's zero tolerance for CBS lesions on exported fruit.

*Phyllosticta citricarpa* produces two sources of inoculum; ascospores and conidia (Kiely 1948; McOnie 1965). Ascospores, produced within asci born within pseudothecia, constitute the primary source of inoculum for CBS (Kiely 1948; McOnie 1965). Pseudothecia do not develop on twigs, leaves and fruits still attached to trees or on harvested fruits; but occur exclusively on leaf litter on the orchard floor (Kiely 1948; McOnie 1964; Kotzé 2000). Although leaves are infected while still on trees, infection remains latent and sexual reproduction is not initiated until after leaf abscission (Kiely 1948; McOnie 1964). Depending on the frequency of wetting and prevailing temperatures, pseudothecia develop on decomposing leaf litter on the orchard floor within 40-180 days after leaf drop (Kiely 1948; Kotzé 1981, 2000). The secondary cycle of CBS involves conidia formed in pycnidia, which occur in fruit lesions and even more abundantly on old dead leaves that would have been infected while still attached on the tree (Kiely 1948; Kotzé 1963).

Since the discovery of the disease, fungicides have been the major means of controlling citrus black spot (Kotzé, 1981). Despite improvements in fungicide efficacy, timing and application, the disease is still a limiting factor in citrus production. This may be, at least in part, a consequence of management programs based exclusively on chemical control and little effort made to implement alternative or complementary strategies. Reliance on fungicides can be reduced by the integration of different control measures that include biological and cultural approaches. An integrated approach will, however, result in a more complex management program that includes major changes in grower practices. Such changes include the manner in which growers manage or handle their pruning debris and leaf litter.

Pruning trees correctly and timely is a generally recommended horticultural practice that enables management of several fungal diseases of citrus by the removal of dead wood as well as enhancing the efficacy of chemical control measures since it opens up the canopy to allow air, light and spray penetration. A general

recommendation to growers from a disease management perspective is that pruning debris, as well as leaf litter, be removed from orchards and be destroyed to reduce the inoculum for CBS. However, physical removal of pruning debris is laborious and consequently, citrus growers often leave shredded pruning debris in the orchard to retain organic matter. Shredding of pruning debris in the orchard is less laborious and more practical for growers. However, whether this practise poses a risk of contributing to CBS inoculum in the orchards and to what extent the contribution would be, is uncertain. Spore trap results have shown that ascospore numbers increased in orchards where pruning debris was not removed (Kobus Serfontein, pers. comm.). It is general knowledge that leaf litter on the orchard floor is the source of the primary inoculum of *P. citricarpa* and it has also been shown that the removal of leaves on the orchard floor can be as effective as fungicide sprays in controlling CBS (Truter, 2010). The aim of this study was to investigate whether chopped pruning debris, used as organic material, has the potential to contribute to CBS inoculum in citrus orchards.

### **Stated objectives**

To compare the relative suitability of intact and chopped pruning debris as substrate for sexual reproduction and ascospore production of *Phyllosticta citricarpa*.

### **Materials and methods**

The trial was conducted over three seasons (2019-2020, 2020-2021 and 2021-2022 seasons) in a 'Eureka' lemon orchard located in Hoedspruit. Chopped (shredded) and un-chopped pruning debris were collected, during the month of July each season, and placed in different plots (400 mm x 300 mm x 150 mm wire frames) under citrus trees to expose them to natural environmental conditions (Fig. 4.3.4.1). Naturally abscised leaves found on the orchard floor were also collected and placed into a separate plot as a control (Fig. 4.3.4.1). Twenty-four wire frames representing six replications of each treatment (naturally abscised leaves, chopped or un-chopped pruning debris) were randomly placed within the orchard each season, with four frames allocated to one replication due to the small size of the frames. At each sampling period, samples for each replication were collected from all four frames and pooled for further analysis using the Kotzé inoculum monitor (KIM) at the DALRRD quarantine facility in Stellenbosch. Sampling occurred monthly from October to March in the 2019-2020 and 2020-2021 seasons, but sampling commenced earlier, in September of the 2021-2022 season.

To analyse samples using the KIM, the protocol described by Truter (2010) was followed. Samples were rinsed for 30 seconds in tap water to remove excess soil and dirt before they were secured with cable ties between two circular plastic grids. The grids were submerged in water at 40°C for 5 minutes and then drained on paper towels for 5 minutes to remove excess water. The grids with samples were placed on the grid support of the KIM. A microscope slide coated with Vaseline petroleum jelly was placed in the slide holder to collect ascospores (Truter 2010). After the two-hour KIM operation at room temperature, the slides were removed and stained with drops of lactophenol before being examined for presence of *Phyllosticta* ascospores (Fig. 4.3.4.2) under a microscope. The number of ascospores on each slide, which represented a replicate of each treatment, were counted. The number of ascospores trapped were then converted to ascospores trapped per cubic meter of air (spores/m<sup>3</sup>) (Fourie *et al.* 2013; Moyo *et al.* 2020).

The aerial release of *Phyllosticta* ascospores from leaf litter was monitored at 3-hourly intervals by use of volumetric spore traps (Interlock Systems, Pretoria, South Africa). The spore trap was operated continuously from October through to March of each growing season (2019-2020, 2020-2021 and 2021-2022 seasons). Environmental conditions including rainfall, temperature and relative humidity as well as the CBS predictions from CRI-PhytRisk were obtained from a nearby iLeaf weather station. The pseudothecium maturation period was determined using CRI-PhytRisk.

### **Results and discussion**

Due to the difficulties in finding the KIM to use for sample analysis, sampling commenced in November for the 2019-2020 season. With the exception of the naturally abscised leaves collected in February during the 2020-

2021 season, no samples were collected after the January sampling for all three seasons (Table 4.3.4.1) because there were not enough samples to collect due to the advanced decomposition of the pruning debris.

Results of the KIM analysis were inconsistent among the different seasons and treatments. Generally, no clear trend of gradual decrease in the number of ascospores as time elapsed from October to March or as leaves decomposed was observed during the trials. The highest number of ascospores was recorded in the 2020-2021 season and the lowest number of ascospores during the 2019-2020 season. Nevertheless, chopping or shredding the pruning debris reduced the ascospore numbers when compared to un-chopped pruning debris (Table 4.3.4.1). For all three seasons, a higher number of ascospores was collected from un-chopped debris compared to chopped debris. Shredding the pruning debris could reduce the leaf surface area available for sexual reproduction by the fungus. No ascospores were collected from the naturally fallen leaves in the 2019-2020 season and although it is not quite clear what the reason for this is, we suspect that the leaves were too decomposed from the onset of sampling.

A higher number of days with possible ascospore infection was predicted by CRI-PhytRisk in the 2020-2021 in comparison to the other seasons (Table 4.3.4.2). The outputs of the CRI-PhytRisk prediction system supported the results obtained with the KIM analysis, which showed the highest number of ascospores in the 2020-2021 season. The biofix date was reached earlier in the 2019-2020 season (28 August 2019), where slightly warmer maximum temperatures were experienced in comparison to other seasons (Table 4.3.4.2). The biofix date refers to the date from which ascospores will progressively be released from maturing pseudothecia and is calculated from the 1<sup>st</sup> of July with the CRI-PhytRisk prediction system. More days with rain of more than 0.1 mm were predicted in the 2020-2021 season, however, the highest total rainfall was recorded during the 2021-2022 season (Table 4.3.4.2).

The results of the spore trapping in the 2019-2020 season could not be used as they were found unreliable. Ascospores were trapped using the volumetric spore trap throughout the duration of the trials from October to March of the 2020-2021 and 2021-2022 seasons. The highest number of ascospores was trapped during the 2020-2021 season in the month of February 2021 (Fig. 4.3.4.3). These results also correspond to the outputs of the CRI-PhytRisk and KIM analysis which showed that the 2020-2021 season had the highest number of ascospores compared to other seasons. This season had slightly more days with rainfall compared to others (Table 4.3.4.2).

### **Conclusion to date**

Shredding/chopping pruning debris in the orchard lead to reduced ascospore inoculum load and can potentially contribute to CBS management.

### **Technology transfer**

The work will be presented at the IPM and Disease management workshops.

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**Table 4.3.4.1.** Total number of ascospores trapped per cubic meter of air (spores/m<sup>3</sup>), extracted from pruning debris in a 'Eureka' lemon orchard in Hoedspruit using the Kotzé inoculum monitor (KIM) during the 2019-2020, 2020-2021 and 2021-2022 growing seasons.

Season/Month of collection of debris	Total ascospore trap numbers (spores/m <sup>3</sup> ) collected from samples		
	Un-chopped pruning debris	Naturally fallen leaves	Chopped (shredded) pruning debris
<b>2019-2020</b>			
October	— <sup>a</sup>	— <sup>a</sup>	— <sup>a</sup>
November	11170.11	0.00	72.07
December	0.00	0.00	72.07
January	72.07	0.00	0.00
February	— <sup>b</sup>	— <sup>b</sup>	— <sup>b</sup>
March	— <sup>b</sup>	— <sup>b</sup>	— <sup>b</sup>
<b>2020-2021</b>			
October	11818.70	46626.19	8864.02
November	3531.20	21619.56	648.59
December	4684.24	864.78	0.00
January	72065.22	0.00	72.07
February	— <sup>b</sup>	144.13	— <sup>b</sup>
March	— <sup>b</sup>	— <sup>b</sup>	— <sup>b</sup>
<b>2021-2022</b>			
September	1297.17	0.00	14773.37
October	12395.22	25727.28	648.59
November	16791.20	1153.04	23132.93
December	0.00	0.00	0.00
January	0.00	0.00	0.00
February			
March	— <sup>b</sup>	— <sup>b</sup>	— <sup>b</sup>

<sup>a</sup> – No samples were collected due to difficulties in finding the KIM to use for sample analysis

<sup>b</sup> – No samples were collected due to unavailability of samples at collection

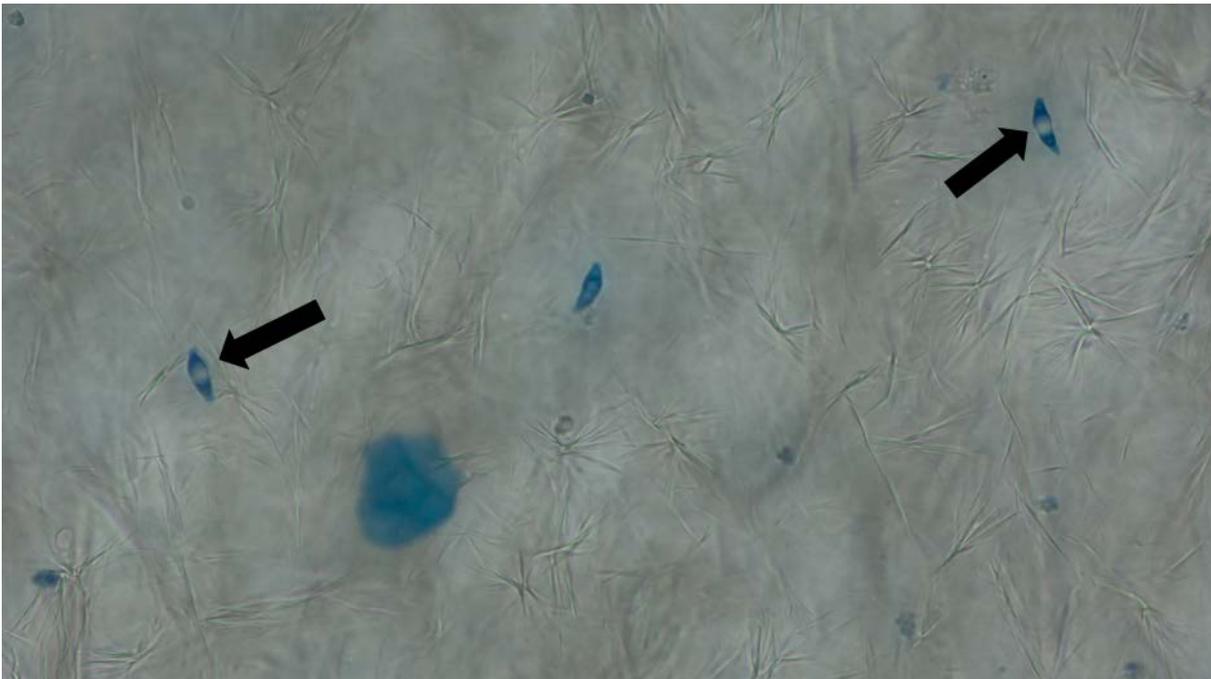
**Table 4.3.4.2.** CRI-PhytRisk predictions of Citrus black spot in in a 'Eureka' lemon orchard in Hoedspruit during the 2019-2020, 2020-2021 and 2021-2022 growing seasons.

	2019-2020 <sup>a</sup>	2020-2021 <sup>a</sup>	2021-2022 <sup>a</sup>
<b>Citrus black spot prediction</b>			
<b>Biofix date</b>	29-08-2019	12-09-2020	13-09-2021
<b>Number of days with possible ascospore infection</b>	4	8	4
<b>Number of days with possible pycnidiospore infection</b>	6	11	5
<b>Number of 3-hr periods with predicted ascospore infection</b>	8	29	6
<b>Number of 3-hr periods with predicted pycnidiospore infection</b>	19	36	10
<b>Weather variables</b>			
<b>Number of days with measurable rainfall (&gt; 0.1 mm)</b>	41	75	71
<b>Total rainfall (mm)</b>	303.30	378.10	554.30
<b>Average temperature (°C)</b>	25.38	24.86	24.27
<b>Average relative humidity (%)</b>	69.33	76.47	75.28

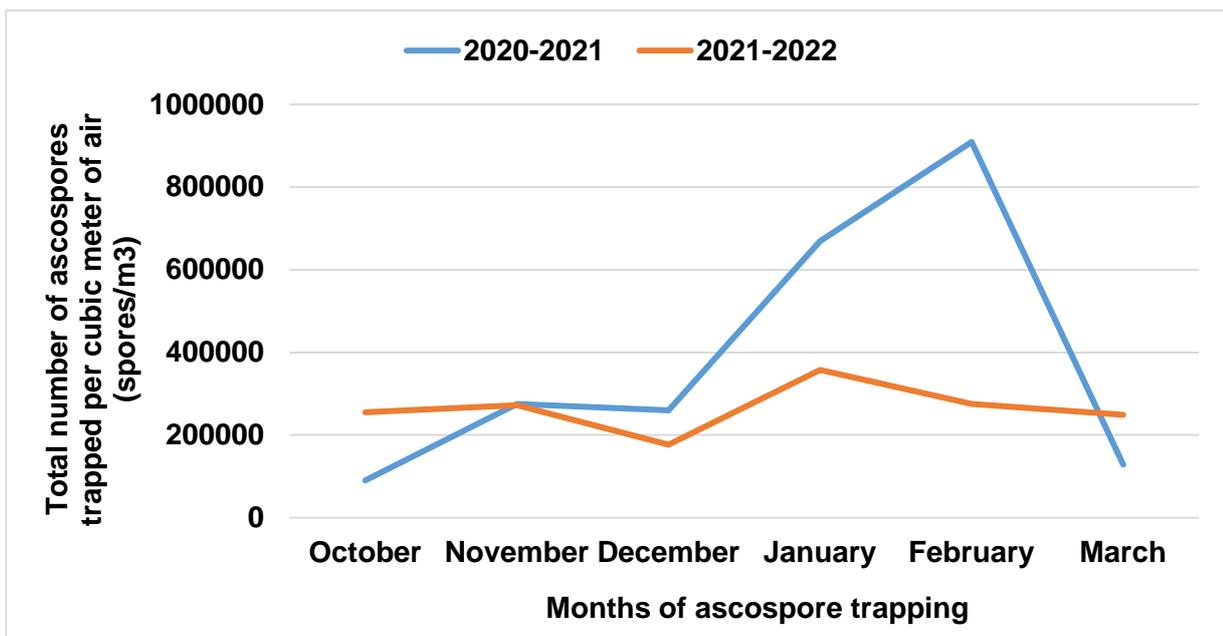
<sup>a</sup> Variables are measured from October to March of each growing season



**Figure 4.3.4.1.** Pruning debris in a 'Eureka' lemon orchard. **A.** Chopped (shredded) pruning debris on the orchard floor. **B.** Chopped (shredded) pruning debris inside the wire frame under lemon trees. **C.** Un-chopped pruning debris on the orchard floor and inside the wire frame (arrow). **D.** Naturally abscised leaves on the orchard floor. **E.** Naturally fallen leaves inside the wire frame under lemon trees.



**Figure 4.3.4.2.** *Phyllosticta* ascospores (arrows), on a Vaseline petroleum jelly coated microscope slide, collected from Eureka lemon pruning debris with the Kotzé Inoculum Monitor.



**Figure 4.3.4.3.** Total number of ascospores trapped per cubic meter of air (spores/m<sup>3</sup>), from a Eureka lemon orchard, using a volumetric spore trap, from October to March of the 2020-2021 and 2021-2022 seasons

**4.3.5 FINAL REPORT: Epidemiology, inoculum potential and infection parameters of Citrus Black Spot**

Project RCE-2-14 (1244) (2019/04 – 2022/03) by Providence Moyo and Paul H. Fourie (CRI)

**Summary**

Citrus Black Spot (CBS) is the most important fungal disease of citrus in South Africa, because of its quarantine status in major export countries. Epidemiology of CBS is not fully understood, since there is currently no method to distinguish between ascospores of the CBS pathogen and those of the non-pathogenic *P. capitalensis*. Furthermore, actual infection has not been measured yet, although generic infection models are

available. The objectives of this project were aimed at improving our understanding of CBS epidemiology, and thus improve spore release and infection models. Many of the technically challenging objectives were not realised due to capacity constraints, mostly as a result of resignations by workers. The project was moved from Stellenbosch University to CRI in Nelspruit. Certain objectives were partially fulfilled. The first pycnidiospore inoculation trial conducted in Nelspruit was evaluated and 21 and 27°C were found to be optimal temperatures for infection of Troyer citrange leaves; lesions were not observed on plants incubated at 15°C. No symptoms developed on the inoculated leaves of the second and third trial done in Nelspruit, or the trial conducted in Stellenbosch. A breakthrough with the production of *P. citricarpa* ascospores in culture was achieved in Nelspruit in 2020, but these were produced only in very low numbers that were insufficient to conduct infection studies. Further trials to produce more ascospores for germination and infection studies were unsuccessful.

## Opsomming

Sitruswartvlek (CBS) is, vanweë sy kwarantynstatus in groot uitvoerlande, die belangrikste swamsiekte van sitrus in Suid-Afrika. Epidemiologie van CBS word nie ten volle verstaan nie, aangesien daar tans geen metode is om tussen askospore van die CBS-patogeen en dié van die nie-patogeniese *P. capitalensis* te onderskei nie. Verder is werklike infeksie nog nie gemeet nie, hoewel generiese infeksie Modelle beskikbaar is. Die doelwitte van hierdie projek was daarop gemik om ons begrip van CBS-epidemiologie te verbeter, en dus om spoorvrystelling- en infeksie Modelle te verbeter. Baie van die tegniese uitdagende doelwitte is nie verwesenlik nie as gevolg van kapasiteitsbeperkings, meestal as gevolg van bedankings deur werkers. Die projek is vanaf die Universiteit Stellenbosch na CRI in Nelspruit geskuif. Sekere doelwitte is gedeeltelik bereik. Die eerste pycnidiospore-inokulasieproef wat in Nelspruit uitgevoer is, is geëvalueer en daar is gevind dat 21 en 27°C die optimale temperatuur vir infeksie van Troyer citrange blare is; letsels is nie op plante wat by 15°C geïnkubeer is, waargeneem nie. Geen simptome het op die geïnkuleerde blare van die tweede en derde proef wat in Nelspruit gedoen is, of die proef wat in Stellenbosch gedoen is, ontwikkel nie. 'n Deurbraak met die produksie van *P. citricarpa* askospore in kultuur is in 2020 in Nelspruit behaal, maar spore is slegs in baie lae getalle geproduseer wat onvoldoende was om infeksie studies uit te voer. Verdere proewe om meer askospore vir ontkiëming- en infeksie studies te produseer, was onsuksesvol.

## Introduction

Citrus Black Spot (CBS), caused by *Phyllosticta citricarpa*, is the most important fungal disease of citrus in South Africa, because of its quarantine status in major export countries. Citrus fruit is susceptible to infection by ascospores (primary inoculum source) and pycnidiospores (secondary inoculum source) during the four to five months after petal fall, but infections typically stay latent until after colour break when fruit mature. This long latent period, as well as the fact that, until recently, ascospores could not easily be produced in culture, made infection studies very difficult. As a result, infection parameters used for CBS infection models are based on germination studies and not infection *per se*. Ascospore trapping studies could also not distinguish between the ascospores of *P. citricarpa* and those of the non-pathogenic *P. capitalensis*, and existing epidemiological models of citrus black spot (Fourie *et al.*, 2013) were developed from datasets of *Phyllosticta* spp. ascospore trappings that did not distinguish between species.

In RCE-6, research was conducted to address these shortcomings. However, the ambitious objectives in RCE-6 were found to be very difficult and progress was slower than expected, as summarised below:

- A. *In planta* effect of temperature and wetness duration on germination, appressorium formation and infection of pycnidiospores of *P. citricarpa*

This objective was mostly addressed in RCE-6, except that appressorium formation and infection could not be assessed. Various methods to assess infection in high throughput assays were developed but none proved effective.

- B. *In planta* effect of temperature and wetness duration on germination, appressorium formation and infection of ascospores of *P. citricarpa*

This objective was not achieved as ascospores could not be produced in culture.

- C. Epidemiology of ascospore release of *P. citricarpa* and *P. capitalensis* in South African orchards

A qPCR technique to distinguish between *P. citricarpa* and *P. capitalensis* was successfully optimised in our laboratories, and hundreds of passive filter paper spore traps adjacent to volumetric spore traps were collected on a weekly basis from orchards in various production regions. Whilst the qPCR technique could detect and quantify DNA extracted from very small quantities of spores from spore suspensions, we found that we could only reliably quantify DNA extracted from larger amounts of spores (>1000 spores). The numbers of spores on the passive spore traps were unfortunately too low to reliably analyse, rendering all the filter paper spore traps redundant. Further improvements were made to increase the sensitivity of the qPCR assay (nested qPCR), but we realised that active spore traps should be used in future in samples where larger quantities of spores are trapped.

Recent research outcomes from Australia have made the attainment of the infection study objectives more realistic. Opposite mating types of *P. citricarpa* were used in mating type studies and ascospores were successfully produced in culture (Tran *et al.*, 2017). As part of the RCE-6 project, various attempts were made to produce ascospores in culture but with no success. Since ascospores are the primary inoculum source, this remained an important objective, and a collaboration with the Australian researchers to optimise this technique in our laboratories was established. Once ascospores could reliably be produced in culture, further studies investigating the germination and infection of ascospores (as in Objective B above) were needed. The Australian researchers have also recently published a method to conduct infection studies on Troyer citrange seedlings (Tran *et al.*, 2018) and this method is valuable in conducting further infection studies.

It was therefore proposed to continue research on the RCE-6 project's objectives, specifically as stated below. Capacity constraints due to resignations by researchers on the project as well as shortage of equipment led to failure in the execution of some objectives in the project. The successful outcomes of this research would have been invaluable to improve our understanding of CBS epidemiology and spore release and infection models.

### **Stated objectives**

- A. Production of *P. citricarpa* and *P. capitalensis* ascospores in culture.
- B. *In planta* effect of temperature and wetness duration on germination and appressorium formation of *P. citricarpa* ascospores.
- C. Infection studies of *P. citricarpa* pycnidiospores and ascospores on citrus seedlings.
- D. Study the ascospore maturation and release parameters of *P. citricarpa* and *P. capitalensis* in South African citrus orchards and optimize the qPCR method to distinguish between ascospores of *P. citricarpa* and *P. capitalensis*.

### **Materials and methods**

#### **Production of *P. citricarpa* and *P. capitalensis* ascospores in culture**

Opposite mating types of *P. citricarpa* (MAT 1 and MAT 2) from Dr. Elma Carstens' culture collection were grown on ½ strength PDA for two weeks. After two weeks, a spore suspension of one mating type was prepared and pipetted onto the culture of an opposite mating type (Tran *et al.*, 2017) and incubated for two weeks at 25°C. A second method referred to as the "sandwich mating technique" was also used. This method entailed placing 2-week-old mycelial plugs of one mating type face down onto a culture of the second mating type. The lids of the Petri dishes were observed after two weeks for the presence of ascospores.

#### ***In planta* effect of temperature and wetness duration on germination and appressorium formation of *P. citricarpa* ascospores**

The same methodology as for pycnidiospore infection studies conducted in RCE-6 was to be followed. The ascospore suspension was to be prepared by washing the Petri dish lids 2 weeks after mating. The suspension was to be filtered through a cheesecloth and washed three times by centrifugation at 2500 rpm for 5 min and a final spore concentration of  $1 \times 10^4$  spores mL<sup>-1</sup> used for inoculation. For the temperature trial (12°C, 18°C, 25°C, 30°C, 35°C and 40°C), detached Eureka lemon leaves were to be inoculated at six different inoculation points, marked with a permanent marker by pipetting a 10 µL drop of the ascospore suspension onto the marked areas and incubated for 24h at the different temperatures listed above.

In a second set of trials, the effects of wetness interruption after different incubation (4h, 6h, 8h, and 12h) periods at 25°C were to be investigated. Each incubation period was to be followed by a 20 min drying period in a laminar flow and incubated for a further 3h. After the 3h incubation period the inoculated areas were to be re-inoculated by pipetting 10 µL autoclaved bottled water onto the marked areas and incubated for a further 20h. After the incubation period for both the germination and wetness interruption trials, two inoculation points per leaf were to be cut out and placed on a microscope slide. The germinating spores on the leaves were to be stained with 20 µL LIVE/DEAD BacLight (LDB) stain, incubated for 5 min and viewed with an epifluorescence microscopy to record the germination percentage.

### **Infection studies of *P. citricarpa* pycnidiospores and ascospores on citrus seedlings (Nelspruit)**

Using the methods published by Tran *et al.* (2018), and as explained in collaboration with Dr Andrew Miles (one of Dr Tran's supervisors), three-month-old Troyer citrange citrus seedlings were inoculated with pycnidiospores of *P. citricarpa*. Three spore concentrations ( $1 \times 10^3$ ,  $1 \times 10^4$  and  $1 \times 10^5$  spores/ml) were prepared as previously described and used for the inoculations. The citrus seedlings were inoculated by pipetting 10 – 20 µl droplets onto the leaves, done inside an incubation room with high humidity (>80% RH). After inoculation, the seedlings were incubated at different temperatures (15, 21, 27 and 33°C) for 24h. After 24h the seedlings were moved to the glasshouse and left to grow for 10 months before evaluating the trial. This was done to determine the most effective method to initiate infection *in planta*. After 10 months, the number of lesions on the inoculated leaves were counted. Koch's postulates were fulfilled through reisolations to establish the ability of the pathogen to cause disease on plant material.

### **Study the ascospore maturation and release parameters of *P. citricarpa* and *P. capitalensis* in South African citrus orchards and optimize the qPCR method to distinguish between ascospores of *P. citricarpa* and *P. capitalensis***

The optimization of the qPCR standard curve was to be done for spores obtained from active volumetric spore traps located within orchards situated in the Sundays' River Valley, Nelspruit, Letsitele and Hoedspruit. The spore samples were received from the service provider (QMS Laboratories) where they were stained with lactophenol cotton blue prior to the spore counts being done on the discs. Further ascospore trapping was done by the CRI team using a Quest volumetric spore trap (Interlock Systems, Pretoria, South Africa) placed in a Eureka lemon orchard in Hoedspruit. Prior to placing the spore trap disc in the spore trap, it was covered with clear adhesive plastic and sprayed with petroleum jelly spray (PJS, Interlock Systems, Pretoria, South Africa). After counting, the ascospores were washed from the spore trap discs with water and the suspension centrifuged three times at 2500 rpm for 5 min. The supernatant was then removed and the pellet re-suspended in H<sub>2</sub>O. The DNA was to be extracted from this suspension using AMPure reagents and beads according to manufacturer's protocol. New standard curves with an exogenous internal positive control (EIPC) were to be set up to detect partial or complete inhibition of the qPCR reaction, as well as potential experimental errors. A multiplex PCR was to be carried out to improve sensitivity of the qPCR assay. Conditions for the 1<sup>st</sup> PCR: Melting step at 94°C for 5 min, followed by 10 cycles of 94°C for 45 s, 66.4°C for 45 s, and 72°C for 45 s and final elongation step of 72°C for 7 min. The 2<sup>nd</sup> qPCR step is the same as mentioned above. For *P. citricarpa*, spore dilutions were to be made from  $10^6$  to 3906 spores, and for *P. capitalensis*, spore dilutions from 400 000 to 1560 spores. The qPCR protocol of Hu *et al.* (2014) was to be adjusted by using Sensimix Probe II and adding 1 µL of 20 mg.mL<sup>-1</sup> BSA to each 25 µL reaction.

## **Results and discussion**

Apart from being a very technically challenging project, progress was hampered by capacity constraints. Firstly, the PhD student on this project terminated her studies. A contract researcher was appointed, but accepted a permanent appointment elsewhere prior to conclusion of the project. The project was moved from the University of Stellenbosch to CRI in Nelspruit, where Dr Moyo could have more active and direct supervision. A contract researcher was again appointed, who was later appointed in a permanent technician position. Unfortunately, this person also resigned just as the project was regaining its momentum.

### **Production of *P. citricarpa* and *P. capitalensis* ascospores in culture**

After many unsuccessful attempts of ascospore production in Stellenbosch, the research project was moved to CRI in Nelspruit. A breakthrough was finally achieved in 2020 also after many attempts and optimisation. The use of the 'sandwich mating technique' as described above resulted in production of ascospores of *P. citricarpa* in culture. The second method, which involving pipetting a spore suspension of one mating type on top of the other did not yield any ascospores and was always marred with contamination, which resulted in the plates being discarded. Optimisation methods that eventually resulted in the production of ascospores included incubating the cultures for longer than 2 weeks (4 to 6 weeks) at temperatures of approximately 27 - 28°C, instead of incubating for 2 weeks at ambient conditions, as reported by Tran et al. (2017). Ascospores were found on the lids of the Petri dishes (Fig. 4.3.5.1) more than 4 weeks after pairing the isolates; however, the spores were produced in very low numbers, which were not sufficient to be used in further experimental work. Due to the longer incubation period, some ascospores were already germinating on the Petri dish lids (Fig. 4.3.5.1). Further attempts at mass production of ascospores involved mating cultures almost every week during the warm months but the number of ascospores produced was always very low. Moreover, efforts at mass ascospore production were also thwarted by problems of contamination and mites.

#### ***In planta* effect of temperature and wetness duration on germination and appressorium formation of *P. citricarpa* ascospores**

This objective involved viewing and recording germinating ascospores on detached Eureka lemon leaves with an epifluorescence microscope. Previous work on germinating pycnidiospores was conducted in Stellenbosch but various attempts at ascospores production in Stellenbosch were unsuccessful and therefore, this part of the project was also moved to the CRI Nelspruit office. After many attempts and optimisation, ascospores were eventually produced in Nelspruit although in very low numbers as explained above. Further attempts at producing sufficient ascospore numbers required for these trials were unsuccessful and therefore, this objective was not fulfilled.

#### **Infection studies of *P. citricarpa* pycnidiospores and ascospores on citrus seedlings**

Due to the unavailability of sufficient ascospores to do any experimental work, infection studies were only conducted using pycnidiospores. Three spore concentrations ( $1 \times 10^3$ ,  $1 \times 10^4$  and  $1 \times 10^5$  spores/ml) were used in the trials and inoculated seedlings were incubated at four different temperatures (15°C, 21°C, 27°C and 33°C) for 24h before being moved to the glasshouse where they grew for 10 months prior to the trial evaluation. Four trials were conducted using pycnidiospores for this objective (one in Stellenbosch and three in Nelspruit), but CBS symptoms were only obtained on seedlings inoculated in one of the three trials conducted in Nelspruit.

Inoculation of 3-month-old Troyer citrange citrus seedlings with pycnidiospores, in one of the trials conducted in April 2019 in Nelspruit, resulted in citrus black spot symptoms. These symptoms were often visible as small lesions often containing pycnidia (Fig. 4.3.5.2). Although the highest mean number of lesions was obtained from seedlings incubated at 27°C, no significant ( $P > 0.05$ ) differences between the number of lesions achieved at 21 and 27°C were observed. No infection was obtained at 15°C and only one lesion was observed on a leaf inoculated with  $1 \times 10^5$  spores/ml at 33°C. The highest mean number of lesions was produced by  $1 \times 10^4$  spores/ml at 27°C (Table 4.3.5.1). *Phyllosticta citricarpa* isolates were recovered from 39 of the 117 CBS lesions (including the lesion obtained at 33°C) examined, but never from the asymptomatic sites of leaves incubated at 15 and 33°C.

#### **Study the ascospore maturation and release parameters of *P. citricarpa* and *P. capitalensis* in South African citrus orchards and optimize the qPCR method to distinguish between ascospores of *P. citricarpa* and *P. capitalensis***

This challenging objective was not fulfilled for to a number of reasons including capacity constraints, as explained above.

In the case of the spore trapping done by the CRI team, several attempts were made to dislodge ascospores from the adhesive tape with no success. This was done by excising the adhesive tape covering the spore disc and placing small pieces into glass bottles containing sterile water (as successfully employed by van Niekerk et al. (2010). These bottles were then heated for 2 min at 50°C in a warm water bath to melt the PJS and place the spores into suspension. However, after many attempts, it was realised that most of the ascospores were moved to the glass cover slips used during the spore trap disc reading, probably by the stain used in the

reading. Thus, more ascospores were obtained by washing the glass cover slips compared to the adhesive tape.

## Conclusions

Capacity constraints due to resignations of staff members, led to failure in the execution of most objectives in this project. Progress was, however, achieved with other objectives. After many attempts, production of ascospores in culture was achieved but in low numbers, which were unfortunately insufficient to do further trials. Optimisation of the method to mass produce sufficient ascospores for further germination and infection studies was unsuccessful. The temperatures of 21 and 27°C were found to be most suitable for infection of Troyer citrange leaves using pycnidiospores of *P. citricarpa*, while no infection was observed at 15°C. Symptom expression in the infection trials was, however, erratic (one in four trials)

## Technology transfer

No technology transfer took place in this reporting period.

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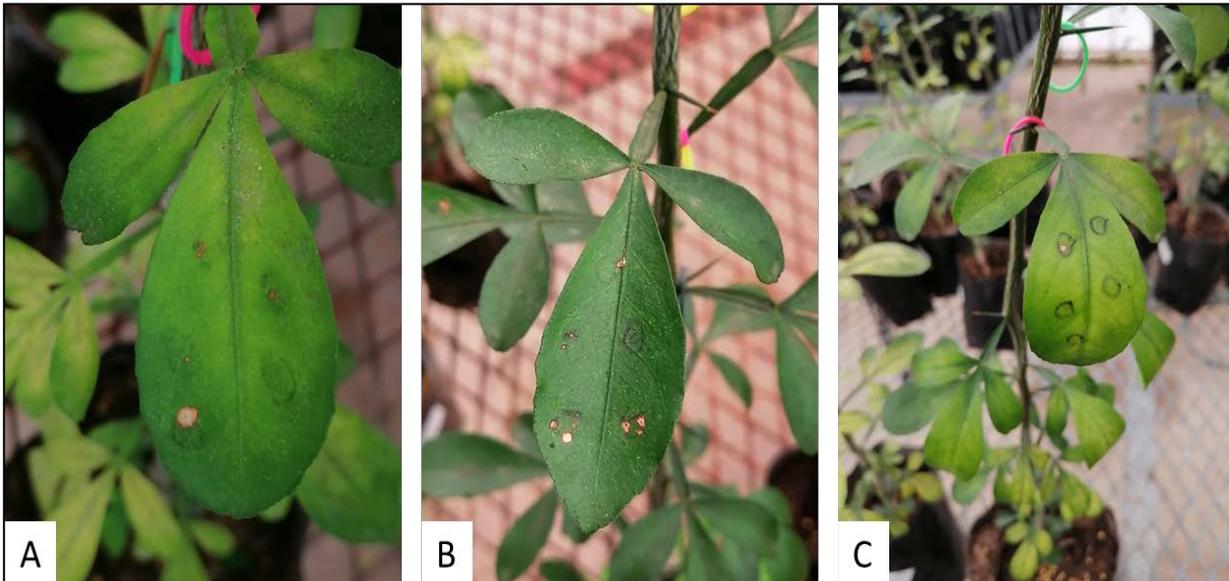
**Table 4.3.5.1.** Mean number of CBS lesions on “Troyer citrange” leaves inoculated with three different spore suspension concentrations and incubated at three different temperatures for 24 hours to allow infection in April 2019

Spore concentration (spores/ml)	Incubation temperature (°C)	Mean lesion number <sup>a</sup>
<b>1x10<sup>3</sup></b>	15	0.000 c
	21	0.320 b
	27	0.000 c
	33	0.000 c
<b>1x10<sup>4</sup></b>	15	0.000 c
	21	0.412 ab
	27	0.632 a
	33	0.000 c
<b>1x10<sup>5</sup></b>	15	0.000 c
	21	0.226 bc
	27	0.225 bc
	33	0.011 c

<sup>a</sup>Means in a column followed by the same letter are not significantly different ( $P > 0.05$ ) according to Tukey’s HSD test.



**Figure 4.3.5.1.** Ascospores of *P. citricarpa* produced through the 'sandwich mating technique'. Ascospores were observed on the lid of a Petri dish under a light microscope. Germinating ascospores are indicated with arrows.



**Figure 4.3.5.2.** Citrus black spot symptoms observed on Troyer citrange leaves inoculated with *P. citricarpa* pycnidiospore suspensions during April 2019. Leaves inoculated with (A)  $1 \times 10^4$  spores/ml and incubated at 27°C, (B)  $1 \times 10^5$  spores/ml and incubated at 27°C and (C)  $1 \times 10^4$  spores/ml and incubated at 21°C.

#### 4.3.6 FINAL REPORT: Development of a rapid molecular tool to detect benzimidazole resistance in field-collected *Phyllosticta citricarpa* isolates

Project 1316 (2021/04 – 2022/03) by Providence Moyo, Glynnis Cook, Elaine Basson, Chanel Steyn, Rachelle Bester, Charmaine Olivier and Paul H. Fourie (CRI)

##### Summary

Citrus black spot (CBS), caused by *Phyllosticta citricarpa*, is an economically important disease, which is effectively controlled by repeated fungicide applications to protect fruit from infection. Systemic fungicides such as benzimidazoles are widely used for controlling CBS in South Africa, but the molecular mechanisms of benzimidazole resistance in *P. citricarpa* had not been investigated. Analysis of the nucleotide sequence of the beta-tubulin gene in benzimidazole-resistant *P. citricarpa* isolates revealed mutations, inducing three amino acid replacements, when compared to that of sensitive strains. The amino acid replacements in benzimidazole-resistant isolates included the change of glutamic acid to either alanine or lysine at codon 198 of the beta-tubulin gene and a change from phenylalanine to tyrosine at codon 200. All three mutations have previously been implicated in benzimidazole resistance in several fungal pathogens. A polymerase chain reaction (PCR) assay was designed to amplify a portion of the beta-tubulin gene, which was subsequently sequenced to identify benzimidazole resistance in *P. citricarpa*. This PCR and sequence assay were found to be a more rapid and reliable method for detecting resistance compared to the fungicide-amended plate test and is valuable for monitoring the occurrence of benzimidazole-resistant *P. citricarpa* isolates and for assessment of the need for alternative CBS management practices.

##### Opsomming

Sitruswartvlek (CBS), deur *Phyllosticta citricarpa* veroorsaak, is 'n ekonomies belangrike siekte, wat effektief beheer word deur herhaalde swamdodertoedienings om vrugte teen infeksie te beskerm. Sistemiese swamdoders soos bensimidasool word wyd gebruik vir die beheer van CBS in Suid-Afrika, maar die molekulêre meganismes van bensimidasool-weerstand in *P. citricarpa* is nog nie ondersoek nie. Ontleding van die nukleotiedvolgorde van die beta-tubulien-geen in bensimidasool-weerstandbiedende *P. citricarpa* isolate, het mutasies aan die lig gebring wat drie aminosuurvervangings veroorsaak, in vergelyking met dié van sensitiewe isolate. Die aminosuurvervangings in bensimidasool-weerstandbiedende isolate het die verandering van glutamiensuur na óf alanien óf lisien by kodon 198 van die beta-tubulien-geen, en die verandering van fenielalanien na tirosien by kodon 200, ingesluit. Al drie mutasies is voorheen by bensimidasool-

weerstandbiedendheid in verskeie swampatogene aangedui. 'n Polimerase-kettingreaksie (PKR)-toets is ontwerp om 'n gedeelte van die beta-tubulien-geen te amplifiseer, waarna volgorde-bepaling gedoen is om bensimidiasool-weerstand in *P. citricarpa* te identifiseer. Daar is gevind dat hierdie PKR en volgorde-bepalingstoets 'n vinniger en meer betroubaar metode is om weerstand op te spoor, in vergelyking met die swamdoder-gewysigde plaattoets, en is waardevol vir die monitering van die voorkoms van bensimidiasool-weerstandbiedende *P. citricarpa* isolate, en vir die bepaling van die behoefte aan alternatiewe CBS-bestuurspraktyke.

## Introduction

Citrus black spot (CBS), caused by the fungal pathogen *Phyllosticta citricarpa*, and is the most economically important disease in commercial citrus orchards in South Africa and other countries (Kiely 1948; Kotzé 2000; Brentu *et al.* 2012; Schubert *et al.* 2012). Management of this disease in South Africa is mainly based on intensive chemical spray programmes combining different chemical classes of fungicides, in mixtures or in alteration. Currently, CBS control is achieved with the use of protective fungicides based on copper, dithiocarbamates or dipotassium phosphate and systemic fungicides (such as benzimidazoles and strobilurins).

Benzimidazole fungicides are the first group of systemic single-site fungicides introduced in agriculture in the early 1970s (Deising *et al.* 2008; Walker *et al.* 2013; Vela-Corcía *et al.* 2018). These fungicides, which include carbendazim, benomyl, thiophanate-methyl and thiabendazole, are registered in numerous countries and play an important role in plant protection. They control a remarkably broad spectrum of phytopathogenic fungi. These molecules are specific inhibitors of microtubule assembly and act by inhibiting germ-tube elongation and mycelial growth (Davidse 1986; Leroux *et al.* 1999). Because of their specificity, the development of resistance has been a major concern in disease management in different pathosystems. Resistance to benzimidazoles has been detected in many phytopathogenic fungi including *Venturia inequalis* (Koenraadt *et al.* 1992), *Colletotrichum* spp. on various crops (Whiteside 1980; Wong *et al.* 2008), *Botrytis cinerea* (Bolton 1976; Tripathi and Schlösser 1982), *Cochliobolus heterostrophus* (Greenaway 1973) and *Podosphaera xanthii* (McGrath 2001).

Benzimidazoles became very attractive to growers because of their pre- and post-infection activity against *P. citricarpa* and other pathogens on various crops. Benomyl has been in use in South Africa for the control of citrus black spot since 1971 (Schutte *et al.* 2003). As early as 1982, roughly a decade after benomyl was first applied commercially, populations of *P. citricarpa* collected from an orchard of Valencia oranges in Mpumalanga were found to have developed resistance to the fungicide (Hebert and Grech 1985). Since the initial report, benomyl resistance in populations of *P. citricarpa* has been observed in all important citrus growing areas in South Africa (Schutte *et al.* 2003). Due to the presence of benomyl resistance, growers are increasingly relying on strobilurin fungicides and multi-site protectant fungicides such as dithiocarbamates to manage CBS. Protectant fungicides need to be applied more frequently and possess no curative action. Strobilurins registered for CBS control in South Africa provide longer protection intervals but have limited curative action, with up to only 3 days for certain products.

Several mutations in the  $\beta$ -tubulin gene have been associated with resistance to in phytopathogenic fungi the benzimidazole group of fungicides (Koenraadt *et al.* 1992; Jung *et al.* 1992; Cooley and Caten 1993; Ma *et al.* 2003, 2005a, b), but the most common mutations occur in codon 198 or 200 (Ma *et al.* 2005b; Peres *et al.* 2004; Chung *et al.* 2006; Kim *et al.* 2007). These point mutations lead to changes in amino acid sequences at the benzimidazole binding site (Koenraadt *et al.* 1992). In many phytopathogenic fungi, glutamic acid is substituted with alanine, valine or glycine at position 198 and phenylalanine is substituted with tyrosine at codon 200 (Fujimura *et al.* 1992; Koenraadt *et al.* 1992; Yarden and Katan 1993; Albertini *et al.* 1999; Peres *et al.* 2004; Ma *et al.* 2005b; Chung *et al.* 2006; Kim *et al.* 2007). Although the molecular mechanisms of benzimidazole resistance have been intensively studied in a variety of pathogens, these have not yet been studied in *P. citricarpa*.

Monitoring of benzimidazole resistance is of practical importance to evaluate the efficiency of these fungicides against CBS and for citrus growers to adjust their management strategies promptly. Current methods for

detection of *P. citricarpa* benzimidazole-resistant isolates in South Africa, rely on *in vitro* tests based on mycelial growth on medium amended with benzimidazole fungicides. As viability of *P. citricarpa* isolates recovered from commercial orchards is usually low, alternative monitoring methods are needed. Molecular diagnosis techniques such as allele-specific polymerase chain reaction (PCR), real-time PCR, PCR-restriction-fragment length polymorphism (PCR-RFLP) and the two-step high-resolution melting (HRM) analysis have been found to be suitable for monitoring fungicide resistance in phytopathogenic fungal populations (Luck and Gillings 1995; Fernández-Ortuño *et al.* 2014; Banno *et al.* 2008; Ziogas *et al.* 2009; Chatzidimopoulos *et al.* 2014). DNA-based diagnosis of benzimidazole resistance in *P. citricarpa* will, therefore, be a reliable and rapid method. This method can also be used to supplement the classical assay methods; i.e. can be used in parallel or even in place of classical assays.

The aim of this study was to develop a rapid molecular tool to detect benzimidazole resistance in field-collected CBS lesions and to use this method to monitor benzimidazole resistance in citrus orchards in South Africa. The successful development of molecular detection methods would allow the rational use of benzimidazole fungicides to control CBS.

### **Stated objectives**

1. Determine sensitivity of *P. citricarpa* isolates to benomyl.
2. Sequence the beta-tubulin gene of resistant and sensitive isolates of the CBS pathogen.
3. Design PCR primers to detect resistant isolates.
4. Test for efficiency and sensitivity of designed primers.

### **Materials and methods**

#### **Sensitivity of *P. citricarpa* isolates to benomyl**

Citrus fruit samples with CBS symptoms were collected from commercial orchards in Southern Africa, with varying levels of benomyl use over the years. Symptomatic fruit sent by citrus growers to the Citrus Research International Diagnostic Centre, to test for presence of benomyl resistance in their orchards, were also included in the study. Depending on the number of lesions per fruit, one to three lesions were excised from each fruit and plated on potato dextrose agar amended with Streptomycin at 0.04 g per litre (PDA<sup>+</sup>). After 7 to 10 days, isolates were plated on Synthetic nutrient-poor agar (SNA) where hyphal tips of the isolates were plated on PDA<sup>+</sup> to obtain clean cultures.

Sensitivity of *P. citricarpa* isolates to benomyl was determined using mycelial growth assays. A stock solution of benomyl was prepared in water and varying volumes of this solution were added to molten PDA to achieve final concentrations ranging from 0 to 100 parts per million (ppm) of benomyl per litre agar. Potato dextrose agar plates were supplemented with benomyl at 0, 2.5, 5.0, 7.5, 10, 25, 50 and 100 ppm. To determine the sensitivity of *P. citricarpa* to the fungicide, 4 mm mycelial plugs were taken from the edge of a 2-week old colony of each isolate and placed at the centre of the PDA plates amended with benomyl at different concentrations. Three replications of each fungicide concentration were used for each isolate. After incubation of the plates at 25 °C for 2 weeks, radial growth of each isolate was measured. Radial growth was determined by two perpendicular measurements of the colony and averaging the diameters. Data were then used to calculate the percent relative growth and expressed as percentage of the inhibition (Nalumpang *et al.* 2010). Isolates that exhibited any growth on PDA amended with benomyl were designated as benomyl-resistant isolates and those in which growth was totally inhibited by the addition of the fungicide in PDA were designated as benomyl-sensitive isolates. In order to further classify the resistant phenotypes, percentage inhibition data for resistant isolates were subjected to suitable regression analysis in order to determine the effective concentration for 50% inhibition of mycelial growth (EC50 value) in XLSTAT (version 2019.4.1).

#### **Sequence the beta-tubulin gene of resistant and sensitive isolates of the CBS pathogen**

Benomyl-resistant and -sensitive isolates of *P. citricarpa*, identified using the bioassays described above, were cultured on PDA plates at 25 °C for 2 weeks before aerial mycelia and conidia were collected for DNA extraction. DNA was extracted and purified using Nucleospin Plant DNA extraction kits (Macherey Nagel)

according to the manufacturer's recommendations. DNA was re-suspended in 20 µL of elution buffer and quantified using a NanoDrop ND-1000 spectrophotometer.

A nucleotide sequence for the beta-tubulin gene was initially obtained by PCR amplification using degenerate primers B1 and B3 (Butters *et al.* 2003) and subsequent Sanger sequencing of the PCR product. A specific reverse primer, PCR1 (5'-GGAGGCAGCCATCATGTTCTT-3'), was designed and used with the TUBF1 primer (Butters *et al.* 2003) to amplify a 1307 bp fragment spanning the codon mutation sites previously reported for other fungi. Reactions were performed in 15 µl reaction volumes using Q5 High fidelity Master Mix (New England BioLabs Inc., Ipswich, UK) and a final concentration of 0.2 µM of each primer. Cycle conditions included an initial denaturation step at 98 °C for 30 s, followed by 35 cycles of 98 °C for 10 s, 55 °C for 30 s, 72 °C for 60 s and a final extension of 72 °C for 2 min. PCR products were gel-purified using the Zymoclean Gel DNA recovery kit (Zymo Research Corporation, Irvine, CA) and bi-directionally Sanger sequenced at Inqaba Biotech (Pretoria, South Africa). DNA sequences were compiled by trimming low-quality bases and overlapping sequences were aligned using BioEdit (Hall 1999).

### **Design PCR primers and test for sensitivity to detect resistant isolates**

Fungal cultures of species such as *P. capitalensis*, *Colletotrichum gloeosporioides* and *Alternaria alternata*, which often cohabit CBS lesions with *P. citricarpa*, and are found in other citrus fruit rind lesion types, were obtained for the purposes of primer design and assay specificity testing. DNA was isolated from pure cultures using the Wizard Genomic DNA Purification Kit (Promega, Madison, USA). Partial beta-tubulin sequences were generated for *P. capitalensis*, *C. gloeosporioides* and *A. alternata* as detailed for *P. citricarpa* above. PCR primers, CBS-BR\_F2 (5'-CCGCAGAAACAACCAATCTG-3') and CBS-BR\_R1 (5'-GTCCTCATGCAAATGTCGTACAG-3'), based on the beta-tubulin gene, were designed to specifically amplify *P. citricarpa*. PCR reactions were performed in 15 µl reaction volumes using GoTaq G2 Master Mix (Promega) and a final concentration of 0.2 µM of each primer. Cycle conditions included an initial denaturation step at 94 °C for 3 min, followed by 40 cycles of 94 °C for 10 s, 57 °C for 30 s, 72 °C for 30 s and a final extension of 72 °C for 2 min.

The ability of the PCR assay to amplify *P. citricarpa* DNA isolated directly from fruit lesions without culturing was also tested. This was performed on DNA extracted directly from 22 fruit lesions, which were previously confirmed to be positive for *P. citricarpa* by real-time PCR, using the protocol of Hu *et al.* (2014), in routine CBS diagnostic tests at the CRI-DC. DNA from fruit lesions was extracted using the Wizard Genomic DNA Purification Kit (Promega). PCR products were gel purified as detailed above and Sanger sequencing was performed at Inqaba Biotech. The proximity of the reverse primer to the mutation sites (approximately 80 bp upstream of the mutation sites) allowed for Sanger sequencing to be conducted using only the reverse primer to determine the sensitivity or resistance to benzimidazole fungicides, based on the nucleotide sequences as listed in Table 4.3.6.1.

In further tests to compare its effectiveness over the traditional bioassays, the PCR assay using primers CBS-BR\_F2 and CBS-BR\_R1 was applied in 70 commercial fruit and leaf samples received for benzimidazole resistance testing at CRI-DC during 2021. CBS lesions excised from fruit and leaf samples were divided into 2 parts: one part was plated onto PDA<sup>+</sup> to obtain *P. citricarpa* cultures for use in benzimidazole resistance bioassays whereas the other half was used to extract DNA directly without culturing and subsequently sequenced to determine the benzimidazole sensitivity based on beta-tubulin nucleotide sequences. The bioassays were based on mycelial growth tests, at a 10 µg per ml benomyl concentration, currently used in the CRI-DC. DNA extraction and PCR as well as sequencing to determine the sensitivity to benzimidazole fungicides were performed as described above.

## **Results and discussion**

### **Sensitivity of *P. citricarpa* isolates to benomyl**

A total of 134 isolates of *P. citricarpa* were analysed and their sensitivity to benomyl characterised. A clear distinction was observed between sensitive and resistant isolates. All resistant isolates grew on PDA amended with benomyl whereas sensitive isolates grew only on PDA without benomyl. The bulk of the isolates (57) were obtained from the Mpumalanga province and benomyl resistance was detected in 39 of these isolates. All the

isolates obtained from two orchards in KwaZulu-Natal were benomyl-resistant, whereas two of the three isolates from one orchard in eSwatini were benomyl-resistant and 5 of the 10 isolates obtained from Zimbabwe were resistant to benomyl. The high frequency of benomyl resistance is cause for concern as the efficacy of benzimidazole fungicides will be reduced in the affected orchards. Benzimidazole resistance is generally not associated with a fitness penalty (Dovas *et al.* 1976) and growers can, therefore, expect resistance frequencies to remain stable in orchards. Use of benzimidazoles should be carefully considered, avoided or replaced with chemically unrelated fungicides. All 20 isolates tested from nine orchards surveyed in the Eastern Cape were sensitive to benomyl. Employing resistance management strategies, such as alternation with alternative chemistry, fungicide mixtures and resistance monitoring remain critical to ensure the sustained and effective use of benzimidazole fungicides against CBS in these orchards.

### **Sequence the beta-tubulin gene of resistant and sensitive isolates of the CBS pathogen**

Analysis of all benomyl-sensitive isolates of *P. citricarpa* revealed glutamic acid and phenylalanine amino acids at codon positions 198 and 200 of the beta-tubulin gene, respectively (Table 4.3.6.1). Changes in the beta-tubulin gene of benomyl-resistant isolates were restricted to codons 198 and 200. Among the 43 benomyl-resistant isolates sequenced, 24 had mutations in codon 198 and 19 in codon 200 of the beta-tubulin gene (Table 4.3.6.1). Two amino acid substitutions occurred at codon 198; a GAG-to-AAG nucleotide mutation that resulted in the substitution of glutamic acid (GAG) with lysine (AAG) was observed in 19 resistant isolates, while a glutamic acid (GAG) to alanine (GCG) substitution was identified in five resistant isolates (Table 4.3.6.1). Codon 200 for phenylalanine (TTC) was changed to tyrosine (TAC) in 19 benomyl-resistant isolates of the pathogen (Table 4.3.6.1). All isolates found to be benomyl-resistant by molecular analysis of the beta-tubulin gene were also found to be resistant in fungicide amended media assays. EC50 values of the codon 198 – lysine genotypes ranged from 28.5 - 89.7 µg/ml, the codon 198 – alanine genotypes from 42.6 - 64.9 µg/ml, and the codon 200 – tyrosine genotypes ranged from 20.4 - 50.4 µg/ml (Table 4.3.6.1).

### **Design PCR primers and test for sensitivity to detect resistant isolates**

Due to the proximity of the multiple mutations detected in the beta-tubulin gene, it was not possible to develop a single, specific PCR to simultaneously detect the range of mutations associated with benzimidazole resistance in *P. citricarpa*. Instead, a PCR primer pair CBS-BR\_F2 and CBS-BR\_R1 was developed to amplify DNA in the beta-tubulin region specifically for *P. citricarpa* isolates. Thereafter, the amplicon was Sanger sequenced using only the reverse primer to determine the sensitivity of isolates to benzimidazole fungicides. This primer pair successfully amplified a 694 bp DNA fragment from *P. citricarpa* isolates only and not from other non-target fungi, including *P. capitalensis*, *C. gloeosporioides* and *A. alternata*. DNA extracted directly from 22 CBS fruit lesions was successfully amplified and 16 lesions were identified as benzimidazole-sensitive and six as benzimidazole-resistant, based on the reverse primer sequence data that indicated the SNPs at codons 198 and/or 200 associated with benzimidazole resistance.

The recovery rate of *P. citricarpa* from CBS lesions through plating was very low (41.4%) from samples obtained in some orchards in Mpumalanga and Limpopo (Table 4.3.6.2). The molecular assay, on the other hand, consistently generated a 694 bp PCR fragment from the DNA extracted directly from all the CBS lesions of the 70 samples (68 fruit and 2 leaf lesions) received for benzimidazole resistance testing at the CRI-DC (Table 4.3.6.2). Bioassay results of the 29 isolates that could be recovered from lesions conformed to the molecular assay results for those isolates (Table 4.3.6.2). In total, 38 of the 70 isolates were designated as benomyl-resistant. The nucleotide mutation resulting in an amino acid change from phenylalanine to tyrosine at codon 200 was the predominant mutation associated with resistance to benzimidazole, with a frequency of 37.1% compared to 11.4% and 5.7% for the glutamic acid to lysine and glutamic acid to alanine mutations at codon 198, respectively (Table 4.3.6.2).

### **Conclusions**

Benzimidazole resistance in *P. citricarpa* is associated with single nucleotide mutations in the beta-tubulin gene, which alter the structure of the fungicide binding sites. Glutamic acid and phenylalanine at codon positions 198 and 200 of the beta-tubulin gene, respectively, seem to be the key sites for benzimidazole binding in *P. citricarpa*. The rapid and efficient benzimidazole testing assay developed in the study will allow growers to determine resistance frequencies in their orchards and also make better informed management decisions.

## Technology transfer

Moyo, P., Cook, G., Basson, E., Steyn, C., Bester, R. Olivier, C. and Fourie, P.H. 2022. Monitoring Benzimidazole Resistance in *Phyllosticta citricarpa* Using a Molecular Assay Targeting Mutations in Codons 198 and 200 of the  $\beta$ -Tubulin Gene. *Plant Disease* 106(5):1374-1380. doi: 10.1094/PDIS-07-21-1459-RE

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**Table 4.3.6.1.** Point mutations and deduced amino acid substitutions in the beta-tubulin gene for 61 isolates of *P. citricarpa* with different sensitivities to benomyl

Benomyl phenotype <sup>a</sup>	Codon substitution	Amino acids in codon		Number of isolates	EC50 value (µg/ml)
		198	200		
<b>Sensitive</b>	None	Glutamic acid	Phenylalanine	18	< 2.5
<b>Resistant</b>	GAG to AAG	Lysine	Phenylalanine	19	28.5 - 89.7
<b>Resistant</b>	GAG to GCG	Alanine	Phenylalanine	5	42.6 - 64.9
<b>Resistant</b>	TTC to TAC	Glutamic acid	Tyrosine	19	20.4 - 50.4

<sup>a</sup>Benomyl phenotypes were determined by growing isolates on potato dextrose agar amended with 2.5 to 100 µg/ml benomyl. Sensitive = no growth at any benomyl concentrations; Resistant = growth at all benomyl concentrations

**Table 4.3.6.2.** Benzimidazole resistance monitoring by bioassays, PCR and sequencing

Origin	Date received	Orchard	Citrus type	Isolates	Fruit /Leaf	Lesion viability on PDA <sup>a</sup>	Resistance Phenotype <sup>b</sup>	PCR reaction <sup>c</sup>	Amino acids in codon		Genotype <sup>d</sup>
									198	200	
Limpopo	25-02-2021	1	Not recorded	21-2211	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
Mpumalanga	02-03-2021	1	Lemon	21-2420	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
Mpumalanga	02-03-2021	2	Lemon	21-2423	Fruit	-	N/A	+	Glu	Phe	Ben <sup>S</sup>
Mpumalanga	02-03-2021	3	Lemon	21-2426	Fruit	+	Sensitive	+	Glu	Phe	Ben <sup>S</sup>
Mpumalanga	02-03-2021	4	Lemon	21-2429-1	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
Mpumalanga	02-03-2021	5	Lemon	21-2432	Fruit	-	N/A	+	Lys	Phe	Ben <sup>R</sup>
Mpumalanga	02-03-2021	6	Lemon	21-2440	Fruit	-	N/A	+	Lys	Phe	Ben <sup>R</sup>
Mpumalanga	02-03-2021	7	Lemon	21-2458-1	Fruit	-	N/A	+	Glu	Phe	Ben <sup>S</sup>
Mpumalanga	02-03-2021	8	Lemon	21-2464	Fruit	-	N/A	+	Glu	Phe	Ben <sup>S</sup>
Mpumalanga	02-03-2021	9	Lemon	21-2465	Fruit	-	N/A	+	Lys	Phe	Ben <sup>R</sup>
Limpopo	05-03-2021	2	Lemon	21-2582	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
Limpopo	05-03-2021	2	Lemon	21-2583	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
Limpopo	05-03-2021	2	Lemon	21-2584	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
Limpopo	05-03-2021	2	Lemon	21-2585	Fruit	+	Resistant	+	Glu	Tyr	Ben <sup>R</sup>
Limpopo	05-03-2021	2	Lemon	21-2586	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
Limpopo	05-03-2021	2	Lemon	21-2587	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
Limpopo	05-03-2021	2	Lemon	21-2588	Fruit	+	Resistant	+	Glu	Tyr	Ben <sup>R</sup>
Limpopo	05-03-2021	2	Lemon	21-2589	Fruit	+	Resistant	+	Glu	Tyr	Ben <sup>R</sup>
Limpopo	05-03-2021	2	Lemon	21-2590	Fruit	+	Resistant	+	Glu	Tyr	Ben <sup>R</sup>
Limpopo	05-03-2021	2	Lemon	21-2591	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
Limpopo	15-03-2021	3	Mandarin hybrid	21-2840	Fruit	-	N/A	+	Glu	Phe	Ben <sup>S</sup>
Limpopo	15-03-2021	3	Mandarin hybrid	21-2841	Fruit	-	N/A	+	Glu	Phe	Ben <sup>S</sup>
Limpopo	15-03-2021	3	Mandarin hybrid	21-2842	Fruit	-	N/A	+	Glu	Phe	Ben <sup>S</sup>
Limpopo	15-03-2021	3	Mandarin hybrid	21-2843	Fruit	-	N/A	+	Glu	Phe	Ben <sup>S</sup>
Limpopo	15-03-2021	3	Mandarin hybrid	21-2844	Fruit	-	N/A	+	Glu	Phe	Ben <sup>S</sup>
Limpopo	31-05-2021	4	Lemon	21-3639	Fruit	+	Resistant	+	Glu	Tyr	Ben <sup>R</sup>
Limpopo	31-05-2021	4	Lemon	21-3640	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
Limpopo	31-05-2021	4	Lemon	21-3641	Fruit	+	Resistant	+	Glu	Tyr	Ben <sup>R</sup>
Limpopo	31-05-2021	5	Lemon	21-3642	Leaf	+	Resistant	+	Lys	Phe	Ben <sup>R</sup>
Limpopo	31-05-2021	5	Lemon	21-3643	Leaf	-	N/A	+	Ala	Phe	Ben <sup>R</sup>

<b>Limpopo</b>	31-05-2021	5	Lemon	21-3644	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
<b>Limpopo</b>	31-05-2021	5	Lemon	21-3645	Fruit	+	Resistant	+	Ala	Phe	Ben <sup>R</sup>
<b>Limpopo</b>	31-05-2021	5	Lemon	21-3646	Fruit	-	N/A	+	Ala	Phe	Ben <sup>R</sup>
<b>Limpopo</b>	31-05-2021	5	Lemon	21-3647	Fruit	-	N/A	+	Lys	Phe	Ben <sup>R</sup>
<b>Limpopo</b>	16-04-2021	6	Lemon	21-4272	Fruit	+	Sensitive	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	16-04-2021	6	Lemon	21-4273	Fruit	+	Sensitive	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	16-04-2021	6	Lemon	21-4274	Fruit	+	Sensitive	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	16-04-2021	6	Lemon	21-4275	Fruit	-	N/A	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	16-04-2021	6	Lemon	21-4276	Fruit	+	Sensitive	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	16-04-2021	6	Lemon	21-4277	Fruit	+	Sensitive	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	21-04-2021	7	Lemon	21-4826	Fruit	+	Sensitive	+	Glu	Phe	Ben <sup>S</sup>
<b>Mpumalanga</b>	21-04-2021	10	Sweet orange	21-4828	Fruit	+	Resistant	+	Glu	Tyr	Ben <sup>R</sup>
<b>Limpopo</b>	29-04-2021	8	Mandarin hybrid	21-5201	Fruit	-	N/A	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	29-04-2021	8	Mandarin hybrid	21-5202	Fruit	+	Sensitive	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	29-04-2021	8	Mandarin hybrid	21-5203	Fruit	+	Sensitive	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	30-04-2021	9	Lemon	21-5456	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
<b>Limpopo</b>	30-04-2021	9	Lemon	21-5457	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
<b>Limpopo</b>	05-05-2021	10	Grape fruit	21-5592-1	Fruit	+	Sensitive	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	05-05-2021	10	Grape fruit	21-5592-2	Fruit	-	N/A	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	06-05-2021	11	Lemon	21-5600-1	Fruit	+	Resistant	+	Lys	Phe	Ben <sup>R</sup>
<b>Limpopo</b>	06-05-2021	11	Lemon	21-5600-2	Fruit	+	Resistant	+	Lys	Phe	Ben <sup>R</sup>
<b>Limpopo</b>	12-05-2021	12	Mandarin hybrid	21-5785	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
<b>Mpumalanga</b>	12-05-2021	11	Sweet orange	21-5829	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
<b>Mpumalanga</b>	12-05-2021	11	Sweet orange	21-5831	Fruit	-	N/A	+	Lys	Phe	Ben <sup>R</sup>
<b>Mpumalanga</b>	12-05-2021	11	Sweet orange	21-5832	Fruit	-	N/A	+	Ala	Phe	Ben <sup>R</sup>
<b>Limpopo</b>	13-05-2021	13	Lemon	21-5833	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
<b>Limpopo</b>	13-05-2021	13	Lemon	21-5834	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
<b>Limpopo</b>	13-05-2021	13	Lemon	21-5835	Fruit	+	Sensitive	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	13-05-2021	14	Lemon	21-5842-1	Fruit	+	Sensitive	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	13-05-2021	14	Lemon	21-5842-2	Fruit	+	Sensitive	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	13-05-2021	14	Lemon	21-5843-1	Fruit	-	N/A	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	13-05-2021	14	Lemon	21-5843-2	Fruit	+	Sensitive	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	13-05-2021	14	Lemon	21-5844-1	Fruit	+	Sensitive	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	13-05-2021	14	Lemon	21-5844-2	Fruit	-	N/A	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	13-05-2021	14	Lemon	21-5845-1	Fruit	+	Resistant	+	Glu	Tyr	Ben <sup>R</sup>

<b>Limpopo</b>	13-05-2021	14	Lemon	21-5845-2	Fruit	-	N/A	+	Glu	Tyr	Ben <sup>R</sup>
<b>Limpopo</b>	13-05-2021	14	Lemon	21-5846-1	Fruit	-	N/A	+	Glu	Phe	Ben <sup>S</sup>
<b>Mpumalanga</b>	17-05-2021	12	Mandarin hybrid	21-5848-1	Fruit	+	Sensitive	+	Glu	Phe	Ben <sup>S</sup>
<b>Mpumalanga</b>	17-05-2021	12	Mandarin hybrid	21-5848-2	Fruit	+	Sensitive	+	Glu	Phe	Ben <sup>S</sup>
<b>Limpopo</b>	18-05-2021	15	Sweet orange	21-5852-2	Fruit	-	N/A	+	Glu	Phe	Ben <sup>S</sup>

<sup>a</sup>A “plus” symbol (+) means that the CBS lesions grew when plated on potato dextrose agar (PDA) and a “minus” symbol (-) means lesions did not grow when plated on PDA.

<sup>b</sup>The resistance phenotypes of CBS lesions that grew on PDA<sup>+</sup>, were determined based on mycelial growth at 10 ppm benomyl; N/A means that mycelial growth tests could not be performed because lesions did not grow when plated on PDA<sup>+</sup>.

<sup>c</sup>The “plus” symbol (+) means that DNA extracted directly from CBS lesions, without culturing, was successfully amplified by the primers CBS-BR\_F2/CBS-BR\_R1.

<sup>d</sup>Isolates have a sensitive type beta-tubulin gene (Ben<sup>S</sup>) or a beta-tubulin gene with codon substitutions that confer resistance to benomyl (Ben<sup>R</sup>).

#### 4.3.7 FINAL REPORT: Unravelling the clonal distribution of *Phyllosticta citricarpa* through a Genotyping-By-Sequencing approach

Project 1235 (2019 - 2022) by Aletta Bester-van der Merwe (US), Paul Fourie; Elma Carstens (CRI); Megan Dewdney (University of Florida, USA)

##### Summary

Knowledge of the population genetic structure of a given pathogen is important for a comprehensive understanding of disease epidemiology. Molecular markers identified from whole-genome sequence data are a valuable resource to determine the distribution of genetic variation and connectivity between isolates and populations, and can assist in the understanding of a pathogen's population dynamics. In this study, we used high throughput sequencing (HTS) to investigate the genetic diversity within and between *Phyllosticta citricarpa* populations, as well as to mine for additional SSR markers.

Seventeen USA isolates have been sequenced of which the genetic diversity has been assessed using *in silico* genotyping and variant calling. A total of 1,939 SSRs have been identified and *in silico* genotyped in the USA isolates. The results obtained confirm the clonal nature of the USA *P. citricarpa* isolates and *in silico* detection of mating types confirmed the presence of only one mating type in the USA isolates. A manuscript describing these results has been published (Coetzee *et al.* 2021): Extending the knowledge of *Phyllosticta citricarpa* population structure in USA with re-sequencing and genome wide analysis. *Physiological and Molecular Plant Pathology*, 113:101591.

Two more marker sets were developed using an isolate from China (1,969 SSRs) and from South Africa (1,987 SSRs). We sequenced and analysed (with the same approaches used for the USA isolates) HTS data from 71 *P. citricarpa* isolates from seven different countries to determine the genetic connectivity between these geographic locations. The three SSR marker sets gave similar results in terms of the genetic relationship/connectivity of the countries as previously published by Carstens *et al.* (2017). We found that isolates from China are most genetically distinct from the other countries, while there is some degree of genetic connectivity among all other countries. The five South African provinces where citrus black spot is present, also show a genetic overlap between isolates sequenced, corroborating the findings of Carstens *et al.* (2017). Unfortunately, the *P. citricarpa* DNA from Guarnaccia *et al.* (2017) (DNA from Italy, Malta and Greece) did not pass quality control and could not be sequenced and included in the analysis. Also no new *P. citricarpa* populations have been collected in South Africa or the USA to test with the newly developed markers.

Further work included the in-depth investigation of the genetic diversity of *P. citricarpa* populations in the Eastern Cape, to investigate the contribution of asexual reproduction in this province. We sequenced six isolates from five orchards each located in the Eastern Cape (Carstens, 2018). We found that the populations from different production areas clustered separately, while populations from the same production area cluster together. These findings correspond with the results of Carstens (2018).

Furthermore, the whole genome sequence data were used to confirm the mutations in the  $\beta$ -tubulin gene conferring benzimidazole resistance (Moyo *et al.*, 2021) in *P. citricarpa*. Highly specific PCR primers that that can be used for rapid detection of benzimidazole resistance in *P. citricarpa*, were designed.

##### Opsomming

Kennis van die genetiese struktuur van 'n patoogenpopulasie is belangrik vir 'n omvattende begrip van siekte-epidemiologie. Molekulêre merkers wat geïdentifiseer word uit heel-genoomvolgorde data is 'n waardevolle bron om die verspreidingspatrone van genetiese variasie en konnektiwiteit tussen isolate en populasies te bepaal, en kan help om die populasie dinamika van 'n patoogen te verstaan. In hierdie studie het ons hoë deurset volgordebepaling ("high throughput sequencing", HTS) gebruik om die genetiese diversiteit binne en tussen *Phyllosticta citricarpa* populasies te ondersoek, asook om vir addisionele "short sequence repeats" (SSR) merkers te myn.

Sewentien VSA-isolate se volgorde is bepaal en hul genetiese diversiteit deur middel van *in silico* genotipering en variant opsporing beoordeel. 'n Totaal van 1,939 SSRs is geïdentifiseer en *in silico* genotipeer in die VSA isolate. Die resultate wat verkry is, bevestig die klonaliteit van die VSA *P. citricarpa* isolate en *in silico* genotipering van paringstipes bevestig dat slegs een paringstipe in die VSA isolate voorkom. 'n Manuskrip wat hierdie resultate beskryf, is gepubliseer (Coetzee *et al.*, 2021): Extending the knowledge of *Phyllosticta citricarpa* population structure in USA with re-sequencing and genome wide analysis. *Physiological and Molecular Plant Pathology*, 113:101591.

Nog twee merkerstelle is ontwikkel deur 'n isolaat van China (1,969 SSRs) en Suid-Afrika (1,987 SSRs) te gebruik. Ons het HTS-data van 71 *P. citricarpa* isolate vanaf sewe verskillende lande verkry en geanaliseer (met die dieselfde metodes as voorheen vir die VSA isolate gebruik) om die genetiese konnektiwiteit tussen hierdie geografiese liggings te bepaal. Die drie SSR merkerstelle het soortgelyke resultate gelewer in terme van die genetiese verwantskap / konnektiwiteit van die lande, wat in ooreenstemming is met gepubliseerde resultate deur Carstens *et al.* (2017). Ons het gevind dat isolate van China geneties onderskei kan word van ander lande, terwyl daar 'n mate van genetiese konnektiwiteit tussen alle ander lande is, wat ook die bevindings van Carstens *et al.* (2017) ondersteun. Die vyf Suid-Afrikaanse provinsies waar sitrus swartvlek voorkom, toon ook 'n genetiese oorvleueling tussen die isolate wat bestudeer is. Resultate stem ooreen met Carstens *et al.* (2017). Ongelukkig het die *P. citricarpa* DNA van Guarnaccia *et al.* (2017) (DNA van Italië, Malta en Griekeland) nie die kwaliteitsbeheer geslaag nie en kon dus nie in die volgordebepaling en analise ingesluit word nie. Geen nuwe *P. citricarpa* populasies is in Suid-Afrika of die VSA versamel om met die nuwe merkers te toets nie.

Verdere werk het 'n diepgaande ondersoek behels van die genetiese diversiteit van *P. citricarpa* populasies in die Oos-Kaap, om die bydrae van ongeslagtelike voortplanting in hierdie provinsie te ondersoek. Ons het die volgordes van ses isolate van vyf boorde elk, wat in die Oos-Kaap geleë is (Carstens, 2018), bepaal. Ons het gevind dat die populasies uit verskillende produksiegebiede afsonderlik groepeer, maar dat populasies van dieselfde produksie gebiede wel saam groepeer. Hierdie bevinding stem ooreen met dié van Carstens *et al.* (2018).

Verder is die hele genomvolgordedata gebruik om die mutasies in die  $\beta$ -tubulieneen te bevestig wat bensimidiasoolweerstand (Moyo *et al.*, 2021) in *P. citricarpa* verleen. Hoogs spesifieke PCR-primers wat gebruik kan word vir vinnige opsporing van bensimidiasoolweerstand in *P. citricarpa*, is ontwerp.

## Introduction

The citrus black spot pathogen, *Phyllosticta citricarpa*, was first discovered in 1895 in Australia (Benson, 1895). *Phyllosticta citricarpa* produces two types of spores, namely waterborne conidia (pycnidiospores) and aerially dispersed ascospores. Symptoms can develop on leaves, twigs and fruit, but symptoms on fruit are the most obvious. *Phyllosticta citricarpa* can reproduce sexually and asexually (Wang *et al.*, 2016; Amorim *et al.*, 2016; Tran *et al.*, 2017). Waterborne, short-lived asexual pycnidiospores, are produced in pycnidia on fruit, leaves and twigs. Ascospores are sexually produced in pseudothecia that develop on leaf litter, but never on fruit. The primary inoculum source for spreading the disease in the orchards is the airborne ascospores that are produced on the fallen leaves (leaf litter) and the critical period for potential fruit infection starts at fruit set with the young fruitlet remaining susceptible for 4 to 5 months after fruit set. Leaves are only susceptible for up to 10 months in age (Kiely, 1948; McOnie, 1965; Kotzé, 1981; Spósito *et al.*, 2007; Truter, 2010; Fourie *et al.*, 2013).

Fifteen simple sequence repeat (SSR) markers were developed and populations from South Africa, Brazil, Australia, USA, and China were genotyped. High levels of connectivity were present between populations from South Africa, Australia, USA and Brazil, which is an indication of the dispersal of a genotype across broad geographic distances. The USA population was found to be clonal, i.e. only asexual reproduction occurs (Carstens *et al.*, 2017; Wang *et al.*, 2016). Putative clonal survival of *P. citricarpa* has also been reported in Portugal, Malta and Italy in Europe by Guarnaccia *et al.* (2017) with high levels of connectivity present between populations from Australia and Portugal. Spatial and temporal analysis of *P. citricarpa* populations in lemon orchards over two seasons in South Africa also indicated that clonal reproduction is important (low genotype

evenness values and dominant multilocus genotypes) (Carstens, 2018). This is in contrast with previous studies conducted in South Africa and Australia, which indicated that pycnidiospores have a relatively minor role in the epidemiology of CBS (Kiely, 1948; McOnie, 1965; Kotzé, 1981). The studies conducted in Australia and South Africa by Kiely (1948) and Kotzé (1981), respectively, concluded that the sexual spores (ascospores) are the main propagule causing infection. However, studies conducted in Brazil concluded that the asexual spores also play an important role in the epidemiology of the pathogen, specifically under their high-rainfall conditions (Spósito *et al.*, 2007; Spósito *et al.*, 2008). More recent studies in Australia (Tran *et al.* 2020) also suggested that inoculum in tree canopies (asexual spores) might contribute to fruit infection as neither the trapped ascospore numbers nor pycnidiospore numbers from leaf litter correlated with fruit infection data. In Florida, pycnidiospores are the only source of fruit infection since only one mating type occurs, precluding sexual reproduction (Wang *et al.*, 2016).

High throughput sequencing and assembly of whole genomes allow for the detection of variation between individuals, marker discovery and *in silico* genotyping. In this project, we performed whole genome sequencing of previously identified isolates (Carstens, 2018; Guarnaccia *et al.*, 2017) and did *in silico* genotyping with the 15 previously developed SSR markers, and developed additional genetic markers to detect genome wide variation with-in and between populations. This will aid in a more comprehensive understanding of the genetic diversity and/or population structure and provide a clearer picture of the genetic composition and connectivity of populations. This information will also allow for a better understanding of the role of pycnidiospores in the epidemiology of the citrus black spot pathogen, the long-distance clonal dispersal of the pathogen and will refine control measures and management programmes for the disease.

Futhermore, the whole genome sequencing data generated in this project was used to study the mutations that give rise to *P. citricarpa* resistance against fungicides. The occurrence of citrus black spot in orchards is mainly controlled by the application of fungicides during the fruit susceptibility period (from October to March in South Africa). Various contact and systemic fungicides are registered for use in South Africa. Systemic fungicides can be absorbed by the plant and then translocated within the plant system. Strobilurins (azoxystrobin, trifloxystrobin and pyraclostrobin) and benzimidazoles (benomyl and carbendazim) are the main classes of systemic fungicides available for the control of citrus black spot (Van Niekerk *et al.*, 2019). Benzimidazole fungicides are widely used in South Africa. Resistance to benomyl was first reported in 1982 in Mpumalanga by Herbert and Grech (1985). Benzimidazole resistance still occurs and over application or misuse can easily result in the pathogen developing resistance to it. Mutations in the B-tubulin gene of fungal pathogens renders them resistance to fungicides (Cai *et al.*, 2015; Koenraad *et al.*, 1992). The HTS data of susceptible and resistant isolates were used to detect the variation in the *P. citricarpa* B-tubulin gene and used to develop primers for easy and fast resistance detection.

### **Stated objectives**

1. Whole genome sequencing and analysis of 20 isolates of the clonal USA populations.
2. Mining and development of additional genetic markers from HTS data.
- 3a. Whole genome sequencing and genotyping of 20 isolates of the clones identified by Carstens *et al.* (2018), Guarnaccia *et al.* (2017) and other countries with the additional genetic markers. The 20 isolates of USA will be used as controls.
- 3b. Whole genome sequencing and genotyping of isolates from Carstens *et al.* (2018), Guarnaccia *et al.*, (2017) with the newly developed genetic markers.
4. Whole genome sequencing and genotyping of isolates from new populations (South Africa, USA) with the newly developed genetic markers.
5. Whole genome sequencing and genotyping of 30 isolates from five *P. citricarpa* populations in the Eastern Cape (Carstens, 2018) with the newly developed markers to investigate the contribution of asexual reproduction in this province.
6. Developing of primers for the *P. citricarpa* B-tubulin gene and screening of isolates.

### **Materials and methods**

#### **Whole genome sequencing and analysis of 20 isolates of the clonal USA populations.**

Whole genome sequencing was performed for 17 USA isolates, using the Ion Torrent sequencing platform. The HTS read data for the 17 isolates were assembled using SPAdes v.3.13.0 (Bankevich *et al.*, 2012), including four reference sequences to guide the assembly (with the SPAdes “untrusted contigs” parameter).

The HTS reads from each of the 17 isolates were mapped to the assembled contig sets of each of the isolates, using Burrows Wheeler Aligner (BWA; Li and Durbin, 2010). Variants were called with Genome Analysis Toolkit (GATK, Broad Institute, <https://software.broadinstitute.org/gatk/>) HaplotypeCaller module, with a ploidy setting of 1. Variants were filtered using the VariantFiltration module (with a window size of 35 and cluster size of 3 to detect SNP clusters, minimum read depth of 5 (DP<5) and quality by depth of 5 (QD<5). The SNPs were selected using the GATK SelectVariant module (--select-type-to-include SNP).

The number of variants that pass the filtration were counted and normalized for number of reads in each library and length of assembly it is mapped against (number of variants/1,000,000 reads in library /1 Mb genome assembly length).

The whole genome sequencing data were mined for additional markers using a customised bioinformatics pipeline, to identify SSRs, design primers and perform *in silico* genotyping. The assembled genome with the best assembly statistic (USA122), were selected for SSR detection using the MlcroSAteLLite identification tool (MISA) (Thiel *et al.*, 2003). The command-line version of Primer3 (Untergasser *et al.*, 2012) was used to design primers for PCR amplification of the mined SSRs. This marker set were used to generate genotypes (length of amplified products) with *in silico* PCR available in iPCReSS 2.2.0 (part of the Exonorate package) (Slater and Birney, 2005) for all sequenced isolates.

Primer sets to amplify the two mating type loci (MAT1-1-1 and MAT1-2-1) from *P. citricarpa* were previously developed (Wang *et al.*, 2016) and used for *in silico* PCR amplification to determine the mating type of the sequenced isolates.

### **Develop additional genetic markers through the mining of the data generated.**

The whole genome sequencing data of two isolates, one from South Africa and one from China were mined for additional markers using the customised bioinformatics pipeline previously designed (objective 1). The primers previously designed on USA122 (objective 1) were also included. These three marker sets were used to generate genotypes for all sequenced isolates. Genotype accumulation curves were drawn for the genotypes generated with the three marker sets using the R package *poppr* version 2.9.2 (Kamvar *et al.*, 2014; Kamvar *et al.*, 2015) to determine the minimum number of loci necessary to discriminate between isolates. Genotype data were imported into R and a principal coordinate analysis (PCoA) was performed using the *ade4* package version 2.3.1 (Jombart, 2008) in R version 4.0.5 (R Core Team, 2021).

### **Whole genome sequencing and genotyping of isolates identified by Carstens (2018) and Guarnaccia *et al.*, (2017).**

Fifty-eight *P. citricarpa* isolates from Carstens *et al.* (2018), including isolates from the five citrus production regions in South Africa where CBS is present, and from, Australia, Brazil, China, and the United States of America, were selected for sequencing. Additionally, 13 isolates from three other countries (Argentina, Cuba and eSwatini) were also included. Unfortunately, the DNA from Guarnaccia *et al.* (2017) did not pass quality control and could not be sequenced.

Genotypes were generated for all isolates based on the SSR marker set developed on the South African isolate (objective 2). Additionally, SNPs were detected relative to a reference sequence. HTS reads from each of the isolates were mapped against the reference genome *P. citricarpa* CBS127454 (Guarnaccia *et al.*, 2019) obtained from the USA Department of Energy Joint Genome Institute (<https://mycocosm.jgi.doe.gov/Phycitr1/Phycitr1.home.html>). Mapping was performed using BWA (Li and Durbin, 2010) and variant calling using Genome Analysis Toolkit (GATK, Broad Institute, <https://software.broadinstitute.org/gatk/>).

Descriptive population statistics, namely the number of alleles, Nei's genetic diversity (Nei, 1973), and evenness (Grünwald et al., 2003; Ludwig and Reynolds, 1988; Pielou, 1975) were calculated for both SSR and the SNP datasets, using *poppr*. Genodive version 3.05 (Meirmans, 2020) was used to assess the distribution of intra- and inter- population variation with an analysis of molecular variance (AMOVA) (Excoffier et al., 1992) and pairwise genetic differentiation (PhiPT, analogue of  $F_{ST}$  (Wright, 1951)) between populations calculated with 999 permutations.

Principal component analysis (PCA) was performed, for both SSR genotype and the SNP datasets, using *ade4* package version 2.3.1 (Jombart, 2008) in R version 4.0.5 (R Core Team, 2021). To improve the distribution of populations' resolution from other areas, the PCA was also performed excluding the Chinese isolates. Nei's pairwise population identity index (Nei, 1978) was calculated in Genodive for the 12 populations, and a dendrogram constructed in R using the package *heatmap* (Kolde, 2019) to illustrate population kinship. Only one Cuban isolate was included in this study, therefore its SSR genotype and SNP score data were duplicated for inclusion in the calculations.

The pairwise read mapping approach for the detection and enumeration of variants between isolates were also performed as described in objective 1.

**Whole genome sequencing and genotyping of isolates from new populations (South Africa and USA) with the newly developed genetic markers.**

This objective could not be completed as no new populations were collected from South Africa or the USA.

**Whole genome sequencing and genotyping of 30 isolates from five *P. citricarpa* populations in the Eastern Cape (Carstens, 2018) with the newly developed markers to investigate the contribution of asexual reproduction in this province.**

Objective 4 has been amended to include an in-depth study of the genetic diversity of *P. citricarpa* populations in the Eastern Cape. We sequenced six isolates from four orchards each, located in the Sunday's River Valley and Gamtoos River Valley, Eastern Cape, South Africa (Carstens, 2018). The HTS data from these isolates were assembled as described in objective 1, and the marker set developed on the South African isolate (objective 2) were used for *in silico* genotyping of these isolates, together with those from the rest of South Africa. This includes six isolates from Patensie (Gamtoos River Valley) in the Eastern Cape, already included in objective 3. Principal component analysis (PCA) was performed as described in objective 3.

**Develop primers for the *P. citricarpa*  $\beta$ -tubulin gene and screen isolates**

Thirteen *P. citricarpa* isolates from five farms in Mpumalanga and North West, South Africa, were randomly selected for inclusion in this study. Two to three fruit samples were collected per farm. Their sensitivity to benzimidazole was tested using a standard fungicide resistance analysis. DNA was extracted from these isolates and submitted for HTS. The HTS data were assembled and the  $\beta$ -tubulin gene sequence retrieved from the assemblies. The  $\beta$ -tubulin gene sequences were aligned and inspected for mutations. PCR primers were developed for a part of the B-tubulin gene of *P. citricarpa* containing the mutations. The developed PCR assays were tested in 12 field isolates with known resistant status.

**Results**

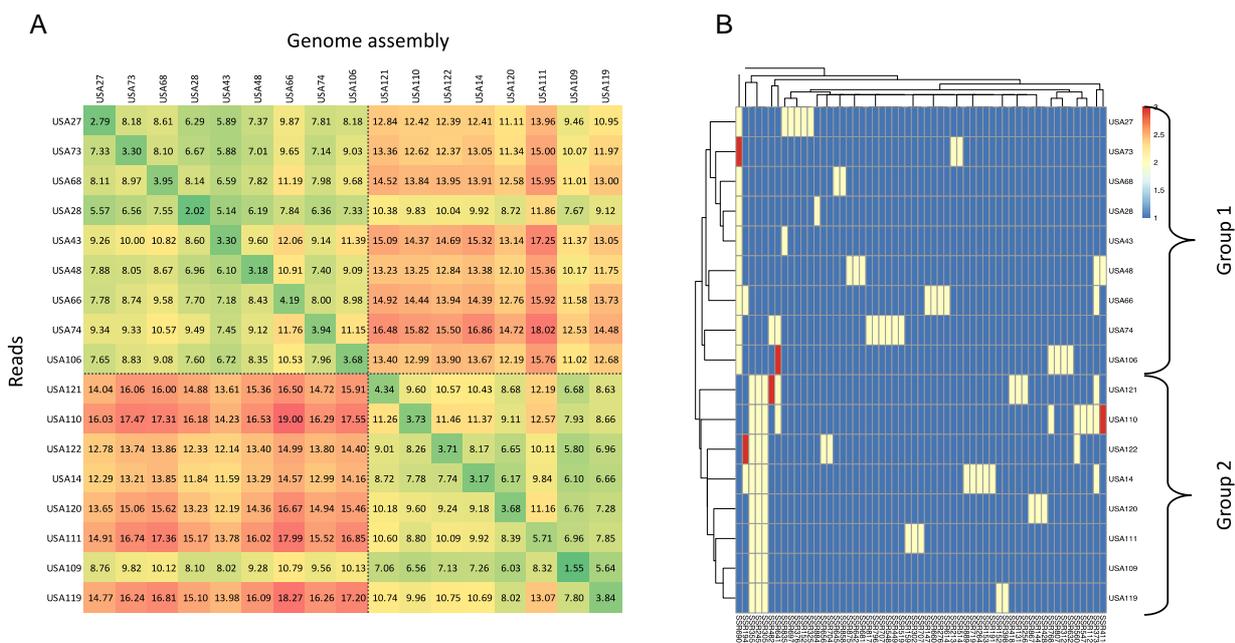
Task table

Objective/milestone	Achievement
<b>Whole genome sequencing and analysis of 20 isolates of the clonal USA populations.</b>	Seventeen USA isolates have been sequenced and their genetic diversity assessed. The USA population was found to be clonal, i.e. only asexual

	reproduction occurs and only one mating type is present. A manuscript describing the results has been published: Coetzee B, Carstens E, Dewdney M, Fourie PH, Bester-van der Merwe AE (2021) Extending the knowledge of <i>Phyllosticta citricarpa</i> population structure in USA with re-sequencing and genome wide analysis: <i>Physiological and Molecular Plant Pathology</i> , 113:101591
<b>Develop additional genetic markers through the mining of the data generated.</b>	This objective has been completed: 1,939 SSRs have been identified and <i>in silico</i> genotyped in the USA and South African isolates. Two more marker sets, using isolates from South Africa (1,987 markers) and China (1,969 markers) have been developed, and genotyped in all isolates.
<b>Whole genome sequencing and genotyping of isolates identified by Carstens et al. (2018) and Guarnaccia et al. (2017).</b>	Fifty-eight <i>P. citricarpa</i> isolates from Carstens <i>et al.</i> (2018) and 13 isolates from three other countries (Argentina, Cuba and eSwatini) have been sequenced and analysed ( <i>in silico</i> genotyping, SNP detection and inter-isolate variant calling). The three approaches in analysing the HTS data gave the same overall population clustering results. Unfortunately, the DNA from Guarnaccia <i>et al.</i> (2017) did not pass quality control and could not be sequenced.
<b>Whole genome sequencing and genotyping of isolates from new populations (South Africa and USA) with the newly developed genetic markers.</b>	This objective will not be completed as no new populations have been collected in South Africa or the USA.
<b>Whole genome sequencing and genotyping of 30 isolates from five <i>P. citricarpa</i> populations in the Eastern Cape (Carstens, 2018) with the newly developed markers to investigate the clonality and contribution of asexual reproduction in this province.</b>	Thirty isolates from Carstens <i>et al.</i> (2018) have been sequenced and analysed ( <i>in silico</i> genotyping and variant calling).
<b>Develop primers for the <i>P. citricarpa</i> <math>\beta</math>-tubulin gene and screen isolates for a genome wide association study in order to link variation to benzimidazole resistance.</b>	Whole genome sequencing and assembly for 13 isolates revealed mutations in the $\beta$ -tubulin gene linked to benzimidazole resistance. Primers assays were designed to detect these mutations.

### **Objective 1: Whole genome sequencing and analysis of 20 isolates of the clonal USA populations.**

Seventeen isolates from the United States of America were sequenced and their genomes assembled. A pairwise mapping and variant calling analysis were performed on all isolates, and a low number of inter-isolate variants detected (Figure 4.3.7.1 A). Furthermore, the HTS data was also used to genotype 1,939 SSRs in the isolates, and only 57 polymorphic markers were found (Figure 4.3.7.1 B). Using the primer set for the MAT1-2-1 region, all 17 isolates amplified a single PCR product, indicating they are all mating type MAT1-2-1. This is consistent with the findings of Carstens *et al.* (2017), Hendricks *et al.* (2017), and Wang *et al.* (2016), suggesting that *P. citricarpa* in Florida, USA reproduce asexually, and that spread of the fungus is most likely only by means of conidia. However, it is not a totally homogeneous group, and it was shown that HTS can detect this fine-scale genetic differentiation. The two different approaches, pairwise mapping and variant calling versus SSR detection and *in silico* genotyping, yielded similar results, dividing the isolates into the same two groups.



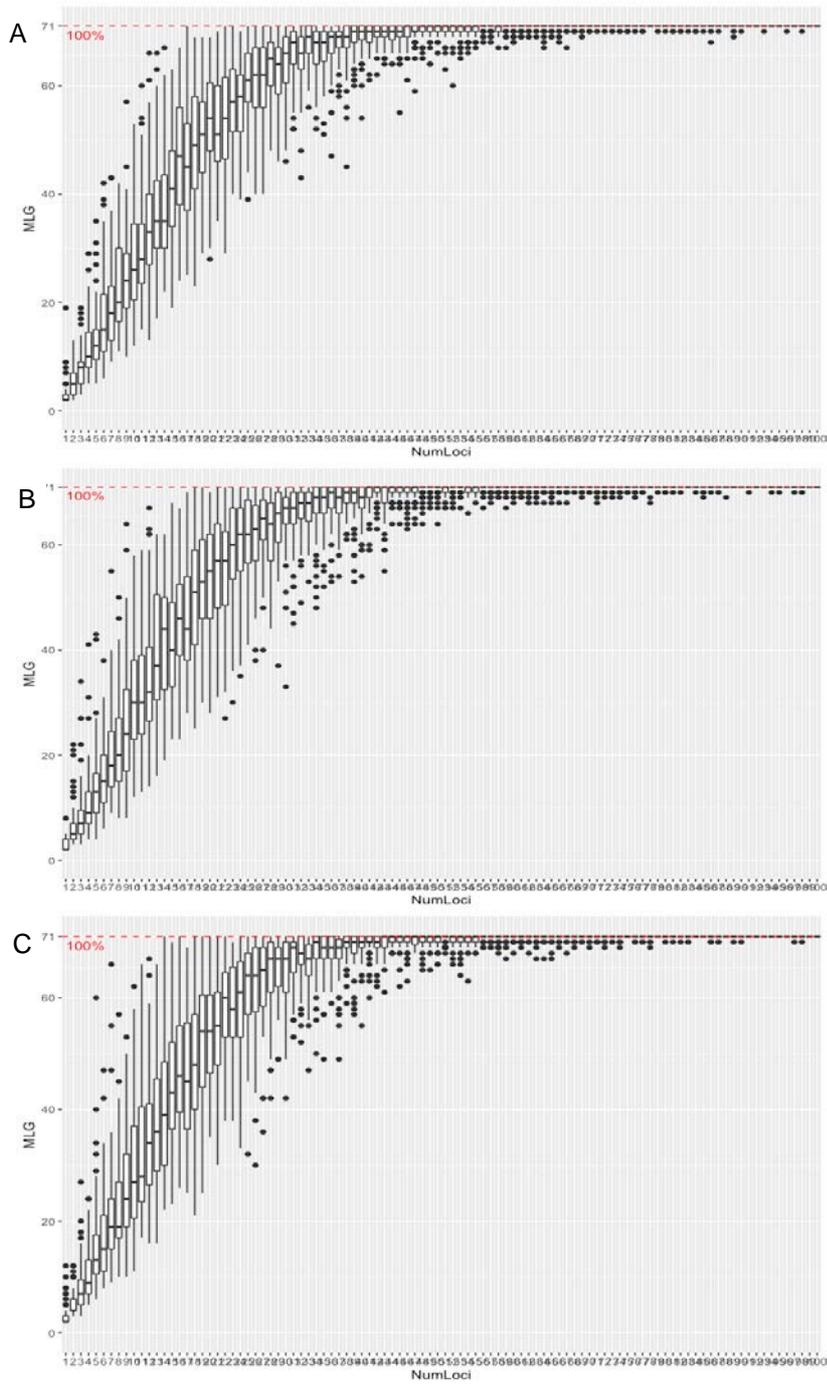
**Figure 4.3.7.1.** Visualization of the read mapping/ variant calling and genotyping data, showing the clustering of the USA *Phyllosticta citricarpa* isolates into two groups. **A:** Pairwise diversity (expressed as number of variants/1,000,000 reads in library /1 Mb genome assembly length) for each isolate's reads mapped to each of the assembled genomes. The colour intensity is proportional to the number of variants detected, green being lower numbers and red higher numbers. **B:** Heatmap of the isolate's genotypes for the 57 polymorphic SSRs. Blue indicates the dominant allele, light yellow indicates the minor allele, and red the third allele. Isolate order is based on hierarchical clustering in the R package *pheatmap*.

**Objective 2: Develop additional genetic markers through the mining of the data generated.**

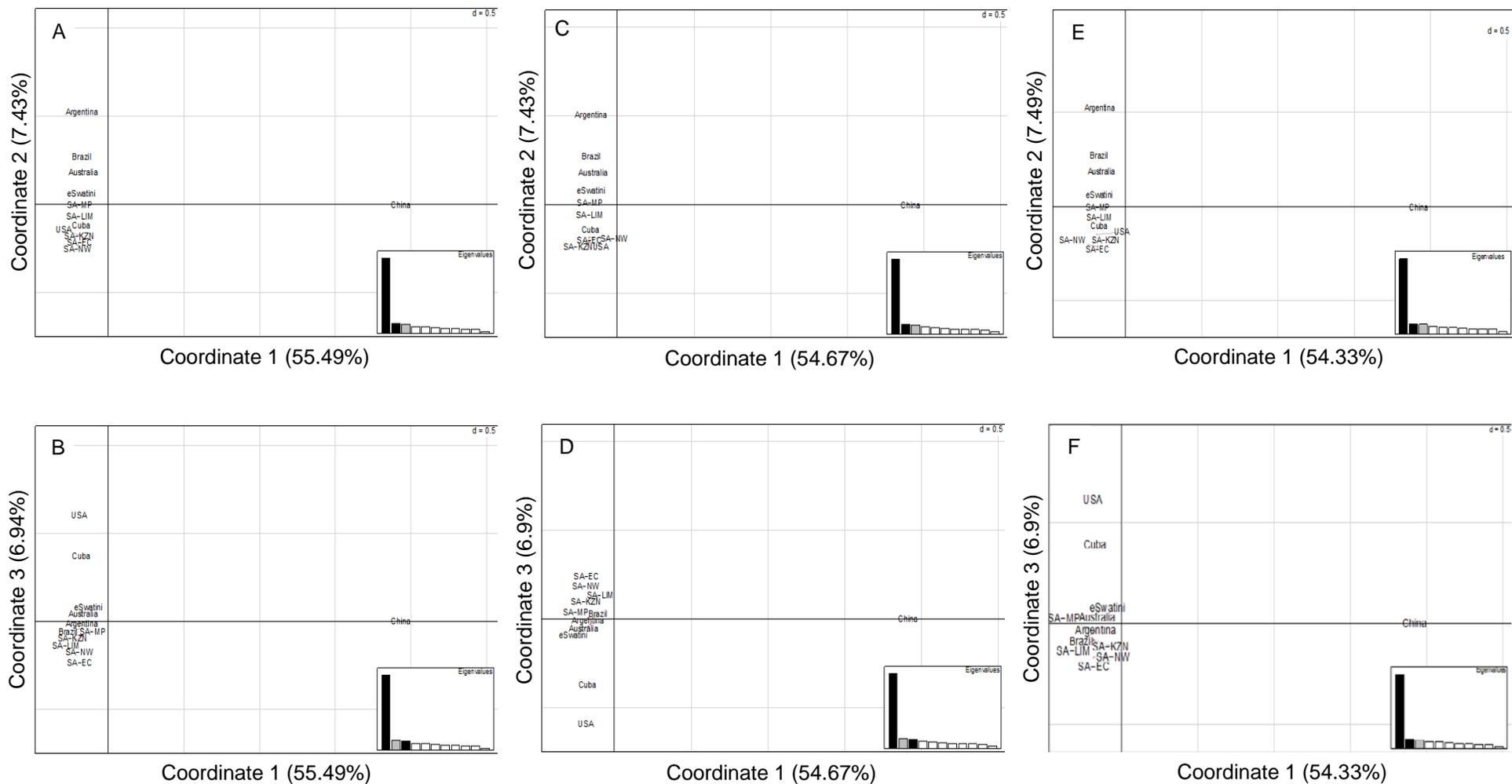
Genotyping resulted in three different SSR genotype datasets, consisting of 1,939 (USA), 1,969 (China) and 1,987 (South Africa) informative markers, respectively (Table 4.3.7.1). The genotype accumulation curves indicated that for all three marker sets, a plateau is reached at approximately 30 markers (Figure 4.3.7.2), indicating that all 71 isolates can be individually distinguished with the more than 1,900 SSR markers in each of the marker sets. Population clustering analysis based on the three marker sets showed similar patterns of genetic relationship between populations (Figure 4.3.7.3) demonstrating that the isolate chosen for marker development will not influence the outcome of the population connectivity analysis.

**Table 4.3.7.1.** Statistics of SSRs detected and primers designed on the representative isolates from three geographic origins, United States of America, China and South Africa. SSR detection and primer design for USA122 were previously described. Number of primer sets producing informative genotypes in the 71 isolates, are also indicated.

	USA122	CH246	SA-LIM3
SSRs detected	9,343	8,719	8,956
SSRs remaining after discarding duplicates, mononucleotide and compound SSRs	5,828	5,677	5,720
Primer sets designed	5,740	5,635	5,689
Primer sets remaining after discarding those with missing genotype information in one or more isolates	4,155	4,190	4,229
Primer sets remaining after discarding uninformative (yielding same genotype for all isolates) SSRs	1,939	1,969	1,987



**Figure 4.3.7.2.** Genotype accumulation curve, showing 100 randomly sampled loci. A: based on genotyping with 1,939 markers designed on the isolate from the USA. B: based on genotyping with 1,969 markers designed on the Chinese isolate. C: based on genotyping with 1,987 markers designed on the South African isolates.



**Figure 4.3.7.3.** Principal coordinate analysis (PCoA) of the 71 isolates. The inset is a scree plot of the eigenvalues for each PCoA. **A:** PCoA based on genotyping with 1,939 markers designed on the isolate from the USA, coordinates 1 and 2. **B:** PCoA based on genotyping with 1,939 markers designed on the isolate from the USA, coordinates 1 and 3. **C:** PCoA based on genotyping with 1,969 markers designed on the Chinese isolate, coordinates 1 and 2. **D:** PCoA based on genotyping with 1,969 markers designed on the Chinese isolate, coordinates 1 and 3. **E:** PCoA based on genotyping with 1,987 markers designed on the South African isolate, coordinates 1 and 2. **F:** PCoA based on genotyping with 1,987 markers designed on the South African isolate, coordinates 1 and 3. EC: Eastern Cape; KZN: KwaZulu-Natal; LIM: Limpopo; MP: Mpumalanga; NW: Northwest, South Africa.

**Objective 3: Whole genome sequencing and genotyping of isolates from Carstens *et al.* (2018), Guarnaccia *et al.*, (2017) with the newly developed genetic markers.**

The draft genomes of the 71 isolates were assembled using the HTS data. The SSR marker set was used to generate *in silico* genotypes for all isolates. A total of 1,987 informative markers were genotyped in all isolates. More than 32,000 SNPs were also identified relative to a reference genome.

Population statistics were calculated for both the SSR genotype and the SNP score datasets (Table 4.3.7.2). Statistics calculated based on the SSR genotype dataset are denoted with superscript a (<sup>a</sup>) and those calculated on the SNP dataset with superscript b (<sup>b</sup>). Pairwise PhiPT values are shown in Table 4.3.7.2.

Principal component analysis (PCA) (Figure 4.3.7.4) and Nei's pairwise population identity index (Figure 4.3.7.5) based on SSR genotypes and SNP datasets, and the hierarchical clustering based on number of inter-isolate variants (Figure 4.3.7.3-3), gave the same overall results. The Chinese population is the most diverse, and is genetically the furthest removed from all other populations, and is therefore the closest to the origin of the pathogen. Isolates from Australia, eSwatini and the South African province Mpumalanga are closely associated and form a cluster together with those from Argentina and Brazil. The Eastern Cape, North West, and KwaZulu-Natal populations in South Africa form another cluster, while isolates from Limpopo are distributed between the two above-mentioned clusters. Southern African populations are also related to populations in North America, and are a possible source of *P. citricarpa* populations that are now found in North America.

**Table 4.3.7.2.** Population genetic statistics for the 11\* populations across 1,987 SSR markers<sup>a</sup> and 32,560 SNPs<sup>b</sup> evaluated.

Population	Isolates	Across 1 987 SSR markers			Across 32 560 SNPs		
		N <sub>a</sub> <sup>a</sup>	H <sub>e</sub> <sup>a</sup>	E5 <sup>a</sup>	N <sub>a</sub> <sup>b</sup>	H <sub>e</sub> <sup>b</sup>	E5 <sup>b</sup>
South Africa Eastern Cape	6	1.391	0.139	0.766	1.088	0.037	0.781
South Africa KwaZulu-Natal	7	1.505	0.175	0.763	1.190	0.076	0.769
South Africa Limpopo	7	1.391	0.138	0.762	1.158	0.067	0.802
South Africa Mpumalanga	7	1.374	0.129	0.756	1.121	0.049	0.771
South Africa North West	8	1.477	0.155	0.760	1.149	0.062	0.800
South Africa (all provinces)	35	2.043	0.164	0.592	1.305	0.080	0.665
Argentina	6	1.442	0.162	0.769	1.165	0.070	0.782
Australia	5	1.335	0.142	0.812	1.189	0.091	0.818
Brazil	6	1.404	0.142	0.756	1.134	0.056	0.777
China	6	1.554	0.211	0.800	1.361	0.165	0.819
eSwatini	6	1.376	0.142	0.778	1.175	0.074	0.778
United States of America	6	1.319	0.117	0.775	1.012	0.005	0.775
<b>Mean</b>	<b>6</b>	<b>1.381</b>	<b>0.138</b>	<b>0.772</b>	<b>1.171</b>	<b>0.069</b>	<b>0.778</b>

N<sub>a</sub>: Number of alleles; H<sub>e</sub>: Nei's diversity; E5: Evenness.

\* Only one isolate from Cuba was included in this study, and therefore it was not included in this analysis.

<sup>a</sup> Used to denote statistics calculated for the SSR markers.

<sup>b</sup> Used to denote statistics calculated for the SNPs.

**Table 4.3.7.3 A.** Pairwise PhiPT<sup>a</sup> values for 12 populations (5 South African provinces and 7 other countries) genotyped with 1,987 SSR markers. Significance (p values) are indicated in brackets.

Population*	SA-EC	SA-KZN	SA-LIM	SA-MP	SA-NW	Argentina	Australia	Brazil	China	eSwatini
SA-KZN	0.076 (0.003)									
SA-LIM	0.117 (0.010)	0.067 (0.004)								
SA-MP	0.234 (0.001)	0.150 (0.001)	0.137 (0.003)							
SA-NW	0.067 (0.003)	0.052 (0.003)	0.093 (0.002)	0.183 (0.001)						
Argentina	0.219 (0.003)	0.149 (0.008)	0.170 (0.002)	0.199 (0.002)	0.196 (0.002)					
Australia	0.211 (0.004)	0.134 (0.001)	0.129 (0.001)	0.155 (0.002)	0.176 (0.001)	0.108 (0.019)				
Brazil	0.216 (0.002)	0.144 (0.002)	0.157 (0.001)	0.194 (0.001)	0.181 (0.001)	0.102 (0.047)	0.126 (0.006)			
China	0.630 (0.004)	0.606 (0.003)	0.634 (0.002)	0.646 (0.002)	0.626 (0.001)	0.611 (0.002)	0.612 (0.002)	0.626 (0.002)		
eSwatini	0.195 (0.006)	0.126 (0.003)	0.099 (0.003)	0.118 (0.001)	0.160 (0.001)	0.131 (0.004)	0.080 (0.005)	0.152 (0.006)	0.622 (0.002)	
USA	0.322 (0.002)	0.223 (0.001)	0.281 (0.002)	0.317 (0.001)	0.287 (0.001)	0.292 (0.002)	0.274 (0.003)	0.294 (0.001)	0.661 (0.004)	0.247 (0.002)

\*EC: Eastern Cape; KZN: KwaZulu-Natal; LIM: Limpopo; MP: Mpumalanga; NW: North West; SA: South Africa.

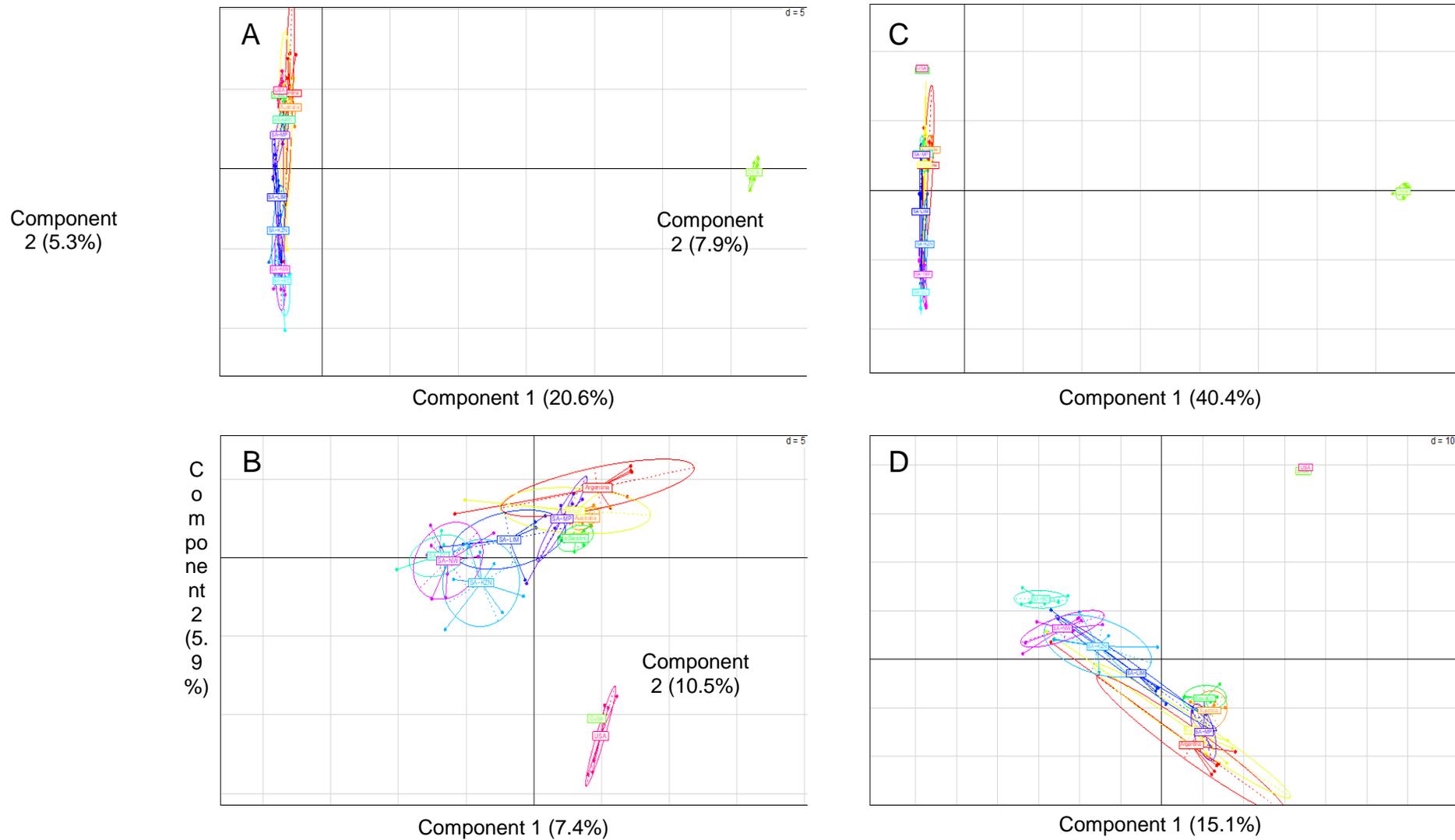
<sup>a</sup> Used to denote statistics calculated for the SSR markers.

**Table 4.3.7.3 B.** Pairwise PhiPT<sup>b</sup> values for 12 populations (5 South African provinces and 7 other countries) genotyped with 32,560 SNP markers. Significance (p values) are indicated in brackets.

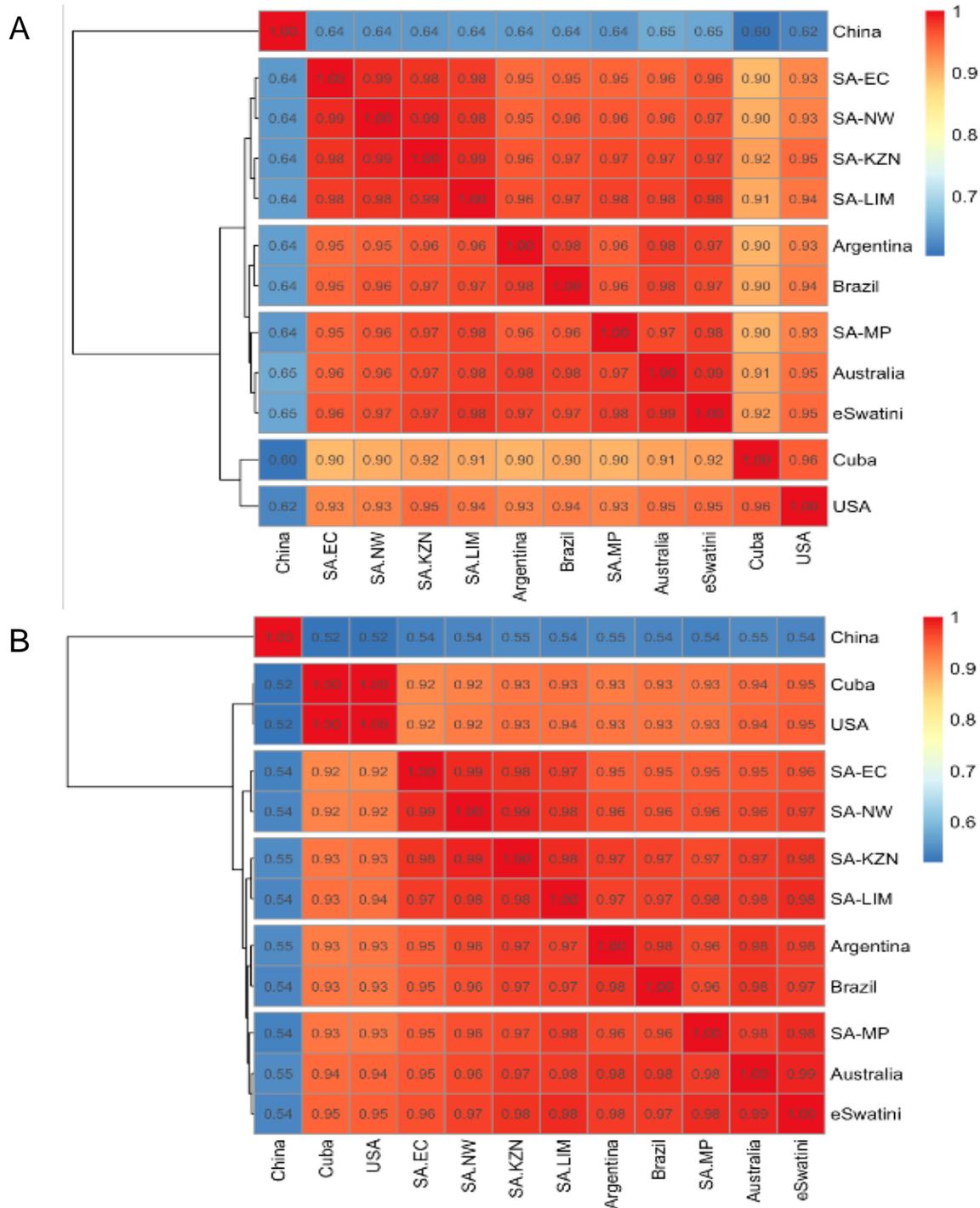
Population*	SA-EC	SA-KZN	SA-LIM	SA-MP	SA-NW	Argentina	Australia	Brazil	China	eSwatini
SA-KZN	0.236 (0.002)									
SA-LIM	0.316 (0.003)	0.164 (0.011)								
SA-MP	0.535 (0.001)	0.336 (0.003)	0.285 (0.001)							
SA-NW	0.208 (0.001)	0.161 (0.001)	0.247 (0.001)	0.416 (0.002)						
Argentina	0.460 (0.002)	0.264 (0.006)	0.286 (0.003)	0.371 (0.002)	0.349 (0.002)					
Australia	0.430 (0.003)	0.238 (0.002)	0.236 (0.002)	0.273 (0.006)	0.322 (0.002)	0.221 (0.023)				
Brazil	0.496 (0.005)	0.293 (0.005)	0.322 (0.004)	0.409 (0.001)	0.381 (0.001)	0.226 (0.059)	0.233 (0.002)			
China	0.805 (0.002)	0.774 (0.001)	0.784 (0.002)	0.803 (0.003)	0.796 (0.001)	0.773 (0.002)	0.748 (0.002)	0.787 (0.001)		
eSwatini	0.420 (0.005)	0.214 (0.002)	0.190 (0.001)	0.208 (0.005)	0.316 (0.001)	0.240 (0.002)	0.125 (0.004)	0.267 (0.002)	0.771 (0.003)	
USA	0.792 (0.005)	0.588 (0.001)	0.610 (0.001)	0.696 (0.001)	0.653 (0.002)	0.643 (0.002)	0.568 (0.002)	0.687 (0.002)	0.838 (0.002)	0.563 (0.007)

\*EC: Eastern Cape; KZN: KwaZulu-Natal; LIM: Limpopo; MP: Mpumalanga; NW: North West; SA: South Africa.

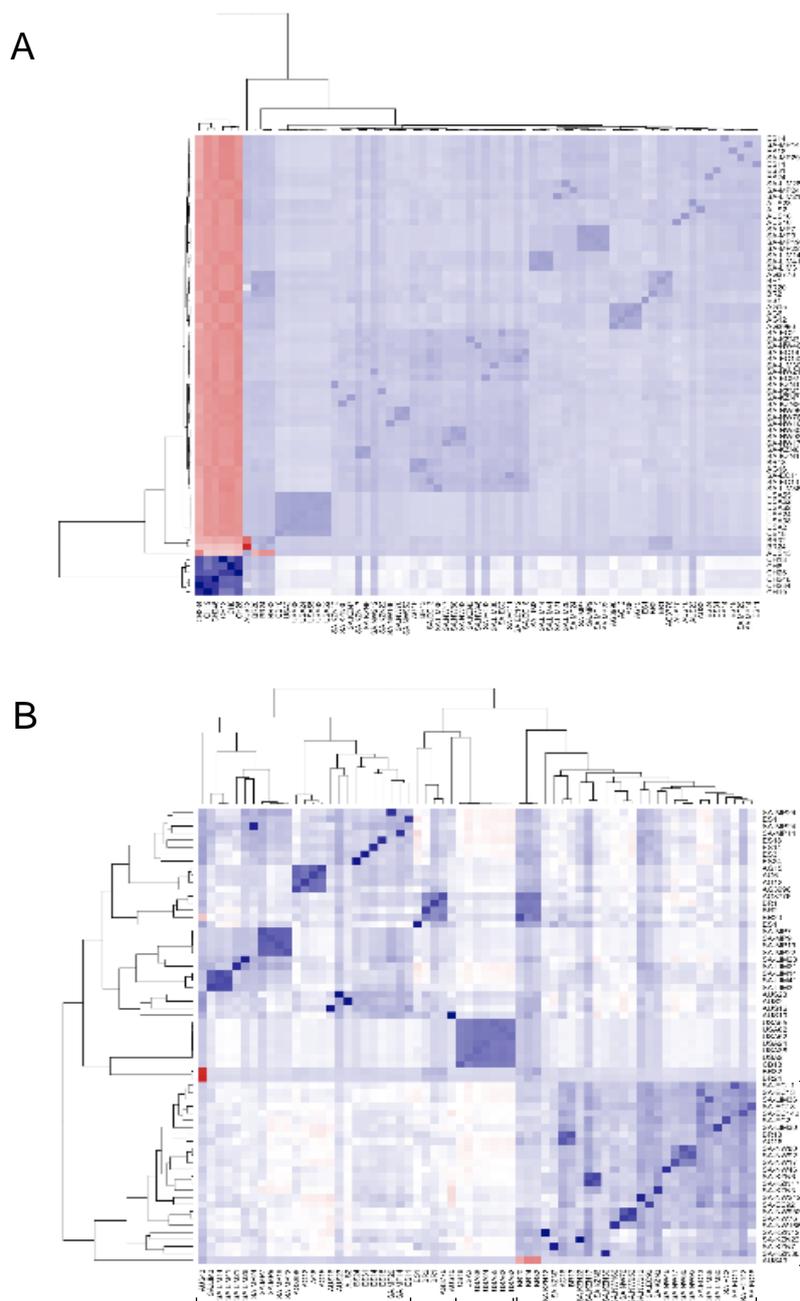
<sup>b</sup> Used to denote statistics calculated for the SNPs



**Figure 4.3.7.4.** Principal component analysis **A:** Based on genotyping with 1,987 informative markers designed on an isolate from South Africa (SA-LIM3), all isolates and **B:** Excluding the Chinese isolates (for ease of viewing). **C:** Based on 32,560 SNP scores, all isolates and **D:** Excluding the Chinese isolates (for ease of viewing). EC: Eastern Cape; KZN: KwaZulu-Natal; LIM: Limpopo; MP: Mpumalanga; NW: North West, SA: South Africa.



**Figure 4.3.7.5.** Pairwise population matrix of Nei's genetic identity index ( $I$ ). A heatmap and dendrogram created in R using the package *pheatmap*, are shown to visually represent the identity values. Blue indicates more dissimilarity, while red indicates more genetic similarity. A: Based on genotyping with 1,987 SSRs B: Based on 32,560 SNP scores. EC: Eastern Cape; KZN: KwaZulu-Natal; LIM: Limpopo; MP: Mpumalanga; NW: North West; SA: South Africa; USA: United States of America.  $I^a$  is used to denote identity values based on SSRs in text, and  $I^b$  values calculated based on SNPs.



**Figure 4.3.7.6.** Hierarchical clustering based on number of variants for each isolate to illustrate individual relationships. A: All isolates B: Excluding the Chinese isolates, for ease of viewing. EC: Eastern Cape; KZN: KwaZulu-Natal; LIM: Limpopo; MP: Mpumalanga; NW: North West; AG: Argentina; AU: Australia; BR: Brazil; CH: China; CB: Cuba; ES: eSwatini; USA: United States of America. The blue/red colour scale is indicative of similarity, blue indicates more similarity, red indicates more dissimilarity.

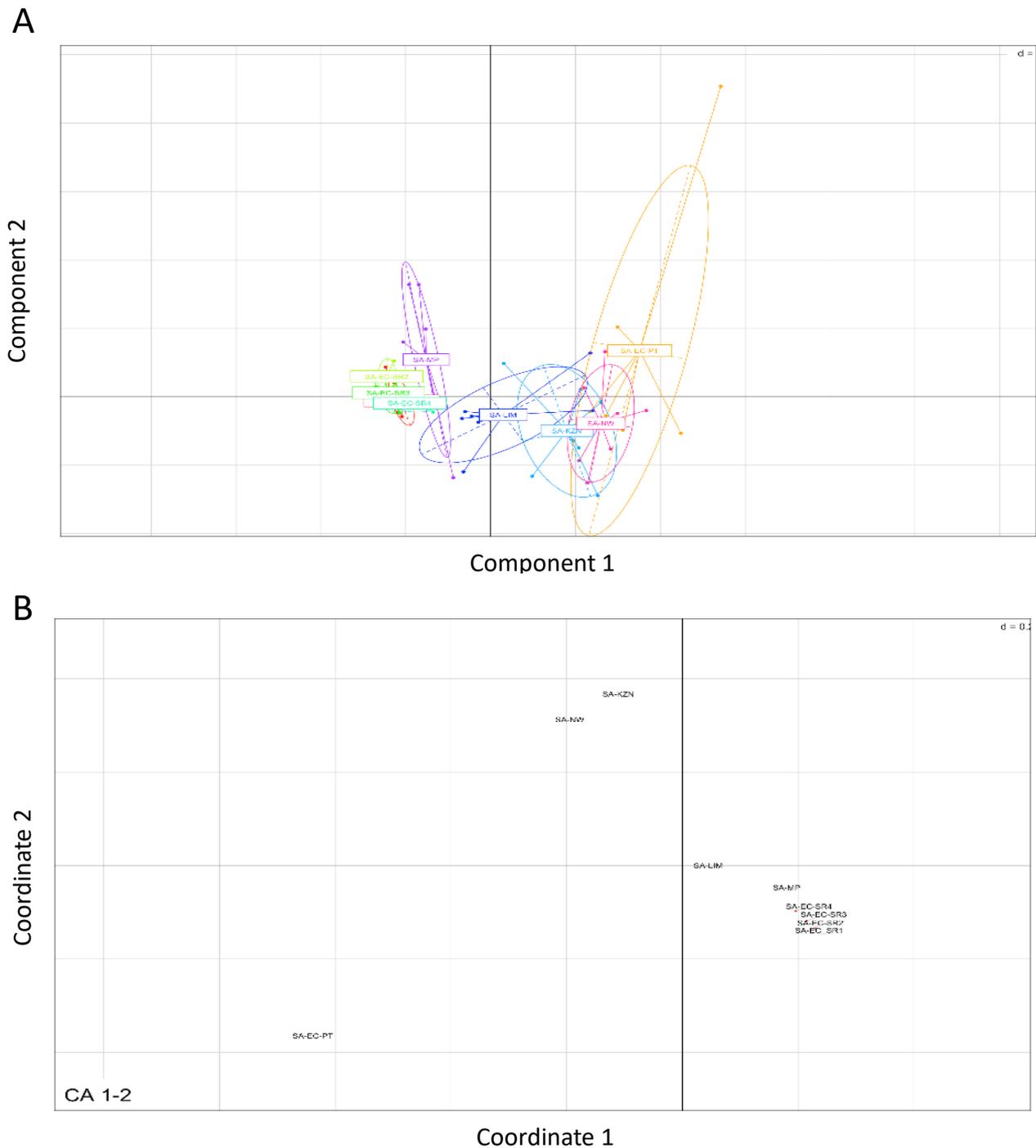
**Objective 4: Whole genome sequencing and genotyping of isolates from new populations (South Africa and USA) with the newly developed genetic markers.**

This objective will not be completed as no new populations have been collected in South Africa or the USA.

**Objective 5: Whole genome sequencing and genotyping of 30 isolates from five *P. citricarpa* populations in the Eastern Cape (Carstens, 2018) with the newly developed markers.**

A total of 2,023 informative markers were genotyped in all the sequenced isolates. From the principal component and principal coordinate analyses (Figure 4.3.7.7 A and B) it is evident that isolates from the Sunday's River Valley are closely related to those from Mpumalanga and Limpopo provinces, while those from

Patensie are genetically the furthest removed from all the provinces in South Africa, and are more related to populations from KwaZulu-Natal and Northwest.



**Figure 4.3.7.7.** A: Principal component analysis of South African isolates, including 5 orchards from the Eastern Cape. B: Principal coordinate analysis of the South African isolates, based on genotyping with 2,023 markers designed on an isolate from the South Africa. SA-EC-SR1: Eastern Cape Sunday's River valley orchard 1; SA-EC-SR2: Eastern Cape Sunday's River valley orchard 2; SA-EC-SR3: Eastern Cape Sunday's River valley orchard 3; SA-EC-SR4: Eastern Cape Sunday's River valley orchard 4; SA-EC-PT: Eastern Cape Patensie orchard; KZN: KwaZulu-Natal; LIM: Limpopo; MP: Mpumalanga; NW: Northwest.

**Objective 6: Developing of primers for the *P. citricarpa* B-tubulin gene and screening of isolates.**

Analysis of the *P. citricarpa*  $\beta$ -tubulin gene sequence revealed three previously described mutations involved in fungicide resistance. The first one was the glutamic acid (GAG) to alanine (GCG) change at amino acid 201

(E198A), conferring benzimidazole resistance. The second mutation was a GAG to AAG substitution resulting in the replacement of glutamic acid with lysine (E198K), while the third at position 203 is a TCC to TAC replacement, substituting phenylalanine for tyrosine (F200Y). The latter two mutations confer resistance to both benzimidazoles to isolates carrying them. Allele-specific primers targeting the three point-mutations at the two sites of the  $\beta$ -tubulin gene were developed. Two primersets detecting the two wild-type genotypes were also designed. PCR assays using the designed allele-specific primers were carried out to confirm the benzimidazole resistance status of the 13 previously sequenced isolates, as well as twelve additional isolates. In each case the primers could successfully detect the benzimidazole resistant isolates, demonstrating the applicability of this rapid PCR assay for high-throughput screening of field isolates.

## Discussion

Due to the importance of CBS as a quarantine disease in citrus, it is imperative to gain extensive knowledge on the epidemiology of *P. citricarpa*. Knowledge of the population genetic structure of a given pathogen is important for a comprehensive understanding of disease epidemiology. This study is the first to use high throughput sequencing (HTS) to study the genetic diversity and connectivity of *P. citricarpa* populations.

The results from this study demonstrate the feasibility of HTS and comparative genome analyses to study the genetic diversity and population structure of this fungus. The genomes of 17 USA isolates were sequenced using HTS and assembled to a high degree of completeness. A pairwise mapping, variant calling and enumeration analysis yielded a low number of inter-isolated variants. A total of 1,939 SSRs were identified from the HTS data and genotyped in the isolates, detecting only 57 polymorphic markers. These results, together with the low number of inter-isolate variants, agree with previously published results (Carstens *et al.*, 2017; Wang *et al.*, 2016) that the Floridian *P. citricarpa* population has low genetic diversity and is most likely clonal. Furthermore, only one mating type was detected, supporting the findings that the fungus spreads and causes new infections through asexual spores (Hendricks *et al.*, 2017).

Two more SSR marker sets based on isolates from geographically distant countries, namely 1,969 on a Chinese isolate and 1,987 on a South African isolate were developed and used for *in silico* genotyping of isolates from the seven countries. Population differentiation and clustering analysis based on the three marker sets show the same genetic relationship/connectivity between the populations (Figures 4.3.7.2-2), showing that the origin of the isolate used for marker development will not influence the outcome of the population clustering analysis.

The SSR marker set based on the South African isolate were chosen for all further analysis. Seventy-one isolates from seven countries, including isolates from Argentina and Eswatini that has not been analysed before, were sequenced. The genomes were assembled and used for *in silico* genotyping with the South African marker set. Furthermore, the HTS data were used for SNP detection relative to a reference genome and inter-isolate variant calling. The three approaches in analysing the HTS data gave the same overall population clustering results. *P. citricarpa* isolates from China are genetically distinct and was the only country that could be significantly differentiated from other countries. There is a degree of genetic connectivity between isolates from all other countries, and all these populations share a high genetic identity ( $> 0.946$  Nei's measure of genetic identity) with each other (Figure 4.3.7.5). Isolates from Argentina, Australia, Brazil, Eswatini, USA and those from Mpumalanga in South Africa cluster most closely together. These countries are also closely associated, as indicated by Nei's measure of genetic identity (Figure 4.3.7.5). Although the dendrogram based on Nei's genetic identity shows that USA is sharing less genetic identity with the populations in this cluster, it still shares the highest identity with Eswatini. The Eastern Cape is the South African province furthest removed from the aforementioned cluster and overlaps mainly with the Northwest and KwaZulu-Natal provinces. This is confirmed by Nei's genetic identity, placing populations from these three provinces closely together (Figure 4.3.7.5). The population from Limpopo are placed between the two groups in the PCA (Figure 4.3.7.4), and have isolates placed in both groups in the variant calling analysis (Figure 4.3.7.6), suggesting that the spread of *P. citricarpa* in South Africa between Mpumalanga and NorthWest/KwaZulu-Natal might have occurred via Limpopo or from Limpopo as the origin, diverging from there to the other provinces.

The genetic connectivity between *P. citricarpa* populations from these geographic locations shown by this study correlates with the findings of Carstens et al. (2017) based on 15 SSR markers for populations from five countries. Populations from these countries having genetic connectivity can be indicative of human-mediated dispersal by means of exchange of infected plant material between countries and orchards and asexual reproduction maintaining the genotypes. These findings provide evidence that clonal reproduction may play an important role in the epidemiology of the disease, as reported by Carstens et al. (2017) and Carstens (2018).

A more comprehensive study of the genetic diversity of *P. citricarpa* on a regional scale included five orchards from the Eastern Cape, South Africa (Figure 4.3.7.7). This supported the findings of Carstens (2018) that there are at least two *P. citricarpa* clusters in South Africa. Mpumalanga and Eastern Cape Sunday's River valley cluster together, while Eastern Cape Patensie, Northwest and KwaZulu-Natal group together. Carstens (2018) also placed the isolates from three Sunday's River valley orchards (ECLE1, ECOR1 and ECOR2, Figure 3, Chapter 4) in a cluster with those from Limpopo and Mpumalanga, while the orchard from Patensie (ECLE2) forms a cluster with KwaZulu-Natal. The PCoA analysis based SSR marker set (Figure 4.3.7.7) showed that Eastern Cape Patensie is further removed genetically from KwaZulu-Natal and NorthWest, and given its low genetic diversity (average number of 2,169 variants detected), might indicate a separate, more recent introduction.

This study represents the largest whole genome sequencing survey of *P. citricarpa* to date and provides a more comprehensive assessment of the population genetic diversity and connectivity of *P. citricarpa* from different geographic origins. Furthermore, this is the first report of the mutations conferring benzimidazole resistance (Moyo *et al.*, 2021) confirmed in *P. citricarpa* isolates from South Africa using HTS. Highly specific PCR primers that can be used for rapid detection of benzimidazole resistance in *P. citricarpa*, were designed. Fungicide resistance still occurs and over application or misuse can easily result in the pathogen developing resistance to it. The occurrence of benzimidazole resistance in *P. citricarpa* should therefore be continuously monitored and spraying regimes amended when necessary.

## Technology transfer

Manuscript published:

Title: **Extending the knowledge of *Phyllosticta citricarpa* population structure in USA with re-sequencing and genome wide analysis**

Authors: Beatrix Coetzee, Elma Carstens, Megan Dewdney, Paul H. Fourie, Aletta E. Bester-van der Merwe  
Physiological and Molecular Plant Pathology (2021) 113:101591

Manuscript submitted to journal:

Title: **Discerning the global phylogeographic distribution of *Phyllosticta citricarpa* by means of whole genome sequencing.**

Authors: Coetzee B, Carstens E, Fourie PH, Dewdney M, Rollins JA, Manzano León AM, Donovan NJ, Glienke C, Miles AK, Li H, Bester-van der Merwe AE

Manuscript in preparation:

Title: **An allele-specific PCR assay for rapid detection of mutations that confer benzimidazole resistance in *Phyllosticta citricarpa***

Authors: Beatrix Coetzee, Elma Carstens, Elaine Basson, Jessica Winn, Mia Groeneveld, Providence Moyo, Tiaan Schutte, Tankiso Mpholo, Jan van Niekerk, Paul H. Fourie, Aletta E. Bester-van der Merwe

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#### 4.3.8 **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 P. Moyo and J. van Niekerk (CRI)

##### **Summary**

Different fungicide spray programmes were evaluated for the control of *Alternaria alternata*, which causes Alternaria brown spot (ABS) on citrus. The spray programmes were evaluated on 'Nova' mandarins in the Kirkwood and Buffeljagsrivier areas in the Eastern Cape (EC) and Western Cape (WC) provinces, respectively. Treatments tested were spray programmes consisting of tank mixtures of various products sprayed at certain spray intervals. None of the treatments or spray programmes was good enough to satisfactorily control the disease for fruit destined for the fresh fruit market. However, a higher level of control was achieved in the WC orchard (between 75 and 82.5% ABS-free fruit) compared to the EC orchard (between 9 and 52.8% ABS-free fruit). The highest amount of ABS free fruit (82.5%) was achieved in the Nova orchard in the Western Cape, with the spray programme involving two applications of azoxystrobin-difenoconazole plus mancozeb plus mineral oil, followed by four applications of mancozeb. None of the tested programmes were effective in the EC orchard. The highest percentage of clean fruit achieved in the EC was 52.8% and was produced by a treatment programme consisting of two copper oxychloride sprays followed by two tank mixtures of mancozeb plus azoxystrobin plus mineral oil and finally two sprays of mancozeb. The lack of efficacy of the treatments in the EC could be due to the high level of ABS inoculum historically present or the complications associated with the change of personnel responsible for applying the treatments.

##### **Opsomming**

Verskillende swamdoder spuitprogramme is vir die beheer van *Alternaria alternata*, wat Alternaria bruinvlek (ABS) op sitrus veroorsaak, geëvalueer. Die spuitprogramme is op 'Nova' mandaryne in die Kirkwood en Buffeljagsrivier areas in onderskeidelik die Oos-Kaap- (OK) en Wes-Kaap-provinsies (WK) geëvalueer. Behandelings wat getoets is, het programme ingesluit wat uit tenkengsels van verskeie produkte bestaan het, wat teen sekere spuit-intervalle gespuit is. Geen van die behandelings of spuitprogramme was goed genoeg om die siekte vir vrugte wat vir die varsvrugtemark bestem is, bevredigend te beheer nie. 'n Hoër vlak van beheer is in die WK boord verkry (tussen 75 en 82.5% ABS-vry vrugte) in vergelyking met die OK boord (tussen 9 en 52.8% ABS-vry vrugte). Die hoogste hoeveelheid ABS-vry vrugte (82.5%) is in die Nova boord in

die Wes-Kaap bereik, met die spuitprogram wat twee toedienings van asoksistrobien-difenokonasool plus mankoseb plus minerale olie, gevolg deur vier toedienings van mankoseb, ingesluit het. Geen van die getoetste programme was effektief in die OK boord nie. Die hoogste persentasie van skoon vrugte wat in die OK bereik is, was 52.8% en is verkry deur 'n behandeling bestaande uit twee koper-oksichloried spuite, gevolg deur twee tenkmengsels van mankoseb plus asoksistrobien plus minerale olie, en laastens twee spuite mankoseb. Die tekort aan doeltreffendheid van die behandelings in die OK kan weens die hoë vlak van ABS inokulum, histories teenwoordig, wees, of die komplikasies wat gepaard gegaan het met die verandering in personeel wat verantwoordelik was vir die toedien van die behandelings.

## Introduction

*Alternaria brown spot (ABS)* is a serious disease of tangerines (*Citrus reticulata*) (Dalikilic *et al*, 2005; Peever *et al*, 2005) and their hybrids in all citrus producing regions of South Africa (Schutte *et al*, 1992). Susceptibility to ABS is a dominant trait that is transferred from 'Dancy' mandarin to its progeny. Dancy mandarin hybrids and some cultivars of unknown origin, such as 'Murcott', 'Emperor' and 'Ponkan', are affected by the disease (Dalikilic *et al*, 2005). The presence of ABS in South Africa is still a serious problem on all cultivars derived from crosses with Dancy tangerine such as the 'Nova', 'Minneola' and 'Mor'.

ABS disease is caused by *Alternaria alternata*. This fungus attacks young leaves, twigs and fruit, causing small black necrotic spots after a 24 to 36 h incubation period. The ABS pathogen sporulates abundantly on lesions on mature leaves remaining in the canopy (Timmer *et al*, 1998, 2003; Reis *et al*, 2006). The pathogen produces a host-specific toxin that causes lesions to expand, often resulting in leaf and fruit drop as well as twig dieback (Pegg 1966; Peever *et al*, 2004, 2005). On more mature fruit, lesions may vary from small necrotic spots to large sunken pockmarks. Leaves are susceptible until they are fully expanded and hardened whereas fruits are susceptible from petal fall until harvest.

Cultural measures, such as wider tree spacing and pruning to allow air movement and drying-off of trees, the elimination of overhead irrigation and avoidance of excess nitrogen fertilizer, can assist in lowering disease severity in some orchards (Dalikilic *et al*, 2005). However, fungicide applications are essential for disease control and production of blemish-free fruit (Schutte *et al*, 1992). In South Africa, it is important to protect fruit and flushes of cultivars such as tangerines and their hybrids with fungicides from September to April/May, often requiring 8+ spray applications. The number of sprays and the products being used are not economically sustainable and may result in unacceptable residues on fruit.

## Objectives

To evaluate different spray programmes on susceptible 'Nova' mandarin orchards for the control of *Alternaria Brown Spot*.

## Materials and methods

Two 'Nova' mandarin orchards, one located in Kirkwood in the Eastern Cape and one located in Buffeljagsrivier in the Western Cape, were used as trial sites for the 2020-2021 season. Spray programmes included alternating mancozeb and dipotassium phosphate and the inclusion of azoxystrobin-difenoconazole as part of a spray programme (Table 4.3.8.1). Similar treatments were applied in both orchards except that the treatment that incorporated a silica drench (treatment 6 in Table 4.3.8.1) was not applied in the Eastern Cape and the treatment that consisted only of alternation of dipotassium phosphate and mancozeb (treatment 5 in Table 4.3.8.1) was not applied in the Western Cape orchard. Each treatment consisted of five single data trees as replicates. Guard trees were located between plots within rows. Unsprayed trees served as controls. The treatments in the Eastern Cape orchard were applied by the grower using his own machinery, but treatments in the Western Cape were applied with a Stihl® SR 420 motorized backpack mist blower.

At fruit maturity in May 2020, 100 and 40 fruit per data tree in the Eastern Cape and Western Cape, respectively, were evaluated according to an infection scale where: 0 = fruit with no brown spot lesions, 1 = fruit with one to five lesions and 2 = fruit with six or more lesions. ANOVA was carried out on the data using

XLSTAT, Version 2014.5.03 (Addinsoft, New York, USA) to determine the efficacy of each treatment on ABS incidence. Tukey's least significant difference (LSD) test ( $P = 0.05$ ) was used to compare means.

## Results and discussion

Task table

Objective / Milestone	Achievement
A. To evaluate different spray programmes on susceptible 'Nova' mandarin orchards for the control of Alternaria Brown Spot.	Spray programmes were successfully evaluated in the Kirkwood and Buffeljagsrivier production areas. None of the programmes tested resulted in more than 90% fruit free from ABS lesions.

The person who always applied the treatments in the EC orchard relocated and someone had to take over the application of the treatments. It is possible that complications associated with the change of personnel negatively impacted the project.

The 2020-2021 season in the Eastern Cape (EC) orchard was characterised by high Alternaria brown spot inoculum pressure and none of the treatments performed well in controlling the disease in this orchard. The ABS inoculum pressure in the Western Cape (WC) orchard was lower compared to the orchard in the EC, as higher percentages (between 75 and 82%) of ABS free fruit were achieved with the treatments applied in comparison to a range of between 9 and 52% ABS free fruit achieved in the EC (Table 4.3.8.1). All the treatments tested in the WC orchard produced statistically similar levels of ABS control, but the spray programme with two applications of azoxystrobin-difenoconazole plus mancozeb plus mineral oil followed by four applications of mancozeb produced the highest amount of ABS free fruit (82.5%) (Table 4.3.8.1). A program consisting of a silica drench applied with mancozeb or copper in the WC produced 76.1% clean fruit, an amount of ABS control statistically similar to other treatments (Table 4.3.10.1). The highest percentage of clean fruit (52.8%) in the EC was achieved by treatment 1 which incorporated copper oxychloride, mancozeb and tank mixtures of azoxystrobin, mancozeb and mineral oil (Table 4.3.8.1). Strangely, the standard spray programme used by the grower (treatment 7) in the EC yielded the lowest amount of clean fruit (1.2%) compared to other fungicide treatments, including the untreated fruit which yield 5% clean fruit (Table 4.3.8.1). The amount of clean ABS free fruit achieved with the treatments applied in both orchards is not high enough for consideration for fruit destined for fresh fruit markets.

A multi-disciplinary strategy is important for effective control of Alternaria brown spot. Cultural practices which allow air movement and drying-off of trees reduce disease pressure (Whiteside, 1979) to some extent, but effective control of the disease is mainly achieved with fungicide spray programmes. Fungicides should, therefore, be applied at spraying intervals that ensure sufficient protection of susceptible host tissues throughout the growing season. Spray programmes such as treatment 2, which involved azoxystrobin-difenoconazole mixed with mancozeb in tank mixtures, should therefore be evaluated further and their spray intervals optimised to achieve better control of the disease.

**Table 4.3.8.1.** Application dates, rates and evaluation of fungicides applied in tank mixtures for the control of *Alternaria* brown spot in Nova mandarin orchards in Kirkwood (Eastern Cape) and Buffeljagsrivier (Western Cape), South Africa, for the period between October 2020 and March 2021.

	Treatments	Dosage (g/ml per 100L water tank mixture)	Mean % of fruit in each class					
			Mean lesions/fruit <sup>v</sup>					
			Kirkwood			Buffeljagsrivier		
			0 lesions	1-5 lesions	≥6 lesions	0 lesions	1-5 lesions	≥6 lesions
1	Copper oxychloride/ copper oxychloride/ azoxystrobin + mancozeb + mineral oil/ azoxystrobin + mancozeb + mineral oil/ mancozeb/ mancozeb	200g/200g/20ml+150g+300ml/20ml+150g+300ml/200g/200g	52.8 a	43.2 d	4.0 e	75.0 a	22.8 b	2.2 b
2	Azoxystrobin-difenoconazole + mancozeb + mineral oil/ azoxystrobin-difenoconazole + mancozeb + oil / mancozeb / mancozeb / mancozeb/ mancozeb	30ml+150g+300ml/30ml+150g+300ml /200g/200g/ 200g/200g	33.0 b	50.4 cd	16.6 d	82.5 a	13.3 c	4.2 b
3	Azoxystrobin + mancozeb + mineral oil/ azoxystrobin+ mancozeb + mineral oil/mancozeb/ mancozeb/ mancozeb/ mancozeb	20ml+150g+300ml/20ml+150g+300ml /200g/200g/200g/200g	10.2 cd	54.0 bc	35.8 b	76.1 a	22.5 b	1.4 b
4	Dipotassium phosphate + mancozeb/ dipotassium phosphate + mancozeb/ azoxystrobin + mancozeb + dipotassium phosphate + mineral oil + azoxystrobin +mancozeb+ dipotassium phosphate + mineral oil/ dipotassium phosphate +mancozeb/ dipotassium phosphate + mancozeb/ dipotassium phosphate + mancozeb	100ml+100g/100ml+100g/20ml+100g +100ml+300ml/20ml+100g+100ml+300ml/100ml+100g/100ml+100g/100ml+100g	13.4 c	62.6 ab	24.0 cd	81.9 a	16.2 bc	1.9 b
5	Dipotassium phosphate + mancozeb/ dipotassium phosphate + mancozeb /dipotassium phosphate + mancozeb / dipotassium phosphate+ mancozeb/ dipotassium phosphate + mancozeb/ dipotassium phosphate + mancozeb/dipotassium phosphate + mancozeb	100ml+100g/100ml+100g/100ml+100g/100ml+100g/100ml+100g/100ml+100g/100ml+100g	9.2 cd	65.2 a	25.6 c	-	-	-
6	Copper oxychloride + silica drench/ copper oxychloride + silica drench/ azoxystrobin + mancozeb + mineral oil + silica drench/ azoxystrobin + mancozeb + mineral oil + silica drench/ mancozeb + silica drench/ mancozeb + silica drench/ mancozeb	200g + 6ml per tree/200g + 6ml per tree/20ml + 150g + 300ml + 6ml per tree/20ml + 150g + 300ml + 6ml per tree/200g + 6ml per tree/200g + 6ml per tree/200g	-	-	-	76.1 a	22.2 b	1.7 b
7	Grower's standard spray program		1.2 e	27.8 e	71.0 a	-	-	-
8	Untreated		5.0 de	67.0 a	28.0 c	48.1 b	41.4 a	10.6 a

<sup>v</sup> Means in a column, based on 500 fruit (100 fruit/data tree), followed by the same letter are not significantly different ( $P > 0.05$ ) according to Tukey's least significant difference (LSD) test

The results clearly show that the efficacy of the different programmes varied greatly. The big difference in results obtained in the Kirkwood versus Buffeljagsrivier trials indicate that the change in personnel at the Kirkwood site probably negatively affected efficacy and therefore there is doubt regarding the results obtained.

Focussing on the Buffeljagsrivier results it can be seen that although all the programmes performed statistically better than the untreated control, only two of the programmes resulted in more than 80% clean fruit. Although good, on a commercial level this level of control is not good enough. Further evaluations and investigation into alternative products are therefore needed.

### **Conclusion to date**

All the experimental programmes performed poorly and could not control the *Alternaria* brown spot disease satisfactorily, especially under high disease pressure situations.

### **Technology transfer**

Moyo, P. and Fourie, P.H. 2019. Epidemiology, prediction and management of *Alternaria*. Presentation at the CRI - IPM and DM workshops, August 2020.

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#### **4.3.9 PROGRESS REPORT: Development of new spray programmes for the control of citrus black spot**

Project 970 (Ongoing) by Providence Moyo, Them bani Nxumalo and Paul Fourie (CRI)

### **Summary**

There is a constant need to evaluate the efficacy of novel control measures, including new fungicides, against citrus black spot (CBS). Experimental fungicides, from different companies, were tested on 'Valencia' oranges

for the control of CBS, according to protocols provided by the different parties. A few treatments resulted in good control of CBS, which was comparable to that produced with fungicides registered for CBS control. Experimental fungicides to note include Product X which gave good CBS control (>99% CBS free fruit) at the highest concentration and when mixed with mineral oil. No phytotoxicity was observed on fruit treated with Product X. The season was, however, marked with low disease pressure. Nonetheless, Product X is a possible candidate for consideration for CBS registration trials and hopefully it will produce clean fruit (CBS free) suitable for the export market, even under high disease pressure. Product S was often associated with phytotoxicity, which was exacerbated by mixing with Product Z in a tank. Testing of novel fungicides and re-evaluation of existing registered fungicides is necessary to improve CBS control.

## Opsomming

Daar is 'n konstante behoefte om die effektiwiteit van nuwe beheermaatreëls, insluitend nuwe swamdoders, teen sitruswartvlek (CBS) te evalueer. Eksperimentele swamdoders, van verskillende maatskappye, is op 'Valencia' lemoene vir die beheer van sitruswartvlek getoets, volgens protokolle wat deur die verskillende partye verskaf is. 'n Paar behandelings het goeie beheer van CBS tot gevolg gehad, wat vergelykbaar was met dié wat verkry is met swamdoders wat vir CBS-beheer geregistreer is. Eksperimentele swamdoders waarop gelet kan word, is Produk X wat goeie CBS-beheer (>99% CBS-vrye vrugte) by die hoogste konsentrasie, en wanneer dit met minerale olie gemeng is, gegee het. Geen fitotoksiteit is waargeneem op vrugte wat met Produk X behandel is nie. Die seisoen is egter deur 'n lae siektedruk gekenmerk. Nietemin is Produk X 'n moontlike kandidaat vir oorweging vir CBS-registrasieproewe en hopelik sal dit skoon vrugte (CBS-vry) produseer wat geskik is vir die uitvoermark, selfs onder hoë siektedruk. Produk S is dikwels met fitotoksiteit geassosieer, wat vererger is deur vermenging met Produk Z in 'n tenk. Toetsing van nuwe swamdoders en herevaluering van bestaande geregistreerde swamdoders, is nodig om CBS-beheer te verbeter.

## Introduction

Citrus Black Spot (CBS), caused by *Phyllosticta citricarpa* (McAlpine) van der Aa, is a major concern for the South African citrus industry. *Phyllosticta citricarpa* is an A1 quarantine pathogen in the European Union and other CBS sensitive markets where there is a zero tolerance for CBS on fruit.

Citrus black spot holds the potential to reduce the South African competitiveness on the global citrus market, due to its phytosanitary status and the impact thereof on trade of citrus fruit. Hence, research has focussed primarily on protecting fruit from infection by the CBS pathogen. Currently, all commercial fungicide applications aimed at protecting fruit from CBS infection begin in mid-October in South Africa, based on research findings from ascospore release and trap data (Kellerman and Kotzé, 1977; Kotzé, 1981). Due to the withdrawal of certain fungicides from the market, as a result of concerns of risks posed to human health and the environment as well as resistance, there is a constant need to evaluate old and new fungicide formulations that may possess activity against CBS, and to structure spray programmes to improve control whilst adhering to permitted maximum residue levels (MRL) and limiting fungicide resistance.

A number of adjuvants are regularly used with systemic fungicide applications in South Africa, to enhance the efficacy of fungicides. However, most systemic fungicides registered for the control of CBS are used in combination with mineral oils. These oils have been shown to enhance the penetration of fungicides into the plant tissues, ultimately increasing the efficacy of the fungicides against CBS (Kellerman and Kotzé 1977). The efficacy of newly developed non-mineral oil adjuvants, as substitutes for mineral oil in standard strobilurin spray programmes, also need to be investigated for the control of *Phyllosticta citricarpa* on citrus in South Africa.

Fungicide manufacturers often develop new fungicide formulations for disease control but they also modify and upgrade old fungicide products to possess new characteristics such as rain fastness and particle size. The evaluation of new fungicide products and re-evaluation of old products for efficacy against CBS remains an integral part of staying globally competitive in the citrus market place.

## Objectives

To evaluate new potential fungicides for the control of citrus black spot.

## Materials and methods

The trial was carried out in a 1.4 ha commercial orchard (Crocodile Valley Citrus Co.) located in Nelspruit (GPS: 25° 28' 16.38" S and 31° 04' 20.70" E). The orchard was planted in 1986 and consisted of Olinda 'Valencia' orange (*Citrus sinensis*) trees grafted on Rough Lemon rootstock (*Citrus jambhiri*), with spacing of 8.3m x 5.6m. The orchard consists of sandy-loam soil.

Different fungicides were applied either alone or in tank mixtures with other fungicides. Fungicide spray applications were conducted at intervals and concentrations determined by the different parties whose products were tested. A few treatments were tested as part of a collaboration with the University of Florida in the United States of America. The efficacy of the different fungicides in tank mixtures was compared with the registered industry standard CBS fungicides.

Commercial fungicide applications against CBS are recommended from fruit set in South Africa and therefore, treatment applications began in mid-October 2020 and were applied using a trailer-mounted, high-volume, high-pressure (2,500-3,000 kPa) sprayer with two hand-held spray guns. Each treatment was replicated four times on single-tree plots arranged in a randomized complete block design. Fungicide volumes varied according to the size and canopy density of the tree, but all trees were sprayed to the point of runoff.

On 1 September 2021 (2 days before harvest), 100 fruit per data tree were evaluated 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. ANOVA was carried out using XLSTAT, Version 2014.5.03 (Addinsoft, New York, USA) to determine the efficacy of each treatment on CBS severity and incidence. Tukey's least significant difference (LSD) test ( $P = 0.05$ ) was used to compare means. The effect of each treatment on the fruit rind (phytotoxicity) was evaluated simultaneously. Phytotoxicity was rated on a scale of 0 to 3: 0 = no dark marks on the rind; 1 = dark marks on one-half of the fruit rind; 2 = dark marks on three-quarters of the fruit rind and 3 = dark marks on the whole fruit rind.

## Results and discussion

Task table

Objective / Milestone	Achievement
<ul style="list-style-type: none"><li>• Evaluation of new fungicides<ul style="list-style-type: none"><li>• Apply fungicides at concentrations determined by different parties</li></ul></li></ul>	All treatments were successfully applied on 'Valencia' oranges. The trial was successfully completed and evaluated in September 2021, where after the data was statistically analysed.

The trial site was characterised by a low incidence of citrus black spot during the 2020-2021 season, with the untreated control trees resulting in an average of 42.25% CBS free fruit (57.75% CBS incidence) (Table 4.3.9.1). Most experimental fungicides significantly reduced CBS infection compared to the untreated control. With the exception of two treatments (16 and 27) involving Product S sprayed either with azoxystrobin or trifloxystrobin without mineral oil, results show that there were no significant differences ( $P < 0.05$ ) between several treatments and the standard registered mancozeb applied in tank mixtures with strobilurin and mineral oil (treatments 9, 11 and 23) (Table 4.3.9.1).

Similar to the industry standard treatments consisting of mancozeb applied with either pyraclostrobin or azoxystrobin and mineral oil in a tank mixture, experimental treatments consisting of copper hydroxide alternated with either azoxystrobin mixed with difenoconazole or pyraclostrobin resulted in 100% CBS control. The standard treatment involving mancozeb applied with trifloxystrobin and mineral oil in a tank mixture,

however, resulted in less CBS control (97% CBS free fruit) (Table 4.3.9.1). No phytotoxicity was observed on fruit treated with any of the registered industry standard fungicides (Table 4.3.9.2).

All treatments involving Product X, from Party A, gave good CBS control (>94% CBS free fruit), which was comparable to that achieved by the industry standard CBS spray programmes. Although not significantly different from other Product X treatments, the two treatments consisting of Product X mixed with mineral oil resulted in the highest percentage of CBS free fruit (99.75%) and least CBS incidence (0.25%) (Table 4.3.9.1). Mineral oils have been reported to enhance penetration of fungicides into plant tissues, ultimately increasing the efficacy of fungicides against CBS (Kellerman and Kotzé 1977). CBS severity was also minimum on fruit treated with Product X in mixture with mineral oil when compared to fruit treated with Product X without the oil (Table 4.3.9.1). An average of 0.25 – 2.75% and 0.25 – 3.25% fruit sprayed with different treatments of Product X had 1-3 lesions and  $\geq 4$  lesions, respectively, but no fruit with  $\geq 4$  lesions were observed with the treatments consisting of Product X in mixture with mineral oil (Table 4.3.9.1).

Product X was shown to be more effective against CBS than Product Y. There were significant differences in the number of fruit without CBS symptoms between treatments consisting of 50 ml/100L of water of Product X and 50 ml/100L of water of Product Y. A 34.0% difference in the number of CBS free fruit was observed between the two treatments. Similar to treatments consisting of Product X, the addition of mineral oil to Product Y improved the efficacy of this product. Ninety-three percent CBS free fruit (7% CBS incidence) was achieved when mineral oil was added in comparison to 61.50% CBS free fruit (38.5% CBS incidence) obtained with the application of Product Y alone without mineral oil (Table 4.3.9.1). High CBS severity was observed on fruit treated with Product Y, with 3.75 - 6.0% and 3.25 - 32.5% of fruit having 1-3 lesions and  $\geq 4$  lesions, respectively (Table 4.3.9.1). No phytotoxicity was evident on fruit treated with any of the treatments from Party A as none of the fruit treated with Products X and Y displayed dark marks on the rind (Table 4.3.9.2).

The replacement of mineral oil with Product Z, at different concentrations (200, 300 and 400 ml), in spray programmes consisting of azoxystrobin mixed with mancozeb (contact fungicide) resulted in good control of CBS (96 - 99% CBS free fruit) but highest control was achieved with the highest concentration of Product Z. Efficacy of Product Z was reduced when it was applied with azoxystrobin and Product S as a contact fungicide, as between 52 and 94% CBS free fruit were achieved. A higher range (63 - 96%) was, however, achieved when Product Z and S were mixed with trifloxystrobin (Table 4.3.9.1). Product S was often associated with some degree of phytotoxicity but the level of phytotoxicity was exacerbated by the addition of Product Z or mineral oil in a tank mixture (Figure 4.3.9.1). An average of between 13.75 and 33% of fruit treated with Product S mixed with azoxystrobin and different concentrations of Product Z were observed to exhibit dark marks (86.25 - 67% incidence of phytotoxicity) but the average ranged between 8.5 and 15.5% (84.5 - 91.5 % incidence of phytotoxicity) when azoxystrobin was replaced with trifloxystrobin in the tank mixtures (Table 4.3.9.2).

Of the fungicides tested as part of a collaboration project with the University of Florida (USA), great control (100% CBS free fruit) of CBS was achieved with the application of treatments involving copper hydroxide alternated with either azoxystrobin + difenoconazole or pyraclostrobin. Fenbuconazole (treatment 35) and fluopyram + tebuconazole were the worst performing products as they resulted in 69 and 60% CBS free fruit, respectively (Table 4.3.9.1). Some degree of phytotoxicity was observed with treatments involving copper hydroxide (Fig. 4.3.9.1), with the highest level of phytotoxicity observed when copper hydroxide was alternated with azoxystrobin + difenoconazole (Table 4.3.9.2). This was, however, not unexpected since copper fungicides cause stippling on fruit (Schutte *et al.* 1997).

**Table 4.3.9.1.** Evaluation of spray programmes for the control of Citrus black spot conducted at Crocodile Valley Co., Nelspruit, Mpumalanga during the 2020-2021 season

Company		Treatments	Dosage (g/ml per 100L water tank mixture)	Average % of fruit with CBS lesions <sup>a</sup>		
				0 lesions	1-3 lesions	≥4 lesions
Party A	1	Product X (x4 applications) <sup>b</sup>	30ml	94,25 j	2,50 abcdef	3,25 ab
Party A	2	Product X (x4 applications) <sup>b</sup>	50ml	95,50 j	2,75 abcdefg	1,75 ab
Party A	3	Product X (x4 applications) <sup>b</sup>	100ml	99,50 j	0,25 a	0,25 a
Party A	4	Product X + mineral oil (x4 applications) <sup>b</sup>	50ml+500ml/50ml+500ml/ 50ml+500ml/50ml+500ml	99,75 j	0,25 a	0,00 a
Party A	5	Product X + mineral oil (x4 applications) <sup>b</sup>	100ml+500ml/100ml+500ml/ 100ml+500ml/100ml+500ml	99,75 j	0,25 a	0,00 a
Party A	6	Product Y (x4 applications) <sup>b</sup>	50ml	61,50 de	6,00 efgh	32,50 hi
Party A	7	Product Y + mineral oil (x4 applications) <sup>b</sup>	50ml+500ml/50ml+500ml/ 50ml+500ml/50ml+500ml	93,00 ij	3,75 abcdefg	3,25 ab
Party A	8	Mancozeb/mancozeb+Product X/mancozeb+Product X/mancozeb <sup>c</sup>	200g/150g+50ml/150g+50ml/ 200g	97,25 j	0,25 a	2,50 ab
Industry standard	9	Mancozeb/mancozeb + pyraclostrobin + mineral oil/mancozeb + pyraclostrobin + mineral oil/mancozeb <sup>c</sup>	200g/ 150g + 10ml + 500ml/ 150g + 10ml + 500ml/ 200g	100,00 j	0,00 a	0,00 a
	10	Untreated control		42,25 a	3,75 abcdefg	54,00 l
Industry standard	11	Mancozeb/mancozeb+azoxystrobin+mineral oil/ mancozeb+azoxystrobin+mineral oil /mancozeb <sup>d</sup>	200g/150g+20ml+300ml/ 150g+20ml+300ml/200g	100,00 j	0,00 a	0,00 a
Party B	12	Mancozeb/mancozeb + azoxystrobin + Product Z/mancozeb + azoxystrobin + Product Z/mancozeb <sup>d</sup>	200g/ 150g + 20ml + 150ml/ 150g + 20ml + 150ml/ 200g	97,50 j	0,75 abc	1,75 ab
Party B	13	Mancozeb/mancozeb + azoxystrobin + Product Z/mancozeb + azoxystrobin + Product Z/mancozeb <sup>d</sup>	200g/ 150g + 20ml + 200ml/ 150g + 20ml + 200ml/ 200g	96,75 j	2,50 abcdef	0,75 ab
Party B	14	Mancozeb/mancozeb + azoxystrobin + Product Z/mancozeb + azoxystrobin + Product Z/mancozeb <sup>d</sup>	200g/ 150g + 20ml + 400ml/ 150g + 20ml + 400ml/ 200g	99,00 j	0,50 ab	0,50 ab
Party B	15	Product S/Product S + azoxystrobin/ Product S + azoxystrobin/Product S <sup>d</sup>	200g/ 200g + 20ml/ 200g + 20ml/ 200g	78,00 gh	4,00 abcdefg	18,00 ef
Party B	16	Product S/Product S + azoxystrobin/ Product S + azoxystrobin/Product S <sup>d</sup>	200g/300g + 20ml/300g + 20ml/ 200g	46,75 ab	3,50 abcdefg	49,75 kl
Party B	17	Product S/Product S + azoxystrobin/ Product S + azoxystrobin/Product S <sup>d</sup>	200g/400g + 20ml/ 200g/400g + 20ml/ 200g	74,25 fg	2,50 abcdef	23,25 fg

<b>Party B</b>	18	Product S/Product S + azoxystrobin + Product Z/ Product S + azoxystrobin + Product Z/Product S <sup>d</sup>	200g/200g + 20ml + 150ml/ 200g + 20ml + 150ml/ 200g	94,25 j	0,75 abc	5.00 ab
<b>Party B</b>	19	Product S/Product S + azoxystrobin + Product Z/ Product S + azoxystrobin + Product Z/Product S <sup>d</sup>	200g/200g + 20ml + 200ml/ 200g + 20ml + 200ml/ 200g	84,75 hi	6,75 gh	8,50 bcd
<b>Party B</b>	20	Product S/Product S + azoxystrobin + Product Z/ Product S + azoxystrobin + Product Z/Product S <sup>d</sup>	200g/200g + 20ml + 400ml/ 200g + 20ml + 400ml/ 200g	52,25 bc	10.00 h	37,75 ij
<b>Party B</b>	21	Product S/Product S + azoxystrobin + mineral oil/ Product S + azoxystrobin + mineral oil/Product S <sup>d</sup>	200g/200g + 20ml + 300ml/ 200g + 20ml + 300ml/ 200g	91,25 ij	0,75 abc	8.00 abc
<b>Industry standard</b>	23	Mancozeb/mancozeb + trifloxystrobin + mineral oil/ Mancozeb + trifloxystrobin + mineral oil /mancozeb <sup>d</sup>	200g/150g+10ml+300ml/ 150g+20ml+300ml/200g	97.00 j	1,50 abcd	1,50 ab
<b>Party B</b>	24	Mancozeb/mancozeb + trifloxystrobin + Product Z/mancozeb + trifloxystrobin + Product Z/mancozeb <sup>e</sup>	200g/ 150g + 10ml + 150ml/ 150g + 10ml + 150ml/ 200g	99,75 j	0,00 a	0,25 a
<b>Party B</b>	25	Mancozeb/mancozeb + trifloxystrobin + Product Z/mancozeb + trifloxystrobin + Product Z/mancozeb <sup>e</sup>	200g/ 150g + 10ml + 200ml/ 150g + 10ml + 200ml/ 200g	82.00 gh	1,75 abcd	16,25 def
<b>Party B</b>	26	Mancozeb/mancozeb + trifloxystrobin + Product Z/mancozeb + trifloxystrobin + Product Z/mancozeb <sup>e</sup>	200g/ 150g + 10ml + 400ml/ 150g + 10ml + 400ml/ 200g	98,75 j	0,75 abc	0,50 ab
<b>Party B</b>	27	Product S/Product S + trifloxystrobin/ Product S + trifloxystrobin/Product S <sup>e</sup>	200g/ 200g + 10ml/ 200g + 10ml/ 200g	51.00 ab	5,00 defg	44.00 jk
<b>Party B</b>	28	Product S/Product S + trifloxystrobin/ Product S + trifloxystrobin/Product S <sup>e</sup>	200g/300g + 10ml/300g + 10ml/ 200g	95,67 j	0,00 a	4,33 ab
<b>Party B</b>	29	Product S/Product S + trifloxystrobin/ Product S + trifloxystrobin/Product S <sup>e</sup>	200g/400g + 10ml/ 200g/400g + 10ml/ 200g	65,50 def	5,00 defg	29,50 gh
<b>Party B</b>	30	Product S/Product S + trifloxystrobin + Product Z/ Product S + trifloxystrobin + Product Z/Product S <sup>e</sup>	200g/200g + 10ml + 150ml/ 200g + 10ml + 150ml/ 200g	81,50 gh	4,75 cdefg	13,75 cde
<b>Party B</b>	31	Product S/Product S + trifloxystrobin + Product Z/ Product S + trifloxystrobin + Product Z/Product S <sup>e</sup>	200g/200g + 10ml + 200ml/ 200g + 10ml + 200ml/ 200g	96.00 j	1,25 abcd	2,75 ab
<b>Party B</b>	32	Product S/Product S + trifloxystrobin + Product Z/ Product S + trifloxystrobin + Product Z/Product S <sup>e</sup>	200g/200g + 10ml + 400ml/ 200g + 10ml + 400ml/ 200g	63,25 de	6,50 fgh	30,25 ghi
<b>Party B</b>	33	Product S/Product S + trifloxystrobin + mineral oil/ Product S + trifloxystrobin + mineral oil/Product S <sup>e</sup>	200g/200g + 10ml + 300ml/ 200g + 10ml + 300ml/ 200g	81,67 gh	4,67 bcdefg	13,67 cde
<b>USA collaboration</b>	35	Fenbuconazole (x6 applications) <sup>f</sup>	50ml	69.00 ef	2,50 abcdef	28,50 gh

<b>USA collaboration</b>	36	Copper hydroxide/azoxystrobin+difenoconazole/ copper hydroxide/azoxystrobin+difenoconazole/ copper hydroxide /azoxystrobin+difenoconazole <sup>f</sup>	335g/96ml/335g/96ml/ 335g/96ml	100,00 j	0,00 a	0,00 a
<b>USA collaboration</b>	37	Fluopyram+tebuconazole (x6 applications) <sup>f</sup>	50 ml	60.00 cd	2.00 abcde	38.00 ij
<b>USA collaboration</b>	38	Copper hydroxide/pyraclostrobin/ copper hydroxide/pyraclostrobin/ copper hydroxide/pyraclostrobin <sup>f</sup>	335g/94ml/335g/94ml/ 335g/94ml	100,00 j	0,00 a	0,00 a

<sup>a</sup>Means followed by the same letter in the same column do not differ significantly ( $P = 0.05$ ) according to Tukey's least significant difference test.

<sup>b</sup>Spray dates were: 9 October 2020; 6 November 2020 (treatments were initially applied on the 5<sup>th</sup> of November but it rained within 2 hours after application and treatments were repeated the next day); 21 December 2020 and 4 February 2021.

<sup>c</sup>Spray dates were: 8 October 2020; 6 November 2020 (treatments were initially applied on the 5<sup>th</sup> of November but it rained within 2 hours after application and treatments were repeated the next day); 22 December 2020 and 4 February 2021.

<sup>d</sup>Spray dates were: 8 October 2020; 29 October 2020; 9 December 2020 and 14 January 2021.

<sup>e</sup>Spray dates were: 9 October 2020; 30 November 2020; 10 December 2020 and 14 January 2021.

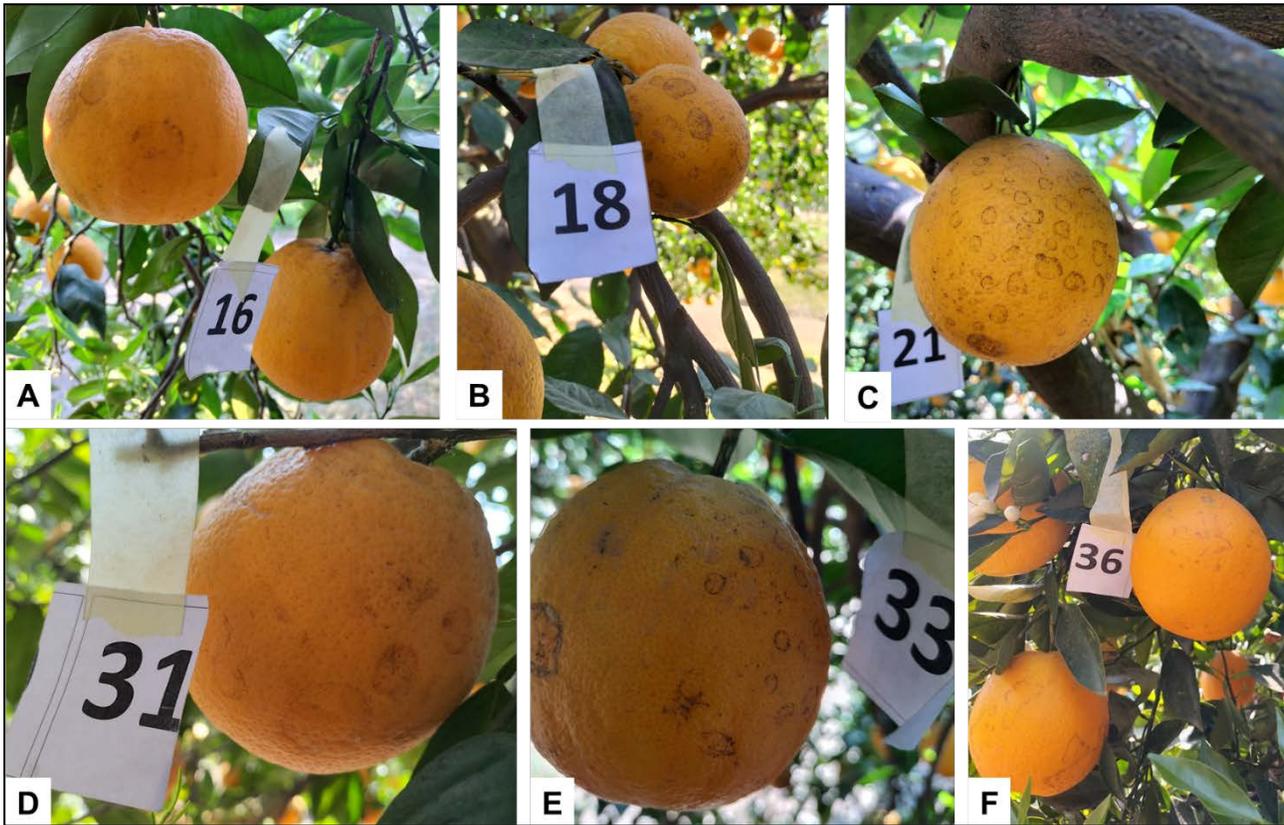
<sup>f</sup>Spray dates were: 17 October 2020; 13 November 2020; 10 December 2020; 5 January 2021; 4 February 2021 and 03 March 2021.

**Table 4.3.9.2.** Evaluation of the effects of citrus black spot spray programmes on the rind (phytotoxicity) of Valencia oranges at Crocodile Valley Co., Nelspruit, Mpumalanga during the 2020-2021 season

	Treatments	Dosage (g/ml per 100L water tank mixture)	Mean % of fruit with dark marks (Phytotoxicity)			
			0=no dark marks	1= dark marks on ½ of the fruit	2= dark marks on ¾ of fruit rind	3=dark marks whole fruit
1	Product X (x4 applications)	30ml	100.00 g	0.00 a	0.00 a	0.00 a
2	Product X (x4 applications)	50ml	100.00 g	0.00 a	0.00 a	0.00 a
3	Product X (x4 applications)	100ml	100.00 g	0.00 a	0.00 a	0.00 a
4	Product X + mineral oil (x4 applications)	50ml+500ml/50ml+500ml/ 50ml+500ml/50ml+500ml	100.00 g	0.00 a	0.00 a	0.00 a
5	Product X + mineral oil (x4 applications)	100ml+500ml/100ml+500ml/ 100ml+500ml/100ml+500ml	100.00 g	0.00 a	0.00 a	0.00 a

6	Product Y (x4 applications)	50ml	100.00 g	0.00 a	0.00 a	0.00 a
7	Product Y + mineral oil (x4 applications)	50ml+500ml/50ml+500ml/ 50ml+500ml/50ml+500ml	100.00 g	0.00 a	0.00 a	0.00 a
8	Mancozeb/mancozeb+Product X/mancozeb+ Product X/mancozeb	200g/150g+50ml/150g+50ml/ 200g	100.00 g	0.00 a	0.00 a	0.00 a
9	Mancozeb/mancozeb +pyraclostrobin + mineral oil/ mancozeb + pyraclostrobin +mineral oil/ mancozeb	200g/ 150g + 10ml + 500ml/ 150g + 10ml + 500ml/ 200g	100.00 g	0.00 a	0.00 a	0.00 a
10	Untreated control		100.00 g	0.00 a	0.00 a	0.00 a
11	Mancozeb/mancozeb+azoxystrobin+mineral oil/ mancozeb+azoxystrobin+mineral oil /mancozeb	200g/150g+20ml+300ml/ 150g+20ml+300ml/200g	100.00 g	0.00 a	0.00 a	0.00 a
12	Mancozeb/mancozeb + azoxystrobin + Product Z/mancozeb + azoxystrobin + Product Z/mancozeb	200g/ 150g + 20ml + 150ml/ 150g + 20ml + 150ml/ 200g	100.00 g	0.00 a	0.00 a	0.00 a
13	Mancozeb/mancozeb + azoxystrobin + Product Z/mancozeb + azoxystrobin + Product Z/mancozeb	200g/ 150g + 20ml + 200ml/ 150g + 20ml + 200ml/ 200g	100.00 g	0.00 a	0.00 a	0.00 a
14	Mancozeb/mancozeb + azoxystrobin + Product Z/mancozeb + azoxystrobin + Product Z/mancozeb	200g/ 150g + 20ml + 400ml/ 150g + 20ml + 400ml/ 200g	100.00 g	0.00 a	0.00 a	0.00 a
15	Product S/Product S + azoxystrobin/ Product S + azoxystrobin/Product S	200g/ 200g + 20ml/ 200g + 20ml/ 200g	99.67 g	0.33 a	0.00 a	0.00 a
16	Product S/Product S + azoxystrobin/ Product S + azoxystrobin/Product S	200g/300g + 20ml/300g + 20ml/ 200g	99.25 g	0.75 a	0.00 a	0.00 a
17	Product S/Product S + azoxystrobin/ Product S + azoxystrobin/Product S	200g/400g + 20ml/ 200g/400g + 20ml/ 200g	98.00 g	2.00 a	0.00 a	0.00 a
18	Product S/Product S + azoxystrobin + Product Z/ Product S + azoxystrobin + Product Z/Product S	200g/200g + 20ml + 150ml/ 200g + 20ml + 150ml/ 200g	27.50 c	21.75 d	21.25 c	29.50 c
19	Product S/Product S + azoxystrobin + Product Z/ Product S + azoxystrobin + Product Z/Product S	200g/200g + 20ml + 200ml/ 200g + 20ml + 200ml/ 200g	13.75 ab	40.25 ef	27.50 d	18.50 d
20	Product S/Product S + azoxystrobin + Product Z/ Product S + azoxystrobin + Product Z/Product S	200g/200g + 20ml + 400ml/ 200g + 20ml + 400ml/ 200g	33.00 c	46.50 fg	17.50 c	3.00 ab
21	Product S/Product S + azoxystrobin + mineral oil/ Product S + azoxystrobin + mineral oil/Product S	200g/200g + 20ml + 300ml/ 200g + 20ml + 300ml/ 200g	9.25 a	57.00 h	16.75 c	17.00 d
23	Mancozeb/mancozeb + trifloxystrobin + mineral oil/ Mancozeb + trifloxystrobin + mineral oil /mancozeb	200g/150g+10ml+300ml/ 150g+20ml+300ml/200g	100.00 g	0.00 a	0.00 a	0.00 a
24	Mancozeb/mancozeb + trifloxystrobin + Product Z/mancozeb + trifloxystrobin + Product Z/mancozeb	200g/ 150g + 10ml + 150ml/ 150g + 10ml + 150ml/ 200g	99.75 g	0.25 a	0.00 a	0.00 a

25	Mancozeb/mancozeb + trifloxystrobin + Product Z/mancozeb + trifloxystrobin + Product Z/mancozeb	200g/ 150g + 10ml + 200ml/ 150g + 10ml + 200ml/ 200g	100.00 g	0.00 a	0.00 a	0.00 a
26	Mancozeb/mancozeb + trifloxystrobin + Product Z/mancozeb + trifloxystrobin + Product Z/mancozeb	200g/ 150g + 10ml + 400ml/ 150g + 10ml + 400ml/ 200g	81.25 e	13.00 c	5.00 ab	0.75 a
27	Product S/Product S + trifloxystrobin/ Product S + trifloxystrobin/Product S	200g/ 200g + 10ml/ 200g + 10ml/ 200g	100.00 g	0.00 a	0.00 a	0.00 a
28	Product S/Product S + trifloxystrobin/ Product S + trifloxystrobin/Product S	200g/300g + 10ml/300g + 10ml/ 200g	89.00 f	9.33 bc	1.67 a	0.00 a
29	Product S/Product S + trifloxystrobin/ Product S + trifloxystrobin/Product S	200g/400g + 10ml/ 200g/400g + 10ml/ 200g	92.25 f	5.50 ab	2.25 a	0.00 a
30	Product S/Product S + trifloxystrobin + Product Z/ Product S + trifloxystrobin + Product Z/Product S	200g/200g + 10ml + 150ml/ 200g + 10ml + 150ml/ 200g	10.25 ab	52.75 gh	31.25 de	5.75 bc
31	Product S/Product S + trifloxystrobin + Product Z/ Product S + trifloxystrobin + Product Z/Product S	200g/200g + 10ml + 200ml/ 200g + 10ml + 200ml/ 200g	15.50 b	43.00 f	33.75 e	7.75 c
32	Product S/Product S + trifloxystrobin + Product Z/ Product S + trifloxystrobin + Product Z/Product S	200g/200g + 10ml + 400ml/ 200g + 10ml + 400ml/ 200g	8.50 a	45.75 f	19.25 c	26.50 e
33	Product S/Product S + trifloxystrobin + mineral oil/ Product S + trifloxystrobin + mineral oil/Product S	200g/200g + 10ml + 300ml/ 200g + 10ml + 300ml/ 200g	55.67 d	33.67 e	9.33 b	1.33 ab
35	Fenbuconazole (x6 applications)	50ml	100.00 g	0.00 a	0.00 a	0.00 a
36	Copper hydroxide/azoxystrobin+difenoconazole/ copper hydroxide/azoxystrobin+difenoconazole/ copper hydroxide /azoxystrobin+difenoconazole	335g/96ml/335g/96ml/ 335g/96ml	89.25 f	10.75 bc	0.00 a	0.00 a
37	Fluopyram+tebuconazole (x6 applications)	50 ml	100.00 g	0.00 a	0.00 a	0.00 a
38	Copper hydroxide/pyraclostrobin/ copper hydroxide/pyraclostrobin/ copper hydroxide/pyraclostrobin	335g/94ml/335g/94ml/ 335g/94ml	99.75 g	0.25 a	0.00 a	0.00 a



**Figure 4.3.9.1.** Valencia orange fruit exhibiting phytotoxicity after treatment with different fungicides. **A** Fruit treated with Product S and azoxystrobin, i.e. 200g Product S/ 300g Product S + 20ml azoxystrobin/ 300g Product S + 20ml azoxystrobin/ 200g Product S. **B.** Fruit treated with Product S, azoxystrobin and Product Z, i.e. 200g Product S/ 200g Product S + 20ml azoxystrobin + 150ml Product Z/ 200g Product S + 20ml azoxystrobin + 150ml Product Z / 200g Product S. **C.** Fruit treated with Product S, azoxystrobin and mineral oil, i.e. 200g Product S/ 200g Product S + 20ml azoxystrobin + 300ml mineral oil/ 200g Product S + 20ml azoxystrobin + 300ml mineral oil/ 200g Product S. **D.** Fruit treated with Product S, trifloxystrobin and Product Z, i.e. 200g Product S/ 200g Product S + 10ml trifloxystrobin + 200ml Product Z/ 200g Product S + 10ml trifloxystrobin + 200ml Product Z / 200g Product S. **E.** Fruit treated with Product S, trifloxystrobin and mineral oil, i.e. 200g Product S/ 200g Product S + 10ml trifloxystrobin + 300ml mineral oil/ 200g Product S + 10ml trifloxystrobin + 300ml mineral oil/ 200g Product S. **F.** Fruit treated with copper hydroxide alternated with azoxystrobin + difenoconazole (3 times), i.e. 335ml copper hydroxide/ 96ml azoxystrobin + difenoconazole.

## Conclusions

Results of this study show that the experimental fungicide, Product X, has the ability to significantly reduce CBS infection, when applied timeously during the fruit susceptibility period. Further trials should be conducted to determine whether excellent CBS control can be achieved consistently with this fungicide, especially under high disease pressure. Further trials on treatments involving the alternation of copper hydroxide with either azoxystrobin mixed with difenoconazole or pyraclostrobin could investigate the effect of extended spray intervals on CBS control and phytotoxicity. Due to market pressures on fungicides such as mancozeb, which could potentially be banned on citrus in the EU, rigorous research with the ultimate goal of registration is needed on any promising fungicide for the control of CBS.

## Future objectives and work plan

Research in the future will consist of further evaluation of fungicides with promising efficacy against CBS.

## Technology transfer

Talks at CRI- IPM and disease management workshops.

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### 4.3.10 **PROGRESS REPORT: The evaluation of different pre-plant products for the control of the citrus nematode, as part of an integrated nematode control approach in citrus replant situations** Project 762 by Jan van Niekerk and Themrani Nxumalo (CRI)

#### Summary

The aim of this project is to identify pre-plant treatments that are effective in keeping orchard soils free from citrus nematode and *Phytophthora* spp. for as long as possible after planting. The trial has been going on since January 2010. The various treatments were applied prior to planting in January 2010 with some treatments still being applied annually in January and November. Tree stem diameter, tree height, nematode soil and root analysis, *Phytophthora* status in the soil, and a visual tree rating, are the parameters that have been monitored yearly since the start of the trial. To date no treatment has stood out in terms of nematode control results. However, based on tree height and trunk diameter measurements, the pre-plant fumigation treatments with 1,3 dichloropropene and metam sodium are beginning to show potential. The trees in these treatments have thicker trunks compared to the cadusafos and control treatments. It is therefore becoming clear that pre-plant soil fumigation in a replant situation does improve tree growth in comparison to no treatment or post-plant treatments.

#### Opsomming

Die doel van hierdie projek is om vóór-plant behandelings te identifiseer wat effektief is om boordgronde vry van sitrus aalwurm en *Phytophthora* spp. te hou vir so lank as moontlik ná plant. Die proef is al sedert Januarie 2010 aan die gang. Die verskeie behandelings is vóór plant in Januarie 2010 toegedien, terwyl sommige behandelings steeds jaarliks in Januarie en November toegedien word. Stamdeursnit, boomhoogte, aalwurm grond- en wortel-analise, *Phytophthora* status in die grond is die parameters wat jaarliks sedert die begin van die proef gemonitor word. Tot op datum het geen behandeling in terme van aalwurmbeheerresultate uitgestaan nie. Gebaseer op boomhoogte en stamdeursnitmetings, begin die vóór-plant berokingsbehandelings met 1,3 dichloropropene en metam natrium potensiaal toon. Die bome in hierdie behandelings het dikker stamme in vergelyking met die cadusafos en kontrole behandelings. Dit word dus duidelik dat vóór-plant grondberoking in 'n herplant situasie boomgroei verbeter in vergelyking met geen behandeling of ná-plant behandelings.

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

Project 1030 (2008 – 2021/2022) by Jan van Niekerk, Charles Stevens, Thembani Nxumalo and Elaine Basson (CRI)

##### Summary

In this project, in the 2021/22 period, further monitoring of tree height, stem diameter en nematode juvenile counts in the soil was done at a preplant fumigation site in the Kirkwood area, Eastern Cape. Results indicated clearly that four years after fumigation and tree planting, trees planted in fumigated soil had significantly thicker stems and were significantly taller compared to trees planted in non-fumigated soil. It was furthermore seen that citrus nematode juvenile counts as well as *Phytophthora* levels were significantly lower in fumigated soil. The beneficial effect of preplant fumigation at this stage is therefore clearly evident. Two potential new, unregistered nematicides were also evaluated at two trial sites, in the Western Cape and Mpumalanga. From results obtained it was seen that the *Paecilomyces lilacinus*-based biological control product has potential to be used in an additive capacity in combination with current chemical control options. It seems that it could provide more long-term control of nematodes but that its efficacy is possibly dependent on soil type. The new chemical nematicide, A22011B, also showed great promise in comparison with currently registered chemical nematicides. It was noted that regardless of soil type, one application of 0.67 ml/tree gave consistent results in the control of nematode females and juveniles, which compared favourably with current registered chemical nematicides.

##### Opsomming

In hierdie projek, in die 2021/22 periode, is verdere monitering van boomhoogte, stamdeursnit en nematode larwe tellings by 'n vóór-plant berokingsperseel in die Kirkwood-area, Oos-Kaap, gedoen. Resultate het duidelik getoon dat bome wat in berookte grond geplant is, vier jaar ná beroking en boomplanting, betekenisvol dikker stamme gehad het en betekenisvol langer was in vergelyking met bome wat in nie-berookte grond geplant is. Daar kon verder gesien word dat sitrus-aalwurmlarwe tellings, sowel as *Phytophthora* vlakke, betekenisvol laer in berookte grond was. Die voordelige effek van vóór-plant beroking is in hierdie stadium dus duidelik sigbaar. Twee potensiele nuwe, ongeregistreerde aalwurmdoders is ook by twee proefpersele, in die Wes-Kaap en Mpumalanga geëvalueer. Uit resultate wat verkry is, is gesien dat die *Paecilomyces lilacinus*-gebaseerde biologiese beheerprodukt potensiaal het om in 'n bykomende kapasiteit in kombinasie met huidige chemiese beheer-opsies gebruik te word. Dit blyk dat dit meer langtermynbeheer van die aalwurms kan verskaf, maar dat die doeltreffendheid daarvan moontlik van grondtipe afhanklik is. Die nuwe chemiese aalwurmdoder, A22011B, het ook groot belofte getoon in vergelyking met huidige geregistreerde chemiese aalwurmdoders. Daar is opgelet dat ongeag grondtipe, een toediening van 0.67 ml/boom konsekwente resultate gelewer het in die beheer van aalwurmwifies en -larwes, wat baie gunstig met huidige geregistreerde chemiese aalwurmdoders vergelyk het.

##### 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 orchards. Yield losses are estimated at about 10% worldwide. The citrus nematode is associated with poor growth of young citrus trees planted in infested orchards 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 (*Diospyros* 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 orchard management practices (Garabedian *et al.* 1984). Threshold values in South Africa have been set at 10 000 juveniles/250 cc soil and at 1000 females/10 g roots in samples.

*Tylenchulus 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 yield 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 nematodes 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 in the 20<sup>th</sup> century, and currently used 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, 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.

## **Objectives**

1. The development and evaluation of new or existing products for the control of soilborne pests and pathogens in citrus orchards.

## **Materials and methods**

*Evaluation of pre-plant soil fumigation of a replant soil on Phytophthora spp. and citrus nematode levels in soil, and on growth of young citrus trees*

In September 2017 an old Midnight Valencia on Carrizo citrange rootstock orchard was removed in the Kirkwood area, Eastern Cape. The aim was to replant immediately with Tango on Carrizo citrange trees. Prior to tree removal, soil and root samples were taken at 40 sites in the old orchard. These were analyzed at the CRI Diagnostic Centre (DC) in Nelspruit. The analyses indicated that the number of citrus nematode juveniles in the soil were on average 2068 per 250 cc soil. In the root samples on average 1532 female nematodes were present per 10 g roots. Both *Phytophthora citrophthora* and *P. nicotianae* were furthermore shown to be present in the orchard soil.

Soil preparation was done and rows pegged out. Certain rows were fumigated with a 60:40 chloropicrin: 1.3 dichloropropene mixture. The fumigation dosage was 60 g/m<sup>2</sup>. For evaluation purposes, in 2019, 10 trees were marked in the fumigated rows and 10 in the non-fumigated rows. In 2019, soil samples were taken at these trees for nematode and *Phytophthora* analyses at the CRI Diagnostic Centre (DC) in Nelspruit. Tree height and stem diameter was also measured. This evaluation was repeated in April 2021. The recorded data were subjected to statistical analyses. Analyses of variance (ANOVA) were done on stem diameter, tree height, percentage *Phytophthora* infested leaf discs and citrus nematode juvenile counts in soil data. This data was obtained from 10 trees planted in fumigated soil and 10 trees planted in non-fumigated (untreated) soil. Means were compared using Tukey's Test at a 90% confidence level.

*Evaluation of a Paecilomyces lilacinus-based biological control product for the control of the citrus nematode, Tylenchulus semipenetrans*

This product was evaluated at two sites in the 2021/22 season. The first site was located in the Jeppes Reef area, Mpumalanga. This site consisted of a navel orchard on C35 rootstock, planted in 2006. At the start of the trial, there were on average 1785 citrus nematode females per 10 g roots and 1222 juveniles per 250 cc soil. Trial treatments for this site are given in Table 4.3.11.1. Each treatment was replicated on eight trees that were split into four randomized blocks with two trees per treatment per block. The first applications were done in October 2021. Soil and root samples for nematode analyses were collected at this site in December 2021 and February 2022, with the final sampling due in May 2022.

The second site was in the Citrusdal area, Western Cape. The 2021/22 season constituted the second season of testing this product at this site. Within the trial orchard, a 43-year old Navel orange orchard with Rough lemon rootstock, the trial was shifted to trees not used in the 2020/21 season. This was to prevent possible effects from the previous season's trials on results of the 2021/22 season. Pre-trial sampling from the new trial trees indicated that on average 7687 citrus nematode females per 10 g roots were present and 4406 nematode juveniles per 250 cc soil. The treatments and application schedule for this trial are given in Table 4.3.11.2. The first applications were done in September 2021. Soil and root sampling at this site were done in November 2021 and January 2022, with a final sampling due in April 2022. Trial layout at this site was similar to the Mpumalanga layout described above.

At both sites, applications were done as a drench around the tree trunk, using a 10 L watering can. After each application, the products were washed into the soil profile with at least 35 mm irrigation.

Results obtained up to March 2022 are presented in this report.

**Table 4.3.11.1.** Treatment details and application schedule followed at the Jeppes Reef trial site, for evaluation of a *Paecilomyces lilacinus*-based biological control product for the control of the citrus nematode

Treatment program	Treatment	Oct	Nov	Dec	Jan	Feb	Mar
1	Untreated	None	None	None	None	None	None
2	Mytech @ 125 g/ha	MyTech	MyTech	MyTech	MyTech	MyTech	MyTech
3	Mytech @ 250 g/ha	MyTech	MyTech	MyTech	MyTech	MyTech	MyTech
4	Cadusafos (10 g/m <sup>2</sup> tree canopy)	Cadusafos					
5	Mytech (125g/ha) + Cadusafos (10 g/m <sup>2</sup> tree canopy)	Cadusafos	MyTech	MyTech	MyTech	MyTech	MyTech
6	Mytech (250g/ha) + Cadusafos (10 g/m <sup>2</sup> tree canopy)	Cadusafos	MyTech	MyTech	MyTech	MyTech	MyTech

**Table 4.3.11.2.** Treatment details and application schedule followed at the Citrusdal trial site, for evaluation of a *Paecilomyces lilacinus*-based biological control product for the control of the citrus nematode

Treatment program	Treatment	Sept	Oct	Nov	Dec	Jan	Feb
1	Untreated	None	None	None	None	None	None
2	Mytech @ 125 g/ha	MyTech	MyTech	MyTech	MyTech	MyTech	MyTech
3	Mytech @ 250 g/ha	MyTech	MyTech	MyTech	MyTech	MyTech	MyTech
4	Fenamiphos (10 ml/m <sup>2</sup> tree canopy)	Fenamiphos					
5	Mytech (125 g/ha) + fenamiphos (10 ml/m <sup>2</sup> tree canopy)	Fenamiphos	MyTech	MyTech	MyTech	MyTech	MyTech
6	Mytech (250 g/ha) + fenamiphos (10 ml/m <sup>2</sup> tree canopy)	Fenamiphos	MyTech	MyTech	MyTech	MyTech	MyTech

*Evaluation of A22011B for the control of the citrus nematode, Tylenchulus semipenetrans*

The product A22011B was provided to CRI by Syngenta to test on citrus for the control of citrus nematode. A trial was therefore initiated in October 2020 in Citrusdal, Western Cape. The site was the same as mentioned above. In the 2021/22 season this trial was repeated at another Citrusdal site, on a new set of trees. At the start of the trial, in September 2021 all trees included in the trial (seven trees per treatment) were sampled for nematode female and juvenile analyses from roots and soil. Results indicated that an average of 7478 nematode females per 10 g roots were present in the trial trees, along with an average of 7365 nematode juveniles per 250 cc soil. The evaluation of this product was also done in the 2021/22 season, at abovementioned Jeppes' Reef trial site. In the trial trees at this site, 2882 nematode females were present per 10 g roots and 1834 nematode juveniles per 250 cc soil.

The trial treatments as provided by Syngenta and followed at both sites, are presented in Table 4.3.11.3 below. Applications were done as a drench around the tree trunk, using a 10 L watering can. After each application, the products were washed into the soil profile with at least 35 mm irrigation. Soil and root samples for nematode analyses were collected 30 days after application (DAA) 1, 60 DAA1, 30 DAA2 and 60 DAA2.

**Table 4.3.11.3.** Treatments for the evaluation of the product A22011B for the control of the citrus nematode in the Citrusdal and Jeppes Reef areas in the 2021/22 season

	TREATMENT	Product / ha Per m <sup>2</sup>	Product (ml) per tree	Time of application
1	Untreated control	-	-	-
2	A22011B	444.4 ml	0.67 ml per tree	Drench in Sept/Oct
3	A22011B	555.5 ml	0.83 ml per tree	Drench in Sept/Oct
4	A22011B	1111.0 ml	1.67 ml per tree	Drench in Sept/Oct

5	A22011B	444.4 ml	0.67 ml per tree and 60 DAA1 0.67 ml per tree	Drench in Sept/Oct and 60 days after application 1; (60 DAA1)
6	A22011B	555.5 ml	0.83 ml per tree and 60 DAA1 0.83 ml per tree	Drench in Sept/Oct and 60 DAA1
7	Fluopyram	0.4125 ml/m <sup>2</sup>		Drench in Drench in Sept/Oct
8	Phenamiphos/Cadusafos	10 ml / m <sup>2</sup> basin area		Drench in Drench in Sept/Oct

## Results and discussion

### Task table

Objective / Milestone	Achievement
Apr –Jun 2021 1. Annual report 2. Nematode trial planning	1. Annual report was written and submitted. 2. Nematode trials were planned and products obtained.
Jul – Sept 2021 1. Commence with nematicide trials in Citrusdal, Western Cape.	1. Nematicide trials were started successfully.
Oct – Dec 2021 1. Commence with nematicide trials at the Jeppes Reef site, Mpumalanga 2. Continue with nematicide trials in the Western Cape as per trial protocol	1. Trials were started successfully 2. Trials were continued in Western Cape as per protocol.
Jan – Mar 2022 1. Evaluation of fumigation trial in Kirkwood 2. Data analysis 3. Continue with nematicide trials as per protocols	1. Trial was evaluated by measuring tree height and trunk diameter and doing nematode analysis from soil samples 2. Data analyses were done. 3. Nematicide trials were continued as per protocols.

### *Evaluation of pre-plant soil fumigation of a replant soil on Phytophthora spp. and citrus nematode levels in soil and growth of young citrus trees*

In 2019 the mean stem diameter of fumigated trees was significantly ( $P = 0.076$ ) more at 26.4 mm, versus the mean for the unfumigated trees of 22.6 mm. In 2021 this trend continued with the mean stem diameter of fumigated trees being significantly ( $P = 0.040$ ) greater at 53.9 mm compared to 49.3 mm of untreated trees (Table 4.3.11.6). Mean tree height of fumigated trees in 2019 was 120.7 cm, significantly ( $P = 0.035$ ) more than the 110.7 cm of the untreated (unfumigated) trees. In 2021, the mean tree height for the fumigated trees was 234.3 cm in comparison to the 226.7 cm mean for the unfumigated trees (Table 4.3.11.4).

In terms of juvenile nematode count, in 2019 soil samples of the fumigated trees had no nematodes (mean of 0.0) which was significantly ( $P = 0.023$ ) lower than the mean of 745.0 per 250 cc soil recorded for the unfumigated trees. In 2021 this trend continued with the mean juvenile count of the unfumigated trees increasing to 3040 per 250 cc soil, significantly more than the 170 recorded for the fumigated trees. The same effect of fumigation was observed for *Phytophthora nicotianae* infestation of soil. In 2019 the mean percentage infested discs was 17.1% for the untreated trees and markedly lower at 0.0% in the fumigated soil. In 2021 the mean percentage in the

unfumigated soil increased to 79.4%, significantly ( $P = 0.021$ ) more than the mean percentage of 25.4% of the fumigated trees (Table 4.3.11.4).

In this trial a 60:40 chloropicrin: 1.3 dichloropropene mixture was used to fumigate soil prior to planting. The results clearly indicate that 4 years after fumigation, the levels of nematodes and also *P. nicotianae* are lower in the treated soil than in the untreated soil. This corresponds to the reported efficacy of chloropicrin and 1.3 dichloropropene against oomycete pathogens and nematodes, respectively (Duniway, 2002; Ruzo, 2006).

**Table 4.3.11.4.** Mean<sup>1</sup> tree stem diameter (mm), tree height (cm), juvenile nematode counts and percentage *Phytophthora nicotianae* infested leaf discs recorded for fumigated and unfumigated trees in the Kirkwood area, Eastern Cape.

Treatment	Stem diameter (mm)		Tree height (cm)		Juvenile nematode counts (250 cc soil)		<i>Phytophthora nicotianae</i> (%)	
	2019	2021	2019	2021	2019	2021	2019	2021
Fumigated	26.4 a <sup>2</sup>	53.9 x	120.7 a	234.3 x	0.0 b	170.0 y	25.4 b	0.0 y
Unfumigated	22.6 b	49.3 y	110.7 b	226.7 y	745.0 a	3040.0 x	79.4 a	17.1 x
LSD	3.47	3.62	7.59	14.83	520.77	2673.0	43.88	21.43
<i>P</i> -value	0.076	0.040	0.035	0.387	0.023	0.079	0.021	0.186

<sup>1</sup>Means derived from 10 replicate trees per treatment.

<sup>2</sup>Means followed by the same letter in a column are not significantly different at a 90% confidence level.

*Evaluation of a Paecilomyces lilacinus (PL)-based biological control product for the control of the citrus nematode, Tylenchulus semipenetrans*

During the November 2021 sampling at the Citrusdal trial site, no significant ( $P = 0.435$ ) treatment effect on mean (eight replicate trees) citrus nematode female counts was noted. However, following a single PL application at 125 g/ha in September 2021, the mean number of females was reduced by 43% in comparison to the untreated control. At this sampling point the only other effective treatment was the single fenamiphos application that reduced the mean by 18% compared to the control. None of the other treatment programmes led to a reduction in mean number of females at this sampling time (Table 4.3.11.5). In terms of mean juvenile counts in November 2021, the fenamiphos treatment performed the best, resulting in a reduction of 57% compared to the untreated control. The second-best treatment was the PL (125 g/ha) application combined with a fenamiphos application. This treatment programme led to a juvenile count reduction of 33% compared to the untreated control. The third-best treatment programme was a combination of fenamiphos with 250 g/ha PL, reducing the mean juvenile counts by 26%. However, none of these reductions were significant despite the significant ( $P = 0.042$ ) treatment effect seen at this sampling point. It is furthermore likely that the success of the last two mentioned treatment programmes November 2021 was due to the fenamiphos treatment applied in September 2021 (Table 4.3.11.5).

At the January 2022 sampling, no treatment effect was seen for either mean female count ( $P = 0.367$ ) or mean juvenile count ( $P = 0.380$ ). In terms of mean female counts, the fenamiphos treatment was again the best, reducing the mean by 39% compared to the untreated control. At this sampling time the application of 125 g/ha PL led to a very slight reduction in female counts of 0.7%. Compared to this, the combination treatment of fenamiphos and 125 g/ha PL reduced the mean by 9%. None of the other treatments reduced the mean female counts at this sampling point (Table 4.3.11.5). At this sampling time all treatment programmes reduced the mean juvenile counts compared to the untreated control. The best programme was the fenamiphos application that reduced the mean count by 67%. This was followed by the combination treatments between PL and fenamiphos. The 125 g/ha rate combined with the fenamiphos reduced the mean count by 51%, followed by the 250 g/ha rate in combination with fenamiphos, which reduced the mean by 37% (Table 4.3.11.5).

From these results at the Citrusdal trial site, it is evident that fenamiphos on its own has the most consistent effect on citrus nematode female and juvenile counts in the sandy soil present at this site. The effect of the PL applications on their own took a while to have an effect on the mean counts. The effect was also better on the juvenile counts compared to the reduction seen in the female counts. This could be due to the motile nature of juvenile nematodes exposing them more to the effects of the biological agent, and also the biological agent taking time to colonize the soil environment under the trees. In sandy soil, using the PL-based treatments could have a better long-term effect on the juvenile counts and consequently also reduce female counts. If growers decide to use this product it would be advisable to start much earlier with treatments and not wait until mean juvenile numbers reach the 10000 per 250 cc soil threshold.

At the Jeppes Reef trial site, the soil was heavier than the sandy soil at the Citrusdal trial site. This could explain the difference in results obtained. At the December 2021 sampling time, the single cadusafos application in October 2021, had the biggest effect on the mean female counts obtained. For this treatment programme the reduction in female mean was 53% compared to the untreated control. Interestingly, both PL application rates reduced the mean female counts at this time point. The 125 g/ha rate after two applications reduced the mean female counts by 45% while two applications of the 250 g/ha rate reduced the counts by 14% (Table 4.3.11.6). The combination treatments did not reduce the mean female counts at this sampling time. Similar results were seen in terms of mean juvenile counts. The cadusafos application reduced the mean by 53% followed by the 250 g/ha PL application with a reduction of 44%, and the 125 g/ha PL application with a 2% reduction. Results from the February 2022 sampling indicated that only the 125 g/ha PL treatment programme reduced the mean female counts. The reduction observed was 70% compared to the untreated control. For the other treatment programmes an increase in mean counts of between 37% and 164% was observed. In the case of the February 2022 juvenile counts the 125 g/ha PL applications again had the best results, reducing the juvenile means by 70%. Here the

second-best treatment was the 125 g/ha PL combined with the cadusafos application that resulted in a 24% reduction (Table 4.3.11.6).

As mentioned above, at this trial site the soil had a heavier texture compared to the Citrusdal site. In this heavier soil the 125 g/ha rate PL application programme gave consistent results by reducing female and juvenile counts at both sampling times. It also showed a reduction in counts at the December 2021 sampling already, indicating that the PL might have a quicker effect in heavier soils. However, this needs to be confirmed with further testing as this trend was not seen for the combination treatment programme with cadusafos. The poor performance of the higher PL rate in February 2022 also needs to be investigated further. The poor performance of the cadusafos application at the second sampling time was surprising. This could indicate degradation of the active ingredient in heavier soils and that a second application is needed on the heavier soils.

**Table 4.3.11.5.** Mean<sup>1</sup> citrus nematode and juvenile counts recorded for various *Paecilomyces lilacinus* (PL) and fenamiphos-based treatment programmes, applied in the Citrusdal area on a 43-year old navel on rough lemon orchard, and sampled in November 2021 and January 2022.

Treatment	Female Nov 21 (10 g roots)	Juvenile Nov 21 (250 cc soil)	Female Jan 22 (10 g roots)	Juvenile Jan 22 (250 cc soil)
Untreated	6738 a <sup>3</sup>	5581 ab	5125 ab	4069 a
PL <sup>2</sup> @ 125 g/ha	3813 (-43) <sup>4</sup> a	6369 (14) ab	5088 (-0.7) ab	3906 (-4) a
PL @ 250 g/ha	9438 (40) a	10600 (90) a	8538 (67) a	3425 (-16) a
Fenamiphos (10 ml/m <sup>2</sup> tree canopy)	5500 (-18) a	2419 (-57) b	3125 (-39) b	1338 (-67) a
PL (125 g/ha) + fenamiphos (10 ml/m <sup>2</sup> tree canopy)	7791 (16) a	3713 (-33) b	4663 (-9) ab	1988 (-51) a
PL (250 g/ha) + fenamiphos (10 ml/m <sup>2</sup> tree canopy)	7663 (15) a	4119 (-26) b	7588 (48) ab	2563 (-37) a
<i>P</i> -value	0.435	0.042	0.367	0.380

<sup>1</sup>Means derived from samples taken from eight replicate trees per treatment

<sup>2</sup>PL = *Paecilomyces lilacinus*

<sup>3</sup>Means followed by the same letter in a column are not significantly different at a 95% confidence level.

<sup>4</sup>Percentage reduction in count in comparison to the untreated control.

**Table 4.3.11.6.** Mean<sup>1</sup> citrus nematode and juvenile counts recorded for various *Paecilomyces lilacinus* (PL) and cadusafos-based treatment programmes, applied in the Jeppes Reef area on a 16-year old navel on C35 orchard, and sampled in December 2021 and February 2022.

Treatment	Female Dec 21 (10 g roots)	Juvenile Dec 21 (250 cc soil)	Female Feb 22 (10 g roots)	Juvenile Feb 22 (250 cc soil)
Untreated	1138 xy <sup>3</sup>	744 y	1150 yz	1038 x
PL <sup>2</sup> @ 125 g/ha	625 (-45) <sup>4</sup> y	725 (-2) y	350 (-70) z	313 (-70) x
PL @ 250 g/ha	975 (-14) xy	413 (-44) y	3038 (164) x	1100 (6) x
Cadusafos (20 g/m <sup>2</sup> tree canopy)	538 (-53) y	350 (-53) y	2263 (97) xy	1169 (13) x
PL (125 g/ha) + cadusafos (20 g/m <sup>2</sup> tree canopy)	1713 (50) x	2669 (259) x	1575 (37) xyz	794 (-24) x
PL (250 g/ha) + cadusafos (20 g/m <sup>2</sup> tree canopy)	1763 (55) x	1225 (65) y	2125 (85) xyz	1113 (7) x
<i>P</i> -value	0.089	0.019	0.078	0.688

<sup>1</sup>Means derived from eight replicate trees per treatment

<sup>2</sup>PL = *Paecilomyces lilacinus*

<sup>3</sup>Means followed by the same letter in a column are not significantly different at a 95% confidence level.

<sup>4</sup>Percentage reduction in count in comparison to the untreated control.

#### *Evaluation of A22011B for the control of the citrus nematode, Tylenchulus semipenetrans*

Results obtained for the 2021/22 season at the Citrusdal trial site with the experimental A22011B nematicide were very promising. At the 30 days after application 1 (30DAA1) sampling time, all treatments reduced the mean female counts in comparison to the untreated control treatment. The best treatment was treatment 2 of a single application of A22011B in September 2021 at a rate of 0.67 ml/tree. This treatment reduced the mean counts by 61%. This was markedly ( $P = 0.642$ ) better than treatment 8, the standard fenamiphos treatment, that reduced the mean by 39%. It was also better than treatment 7, the fluopyram application in September 2021, which reduced the mean count by 47%. The remaining treatments (3 – 6) that included A22011B, reduced the mean female count at the 30DAA1 sampling time by between 16% and 46% (Table 4.3.11.7). At this time point, all treatments also reduced the mean juvenile counts compared to the untreated control. In this case the best treatment was 4, reducing the mean juvenile count by 79% and consisting of A22011B applied at a rate of 1.67 ml/tree in September 2021. This was followed by a 78% reduction by treatment 6. This treatment consisted of an application of 0.83 ml/tree in September 2021 and again 60DAA1, although the result is probably due to the first application of A22011B. Treatment 2 again performed well and reduced the mean juvenile count by 67%. In comparison the fluopyram application in September 2021 reduced 30DAA1 the mean juvenile count by 46% and the fenamiphos application at the same time caused a reduction of only 9% (Table 4.3.11.7).

At 60DAA1 it was again seen that all treatments reduced the citrus nematode female counts in comparison to the untreated control. At this time point the best treatment was number 8, the fenamiphos treatment that reduced the mean count by 70%. This was followed by treatment 4, a single application of A22011B applied in September 2021, which reduced the mean count by 62%. Third best was treatment 6, application of 0.83 ml/tree, which at that stage had only been applied once, but reduced the count by 54%. The remaining treatments reduced the mean by between 27% and 46% (Table 4.3.11.7). Juvenile analysis at this point indicated that the best treatment was treatment 4 that reduced the mean juvenile count by 96%. This was followed by treatment 7, the fluopyram application, that reduced the mean by 82%. Treatments 5 and 6 also performed well with reductions of 60% and 62%, respectively (Table 4.3.11.7).

At the third soil and root sampling, 30DAA2, in terms of mean female count, the best treatment was treatment 7, fluopyram applied in September 2021, which reduced the count by 52%. This was followed by treatment 2 with a reduction of 47% and the fenamiphos application, treatment 8, with a 52% reduction. In terms of mean juvenile count, all treatments reduced the counts in comparison to the untreated control. The best two treatments were 5 and 7, both with a reduction of 89%. Treatment 5 consisted of 0.67 ml/tree applied in September 2021 and again 60DAA1, while treatment 7 was a single fluopyram application in September 2021. Treatment 6 also performed very well with a reduction of 87%. This consisted of two applications of 0.83 ml/tree. Treatments 3 and 4 also reduced the mean counts by more than 60% compared to the untreated control (Table 4.3.11.7).

The last soil and root samples were collected 60DAA2. At this sampling time the best treatment was treatment 5 that reduced the mean female count by 64%. In this treatment A22011B was applied twice at 0.67 ml/tree. In terms of performance, treatment 5 was followed by treatment 7 (fluopyram in September 2021) with a 50% reduction, and treatment 8 (fenamiphos in September 2021) with a 36% reduction. At the last sampling time all treatments again reduced the mean juvenile counts. Here treatment 6 performed the best with a reduction of 83%. Treatments 5 and 7 both led to reductions of 71%. Reduction caused by the other treatments were all below 50% (Table 4.3.11.7).

In the soil at the Citrusdal trial treatment 2 performed consistently over the four sampling times. This treatment consisted by a single application of 0.67 ml/tree in spring (September) of 2021. In many cases it performed the same or better compared to the floupyram or fenamiphos applications. Treatment 6 also performed very well over the trial period. This treatment consisted of two applications of 0.83 ml/tree. The first application was applied in spring 2021 and then again 60DAA1. The good performance of this treatment during the last two sampling times was most likely due to the second application. The good results obtained by specifically the single application in treatment 2, could probably be attributed to the good distribution of the active in the sandy soil.

At the Jeppes Reef trial site at 30DAA1, in terms of mean female counts, the best treatment was treatment 2 with a reduction of 19%. This was followed by treatment 5 with a reduction of 17%. These were the only two treatments causing a reduction in female counts at this time point. However, in terms of mean juvenile counts at this time point, all treatments, except treatment 8 (cadusafos) reduced the mean counts in comparison with the untreated control. The best treatment was again treatment 2 causing a reduction of 77%. This was followed by treatments 3 to 7 that caused reductions of between 26% (fluopyram, treatment 7) and 76% (treatment 5) (Table 4.3.11.8).

Also, at 60DAA1, treatment 2 performed well with a reduction of 45% that was only slightly poorer than treatment 4 with a reduction of 46%. Both these treatments were furthermore slightly better than treatment 7 that caused a reduction of 44%. However, in terms of mean juvenile counts at this timepoint, treatments 3 to 7 performed extremely well with reductions of between 94% and 98% compared to the untreated control. Treatment 2 in this case caused a reduction of only 36%, while treatment 8 caused a 49% reduction (Table 4.3.11.8).

Thirty days after application 2 (30DAA2) results indicated that only treatments 8 (44% reduction), 3 (16% reduction) and 2 (11% reduction) reduced the mean female nematode counts. In terms of juvenile counts at this point, none of the treatments caused a reduction in mean juvenile counts (Table 4.3.11.8). However, the results from the 60DAA2 sampling painted a different picture. Here again treatment 2 performed the best with a 79% reduction in mean female count. All other treatments similarly caused a reduction in mean female counts. The reductions caused by these treatments varied between 46% and 75%. The juvenile count results also showed that all treatments reduced the mean juvenile counts compared to the control. Treatment 4 performed the best with a 97% reduction. This was followed by treatment 6 with an 84% reduction. Both treatments 2 and 5 caused a 77% reduction (Table 4.3.11.8).

Even at this trial site, with a heavier soil, treatment 2 performed remarkably well over the trial period. Other treatments containing the experimental nematicide performed well at certain sampling points but treatment 2 stood out as one of the best treatments at almost all the time points and in terms of female and juvenile counts. It would therefore seem that one application of 0.67 ml/tree would be sufficient in heavier soils to control the citrus nematode.

**Table 4.3.11.7.** Mean citrus nematode female and juvenile counts obtained after applications of either fenamiphos and fluopyram or the experimental nematicide, A22011B, applied according to different application programmes. Results obtained from root and soil samples taken 30 days after application 1 (30DAA1), 60DAA1, 30DAA2 and 60DAA2 from trees at the Citrusdal trial site for the 2021/22 season.

Treatment	Female 30DAA1 (10 g soil)	Juvenile 30DAA1 (250 cc soil)	Female 60DAA1 (10 g soil)	Juvenile 60DAA1 (250 cc soil)
1	12186 a <sup>1</sup>	9836 a	10443 a	5564 a
2	4757 (-61) <sup>2</sup> a	3268 (-67) a	5657 (-46) ab	5643 (1) a
3	10229 (-16) a	7086 (-28) a	7614 (-27) ab	5264 (-5) ab
4	6929 (-43) a	2050 (-79) a	3971 (-62) b	243 (-96) b
5	8557 (-30) a	7900 (-20) a	6700 (-36) ab	2229 (-60) ab
6	6614 (-46) a	2129 (-78) a	4800 (-54) b	2100 (-62) ab
7	6485 (-47) a	5307 (-46) a	7028 (-33) ab	1029 (-82) ab
8	7429 (-39) a	8971 (-9) a	3129 (-70) b	4364 (-22) ab
<i>P</i> -value	0.642	0.335	0.197	0.196

Treatment	Female 30DAA2 (10 g soil)	Juvenile 30DAA2 (250 cc soil)	Female 60DAA2 (10 g soil)	Juvenile 60DAA2 (250 cc soil)
1	8943 a	6107 a	5314 ab	2807 a
2	4714 (-47) a	3900 (-36) ab	5157 (-3) ab	2093 (-25) a
3	6157 (-31) a	2471 (-60) bc	5900 (11) ab	1614 (-43) a
4	9614 (7) a	1621 (-73) bc	6142 (15) a	2571 (-8) a
5	5986 (-33) a	657 (-89) c	1929 (-64) b	821 (-71) a
6	6071 (-32) a	786 (-87) bc	4371 (-18) ab	464 (-83) a
7	4314 (-52) a	643 (-89) c	2657 (-50) ab	821 (-71) a
8	5543 (-38) a	3050 (-50) abc	3400 (-36) ab	1514 (-47) a
<i>P</i> -value	0.575	0.010	0.362	0.845

<sup>1</sup>Means followed by the same letter in a column are not significantly different at a 95% confidence level.

<sup>2</sup>Percentage reduction in count in comparison to the untreated control.

**Table 4.3.11.8.** Mean citrus nematode female and juvenile counts obtained after applications of either fenamiphos and fluopyram or the experimental nematicide, A22011B, applied according to different application programmes. Results obtained from root and soil samples taken 30 days after application 1 (30DAA1), 60DAA1, 30DAA2 and 60DAA2 from trees at the Jeppes Reef trial site for the 2021/22 season.

Treatment	Female 30DAA1 (10 g soil)	Juvenile 30DAA1 (250 cc soil)	Female 60DAA1 (10 g soil)	Juvenile 60DAA1 (250 cc soil)
1	1429 y	1736 xy	1771 xy	1450 x
2	1157 (-19) y	407 (-77) y	971 (-45) y	933 (-36) y
3	1814 (27) y	1221 (-30) xy	1700 (-4) xy	57 (-96) y
4	1485 (4) y	507 (-71) y	957 (-46) y	43 (-97) y
5	1186 (-17) y	421 (-76) y	1514 (-15) xy	93 (-94) y
6	3900 (173) x	578 (-67) y	2386 (35) x	36 (-98) y
7	2071 (45) y	1279 (-26) xy	1000 (-44) y	29 (-98) y
8	1929 (35) y	2114 (22) x	1357 (-23) xy	743 (-49) xy
<i>P</i> -value	0.038	0.138	0.353	0.002

Treatment	Female 30DAA2 (10 g soil)	Juvenile 30DAA2 (250 cc soil)	Female 60DAA2 (10 g soil)	Juvenile 60DAA2 (250 cc soil)
1	1414 xy	121 y	3271 x	1514 x
2	1257 (-11) xy	336 (178) xy	700 (-79) b	343 (-77) xy
3	1186 (-16) xy	407 (236) xy	1757 (-46) b	471 (-69) xy
4	1743 (23) xy	571 (372) xy	843 (-74) b	43 (-97) y
5	2243 (59) x	629 (420) xy	814 (-75) b	343 (-77) xy
6	1429 (1) xy	1236 (921) x	1143 (-65) b	235 (-84) y
7	1443 (2) xy	364 (200) xy	929 (-72) b	1250 (-17) xy
8	786 (-44) y	21 (83) y	1057 (-68) b	857 (-43) xy
<i>P</i> -value	0.652	0.484	<0.0001	0.195

<sup>1</sup>Means followed by the same letter are not significantly different at a 95% confidence level.

<sup>2</sup>Percentage reduction in count in comparison to the untreated control.

## Conclusions

Results from the *Paecilomyces lilacinus* testing indicated that at the Citrusdal site, the fenamiphos treatment gave the most consistent results across the two samplings done to date. The effect of this chemical nematicide probably also explains why the two treatments where the *Paecilomyces lilacinus* product was combined with the chemical showed some effect. However, it would be interesting to see if the effect of the biological product becomes more prominent with increased time after fenamiphos application. In the end it could indicate that once the effects of the fenamiphos wear off, the biological agent would have colonized the rhizosphere area, giving an additive, more long-term control of citrus nematodes.

At the Jeppes Reef site it was interesting to note that from the first sampling in December 2021, the application of *Paecilomyces lilacinus* already led to a decrease in female and juvenile counts. This treatment also led to a reduction at the February 2022 sampling. This effect was interestingly not seen for the higher rate of application. It would therefore seem that the biological agent colonized the root zones faster at this trial site with a heavier soil type compared to the Citrusdal site where the agent took longer to have an effect. To this same end, in the two samplings to date, it seems that after the December sampling, the effect of cadusafos declined. This trend will hopefully be confirmed by results from the last sampling.

Based on the results to date it therefore seems that the efficacy of *Paecilomyces lilacinus* in the control of citrus nematode is affected by soil type and characteristics. However, it could still be a valuable long-term tool to support chemical nematicide applications.

Evaluation of the A22011B experimental nematicide indicated that this has potential for control of citrus nematodes. As stated above, even one application of 0.67 ml/tree gave very consistent results and compared favourably with the current registered chemicals fenamiphos, cadusafos and fluopyram.

## Technology transfer

Where appropriate, results from these tests will be communicated with growers using various platforms.

## Further research

Continue to search for alternative products and methods for the control of the citrus nematode and *Phytophthora* spp. in citrus orchards. Any reports of phytotoxic damage caused by existing applications to control *Phytophthora* on new cultivars will be investigated along with any new products to use in the citrus nursery industry for the control of soilborne pathogens.

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#### 4.3.12 **PROGRESS REPORT: Characterization and management of Valley Bushveld Citrus Decline** Project 1068 (2012/13 – 2022/23) by Jan van Niekerk (CRI) and Elodie Stempien (USPP)

##### **Summary**

The Valley Bushveld Citrus Decline syndrome has been studied extensively in the last five years. Characterization of the pathogens involved identification of several pathogens, including four new *Neocosmospora* species, associated with the internal root and trunk decay symptoms seen in declining trees. As these pathogens are known to infect predisposed hosts, predisposing biotic and abiotic factors were investigated. These investigations revealed that high soil pH and electrical conductivity (EC) probably plays a role in predisposing trees planted on Carrizo citrange rootstock, which are sensitive to high soil pH and EC. This could also explain why orchards planted to Rough lemon rootstock do not suffer from decline problems. Finding alternative rootstocks is therefore seen as a long-term solution to the decline syndrome. A rootstock trial, including 10 different rootstocks, was therefore planted in the Sunday's River Valley in March 2021. However, maintaining tree health in existing young orchards where the decline is expected to appear at some stage, is also important. Several treatment programs with chemicals and natural plant extract products were therefore tested and it was seen that a benomyl drench treatment or a foliar spray treatment with specific plant extract products, are able to maintain or improve tree health to the same level as trees grown in soil fumigated preplant. These treatments will therefore be evaluated further in the rootstock trial. In the end management of the Valley Bushveld decline will be a combination of specific rootstocks combined with treatments such as soil drench or foliar sprays. The bacterial communities in the roots of diseased and healthy trees were furthermore compared. It was determined that certain bacterial genera are only associated with diseased, declining trees. Their significance needs to be investigated, especially their interaction with the associated pathogens.

## Opsomming

“Valley Bushveld Citrus Decline” is in die laaste vyf jaar intensief bestudeer. Karakterisering van die patogene betrokke, het verskeie *Neocosmospora* spesies, insluitend vier nuwe spesies, identifiseer wat met interne wortel-en stamverrotting simptome geassosieer word. Omdat hierdie patogene bekend is om verswakte gashere te infekteer, is potensiële predisponerende biotiese en abiotiese faktore ondersoek. Hierdie ondersoek het aangedui dat grond pH en elektriese konduktiwiteit (EK) waarskynlik 'n rol speel om bome wat op Carrizo citrange onderstamme geplant is, te verswak en predisponeer. Hierdie onderstam is sensitief vir hoë grond pH en EK en dit kan ook verklaar hoekom boorde geplant met Growweskiisuurlemoen onderstam, nie probleme toon nie. 'n Alternatiewe onderstam word dus beskou as die langtermyn oplossing van die probleem. 'n Onderstam proef wat 10 verskillende onderstamme insluit is dus in Maart 2021 in die Sondagsriviervallei geplant. Die handhawing van boordgesondheid in bestaande jong boorde, waar die “decline” moontlik in die toekoms kan ontwikkel, is egter ook belangrik. Verskeie programme, gebaseer op chemiese en natuurlike plantekstrakprodukte, is dus getoets. Daar is bevind dat 'n benoemde grondbehandeling of 'n blaarbespuitingsprogram met spesifieke plantekstrakprodukte, boomgesondheid op dieselfde vlak as bome geplant in beroekte grond, kan hou. Hierdie behandelings sal dus verder in die onderstamproef getoets word. Op die uiteinde sal die bestuur van “Valley Bushveld Citrus Decline” 'n kombinasie van die gebruik van spesifieke onderstamme en boordbehandelings soos 'n grondtoediening of blaarbespuiting wees. Die bakteriese populasies in die wortels van siek en gesonde bome is ook vergelyk. Dit is gevind dat sekere bakterie genera slegs met siek bome geassosieer word. Hulle belangrikheid moet bestudeer word, veral die interaksie met die geassosieerde patogene.

### 4.3.13 **PROGRESS REPORT: Further validation and improvements of CRI-PhytRisk**

Project 1238 (2019/04 – 2024/03) by Providence Moyo and Paul Fourie (CRI)

#### Summary

CRI-PhytRisk is a CBS-risk management platform that provides decision support to citrus growers in CBS areas, to improve fungicide spray timing, and indicates CBS-risk on fruit destined for export, based on the past season's weather conditions and CBS infection predictions. The CBS models were again validated in a collaborative project with Brazilian researchers. Forecasts based on the predicted weather data from YR.no were much more aggressive than those predicted from measured weather data (iLeaf); this was attributed to much higher predicted than measured rainfall figures. The CRI-PhytRisk platform has been completely upgraded to newer, more efficient technology. Programming has also started to allow other weather data platforms to import data to PhytRisk. The *myPhytRisk* portal has been programmed and will allow CRI-PhytRisk users the opportunity to self-assess their orchards' CBS risk, based on spray records and modelled infection periods. The *myPhytRisk* portal will be launched in August 2022. CRI-PhytRisk reporting functionality in Power BI is being developed and will allow real-time and comprehensive reporting. These reports will also allow us to better calibrate the model thresholds, particularly those in the Botrytis models. It is proposed that CRI-PhytRisk continue as a research service project, with funding allocated for maintenance and continuous improvement.

#### Opsomming

CRI-PhytRisk is 'n CBS-risikobestuursplatform wat besluitnemingsondersteuning aan sitrus-produisente in CBS-gebiede bied, om beplanning van swamdodersspuite te verbeter, asook om CBS-risiko aan te dui op vrugte wat vir uitvoer bestem is, gebaseer op die afgelope seisoen se weerstoestand en CBS-infeksievoorspellings. Die CBS-modelle is weer bekragtig in 'n samewerkingsprojek met Brasiliaanse navorsers. Voorspellings gebaseer op die voorspelde weerdata van YR.no was baie meer aggressief as dié wat uit gemete weerdata voorspel is (iLeaf); dit is toegeskryf aan veel hoër voorspelde as gemete reënvallyfers. Die CRI-PhytRisk platform is heeltemal na nuwer, meer doeltreffende tegnologie opgegradeer. Programmering het ook begin om ander weerdataplatforms toe te laat om data na PhytRisk oor te dra. Die *myPhytRisk* portaal is geprogrammeer en sal CRI-PhytRisk gebruikers die geleentheid bied om self hul boorde se CBS-risiko te evalueer, gebaseer op spuitrekords en gemodelleerde infeksieperiodes. Die *myPhytRisk* portaal sal in Augustus 2022 bekend gestel word. CRI-PhytRisk verslagdoeningsfunktionaliteit in Power BI

word ontwikkel en sal intydse en omvattende verslagdoening moontlik maak. Hierdie verslae sal ons ook in staat stel om die modeldrempels beter te kalibreer, veral dié in die Botrytis-modelle. Daar word voorgestel dat CRI-PhytRisk voortgaan as 'n navorsingsdiensprojek, met befondsing wat vir instandhouding en voortdurende verbetering toegeken word.

#### 4.3.14 **PROGRESS REPORT: New oomycete fungicide-based approaches for managing *Phytophthora nicotianae* in citrus orchards**

Project number 1302 (April 2021 – March 2023) by L Reinecke, J Bisschoff, KN Bophela, TA Coutinho (UP) and JE van der Waals (CRI)

##### **Summary**

The sustainability of citrus production is threatened by *Phytophthora nicotianae*, the pathogen that causes root rot of infected plants, resulting in severe yield losses. Oomycides commonly applied to control this pathogen are phenylamides and phosphonates. However, there are reports of insensitivity development against these oomycides by the pathogen. New oomycides have been developed, but it is important to screen the South African *P. nicotianae* population for possible sources of insensitivity to these products. Four new active ingredients will be screened in this project, namely amisulbrom, ethaboxam, mandipropamid, and zoxamide. Biological control agents are also available for management of *P. nicotianae*, most of which contain species of *Trichoderma*. Three such products will be tested. Soil samples were collected from within the dripline of citrus trees in citrus orchards and nurseries, and *P. nicotianae* was isolated through baiting techniques for subsequent use in *in vitro* and *in vivo* trials. The *in vivo* trials will be done on rough lemon rootstock seedlings, which is used commonly in the citrus industry. The results from this study will provide an indication of the sensitivity of the South African *P. nicotianae* population from citrus to novel oomycides, as well as the efficacy of three biological control agents in suppressing this pathogen.

##### **Opsomming**

Die sitrusindustrie word bedreig deur *Phytophthora nicotianae*, 'n patogeen wat wortelvrot veroorsaak en tot groot verliese in opbrengs kan lei. Swamdoders wat algemeen aangewend word om die patogeen te beheer is fosfonate en fenielamiede, maar verslae dui aan dat daar 'n toename in verminderde sensitiwiteit is deur die patogeen teen hierdie swamdoders. Nuwe oomiseetdoders moet getoets word om te bepaal of hulle doeltreffend sal wees teen *P. nicotianae*. Vier nuwe aktiewe bestanddele sal getoets word teen *P. nicotianae*: amisulbrom, ethaboxam, mandipropamid, en soksamied. Biologiese beheermiddels is ook beskikbaar vir die moontlike beheer van *P. nicotianae*, waarvan baie uit die genus *Trichoderma* bestaan. Drie sulke produkte gaan in hierdie projek getoets word. Monsters was geneem van geïnfesteerde boorde en kwekerie en *P. nicotianae* was daarna geïsoleer vir gebruik in *in vitro* en *in vivo* proewe. Die *in vivo* proewe word op growweskil suurlemoen onderstamsaailinge gedoen aangesien dit algemeen in die industrie gebruik word. Die resultate van hierdie proewe sal 'n aanduiding gee van die doeltreffendheid van die nuwe swamdoders sowel as die biologiese beheermiddels en die bevindinge sal aan die industrie weergegee word.

#### 4.3.15 **PROGRESS REPORT: Effect of citrus rootstocks on phosphonate applications for *Phytophthora* disease management in citrus**

Project 1303 (2021/2 – 2022/3) by JM van Niekerk (CRI) and M Tobias (USPP)

##### **Summary**

The aim of this study was to investigate the effect of different rootstocks on phosphite translocation and persistence in trees after phosphonate foliar applications for *Phytophthora* disease control. In June 2021, Delta Valencia trees on either rough lemon (RL), Carrizo Citrange (CC) or X639 rootstocks, located at Letaba Estates, were sprayed with potassium phosphite at the registered dosage. For residue analyses, fruit samples were collected from sprayed trees before spraying, and 7, 14, 21, and 28 days after spraying. These same trees were again sprayed with potassium phosphite in October 2021 and roots were sampled for residue analyses at 7-day intervals up to 56 days after spraying. In Citrusdal, in July 2021, Delta Valencia trees on either RL or CC rootstocks were sprayed with the same chemical. Fruit was collected at the previously

mentioned timepoints for residue analyses and *Phytophthora nicotianae* inoculations. Results at Letaba Estates indicated that the phosphite residues in fruit from RL trees peaked faster after spraying, and to a higher level, than those in fruit from CC or X639 trees. At this site, root phosphite residues in RL also peaked earlier compared to CC and X639. In all three rootstocks the residues persisted in the roots for 56 days. However, residue levels in X639 roots were much lower compared to CC and RL. At the Citrusdal site phosphite residues in fruit from RL trees were also observed to increase faster and to a higher level compared to residues in fruit from CC trees. Good control of *Phytophthora* brown rot was therefore achieved much more rapidly on RL tree fruit. It was noted that a phosphite residue of 6 mg/kg fruit tissue was needed for brown rot control. It therefore seems that phosphite is translocated faster with less breakdown in RL trees compared to the other rootstocks in the study.

## Opsomming

Die doel van hierdie studie was om die effek van verskillende onderstamme op fosfiettranslokasie en volharding in bome, ná fosfonaatblaartoedienings vir *Phytophthora* siektebeheer, te ondersoek. In Junie 2021 is Delta Valencia bome op óf growwe suurlemoen (RL), Carrizo Citrange (CC) óf X639 onderstamme, geleë by Letaba Estates, met kaliumfosfiet teen die geregistreerde dosis gespuit. Vir residu-analises, is vrugmonsters van gespuite bome vóór bespuiting en 7, 14, 21 en 28 dae ná bespuiting, versamel. Hierdie selfde bome is weer in Oktober 2021 met kaliumfosfiet gespuit en wortelmonsters is met 7-dae-intervalle tot 56 dae ná bespuiting, vir residu-analises geneem. In Citrusdal, in Julie 2021 is Delta Valencia bome op óf RL óf CC onderstamme met dieselfde middel gespuit. Vrugte is op bogenoemde tye vir residu-analises en *Phytophthora nicotianae* inokulasies versamel. Resultate van Letaba Estates het aangetoon dat die fosfietresidu in vrugte van RL bome vinniger ná bespuiting gepeik het, en tot 'n hoër vlak, as in vrugte van CC of X639 bome. By hierdie perseel het wortelfosfietresidu in RL ook vroeër gepeik in vergelyking met CC en X639. In al drie onderstamme het die residu vir 56 dae in die wortels gebly. Residuvlakke in X639 wortels was egter baie laer in vergelyking met CC en RL. By Citrusdal is ook opgelet dat fosfietresidu in vrugte van RL bome vinniger en tot 'n hoër vlak toegeneem het in vergelyking met residu in vrugte van CC bome. Goeie beheer van *Phytophthora* bruinvrot is dus baie vinniger op RL boomvrugte behaal. Daar is opgemerk dat 'n fosfietresidu van 6 mg/kg vrugweefsel nodig is vir bruinvrotbeheer. Dit blyk dus dat fosfiet vinniger in RL bome getranslokeer word, met minder afbraak, in vergelyking met die ander onderstamme in die studie.

### 4.3.16 PROGRESS REPORT: Sources of *Phytophthora* spp. infestation in citrus nurseries

Project 1304 (2021/22 – 2022/23) by K N Bophela, T A Coutinho (UP) and J E van der Waals (CRI)

## Summary

*Phytophthora* root rot is one of the most devastating diseases in citrus production. Sporadic outbreaks of *Phytophthora* root rot are often reported in citrus nurseries. The occurrence of outbreaks can be attributed to several factors including the presence of *Phytophthora* propagules in growth media and/or irrigation water, over-seasoning inoculum present in asymptomatic nursery stock and wind-blown particles carrying inoculum. *Phytophthora nicotianae* is commonly found in nurseries of potted ornamental fruit trees and is widespread in South Africa. *Phytophthora citrophthora* is frequently isolated together with *P. nicotianae* and typically occurs in cooler production areas. These pathogens cause severe damage to citrus production. They can also produce large amounts of secondary inoculum in a single growing season. The frequent association of these pathogens with asymptomatic plants make their detection difficult. The main objective of this study was to identify novel sources of *Phytophthora* contamination in citrus nurseries. Surveys were conducted in 19 citrus nurseries and three orchards located in the Limpopo, Mpumalanga, North West, Eastern- and Western Cape provinces. Samples from water, soil, and potting media were collected for isolation of *Phytophthora* species. Samples were also collected from additional sources including offsite (truck, seedling trays/containers), production line (already filled seedling trays, sowing seeds, wheelbarrows, tyres, moving trays), workers (gloves, hands, footwear), and environmental (cement pad, rubbish dump, litter, moss). DNA was extracted from putative *Phytophthora* isolates using a CTAB DNA extraction protocol for species identification by ITS sequencing using oomycete specific ITS primers, namely ITS4 and ITS6. A total of 1355 soil samples, inclusive of potting media, and 101 water samples were collected from the nurseries and orchards, collectively. From the processed samples, 95 putative *Phytophthora* isolates have been recovered and identified microscopically,

and of the 28 putative isolates whose ITS was sequenced, three isolates were confirmed as *Phytophthora* spp. The other fungal genera recovered were *Fusarium* (12), *Phytophthora* (5), *Pythium* (6), *Neocosmospora* (1) and *Aspergillus* (1). To date, most putative *Phytophthora* isolates were recovered from potting media collected from asymptomatic potted citrus seedlings, indicating a potential contamination source in nurseries, although it is too early to state definitively. The picture may change as more samples are processed and putative *Phytophthora* isolates are identified molecularly.

## Opsomming

Phytophthora wortelvrot is een van die mees verwoestende siektes in sitrusproduksie. Sporadiese uitbrake van Phytophthora wortelvrot word dikwels in sitruskwekerie aangemeld. Die voorkoms van uitbrake kan toegeskryf word aan verskeie faktore, insluitend die teenwoordigheid van *Phytophthora* propagules in groeimedia en/of besproeiingswater, inokulum wat óór seisoene in asimptomatiese kwekerymateriaal teenwoordig is, en windgewaaide partikels wat inokulum dra. *Phytophthora nicotianae* word algemeen in kwekerie van gepotte ornamentele vrugtebome aangetref, en kom wydverspreid in Suid-Afrika voor. *Phytophthora citrophthora* word dikwels saam met *P. nicotianae* geïsoleer en kom tipies in koeler produksiegebiede voor. Hierdie patogene veroorsaak ernstige skade aan sitrusproduksie. Hulle kan ook groot hoeveelhede sekondêre inokulum in 'n enkele groeiseisoen produseer. Die gereelde assosiasie van hierdie patogene met asimptomatiese plante maak hul opsporing moeilik. Die hoofdoel van hierdie studie was om nuwe bronne van *Phytophthora*-kontaminasie in sitruskwekerie te identifiseer. Opnames is in 19 sitruskwekerie en drie boorde in die Limpopo, Mpumalanga, Noordwes, Oos- en Wes-Kaap provinsies gedoen. Water-, grond- en potmediummonsters is vir isolasie van *Phytophthora* spesies versamel. Monsters is ook vanaf addisionele bronne versamel, insluitend die perseel (vragmotor, saailinghouers), produksielyn (reeds gevulde saailinghouers, saaisaad, kruiswaens, bande, bewegende houers), werkers (handskoene, hande, skoene) en omgewing (sementblad, vullishoop, rommel, mos). DNS is uit moontlike *Phytophthora*-isolate geëkstraheer deur gebruik te maak van 'n CTAB DNS-ekstrasieprotokol vir spesie-identifikasie deur ITS-volgordebepaling, deur gebruik te maak van oömiseet-spesifieke ITS-inleiers, naamlik ITS4 en ITS6. Altesaam 1355 grondmonsters, insluitend potmedia, en 101 watermonsters is uit die kwekerie en boorde versamel. Van die verwerkte monsters is 95 moontlike *Phytophthora*-isolate herwin en mikroskopies geïdentifiseer, en van die 28 moontlike isolate waarvan die ITS-volgordebepaling gedoen is, is drie isolate as *Phytophthora* spp. bevestig. Die ander swamgenera wat herwin is, was *Fusarium* (12), *Phytophthora* (5), *Pythium* (6), *Neocosmospora* (1) en *Aspergillus* (1). Tot op hede is die meeste moontlike *Phytophthora*-isolate vanuit potmedia herwin wat vanuit asimptomatiese gepotte sitrussaailinge versamel is, wat op 'n potensiele besmettingsbron in kwekerie dui, alhoewel dit te vroeg is om definitief te verklaar. Die prentjie kan verander soos meer monsters verwerk word en moontlike *Phytophthora*-isolate molekulêr geïdentifiseer word.

### 4.3.17 PROGRESS REPORT: Phosphonate sensitivity of *Phytophthora nicotianae* in South African citrus orchards and nurseries

Project 1305 (April 2021 – March 2023) by E Theron, KN Bophela, TA Coutinho (UP), J E van der Waals and J van Niekerk (CRI)

## Summary

Fibrous root rot in citrus is caused by *Phytophthora nicotianae* and to a lesser extent by *Phytophthora citrophthora* in South Africa. Phosphonate fungicides have been effectively used for the management of this disease. Recently, reports of reduced sensitivity to these fungicides have emerged for *Phytophthora*. This project aims to characterise the *in vitro* and *in planta* phosphonate sensitivity of *P. nicotianae* isolates from South African citrus production areas. Additional aims include, investigating potential fitness costs associated with reduced sensitivity and searching for possible associations between different phosphonate exposure histories and the development of reduced sensitivity. For this, soil and feeder root samples were collected from citrus orchards and nurseries across the country. Soil baiting and plating on NARPH selective media, yielded pure *Phytophthora* isolates which were identified to species level by PCR-RFLP analysis of the ITS region. Of the 305 isolates obtained, 294 were identified as *P. nicotianae* and 10 as *P. citrophthora*. To determine the sensitivity of the *P. nicotianae* isolates to potassium phosphite (Phosphite 400SL, Villa Crop Protection) and ammonium phosphite (Brilliant SL, UPL), *in vitro* phosphonate sensitivity trials were conducted by an agar

dilution method. The isolates displayed a continuous range of sensitivity values, without any clear sensitivity groupings, that did not differ significantly between products. Results from these trials enabled the selection of the three least sensitive and three most sensitive isolates for use in subsequent *in planta* phosphonate sensitivity trials on rough lemon seedlings. Foliar applications of the above-mentioned phosphonates have commenced for these trials, whereafter inoculation will occur and subsequently, disease assessment. The fitness of these resistant and sensitive isolates will be compared *in vitro* and *in planta* to determine whether there are costs associated with reduced sensitivity and hence, whether resistance will remain or rescind in the absence of phosphonate exposure. Knowledge gained from this study will inform industry on the current efficacy of phosphonates for the management of *Phytophthora* diseases in citrus and facilitate informed resistance management strategies.

## Opsomming

Phytophthora wortelvrot word hoofsaaklik in Suid-Afrika veroorsaak deur *Phytophthora nicotianae* en soms deur *Phytophthora citrophthora*. Fosfonaat swamdoders is 'n effektiewe behandeling vir hierdie siekte, alhoewel onlangse bevindinge dui op moontlike verminderde sensitiviteit in sommige isolate. Die doel van hierdie projek is om die huidige *in vitro* en *in planta* fosfonaat sensitiviteit van *P. nicotianae* isolate vanuit sitrus boorde en kwekerye te bepaal ten einde vas te stel of die geregistreerde dosisse nog effektief is. Verder word daar ondersoek of verskillende fosfonaat blootstellings in die verlede 'n rol kan speel in die variasie in sensitiviteit van isolate, en of verminderde sensitiviteit geassosieer word met verminderde fiksheid in die afwesigheid van fosfonaat blootstelling. Grond- en -wortelmosters is versamel uit sitrusboorde en kwekerye van reg oor die land. Suiwer isolate van *Phytophthora* is verkry vanaf hierdie monsters nadat dit op NARPH selektiewe media uitgeplaat is. Die isolate is identifiseer deur middel van "ITS" volgordebepaling en "RFLP" analise. Proewe is uitgevoer om die *in vitro* fosfonaat sensitiviteit van die isolate teen kalium fosfiet (Phosphite 400SL van Villa Crop Protection) en ammonium fosfiet (Brilliant SL from UPL) te bepaal deur middel van 'n agar verdunnings metode. 'n Wye reeks van sensitiviteitswaardes is gevind vir die verskillende isolate, sonder enige duidelike groeperings of merkwaardige verskille tussen die twee produkte. Drie verminderde sensitiviteit en drie sensitiewe isolate is geselekteer om getoets te word vir fosfonaat sensitiviteit in potproewe op growweskil suurlemoen. Die eerste van drie blaartoedienings van die bogenoemde produkte is reeds voltooi. Na die derde toediening, gaan die plante inokuleer word en siekte assesserings gedoen word. Verminderse sensitiviteit en sensitiewe isolate gaan vergelyk word in terme van hul fiksheid in die afwesigheid van fosfonate om te bepaal of verminderde sensitiviteit voortduur onder hierdie omstandighede. Die kennis verkry deur hierdie studie, kan die sitrusbedryf inlig oor die huidige effektiwiteit van fosfonate teen *Phytophthora*, en help met die ontwikkeling van effektiewe strategieë teen verminderde sensitiviteit.

### 4.3.18 PROGRESS REPORT: Epidemiology and control of *Colletotrichum* species associated with anthracnose on citrus in South Africa

Project 1306 (2021/4 – 2024/3) by F. Halleen, L. Mostert (SU), P. Moyo and J. van Niekerk (CRI)

## Summary

*Colletotrichum* is one of the most important genera of plant pathogenic fungi, causing several diseases including citrus anthracnose. In recent years, postharvest anthracnose symptoms have become common on citrus fruit in production areas such as Citrusdal and Swellendam, in the Western Cape. In these areas this pathogen has become such a problem that it was listed as a research priority by growers. In the northern production areas, *Colletotrichum* is often isolated from citrus fruit when isolations are done for Citrus Black Spot. Anthracnose could become a serious limiting factor in these citrus production areas. Because of the potential negative impact that *Colletotrichum* infections may have on citrus production, new surveys are required to study inoculum sources (i.e. flowers, leaves, fruit and twigs) to identify which *Colletotrichum* species are associated with which citrus diseases in Southern Africa, to determine when fruit becomes infected and what the best chemical options would be to manage them. The current study therefore aims to provide new insight into the epidemiology of *Colletotrichum* species associated with anthracnose-like symptoms and to establish which registered fungicides are effective in managing these species. Between April 2021 and April 2022, 861 *Colletotrichum* isolates were obtained from leaf symptoms, dieback twigs, flowers, fruit, fruitlets and aborted fruit stems from various citrus varieties across the Western Cape Province. A collection of 45

*Colletotrichum* isolates, originating from the Eastern Cape, Limpopo, Mpumalanga and Zimbabwe, were also obtained from the culture collection of CRI. Preliminary identifications, based on beta-tubulin sequences only, grouped a subset of samples into four groups, namely *C. gloeosporioides* (69% of the samples), *C. karstii* (29%), *C. cigarro* (1%) and *C. novae-zelandiae* (1%). Fungal identifications will be finalised by Aug/Sept 2022 where after representative isolates of the identified species will be inoculated onto orchard fruit to determine when infections occur.

## Opsomming

*Colletotrichum* is een van die belangrikste swam plantpatogeengenera en veroorsaak verskeie siektes insluitende sitrus antraknose. Na-oes antraknose simptome op vrugte het die laaste paar jaar redelik algemeen voorgekom in produksie areas soos Citrusdal en Swellendam. Die siekte het in hierdie areas tot so 'n mate toegeneem dat dit as 'n navorsings prioriteit geïdentifiseer is deur produsente. In die noordelike produksie areas word *Colletotrichum* dikwels gevind wanneer daar vir sitrus swartvlek geïsoleer word. Antraknose kan 'n ernstige beperkende faktor word in hierdie produksie areas. Weens die potensiele negatiewe impak wat *Colletotrichum* infeksies op sitrus produksie kan hê, is nuwe opnames nodig om inokulum bronne te ondersoek (soos blomme, blare, vrugte en lootjies) om *Colletotrichum* spesies wat met sitrus siektes in Suid-Afrika assosieer word te identifiseer, om te bepaal wanneer vrug infeksies plaasvind en watter chemiese opsies die beste is vir beheer. Die doel van die huidige studie is om nuwe lig te werp op die epidemiologie van *Colletotrichum* spesies geassosieër met antraknose-tipe simptome en om vas te stel watter geregistreerde swamdoders hierdie spesies effektief beheer. Tussen April 2021 en April 2022, is 861 *Colletotrichum* isolate verkry vanuit blaarsimptome, terugsterf lootjies, blomme, vrugte, jong vrugte en vrugsteeltjies waar vrugte aborteer van verskeie sitrus varieteite regoor die Wes-Kaap produksie areas. 'n Versameling van 45 *Colletotrichum* isolate vanaf die Oos-Kaap, Limpopo, Mpumalanga en Zimbabwe is bekom van CRI se kultuurversameling. Voorlopige identifikasies baseer op beta-tubulin volgordes alleen groepeer 'n substel isolate in vier groepe, naamlik *C. gloeosporioides* (69% van die isolate), *C. karstii* (29%), *C. cigarro* (1%) en *C. novae-zelandiae* (1%). Swam identifikasies sal teen Aug/Sept 2022 afgehandel wees waarna verteenwoordigende isolate van die verskillende spesies op boord vrugte geïnkuleer sal word om te bepaal wanneer vrug infeksies plaasvind.

### 4.3.19 PROGRESS REPORT: Factors affecting results of citrus nursery *Phytophthora* testing

Project 1337 (2021/22) by Jan van Niekerk (CRI), Teagan Crament and Elodie Stempien (USPP)

## Summary

In this study, the effects of nursery media sample storage time, storage temperature or exposure to sunlight on *Phytophthora* analysis results, were investigated. Results indicated that if coir and Klasmann peat samples were stored at 4°C, the positivity percentage declined significantly with increasing storage time. When *Phytophthora nicotianae* infested samples were stored at 25°C or 30°C, the mean percentage of positive samples remained constant at nearly 100%. Results obtained from sand were disappointing and due to very low infestation levels in the sand, no meaningful conclusions could be made for this medium. The sun exposure experiment was conducted in winter and consequently, it was only when samples were exposed to sun for 240 minutes, that the mean percentage of positive samples declined. This experiment therefore needs to be repeated during summer. Comparison of the different testing techniques showed that the traditional soil baiting gave the most accurate results and that the plating of baiting liquid and species-specific PCR gave high a percentage of false negative results. The latter two methods are therefore not suitable for use as validation methods for the soil baiting method. From this study it is evident that when samples from nurseries are collected, they can safely be stored at 25°C if long term storage is required. However, when stored at 4°C, storage should not exceed 7 days. In future studies the sun exposure experiment should be repeated during summer months. The sensitivity of soil baiting furthermore also needs to be determined.

## Opsomming

In hierdie studie is die effekte van kwekerymediummonster opbergingstyd, opbergingstemperatuur of blootstelling aan sonlig op *Phytophthora* analise resultate ondersoek. Resultate het aangedui dat indien kokos-

en Klasmann-veenmonsters by 4°C gestoor is, die persentasie positiewe monsters betekenisvol met toenemende opbergingstyd afgeneem het. Wanneer *Phytophthora nicotianae* besmette monsters by 25°C of 30°C gestoor is, het die gemiddelde persentasie positiewe monsters konstant gebly op byna 100%. Resultate verkry uit sand was teleurstellend en weens baie lae besmettingsvlakke in die sand, kon geen sinvolle gevolgtrekkings vir hierdie medium gemaak word nie. Die sonblootstellingseksperiment is in die winter uitgevoer en gevolglik was dit eers wanneer monsters vir 240 minute aan die son blootgestel is, dat die gemiddelde persentasie positiewe monsters afgeneem het. Hierdie eksperiment moet dus gedurende die somer herhaal word. Vergelyking van die verskillende toetstegnieke het getoon dat die tradisionele grondlokaastegniek die mees akkurate resultate gelewer het en dat die uitplaat van lokaasvloeistof en spesie-spesifieke PKR 'n hoë persentasie vals negatiewe resultate gegee het. Laasgenoemde twee tegnieke is dus nie geskik vir gebruik as valideringsmetodes vir die grondlokaastegniek nie. Uit hierdie studie is dit duidelik dat wanneer monsters van kwekerye versamel word, dit veilig by 25°C gestoor kan word indien langtermyn-opberging vereis word. Wanneer dit egter by 4°C gestoor word, moet opberging nie 7 dae oorskry nie. In toekomstige studies moet die sonblootstellingseksperiment gedurende somermaande herhaal word. Die sensitiwiteit van die grondlokaastegniek moet verder ook bepaal word.

#### 4.3.20 PROGRESS REPORT: Further validation of the current CBS diagnostic protocols

Project 1343 (2021/04 – 2023/03) by Providence Moyo, Paul Fourie, Hano Maree, Rachelle Bester, Jan van Niekerk, Elma Carstens and Elaine Basson (CRI)

##### Summary

The efficacy of the Promega Wizard DNA Purification kit, currently used by the CRI-DC in CBS diagnosis to extract good quality DNA, was tested. The cycle threshold (Ct) values of the internal control real-time assay, targeting the citrus mitochondrial Cytochrome C oxidase sub unit I, were used to compare the efficacy of the current extraction kit to that of four silica membrane-spin column kits. The Zymo Fungal/Bacterial Microprep kit, Promega Wizard DNA Purification kit and Qiagen Mini-Plant kit produced equivalent DNA yield and purity, based on Ct values. The currently used kit will continue to be used because it is cheaper than the other two mentioned kits. High-throughput sequencing analyses were conducted on five *Phyllosticta citricarpa* DNA samples, to evaluate primer regions. The initial sequencing depth was not sufficient to evaluate primer regions and additional data was generated per sample to increase genome coverage. Evaluation of the qPCR primer set region currently used by the CRI-DC, revealed that this primer set has the potential to cross react with other fungi and therefore, the conventional PCR primer set was identified as the more reliable primer set.

##### Opsomming

Die doeltreffendheid van die *Promega Wizard DNA Purification kit*, wat tans deur die CRI-DC in CBS-diagnose gebruik word om goeie kwaliteit DNS te onttrek, is getoets. Die siklus drempelwaardes (Ct) van die interne beheer *real-time* toets, wat die sitrus mitokondriale Sitokroom C oksidase sub-eenheid I teiken, is gebruik om die doeltreffendheid van die huidige ekstraksiestel met dié van vier silika membraan-spin kolomstelle te vergelyk. Die *Zymo Fungal/Bacterial Microprep kit*, *Promega Wizard DNA Purification kit* en *Qiagen Mini-Plant kit* het ekwivalente DNS-opbrengs en suiwerheid geproduseer, gebaseer op Ct-waardes. Die stel wat tans gebruik word, sal steeds gebruik word omdat dit goedkoper is as die ander twee genoemde stelle. Hoë-deurset volgorde-bepaling-analises is op vyf *Phyllosticta citricarpa* DNS monsters uitgevoer, om inleier-areas te evalueer. Die aanvanklike volgorde-bepalingsdiepte was nie voldoende om inleier-areas te evalueer nie, en bykomende data is per monster gegenereer om genoomdekking te verhoog. Evaluering van die qPCR inleierstel-area wat tans deur die CRI-DC gebruik word, het aan die lig gebring dat hierdie inleierstel die potensiaal het om met ander swamme te kruisreageer, en daarom is die konvensionele PKR inleierstel as die meer betroubare inleierstel geïdentifiseer.

#### 4.3.21 PROGRESS REPORT: Sensitivity profiles of *Phyllosticta citricarpa* to quinone outside inhibitors and methyl benzimidazole carbamates

Project 1402 (2022/04 – 2024/03) by Providence Moyo, Thembanani Nxumalo and Paul Fourie (CRI)

##### Summary

Citrus black spot (CBS), caused by *Phyllosticta citricarpa*, is an economically important disease, which is effectively controlled by repeated fungicide applications to protect fruit from infection. Systemic fungicides such as methyl benzimidazole carbamates (MBCs) and strobilurins (quinone outside inhibitors, QoIs) are widely used to control CBS in South Africa, but the frequency of MBC resistance at farm level remains unclear. Resistance to QoIs by the CBS pathogen has yet to be detected. Nonetheless, *P. citricarpa* is classified as having a medium risk of developing resistance to fungicides and therefore, monitoring of QoI sensitivity is an extremely important part of resistance management strategies. Testing of *P. citricarpa* isolates using bioassays for sensitivity to benzimidazole and strobilurins (azoxystrobin, pyraclostrobin and trifloxystrobin) fungicides is currently ongoing.

## Opsomming

Sitruswartvlek (CBS), wat deur *Phyllosticta citricarpa* veroorsaak word, is 'n ekonomies belangrike siekte, wat effektief beheer word deur herhaalde swamdodertoedienings om vrugte teen infeksie te beskerm. Sistemiese swamdoders soos metielbensimidiasoolkarbamate (MBC's) en strobiluriene (kinoon buite-inhibeerders, QoI's) word wyd gebruik vir die beheer van CBS in Suid-Afrika, maar die frekwensie van MBC-weerstand op plaasvlak, bly onduidelik. Weerstand teen QoI's deur die CBS-patogeen, moet nog opgespoor word. Nietemin, *P. citricarpa* word geklassifiseer as medium-risiko om weerstand teen swamdoders te ontwikkel, en daarom is monitering van QoI-sensitiwiteit 'n uiters belangrike deel van weerstand bestuurstrategieë. Toetsing van *P. citricarpa* isolatemet behulp van biotoetse vir sensitiwiteit vir bensimidiasool en strobiluriene (azoksistrobien, piraklostrobien en trifloksistrobien) swamdoders, is tans aan die gang.

### 4.4 PROGRAMME: POSTHARVEST DISEASES

Programme coordinator: Wilma du Plooy (CRI)

#### 4.4.1 Programme summary

Project 123 (4.4.2) saw a total of eight newly proposed products evaluated in various postharvest applications. This included proposed replacements for an industry standard sanitising formulation of calcium hypochlorite, and GRAS-type formulations that, through their sanitation capabilities, can assist in sour rot incidence being limited. The most successful of the sanitising products was a new technology product, namely Zoono, a non-residue forming, novel application of a quaternary ammonium compound. Sani-D as an *in situ* blended product was effective as a sanitiser in the control of sour rot. All of these products still have to address regulatory hurdles before commercialisation in the citrus industry. The results obtained, however, do encourage further development of the actives. No ring tests were conducted, but the tests for resistance monitoring in collaboration with the Diagnostic Center were successful. The results from the *ad hoc* products tests were reported at the 2021 postharvest workshops.

In project 1250 (4.4.3) the study of possible alternatives to imazalil (IMZ) is still ongoing. The efficacy of the active places it as the foremost tool for the in controlling *Penicillium digitatum*. The application of alternative chemicals that are usually considered as resistance management strategies was concluded as far as stand-alone actives are concerned, with combinations of actives and combinations with soft chemicals currently being investigated. Currently registered postharvest actives are pyrimethanil (PYR), fludioxonil (FLU), 2-orthophenyl phenol (OPP) and thiabendazole (TBZ). Azoxystrobin (AZO) was registered as a postharvest fungicide in 2018, and has had limited use in packhouses since then. While none of these five actives are as effective as IMZ in stand-alone applications, combinations are proving to have excellent synergism. There are indications that single actives may have similar synergistic effects when combined with natural or GRAS products. All the tests are done on lemons, navels and Nadorcotts, at three different temperatures and four exposure times each.

Consequential to the study in project 1250, a complaint often received from industry, namely mandarins not responding to postharvest remedies, were confirmed. This prompted project 1325 (4.4.4) wherein the issue that mandarin (*Citrus reticulata*) apparently exhibits inconsistent reactions towards applied fungicides, are investigated. This study is therefore aimed at finding possible phytochemical links in the inconsistent fungicide behaviour on mandarin rinds. The first phase of this study investigates the mandarin rind phytochemistry and

morphological structure, as well as the chemical and biological activities of the rind extracts on three postharvest fungal pathogens. In a concurrent study, the second phase into the influence of different drying methods on the levels of secondary metabolites in colour break and ripe Clementines, are being determined. The drying methods used were solar, oven, air and freeze drying, with the highest levels of metabolites were identified through solar or oven drying. Air dry delivered the lowest levels. Characterization of the morphology and ultrastructure of the rind using electron microscopy is currently being done.

Stem-end saprophytes are creating increasing concerns regarding devaluation of export fruit as well as market access. Investigations in project 1326 (4.4.5) has indicated that wax application may assist to a point, but it fails to control the saprophytic growth more often than not. This happened more where there were delays in transit times and shipping. Similarly, to very low temperatures, it contributed to increased humidity, which in turn resulted in condensation. The additional free water on the woody stem-ends accommodates the establishment and growth of the saprophytes. Using exporters' reports and packhouse information, a direct correlation has been made between lowering of the postharvest residue limits to below what is scientifically proven to be effective and the incidence of the stem end saprophytes. A possible of contamination was investigated, namely the wooden pallet bases that have been problematic due to fungal soiling in the last few seasons. No clear correlation between the incidence of saprophytes on the pallets and the stems could be made. An additional concern is the possible contribution of netting and high humidity in the orchard to the increased prevalence of the saprophytes. This possibility still needs to be investigated. Novel formulations offering possible remedies to the problem, are included in the study.

With increasing pressure to reduce the use of postharvest chemicals, practices in postharvest handling and processing of citrus are being re-evaluated in project 1327 (4.4.6). With sour rot (due to *Galactomyces citri-aurantii*) is believed to originate from the orchard, increased attention is paid to near-harvest preventative action. To date three peroxyacetic acid (PAA) products were evaluated in near-harvest sprays on mandarins. Five trees with sprayed with each product, with 50 fruit inoculated per treatment. No phytotoxicity was observed in any of the treatments, however, the powder formulation was much easier to work with due to the astringent nature of the liquid formulations. The inoculation results were not significant, with low infection from the untreated control. The trial needs to be repeated with more fruit and more trees included per treatment, but focussing on natural infection only, since the inoculation needed to establish sour rot infection in the orchard, is impractical.

Newly developing regulations of chemical use on fresh fruit necessitates an integrated disease management approach. The aim of the study in project 1366 (4.4.7) is to evaluate essential oil volatiles for use in degreening chambers. Essential oil volatiles were tested *in vitro* against *Penicillium digitatum*- and *Galactomyces citri-aurantii*-growth. Cinnamon bark, oregano and thyme essential oil volatiles inhibited the fungal growth the most and were therefore selected to be tested against spore germination. In these trials, the pathogens were exposed to the essential oil volatiles for 72 h after which the filter paper was removed and the growth of the fungi monitored for an additional 11 days (total 2 weeks). Combining cinnamon (4 µl) with oregano (4 µl) inhibited spore germination by 91.00-94.39% and 100% for *P. digitatum* and *G. citri-aurantii*, respectively, whilst inhibiting sporulation of *P. digitatum*. Cinnamon bark and a combination of cinnamon bark with oregano was therefore selected to be tested *in vivo* in future work.

## **Programopsomming**

Agt nuut-voorgestelde produkte was getoets om hulle te evalueer in verskillende na-oes situasies. Dit het ingesluit aanwendings as saniteermiddels teen die industrie se standaard kalsiumhipochloriet en GRAS-tipe produkte wat, deur hulle saniteervermoë kan bydra om die voorkoms van suurvrot in toom te hou (4.4.2). Die mees suksesvolle saniteermiddel was 'n nie-residuvormende, nuwe formulاسie van 'n kwaternêre ammoniumverbinding. Die Sani-D *in situ* vermengingskombinasie het as 'n saniteermiddel goeie resultate in suurvrotbeheer gelewer. Al die produkte moet regulatories struikelblokke oorkom voordat hulle kommersieël beskikbaar gestel kan word. Die resultate van die produkte regverdig egter die verdere ontwikkeling van die produkte. Geen ringtoetse was gedoen nie.

In projek 1250 (4.4.3) is die studie na moontlike alternatiewe vir imasaliel (IMZ) steeds aan die gang. Die effektiwiteit van die aktief maak dat dit steeds die belangrike gereedskapstuk is in die beheer van *Penicillium digitatum*. Die aanwendings van chemikalieë wat tevore gesien was as opsies vir weerstandsbeheer is voltooi en sover dit die enkelstaande aktiewes betref, word hulle tans in kombinasies met mekaar en met GRAS chemikalieë getoets. Huidige na-oes fungisiedes wat registrasie het is imasaliel (IMZ), azoxystrobien (AZO), fludioksonil (FLU), ortofeniel fenaat (OPP), propikonasool (PPZ), and pyrimetaniël (PYR). Die aktiewes was in die fungisiedebad aangewend, maar geen enkel aktief kon die effektiwiteit van IMZ ewenaar nie. In kombinasies word daar egter baie goeie sinergismes gesien. Daar is aanduidings dat enkel aktiewes ook sinergistiese werking mag hê met natuurlike en GRAS chemikalieë. Al die toetse was gedoen met suurlemoene, nawels en Nadorkot sagte sitrus, by drie temperature en vier infeksietye.

Deur die werk wat gedoen is op projek 1250, was die geldigheid van klagtes rondom mandaryne wat nie na wense reageer op na-oesbehandelings nie, bevestig. Dit het gelei tot projek 1325 (4.4.4) waarin mandaryne se oënskynlike onvoorspelbare opname van siektebeheerprodukte, ondersoek word. Die studie is gemik daarop om 'n moontlike fitochemiese verband in hierdie ongelykmatige gedrag van die skil te vind. Die eerste fase van die projek behels 'n ondersoek na die skil fitochemie en die morfologie, sowel as die chemiese en biologiese aktiwiteit van die skilekstrakte op drie na-oespatogene. Die tweede fase van die projek word in 'n gelyktydige studie hanteer, waar die invloed van droogtegnieke op die skilekstrakte van kleurbreek en volryp Clementines ondersoek word. Son-, lug, oond- en vriesdroging was ondersoek. Die hoogste vlakke van metaboliete was verkry met son- en oonddroging. Lugdroging het die laagste opbrengs gelewer. Karakterisering van die morfologie en ultrastruktuur van die skil word met elektronmikroskopie gedoen.

Stingelentsaprofiete skep toenemend kommer weens probleme met waardevermindering van uitvoervrugte en marktoegang. Alhoewel suurlemoene meestal deur die probleem getref was, word dit deesdae op alle sitrustipes aangetref. Omsigtige studies in projek 1326 (4.4.5) wys daarop dat waksaanwending die probleem help verlig, maar dat dit gereeld faal in die beheer van die saprofiete. Dit gebeur veral waar daar verdragings in die vervoer- en verskepingstye was. Verder was daar 'n direkte korrelasie gemaak tussen die verlaging van na-oesfungisiedresidue tot vlakke onder die wetenskaplik bewysde effektiwiteitsvlakke en die voorkoms van die saprofiete. Dit is ook gekoppel aan verminderde gebruik van 2,4D en ontgroening. Palletbassisse met swamgroei soos in die in die laaste paar seisoene was ook ondersoek, maar geen duidelike korrelasie kon gemaak word tussen die voorkoms van die saprofiete op die pallette en die stingelente nie. Die bydrae van nette op die voorkoms van saprofiete op stingelente moet ondersoek word. Nuwe formulasies wat moontlike oplossings bied, word tans ondersoek.

Nuwe regulasies teen die gebruik van chemikalieë op vars vrugte maak die ondersoek na gintegreerde siektebestuur nodig. In projek 1366 (4.4.7) word vlugtige essensiële olies geëvalueer vir gebruik in ontgroeningskamers. Vlugtige essensiële olies is *in vitro* getoets teen *Penicillium digitatum*- en *Galactomyces citri-aurantii*-groei. Kaneelbas, oreganum en tiemie het die swamgroei die meeste gestrem en is dus gekies om teen spoorontkieming getoets te word. In hierdie proewe is die patogene vir 72 uur aan die vlugtige essensiële olie blootgestel waarna die filterpapier verwyder is en die groei van die swamme vir 'n addisionele 11 dae (totaal 2 weke) gemonitor is. Die kombinasie van kaneelolie (4 µl) met oreganumolie (4 µl) het spoorontkieming geïnhibeer met 91.00-94.39% en 100% vir *P. digitatum* en *G. citri-aurantii* onderskeidelik, terwyl sporulering van *P. digitatum* geïnhibeer was. Kaneelbas en 'n kombinasie van kaneelbas met oreganum is gekies vir *in vivo* toetse vir toekomstige werk.

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

Project 123 (Ongoing) by W du Plooy and L Mamba (CRI)

#### **Summary**

A total of eight newly proposed products were evaluated in various postharvest applications. This included proposed replacements for an industry standard sanitising formulation of calcium hypochlorite, and GRAS-type formulations that, through their sanitation capabilities, can assist in sour rot incidence being limited. The most

successful of the sanitising products was a new technology product, consisting of a non-residue forming, novel application of a quaternary ammonium compound. Sani-D as an *in situ* blended product was effective as a sanitiser in the control of sour rot. All of these products still have to address regulatory hurdles before commercialisation in the citrus industry. The results obtained, however, do encourage further development of the actives. No ring tests were conducted, but the tests for resistance monitoring in collaboration with the Diagnostic Center were successful.

## Opsomming

Agt nuut-voorgestelde produkte was getoets om hulle te evalueer in verskillende na-oes situasies. Dit het ingesluit aanwendings as saniteermiddels teen die industrie se standaard kalsiumhipochloriet en GRAS-tipe produkte wat, deur hulle saniteervermoeë kan bydra om die voorkoms van suurvrot in toom te hou. Die mees suksesvolle saniteermiddel was 'n nie-residuvormende, nuwe formulase van 'n kwaternêre ammoniumverbinding. Die Sani-D *in situ* vermengingskombinasie het as 'n saniteermiddel goeie resultate in suurvrotbeheer gelewer. Al die produkte moet regulatoriese struikelblokke oorkom voordat hulle kommersieël beskikbaar gestel kan word. Die resultate van die produkte regverdig egter die verdere ontwikkeling van die produkte. Geen ringtoetse was gedoen nie, maar die weerstandsmonitering in samewerking met die diagnostiese sentrum het voortgegaan.

## Introduction

This ongoing project offers an industry service to evaluate potential new postharvest disease control products or options, as well as to conduct ad hoc experiments. Products are mostly submitted from private companies on a voluntary basis, or projects/products are selected by the researchers involved. Given limited time and resources, requests are screened based on industry priorities.

## Objectives

1. Testing new potential products as fungicides, as well as evaluate possible synergistic reactions between chemicals, with specific focus on sour rot.
2. Evaluate available chemistries for use in the heated flooder – the effect of temperature, pH and exposure time, as well as combinations thereof to be evaluated.
3. Introduce and implement the application of GRAS chemicals and sanitizers into the citrus postharvest industry.
4. Analytical lab focus – ring test with the aim to reduce variability.
5. Assessment of fungicide resistance in citrus packhouses.
6. Technology transfer – primarily at the workshops and through collaboration with extension.

## Materials and methods

Trial protocol for curative control

1. *Ad hoc* tests are performed on products that may be of postharvest use to the citrus industry. Fresh, untreated fruit is collected from a reputable commercial packhouse, sanitised and stored at  $\approx 23$  °C for two days before the trial commences. The fruit is removed from cold storage and allowed to reach ambient temperature before use in the trial.
2. Each treatment has five replicates with 10 -12 fruit in each repeat, except where residue samples are collected, in which case the appropriate repeat has six extra fruit prepared for residue determination.
3. For all trials a sensitive strain of *Penicillium digitatum* (PD) and/or *Galactomyces citri-aurantii* (GCA) are used.
4. A  $10^6$ -spore suspension of PD, and a  $10^8$ -spore suspension GCA are prepared using the standard in-house laboratory technique.
5. Inoculation are done 6 hours prior to treatment.
6. Fruit are treated according to the label instructions of each of the various remedies that are being tested.
7. Lesions are evaluated 4-6 days after inoculation, once >80% of the untreated controls show positive lesion development.

#### Trial protocol for testing water sanitation products

1. Freshly picked, mature Valencia fruit were collected from a commercial packhouse, rinsed in chlorine (150 ppm total chlorine, pH 6 for 60 seconds), and allowed to air dry.
2. The fruit was stored at ~4°C for three days before the trial commenced. The day before the trial commenced the fruit was moved into ambient temperature (22°C) in order for fruit temperature to also reach ambient temperature, and to allow any possible condensation to evaporate.
3. Dip solutions were prepared in 5 L buckets, using tap water corrected to temperature immediately before the fruit was dipped, with a temperature of ~30°C. The temperature at which water enters the laboratory is about 20°C, and adjustments were made to closely resemble typical temperatures found in a postharvest packhouse.
4. Each treatment had 3 replicates with 10 fruit in each repeat.
5. A 5L *Penicillium digitatum* spore suspension, at a 10<sup>6</sup>-spore concentration, was prepared from a virulent, purified isolate harvested from fruit.
6. Control treatments were a standard chlorine treatment and clean water.
7. Products tested were added to the spore solution and agitated for 3 minutes before fruit dipping.
8. Fruits were inoculated by rolling a blunt 9 prong wounding tool over 2 sides of a fruit.
9. After wounding the fruit, they were submerged into the treatment solutions for 1 minute.
10. Fruit were removed and packed into lock back grape cartons, covered with transparent polyethylene bag with 4 holes to facilitate gaseous exchange and prevent build-up of CO<sub>2</sub> and ethylene.
11. The treatments were incubated until >80% decay is visible on the water controls.

#### Results

Objective / Milestone	Achievement
1. New potential products will be tested as sanitation agents and/or fungicides.	A total of eight products were tested, with some at two different concentrations. The results are available in the full reports attached herewith.
2. Introduce and implement the application of GRAS chemicals into the citrus postharvest industry	Products with GRAS actives were tested, with all of them being effective as water sanitizing options. An <i>in situ</i> blending product with actives derived from natural extracts in a commercial form were successful.
3. Assist CRI DC with packhouse resistance testing	Swabs are either collected by extensionists visiting packhouses, or sent to the DC. Although most do not indicate shifts in sensitivity, there were a number of serious concerns with 100% detection from the swabs sent to the DC. The implicated packhouses were consulted on remedial action, with one packhouse visited for closer inspection due to the widespread and serious loss of sensitivity from the sit.
4. Analytical lab focus – ring test with the aim to reduce variability	No ring tests were conducted in 2021.

#### Discussion

##### Alternative products for water sanitation

Sani-D and Sani-D Plus is a plant extract-based sanitizing product that is mixed *in situ* due to the loss of shelf life in a premix. The blended product gave excellent results in curbing sour rot in drench and dump water. Individually, each component has a low kill action. The second plant-extract was a fulvic acid blend called Exceed, which had a very good kill action of spores in the test solution. Two silver solutions were tested, with an unnamed nano-silver giving acceptable kill-action in spore suspensions, but the residue levels were questioned and needs to be resolved by the product owner. The second product (Plant Defense) failed at killing off spores in the test solution at both 0,5 and 1%. Two GRAS solutions (ATM and ADI Knapsack) both exhibited very good spore kill action in the test solution.

### Packhouse surface sanitation

Initially, Zoono was investigated as a pallet preservative, but was included as a sanitiser in the packhouse. The product excelled in controlling re-introduction of infective material on the packline. Similarly, G-Cide at different concentrations, mostly gave excellent cleaning action, but these products do not have a prolonged action period and needs to be reapplied regularly. The 20% solution was less effective than the 10% solution, due to formulation particulars. The owners of the molecule are working on a nano-model to improve the long-term action of the products. G-Cide leaves no residue, as it is reduced to water and non-discernible carbohydrates. Supershot Citrus, a surface sanitiser, was tested, but the results were disappointing in terms of the spore-kill-action within 1 min. The surface cleaning action of the product was excellent, and follow-up swabs on the surfaces revealed very low spore-counts, indicating that the product needs longer exposure time to contaminated surfaces for it to be effective. The product is already widely applied as a crate wash aid.

### Resistance monitoring

Swabs from actively working packhouses were tested regularly throughout the season. More incidences of sensitivity shift in pathogen resistance was detected than in previous years. This is due to the constant lowering of the effective fungicide dosages used, which results in a shift in sensitivity towards the actives. The packhouses concerned were consulted about the issue, with the packhouses where 100% of the swabs were found to represent loss of sensitivity, visited. Suitable measures to curb this problem was suggested in cooperation with the extensionists.

### **Technology transfer**

The work done in 2021 was presented at the online Packhouse Workshop in 2022 and was received very well.

### **Further objectives (milestones) and work plan**

1. New potential products will be tested as sanitation agents and/or fungicides; this specifically include seeking actives for the control of sour rot
2. Introduce and implement the application of GRAS chemicals into the citrus postharvest industry
3. Seek effective products and technologies for water sanitation in citrus packhouses
4. Analytical lab focus – ring test with the aim to reduce variability
5. Assist CRI DC with packhouse resistance testing if required.

### **APPENDIX A**

*The following is an example of the standardised reports that are shared with the various product owners*

#### **TRIAL: Sanitation of water used in aqueous application in citrus packhouses**

PRODUCT: Various chemicals tested  
BY: Dr Wilma du Plooy, Lindokuhle Mamba and Thabang Mgwenya  
DATE OF TRIAL: 2022\_05\_23 (e.g.)

#### **Objectives**

Water sanitizers are believed to enable more effective action of the fungicides and other products used against postharvest pathogens in the packhouse. Although *Penicillium digitatum* (PD) is the most prevalent of the two major postharvest pathogens, there are enough proof that several current remedies will control the organism. Subsequently, it was decided to use *Galactomyces citri-aurantii* as test organism, due to the fact that no effective alternative measures are available as yet.

**Crop:** Lemons and Valencia

**Origin:** Mbombela District

**Trial site:** CRI, 2 Baker Street, Nelspruit, 1200

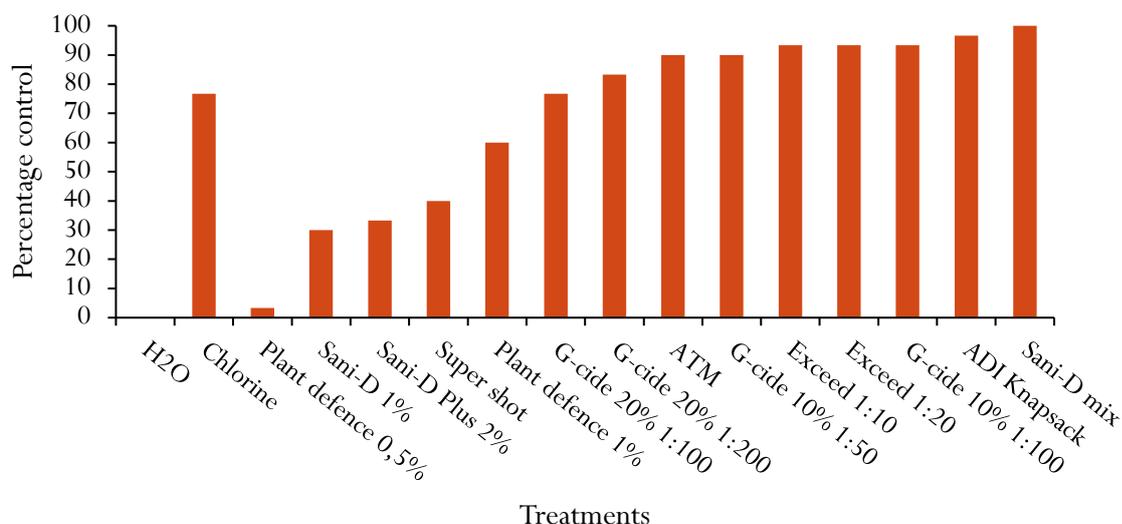
## Materials and Methods

1. Freshly picked, mature Valencia fruit were collected from a commercial packhouse, rinsed in chlorine (150 ppm total chlorine, pH 6 for 90 seconds), and allowed to air dry.
2. The fruit was stored at  $\sim 8^{\circ}\text{C}$  for three days before the trial commenced. The day before the trial commenced, the fruit was moved into ambient temperature ( $22^{\circ}\text{C}$ ) in order for fruit temperature to also reach ambient, and to allow any possible condensation to evaporate.
3. pH adjustments were made to get to a pH of 6.5.
4. Solutions were prepared with tap water and the temperature corrected immediately before the fruit was dipped ( $\sim 30^{\circ}\text{C}$ ). The temperature at which water enters the laboratory is about  $20^{\circ}\text{C}$ , and adjustments were made to more closely resemble typical temperatures found in a postharvest packhouse.
5. Each treatment had 3 replicates with 10 fruit in each repeat.
6. A *Galactomyces citri-aurantii* spore suspension was prepared from a virulent, purified isolate harvested from fruit, at a concentration of  $10^6$  spores/ml.
7. Buckets with 50 L of a 2% Peroxil solution were seeded with 50 ml of abovementioned *Galactomyces citri-aurantii* suspension to get to a final spore count of  $\sim 10^4$ .
8. Control treatments were a standard 150 ppm chlorine solution (FREXUS Chlorine) and clean water to which spore suspensions were added.
9. The solutions were left for 3 minutes to react with the spores.
10. Immediately before treatment, fruit were wounded using a metal plate with 9 x 7mm spikes that were rolled across the cheek section of the fruit, on two sides.
11. Injured fruit was placed in the sanitiser solution with the spores, and left in the solution for 3 minutes while slightly agitating.
12. Treated fruit were removed and dried before placing the 10 fruit from individual replicates in a nectarine liner to separate fruit during incubation. The liners are placed in an open top carton, slipped into a transparent polyethylene bag and closed. The bags were punctured four times on the sides to allow gaseous exchange and prevent excessive moisture build-up.

## Results and Discussion

All the products were compared to a commercially available calcium hypochlorite formulation (Frexus). Most of the products gave curative control of  $> 80\%$ . The continued presence of infection is due to the high inoculum used, which increases the disease pressure as a result of the many visible wounds and prolonged incubation. This is done in order to challenge both the trial product and the standard currently in use.

### Water sanitation



**Graph 1.** Evaluation of different sanitising options against postharvest spread of sour rot spores in aqueous applications on the packline.

## Conclusion

Several products were shown to have excellent potential as aqueous sanitisers and further development and registration should be pursued by the individual product owners.

The following disclaimer is used on all reports generated in the postharvest research group:

### DISCLAIMER

This report contains information and results from a confidential trial to determine any combination of product efficacy and/or compatibility and/or phytotoxicity. Results from this trial is indicative of the potential of a product only. By conducting these trials the CRI is assisting the citrus industry in finding postharvest sanitation and disease control options. Any successful interaction still requires that the necessary accreditations be acquired through the relevant regulatory body (Act 5 or Act 36), and does not imply any product endorsement by the CRI.

THIS REPORT MAY NOT BE USED IN ANY WAY AS PART OF THE MARKETING MATERIAL OF THE PRODUCT OWNER OR DISTRIBUTORS.

#### 4.4.3 PROGRESS REPORT: Optimising available alternative postharvest remedies as replacement for imazalil use on citrus exported to Europe

Project 1250 (2019/20 – 2021/22) by W du Plooy, L Mamba, and P H Fourie (CRI)

### Summary

Combinations of currently registered fungicides against *Penicillium digitatum* were evaluated in 2021, each included in a combination using the registered label dose. The actives included in the trial were imazalil (control), azoxystrobin (AZO), fludioxonil (FLU), orthophenyl phenate (OPP), propiconazole (PPZ), and pyrimethanil (PYR). The actives were applied in a drench, with lemon, orange and soft citrus varieties tested in terms of both curative control and sporulation inhibition. The combination of actives known to have potentially improved efficacy due to synergistic effects, are being explored. Two registered combinations, namely PPZ + PYR (Propirly®), and AZO and FLU (Evolve®) are available. These registered products were included the trials to be able to draw correlations between all the combinations tested. The effect of exposure time and temperature on green mould curative control and sporulation was investigated. Lemon, navel and soft citrus fruit were inoculated with a sensitive *Penicillium digitatum* isolate. Fruit were dipped for 1 minute in aqueous fungicide solutions within 8, 12, 18 and 24 hours after inoculation. Solution temperatures were 25°C, 35°C, and 45°C. In terms of disease control, none of the combinations tested were able to give 100% sporulation inhibition (vs IMZ control), but were significantly more successful than the stand-alone actives. The 2022 studies will focus on finding more effective combinations of the currently registered actives. An attempt will also be made to combine some of these actives with GRAS chemicals. It is believed that the synergistic effects of some of the combined actives will be emulated in terms of curative and anti-sporulation testing with alternative compounds as well. No final conclusion about the specific efficacy of the fungicide combinations can be made at this stage. However, they were all more effective than standalone fungicides across all temperatures and infection times.

### Opsomming

Kombinasies van fungisied aktiewe wat huidiglik geregistreer is teen *Penicillium digitatum* was in 2021 geëvalueer, met elkeen ingesluit teen die geregistreerde dosis soos aangedui op die etiket. Die aktiewes wat ingesluit was, was imasaliel (kontrole), azoxystrobien (AZO), fludioksonil (FLU), ortofeniel fenaat (OPP), propikonasool (PPZ), and pyrimetaniel (PYR). Die aktiewes was in die stortbad aangewend, met suurlemoen, soetlemoen en sagte sitrus variëteite wat getoets was in terme van kuratiewe beheer en sporulasieonderdrukking. Die kombinerings van aktiewes wat bekend is daarvoor dat dit sinergistiese werking het, word spesifiek ondersoek. Twee geregistreerde kombinasies, naamlik PPZ + PYR (Propirly®), en AZO + FLU (Evolve®) is beskikbaar. Hierdie geregistreerde produkte was ingesluit ten einde korrelasies te kon trek tussen al die kombinasies wat getoets was. Die effek van blootstellingstyd en temperatuur op die kuratiewe

beheer en sporulasieonderdrukking van groenskimmel was ondersoek. Suurlemoen, soetlemoen en sagte sitrus vrugte was met 'n sensitiewe isolaat van *Penicillium digitatum* geïnkuleer. Die vrugte was vir 1 min in 'n waterige fungisiedoplossing gedompel binne 8, 12, 18 and 24 uur ná inokulasie. Die oplossingstemperatuur was 25°C, 35°C, 45°C. In terme van siektebeheer kon nie een van die kombinasies 100% sporulasie onderdrukking (vs IMZ kontrole) gee nie, maar was dit wel noemenswaardig beter as die alleenstaande produkte. Die 2022 studies fokus op die ondersoek van meer effektiewe kombinerings van die huidige geregistreerde aktiewes. Daar sal ook gekyk word na kombinerings van hierdie aktiewes met sogenaamde GRAS chemikalieë. Dit word aanvaar dat die sinergisme van alleenstaande aktiewes herhaal sal kan word in terme van kuratiewe werking en sporulasieonderdrukking. Geen finale afleidings kan tans gemaak word oor die effektiwiteit van die kombinasies nie. Hulle was egter almal meer effektief as die alleenstaande aktiewes oor al die blootstellingstye en temperatuurreekse heen.

#### 4.4.4 PROGRESS REPORT: Investigating rind aspects of mandarins, an important commercial citrus type, which affects efficacy of chemical application during postharvest treatments

Project 1325 by J Hakizimana, Q Kritzinger (UP), W Augustyn (TUT) and W du Plooy (CRI)

##### Summary

Postharvest diseases caused by fungal pathogens contribute to major losses in the citrus industry. Synthetic fungicides have typically been used in disease control. However, mandarin (*Citrus reticulata*) in particular have exhibited different reactions towards applied fungicides. The first phase of this study investigated the mandarin rind phytochemistry and morphological structure, as well as the chemical and biological activities of the rind extracts on three postharvest fungal pathogens. Acetone, hexane and methanol were as solvents used to obtain crude rind extracts from mandarin. Methanol extracts showed the highest yield of these extracts. Thin layer chromatography (TLC) separated chemical content resulting in different retention factor (*R<sub>f</sub>*) values as an indication of various secondary metabolites. The *in vitro* antifungal activity of the rind extracts was evaluated against the pathogens using the minimum inhibitory concentration (MIC) assays. The mandarin rind extract seemed to promote the spore germination and fungal growth for all three isolates. In the concurrent second phase of the study, the influence of different drying methods on the levels of secondary metabolites in colour break and ripe Clementines was determined. The drying methods used were solar, oven, air and freeze drying. Peels were removed from the fruit and dried to constant mass, ground to a fine powder and sieved through a 250 µm sieve. A mass of 1.00 g was extracted with methanol: acetone: water (7:7:1), by ultrasonication for 30 min. Extract metabolic profiles were analysed using high pressure reversed phase liquid chromatography (HPLC). The profiles of the fruit at the different stages were very similar, but higher levels of compounds were found in the fully developed (ripe) extracts. The two major peak areas occur at retention time 3.797 and 18.857 min. Characterization of the morphology and ultrastructure of the rind using electron microscopy is currently being done.

##### Opsomming

Na-oessiektes as gevolg van swampatogene lewer 'n noemenswaardige bydrae tot verliese in die sitrusindustrie. Sintetiese fungisiede word tipies gebruik in siektebeheer. Ongelukkig toon mandaryntipes (*Citrus reticulata*) in besonder, afwykende reaksies teenoor die fungisied wat aangewend word. In die eerste fase van hierdie projek word mandarynse fitochemie sowel as die morfologiese struktuur, ondersoek. Dit sluit in die chemiese en biologiese aktiwiteit van die skilstrakte op die na-oespatogene. Asetoon, heksaan en metanol was as oplosmiddels gebruik om growwe ekstrakte uit die skil te verkry. Metanol ekstrakte het die hoogste opbrengs van die drie oplosmiddels gehad. Die chemiese inhoud is met dunlaagchromatografie (TLC) geskei, met die retensie faktor (*R<sub>f</sub>*) waardes 'n aanduiding van verskeie metaboliete. Die *in vitro* antifungus aktiwiteit van die skilstrakte teen die patogene was geëvalueer deur middel van minimum inhiberingskonsentrasie toetse (MIC). Die mandaryn skilstrakte lyk asof dit spoorkieming en fungusgroei van al drie patogene aanwakker. In die gelyktydige tweede fase van die studie was die effek van verskillende drogingstegnieke op die metabolietvlakke van kleurbreek en volryp Clementine skille ondersoek. Die drogingsmetodes was son-, oond-, lug en vriesdroging. Skille was versamel en gedroog tot 'n konstante massa, waana dit gemaal was tot 'n fyn poeier en deur 'n 250 µm sif gesit is. Een gram materiaal was vir 30 minute ultrasonies geëkstraheer met methanol:asetoon:water (7:7:1). Die metaboliese profiele van die ekstrakte

was met behulp van hoë druk omgekeerde fase vloeistofekstraksie (HPLC) bepaal. Die profiele van die verbindings in die vrugte by die verskillende fases het ooreengestem, maar was teenwoordig in hoër vlakke in die volryp ekstrakte. Die twee hoofpieke het by retensietye van 3.797 and 18.857 min uitgekome. Karakterisering van die morfologie en ultrastruktuur van die skille word tans met elektronmikroskopie gedoen.

#### **4.4.5 PROGRESS REPORT: Increasing occurrence of saprophytic stem end growth - investigating the causes and species involved**

Project 1326 (2021/22-23) by W du Plooy, L Mamba and E Basson (CRI)

##### **Summary**

Stem-end saprophytes are creating increasing concerns regarding devaluation of export fruit and market access. Although lemons were most often prone to stem-end mould, saprophytes recently occurred on all citrus types. Careful investigation indicated that wax application may assist, but it fails to control the saprophytic growth more often than not. This happened more where there were delays in transit times and shipping. Like with low temperatures, these situations contribute to increased humidity, which in turn result in increased condensation on the fruit. The additional free water on the woody stem-ends accommodates the establishment and growth of the saprophytes. Using exporters' reports and packhouse information, a direct correlation has been made between lowering of the postharvest residue limits to below what is scientifically proven to be effective and the incidence of the stem end saprophytes. It also seems to be related to omission of 2,4D and degreening. The possibility of contamination was investigated, namely the wooden pallet bases that have been problematic due to fungal soiling in the last few seasons. No clear correlation between the incidence of saprophytes on the pallets and the stems could be made. An additional concern is the possible contribution of netting and high humidity in the orchard to the increased prevalence of saprophytes. This possibility still needs to be investigated. Two novel molecules and a GRAS chemical will be applied in various tests: Preharvest applications as a single application at one and two weeks before harvest, also a double application at one and two weeks before harvest. Postharvest products that are compatible with postharvest fungicides are being investigated. Products with lipophobic qualities and that can be applied in the drench have been included in the trials. A moisture chamber has been constructed, but with no final result as saprophytic growth has not been simulated successfully.

##### **Opsomming**

Stingelentsaprofiete skep toenemend kommer weens probleme met waardevermindering van uitvoervrugte en marktoegang. Alhoewel suurlemoene meestal deur die probleem getref was, word dit deesdae op alle sitrustipes aangetref. Omsigtige studies wys daarop dat waksaanwending die probleem help verlig, maar dat dit gereeld faal in die beheer van die saprofiete. Dit gebeur veral waar daar vertraging in die vervoer- en verskepingstye was. Soos met lae temperature, dra hierdie situasies by tot hoër humiditeit wat dan weer lei tot verhoogde kondensasie op die vrugte. Die addisionele vrywater op die houtagtige stingelente maak die vestiging en groei van die saprofiete moontlik. Deur die inligting van uitvoerders en pakhuisse te gebruik, was daar 'n direkte korrelasie gemaak tussen die verlaging van na-oesfungisiedresidue tot vlakke onder die wetenskaplik bewysde effektiwiteitsvlakke en die voorkoms van die saprofiete. Dit is ook gekoppel aan verminderde gebruik van 2,4D en ontgroening. Die moontlikheid van kontaminasie vanaf palletbassisse met swamgroei in die laaste paar seisoene was ook ondersoek. Daar kon geen duidelike korrelasie gemaak word tussen die voorkoms van die saprofiete op die pallette en die stingelente nie. 'n Verdere bekommernis is die moontlike bydrae van nette en verhoogde humiditeit op die voorkoms van saprofiete in die boorde. Hierdie aspek moet nog ondersoek word. Twee nuwe molekules en 'n GRAS chemikalie sal in verskillende proewe gebruik word in vooroes toepassings: enkel- sowel as dubbele toepassings (beide toepassings gedoen een en twee weke vòr oes). Vir na-oes word produkte wat verenigbaar is met die geregistreerde fungisiede, ondersoek. Produkte wat lipofobiese eienskappe het en wat die stortbad gebruik kan word, word ingesluit. 'n Vogkamer was gebou, maar die ontwikkeling van die saprofiete kon nie gesimuleer word nie en geen finale resultaat is beskikbaar nie.

#### 4.4.6 PROGRESS REPORT: Management of postharvest diseases in the near-harvest period

Project 1327 by L Mamba and W du Plooy (CRI)

##### Summary

With increasing pressure to reduce the use of postharvest chemicals, practices in postharvest handling and processing of citrus are being re-evaluated. With sour rot (due to *Galactomyces citri-aurantii*) believed to originate from the orchard, increased attention is paid to near-harvest preventative action. To this end, the use of postharvest sanitisers in the orchard during the near-harvest period is being investigated. To date three peroxyacetic acid (PAA) products were evaluated: two liquid formulations and a buffered, powder formulation. Mandarin orchards in the Larten area were used, with a high-volume spray application done at colour break, one week before harvest. Five trees with sprayed with each product, leaving three trees between each treatment as a buffer to eliminate the effect of spray drift. In order to determine if there was successful control, an attempt was made to inoculate fruit with sour rot while still on the tree, using a  $10^4$ -spore suspension of *Galactomyces citri-aurantii*. Fifty fruit were inoculated per treatment, using picture hooks each with 5 mm long nails as wounding instruments. Two wounds were made per fruit: one on each side of the fruit in an equatorial position. Control trees were inoculated, but left untreated. No phytotoxicity was observed in any of the treatments, however, the powder formulation was much easier to work with due to the astringent nature of the liquid formulations. The inoculation results were not significant, with low infection from the untreated control. The trial needs to be repeated with more fruit and more trees included per treatment, but focussing on natural infection only, since the inoculation needed to establish sour rot infection in the orchard, is impractical.

##### Opsomming

Met toenemende druk om die gebruik van na-oes chemikalië te verminder, word die praktyke in die na-oes hantering en prosessering van sitrus heroorweeg. Die vermoede bestaan dat suurvrot (*Galactomyces citri-aurantii*) in die boord ontstaan, met gevolglike aandag wat dus gegee word aan voorkomende aksies in die naby-oes tydperk. Drie peroksieasynsuur (PAA) produkte was ondersoek: twee vloeistof formulasies en een gebufferde poeier formulasie. Mandarynboorde in die Larten omgewing was gebruik om 'n hoë volume spuitprogram te toets tydens kleurbreek, een week voor oes. Vyf bome per produk was gespuit, met drie bome oopgelaat tussen elke behandeling om as 'n buffer teen oordraging van spore te dien. Ten einde te bepaal of daar effektiewe beheer was, was 50 vrugte aan elke proefboom met suurvrot geïnkuleer met 'n  $10^4$  spoorsuspensie van *Galactomyces citri-aurantii*. Portrethakkies met drie 5 mm lang spykertjies elk, was gebruik om mee te inokuleer. Die kontrole bome was geïnkuleer, maar nie behandel nie. Geen fitotoksisiteit was in enige van die behandelings opgemerk nie, maar die poeierformulasie was makliker om mee te werk weens die vlugtige suurgehalte van die vloeibare formulasies. Die resultate van die inokulaieproewe was nie noemenswaardig verskillend van dié van die kontroles nie. Die proewe moet herhaal word met meer vrugte en meer bome per behandeling, maar slegs gefokus op natuurlike infeksie, aangesien die boord inokulasies om suurvrot te vestig, onprakties is.

#### 4.4.7 PROGRESS REPORT: Evaluation of essential oil volatiles in degreening chambers for the management of *Penicillium* decay and Sour rot

Project 1366 (2021/7 - 2022/4) by M van Dyk (CRI)

##### Summary

With the newly developing stringent regulations of chemical use on fresh fruit intended for European exports, an integrated disease management approach involving steps within the degreening chambers may be necessary to further reduce the loss of fruit due to decay. The aim of this study is to evaluate essential oil volatiles for use in degreening chambers for the management of *Penicillium*-decay and Sour rot. The volatiles of 15 essential oils were tested *in vitro* against *Penicillium digitatum*- and *Galactomyces citri-aurantii*-growth. For these studies, essential oils were applied at 2, 4, 8 and 16  $\mu$ l to filter paper that was attached to the inside of the lid of 90-mm Petri dishes containing 20 ml PDA, while *P. digitatum* and *G. citri-aurantii* plugs were applied to the PDA medium. Cinnamon bark, Oregano and Thyme essential oil volatiles inhibited the fungal growth the most and were therefore selected to be tested against spore germination. In these trials, the

pathogens were exposed to the essential oil volatiles for 72 h after which the filter paper was removed and the growth of the fungi monitored for an additional 11 days (total 2 weeks). Cinnamon bark (8 µl) essential oils inhibited spore germination by 98.60-100% and 98.69-100% for *P. digitatum* and *G. citri-aurantii*, respectively, over the 2-week period. However, sporulation of *P. digitatum* occurred within 7 days in all plates where spores had germinated. Thyme (4 µl) and oregano (4 µl) on the other hand inhibited sporulation of the newly formed colonies, but with a lower efficacy against spore germination. Combining Cinnamon (4 µl) with Oregano (4 µl) inhibited spore germination by 91.00-94.39% and 100% for *P. digitatum* and *G. citri-aurantii*, respectively, whilst inhibiting sporulation of *P. digitatum*. Cinnamon bark and a combination of cinnamon bark with oregano was therefore selected to be tested *in vivo*.

## Opsomming

Met die nuut ontwikkelde streng regulasies van chemiekalië gebruik op vars vrugte wat vir Europese uitvoere bedoel is, moet 'n meer geïntegreerde siektebestuursbenadering toegepas word. Dit mag stappe binne die ontgroeningkamers behels, om die verlies van vrugte as gevolg van na-oes siekte te verminder. Die doel van hierdie studie is om vlugtige essensiële olies te evalueer vir gebruik in ontgroeningkamers vir die bestuur van Groenskimmel en Suurvrot. Die vlugtige stowwe van 15 essensiële olies is *in vitro* getoets teen *Penicillium digitatum*- en *Galactomyces citri-aurantii*-groei. Vir hierdie studies is essensiële olies teen 2, 4, 8 en 16 µl toegedien op filtreerpapier wat aan die binnekant van die deksel van 90-mm Petri-bakkies wat 20 ml PDA bevat het, terwyl *P. digitatum* en *G. citri-aurantii* propies op die PDA-medium gedek was. Vlugtige essensiële olies van kaneelbas, oregano en tiemie het die swamgroei die meeste gestrem en is dus gekies om teen spoorontkieming getoets te word. In hierdie proewe is die patogene vir 72 uur aan die vlugtige essensiële olie blootgestel waarna die filtreerpapier verwyder is en die groei van die swamme vir 'n addisionele 11 dae (totaal 2 weke) gemonitor is. Kaneelbas (8 µl) essensiële olie het spoorontkieming met 98.60-100% en 98.69-100% vir *P. digitatum* en *G. citri-aurantii* onderskeidelik geïnhibeer oor die 2-week tydperk. Sporulering van *P. digitatum* het egter binne 7 dae plaasgevind in alle plate waarin die spore ontkiem het. Tiemie (4 µl) en oreganum (4 µl) aan die ander kant het sporulering van die nuutgevormde kolonies geïnhibeer, maar met 'n laer doeltreffendheid teen spoorontkieming. Die kombinasie van Kaneel (4 µl) met oreganum (4 µl) het spoorontkieming met 91.00-94.39% en 100% vir *P. digitatum* en *G. citri-aurantii* onderskeidelik geïnhibeer, terwyl sporulering van *P. digitatum* geïnhibeer was. Kaneelbas en 'n kombinasie van kaneelbas met oreganum is gekies vir *in vivo* toetse.

### 4.5 **PROGRAMME: HLB (Huanglongbing)** Programme coordinator: Hano Maree (CRI)

#### 4.5.1 **Programme summary**

The HLB-RP was established in April 2021 to increase research capacity on the HLB pathosystem. The programme facilitates interdisciplinary research to cover different facets of this complex disease through coordinated research activities across research portfolios engaging actively with the Extension and Biosecurity divisions within CRI. The HLB-RP provides a focal point that enables the coordination and long-term planning of research activities to address the industry needs for the challenges that Greening and HLB brings.

In this summary updates are provided for projects that sort directly in the HLB-RP as well as projects in other programmes that deal with aspects of the HLB pathosystem that might also be reported on in their respective programmes. The HLB-RP consists of short-, medium- and long-term projects. Short-term projects tend to focus on basic detection and identification assays that forms the cornerstone of our monitoring efforts for Greening and HLB and their vectors. Medium to long-term projects tackle the more complex task of disease and vector characterisation and investigate current and future disease management strategies. The long-term project evaluates HLB resistant planting material for performance under South African conditions.

#### Pathogen

CRI Projects: 1265, 1322, 1346, 1380, 1381

#### Diagnostics

A Loop-mediated isothermal amplification (LAMP) detection assays were developed in Project 1265 (4.5.3). These assays target the *nrdB* gene region of CLAs and CLaf and were shown to be specific with no cross-amplification occurring with the CLAs assay with any of the other known citrus-infecting Liberibacters, whereas the CLaf assay did additionally amplify CLafCI bv citrus. These LAMP assays were further shown to be as sensitive as the SYBR real-time PCR assay developed by CRI and the ARC targeting the same gene region, but less sensitive than published real-time PCR assays targeting the 16S gene region of citrus-infecting Liberibacters. Furthermore, a crude extraction method for field application was tested and initial results were promising, but the system requires further validation before implementation. We are aiming to field test these assays in the proposed Buffeljags River greening project (1407). A new project (1380) started in 2022 that will build on this work by converting it to a CRISPR detection assay that will increase the sensitivity and provide options for point of care devices.

To inform detection assays accurate sequence data is required that captures the genetic diversity of the target pathogens. In project 1322 (4.5.9) Liberibacter samples are sequenced by high-throughput sequencing (HTS) to gather genome information that can be used to further optimize detection assays. The DNA of 20 samples (4 CLaf CIs: 2 Uganda, 1 Kenya, 1 Knysna and 16 CLafs: 1 Uganda, 15 SA) were sent for HTS on the Ion Torrent platform. A CLaf genome was generated for each of the 20 samples using a reference read mapping strategy. Success of genome construction was dependent on liberibacter concentration and a genome coverage range of 15%-99% was obtained. More data were generated for samples with low genome coverage. The constructed genomes are currently being evaluated for diversity with a specific focus on the regions in the genome used for the current detection assays. Other regions will be evaluated for the design/optimisation of a new validation assay.

#### *Disease characterisation*

As new information is generated, and new technology is developed we can further characterise different aspects of HLB. In project 1346 (4.5.10) the influence of the co-infection of CLaf and CTV will be investigated. This is of particular interest to countries like South Africa that employs a CTV mild strain cross-protection strategy. Plant material (seedlings) have been generated and will be graft infected with CLaf, CTV or CLaf/CTV when they have grown to the appropriate size.

In a new project 1381 (started 2022) an experimental orchard was established for long term Greening disease characterisation. 400 Nadorcott trees were established in pots under insect proof conditions and graft inoculated with CLaf April 2022.

#### Vector

CRI Projects: 1160, 1148, 1255, 1315, 1348, 1362, 1396

#### *Identification*

Project 1255 (4.5.7) aims to distinguishing *Diaphorina* spp. and *Trioza* spp. from other psylloids that may also be captured on yellow sticky traps. The taxonomy of psylloids in the Afrotropical region has been largely ignored and require that we develop our own morphological keys and barcodes for identification of indigenous species. Collected specimens from the field surveys were identified to the closest possible genus and/or species using an unpublished taxonomic key prepared by collaborator Dr Daniel Burckhardt. Specimen collection is continuing in and around citrus environments in Limpopo, Mpumalanga, and Eastern Cape provinces. Indigenous host plants were confirmed for *Diaphorina* species collected. Indigenous *Trioza* sp.(16) and *Diaphorina* sp. (36) including a *D. citri* lookalike have been DNA barcoded. Barcodes of *Diaphorina* specimens grouped closer to *Diaphorina lycii* rather than to *D. citri* or *Diaphorina communis* sequences available on GenBank. A barcode sequence for a *Diaphorina* specimen, collected in Colchester, Eastern Cape and morphologically a lookalike to *D. citri*, showed that the specimen was **not *D. citri***, but an indigenous lookalike occurring on the indigenous tree *Harpephyllum caffrum* commonly known as African Plum tree. The triozaid specimens were morphologically identified as either *Trioza erytrae* or *Trioza litseae* or *Trioza* sp. (*Litseae*-group) and were barcoded. Nucleotide sequence diversity was observed between these specimens and sequences broadly grouped into 5 clusters. Thus far, 'Candidatus Liberibacter africanus' (CLaf) was detected in specimens of two clusters.

#### *Control - Chemical*

New systemic insecticides were evaluated in project 1148 (4.5.5) in preparation for the arrival of *Diaphorina citri* in South Africa. The brown citrus aphid *Aphis citricidus* was used as an indicator pest for screening systemic insecticides. The registered imidacloprid drench and recently registered acephate (Spectra Stem) stem treatment gave promising results. Results from an unregistered product, used as a soil drench for vegetables, were disappointing and a higher dosage will be required. However, another unregistered systemic used on citrus in the USA gave promising results as a soil drench. A field trial against cotton aphid on young citrus was attempted but populations were sporadic and results variable. A contained experiment using a *D. citri* culture on potted citrus plants in an insect-proof structure in SE Kenya in collaboration with ICIPE, using the same set of chemicals was attempted. The *D. citri* culture was, however inadequate for trials, despite mitigation steps like adding *Murraya koenigii* plants to the culture. P. Nderitu did conduct a survey of the citrus production areas in Kenya in search of *D. citri* and possible HLB infections in hot regions. Of the 18 sites visited, *D. citri* was only recovered from Lungalunga in SE Kenya close to the Tanzania border.

#### *Control - Biotechnological*

Project 1160 (4.5.2) was a proof of principle project completed in 2022. The project aimed to establish a suite of CTV infectious clones with a range of 'payloads' that have the potential to slow the spread, or eradicate, HLB in Southern African orchards. Four candidate genes, *arginine kinase*, *abnormal wing disc*, *chitin synthase* and *ran GTPase*, were selected as potential RNAi targets. Three candidate clones were successfully constructed, two *Trioza erytreae* insect-specific (all four genes of *T. erytreae*) and one abnormal wing disc gene-specific (one gene of both *T. erytreae* and *Diaphorina citri*). The replication competency and the production of small RNAs were evaluated in *Nicotiana benthamiana* and served as a proof of principle for the successful delivery of RNAi constructs targeted at the insect vectors within plants. Inoculation into Mexican lime was attempted for the *T. erytreae* specific CTV VIGS vectors. If these citrus plants test positive for CTV, the efficacy of the CTV RNAi strategy can potentially be tested on a *Trioza* colony as part of project 1315 (4.5.8).

#### *Monitoring*

Detection and surveillance of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) and *Trioza erytreae* (Del Guercio) (Hemiptera: Triozidae) are essential in our efforts to prepare for a possible HLB incursion. Current techniques are however ineffective at low pest density, require skilled labour and are time consuming. In project 1315 (4.5.8) novel monitoring and controls tools for suppression of the vector spread are being investigated. In this project odour-based attractants will be evaluated as well as an automated vision-based system for identification of *T. erytreae* and *D. citri* on traps. The efficacy of the CTV RNAi constructs (from project 1160) to suppress a psyllid population will also be evaluated. The first objective is behind schedule due to unforeseeable circumstances. Considerable progress was however made with the second objective. Sticky traps have been inspected for psyllids, photographed, and the images annotated and sent to collaborators in Florida to develop and train artificial intelligence algorithms that identify psyllids. The system is producing promising results for *T. erytreae* identification but more sticky trap samples are being collected to improve accuracy and the ability to detect *Diaphorina citri* and *Diaphorina* species native to South Africa. The third objective has not yet started; access to facilities with biosecurity accreditation is still being negotiated.

#### *Vector characterisation*

Three new projects were started in 2022 to further characterise the vectors of HLB and their related indigenous species.

1348- Determination of citrus feeding by indigenous psyllid species colonizing commercial citrus environments.

1396- Predicting the pathways and entry points to the southern African citrus production areas, for the Asian Citrus Psyllid, *Diaphorina citri*.

1362- An assessment of Citrus psyllid passage risk through damaged insect secure structure

#### Plant

CRI Projects: 1246

#### *Rootstocks*

The UF/CREC rootstock program has been breeding rootstocks for 30+ years and due to the HLB epidemic in Florida potential tolerant/resistant rootstocks, with commercial potential, were identified (rescue trees). These

rootstocks are being evaluated in project 1246 (4.5.6). Seeds of 20 new selections from this breeding program imported and submitted to DALRDD/Plant Health for pathogen screening in 2021. Thereafter the seeds were delivered to Du Roi nursery at Letsitele for germination to develop seedlings. Rootstock seedlings were assessed uniformity and a standard photo ID chart of distinguishing characteristics i.e., leaf types, growth pattern and root development were made to assist in future propagation from seeds in commercial nurseries. It was observed, that even at this early stage of the project, differences in the selections are evident and are expected to result in significant changes in nursery practices, canopy development and fruiting potential. After selecting seedlings based on the selection criteria, they were budded with 'Nadorcott LS' mandarin and 'Midnight' Valencia to allow planting in March 2023 as part of two new orchards at Crocodile Valley Estate in Nelspruit.

## Program-opsomming

Die HLB-NP is in April 2021 gestig om navorsingskapasiteit rakende die HLB-patosisteem te verhoog. Die program fasiliteer interdisiplinêre navorsing om verskillende fasette van hierdie komplekse siekte te dek, deur gekoördineerde navorsingsaktiwiteite oor navorsingsportefeuljies heen wat aktief by die Voorligting- en Biosekuriteit-afdelings binne CRI betrokke is. Die HLB-NP bied 'n fokuspunt wat die koördinerende en langtermynbeplanning van navorsingsaktiwiteite moontlik maak om die industrie se behoeftes aan te spreek vir die uitdagings wat Vergroening en HLB bring.

In hierdie opsomming word opdaterings verskaf vir projekte wat direk in die HLB-NP sorteer, sowel as projekte in ander programme wat handel oor aspekte van die HLB-patosisteem waarvoor ook in hul onderskeie programme gerapporteer kan word. Die HLB-NP bestaan uit kort-, medium- en langtermynprojekte. Korttermynprojekte neig om te fokus op basiese opsporing- en identifikasietoetse wat die hoeksteen vorm van ons moniteringspogings vir Vergroening en HLB en hul vektore. Medium- tot langtermynprojekte pak die meer komplekse taak van siekte- en vektorkarakterisering aan, en ondersoek huidige en toekomstige siektebestuurstrategieë. Die langtermynprojek evalueer HLB-weerstandbiedende plantmateriaal vir gedrag onder Suid-Afrikaanse toestande.

### Patogeen

CRI Projekte: 1265,1322,1346, 1380, 1381

### *Diagnostiek*

'n Lus-bemiddelde isotermiese amplifikasie (LAMP) opsporingstoets is in Projek 1265 ontwikkel ((4.5.3)). Hierdie toetse teiken die *nrdB*-geenstreek van CLas en CLaf en het getoon dat dit spesifiek is met geen kruis-amplifisering wat met die CLas-toets met enige van die ander bekende sitrus-infekterende Liberibacters plaasvind nie, terwyl die CLaf-toets CLafCI bv sitrus addisioneel geamplifiseer het. Daar is verder getoon dat hierdie LAMP-toetse net so sensitief is as die SYBR-intydse PKR-toets wat deur CRI en die LNR ontwikkel is, wat dieselfde geenstreek teiken, maar minder sensitief is as gepubliseerde intydse PKR-toetse wat die 16S-geenstreek van sitrus-infekterende Liberibacters teiken. Verder is 'n kru-ekstraksiemetode vir veldtoediening getoets en aanvanklike resultate was belowend, maar die stelsel vereis verdere validering voor implementering. Ons beoog om hierdie toetse in die veld te toets in die voorgestelde Buffeljagsrivier-vergroeningsprojek (1407). 'n Nuwe projek (1380) het in 2022 begin wat op hierdie werk sal voortbou deur dit na 'n CRISPR-opsporingstoets om te skakel wat die sensitiwiteit sal verhoog en opsies vir punt-van-sorg-toestelle sal bied.

Ten einde opsporingstoetse in te lig, word akkurate volgordedata vereis wat die genetiese diversiteit van die teikenpatogene vaslê. In projek 1322 (4.5.9) word volgorde-bepaling van Liberibacter-monsters deur hoë-deurset-volgordebepaling (HTS) gedoen om genoom-inligting in te samel wat gebruik kan word om opsporingstoetse verder te optimaliseer. Die DNS van 20 monsters (4 CLaf CI's: 2 Uganda, 1 Kenia, 1 Knysna en 16 CLafs: 1 Uganda, 15 SA) is vir HTS op die Ion Torrent-platform gestuur. 'n CLaf-genoom is vir elk van die 20 monsters gegenereer deur van 'n verwysingslees-karteringstrategie gebruik te maak. Sukses van genoomkonstruksie was afhanklik van Liberibacter konsentrasie, en 'n genoomdekkingreeks van 15%-99% is verkry. Meer data is vir monsters met lae genoomdekking gegenereer. Die gekonstrueerde genome word tans vir diversiteit geëvalueer met 'n spesifieke fokus op die streke in die genoom wat vir die huidige

opspringstoetse gebruik word. Ander streke sal geëvalueer word vir die ontwerp/optimering van 'n nuwe valideringstoets.

### Siekte-karakterisering

Soos nuwe inligting gegeneer word, en nuwe tegnologie ontwikkel word, kan ons verskillende aspekte van HLB verder karakteriseer. In projek 1346 (4.5.10) sal die invloed van die mede-infeksie van CLaf en CTV ondersoek word. Dit is veral interessant vir lande soos Suid-Afrika wat 'n CTV milde ras kruisbeskermingstrategie gebruik. Plantmateriaal (saailinge) is gegeneer en sal ent-geïnkuleer word met CLaf, CTV of CLaf/CTV wanneer hulle tot die gepaste grootte gegroei het.

In 'n nuwe projek 1381 (begin 2022) is 'n eksperimentele boord gevestig vir langtermyn Vergroeningsiekte-karakterisering. 400 Nadorcott-bome is in potte onder insekbestande toestande gevestig en ent-geïnkuleer met CLaf in April 2022.

### Vektor

CRI Projekte: 1160, 1148, 1255, 1315, 1348, 1362, 1396

#### *Identifikasie*

Projek 1255 (4.5.7) het ten doel om *Diaphorina* spp. en *Trioza* spp. van ander psilloïede wat ook op geel kleeflokvalle vasgevang kan word, te onderskei. Die taksonomie van psilloïede in die Afro-tropiese streek is grootliks geïgnoreer en vereis dat ons ons eie morfologiese sleutels en DNS-strepieskodes ontwikkel vir identifikasie van inheemse spesies. Versamelde monsters van die veld-opnames is tot die naaste moontlike genus en/of spesie geïdentifiseer met behulp van 'n ongepubliseerde taksonomiese sleutel wat deur medewerker Dr Daniel Burckhardt voorberei is. Monsterversameling gaan in en om sitrus-omgewings in Limpopo-, Mpumalanga- en Oos-Kaap-provinsies voort. Inheemse gasheerplante is vir *Diaphorina* spesies wat versamel is, bevestig. Inheemse *Trioza* sp.(16) en *Diaphorina* sp. (36), insluitend 'n monster wat soos *D. citri* lyk, is DNS-strepieskodes gegee. DNS-strepieskodes van *Diaphorina*-monsters het nader aan *Diaphorina lycii* gegroep as aan *D. citri*- of *Diaphorina communis*-volgordes beskikbaar op GenBank. 'n DNS-strepieskode-volgorde vir 'n *Diaphorina*-monster, versamel in Colchester, Oos-Kaap, en morfologies soortgelyk aan *D. citri*, het getoon dat die monster **nie *D. citri* was nie**, maar 'n inheemse spesie wat soortgelyk in voorkoms is en wat op die inheemse boom, *Harpephyllum caffrum*, algemeen bekend as Afrika-pruimboom, voorkom. Die triosiedmonsters is morfologies geïdentifiseer as óf *Trioza erythrae* óf *Trioza litseae* óf *Trioza* sp. (Litseae-groep) en is 'n DNS-strepieskodes gegee. Nukleotiedvolgorde-diversiteit is waargeneem tussen hierdie monsters en volgordes is breedweg in 5 groepe gegroep. Tot dusver is '*Candidatus Liberibacter africanus*' (CLaf) in monsters van twee groepe opgespoor.

#### *Beheer - Chemies*

Nuwe sistemiese insekdoders is in projek 1148 (4.5.5) geëvalueer ter voorbereiding van die aankoms van *Diaphorina citri* in Suid-Afrika. Die bruin sitrusplantluis, *Aphis citricidus*, is as 'n indikatorplaag gebruik vir die sifting van sistemiese insekdoders. Die geregistreerde imidakloprid grondbehandeling en onlangs geregistreerde asefaat (Spectra Stem) stambehandeling het belowende resultate gelewer. Resultate van 'n ongeregisteerde produk, wat as 'n grondbehandeling vir groente gebruik word, was teleurstellend en 'n hoër dosis sal vereis word. Nog 'n ongeregisteerde, sistemiese produk wat op sitrus in die VSA gebruik word, het egter belowende resultate as 'n grondtoediening gegee. 'n Veldproef teen katoenplantluis op jong sitrus is gepoog, maar populasies was sporadies en resultate wisselvallig. 'n Ingeslote eksperiment deur gebruik te maak van 'n *D. citri*-kultuur op gepotte sitrusplante in 'n insekbestande struktuur in SO Kenia, in samewerking met ICIPE, met dieselfde stel chemikalieë, is gepoog. Die *D. citri*-kultuur was egter onvoldoende vir proewe, ten spyte van stappe soos die byvoeging van *Murraya koenigii*-plante by die kultuur. P. Nderitu het wel 'n opname van die sitrusproduksiegebiede in Kenia gedoen op soek na *D. citri* en moontlike HLB-infeksies in warm streke. Van die 18 terreine wat besoek is, is *D. citri* net in Lungalunga in SO Kenia, naby die Tanzanië-grens, gevind.

#### *Beheer - Biotegnologies*

Projek 1160 (4.5.2) was 'n bewys van beginsel projek wat in 2022 voltooi is. Die projek het ten doel gehad om 'n reeks CTV-aansteeklike klone te vestig met 'n reeks loonvragte wat die potensiaal het om die verspreiding te vertraag, of HLB in Suider-Afrikaanse boorde uit te wis. Vier kandidaatgene, *arginienkinase*, *abnormale vlerkskyf*, *kitiensintase* en *Ran GTPase*, is as potensieële RNAi-teikens gekies. Drie kandidaatklone is

sukcesvol gekonstrueer, twee *Trioza erytreae* insek-spesifiek (al vier gene van *T. erytreae*) en een abnormale vlerkskyf geen-spesifiek (een geen van beide *T. erytreae* en *Diaphorina citri*). Die replikasie bevoegdheid en die produksie van klein RNS'e is in *Nicotiana benthamiana* geëvalueer en het gedien as 'n bewys van beginsel vir die suksesvolle aflewering van RNSi-konstrukte, gerig op die insekvektore binne plante. Inokulasie in Mexikaanse kalk is gepoog vir die *T. erytreae*-spesifieke CTV VIGS vektore. Indien hierdie sitrusplante positief toets vir CTV, kan die doeltreffendheid van die CTV RNSi-strategie moontlik op 'n *Trioza*-kolonie getoets word as deel van projek 1315 (4.5.8).

#### *Monitering*

Opsporing en waarneming van *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) en *Trioza erytreae* (Del Guercio) (Hemiptera: Triozidae) is noodsaaklik in ons pogings om vir 'n moontlike HLB-inval voor te berei. Huidige tegnieke is egter ondoeltreffend teen lae plaagdigtheid, vereis geskoolde arbeid en is tydrowend. In projek 1315 (4.5.8) word nuwe moniterings- en beheer-instrumente vir die onderdrukking van vektorverspreiding ondersoek. In hierdie projek sal reuk-gebaseerde lokmiddels geëvalueer word sowel as 'n outomatiese visie-gebaseerde stelsel vir identifikasie van *T. erytreae* en *D. citri* op lokvalle. Die doeltreffendheid van die CTV RNSi konstrukte (van projek 1160) om 'n bladvlooiopulasie te onderdruk, sal ook geëvalueer word. Die eerste doelwit is agter skedule weens onvoorsiene omstandighede. Aansienlike vordering is egter gemaak met die tweede doelwit. Kleeflokvalle is vir bladvlooi geïnspekteer, gefotografeer, en die beelde is geannoteer en aan medewerkers in Florida gestuur om kunsmatige intelligensie-algoritmes te ontwikkel en op te lei wat bladvlooi identifiseer. Die stelsel lewer belowende resultate vir *T. erytreae*-identifikasie, maar meer kleeflokvalmonsters word ingesamel om akkuraatheid en die vermoë om *Diaphorina citri* en *Diaphorina* spesies inheems aan Suid-Afrika op te spoor, te verbeter. Die derde doelwit het nog nie begin nie; toegang tot fasiliteite met biosekuriteit-akkreditasie word steeds onderhandel.

#### *Vektor karakterisering*

Drie nuwe projekte is in 2022 begin om die vektore van HLB en hul verwante inheemse spesies verder te karakteriseer.

1348- Bepaling van sitrusvoeding deur inheemse psilloïedspesies wat kommersiële sitrus-omgewings koloniseer.

1396- Voorspelling van die paaie en toegangspunte na die Suider-Afrikaanse sitrusproduksiegebiede, vir die Asiatiese Sitrusbladvlooi, *Diaphorina citri*.

1362- 'n Beoordeling van Sitrusbladvlooi deurgang risiko deur beskadigde insek-veilige struktuur

#### Plant

CRI Projekte: 1246

#### *Onderstamme*

Die UF/CREC-onderstamprogram teel al 30+ jaar onderstamme en as gevolg van die HLB-epidemie in Florida, is potensiële bestande/weerstandbiedende onderstamme, met kommersiële potensiaal, geïdentifiseer (ontsnappingsbome). Hierdie onderstamme word in projek 1246 geëvalueer. Sade van 20 nuwe seleksies uit hierdie teelprogram is ingevoer en by DALRDD/Plantgesondheid vir patogeen-sifting in 2021 ingedien. Daarna is die saad by Du Roi kwekery by Letsitele gelewer vir ontkieming om saailinge te kweek. Onderstamsaailinge is eenvormig geassesseer en 'n standaard foto ID-kaart van onderskeidende kenmerke d.w.s. blaartipes, groeipatroon en wortel-ontwikkeling, is gemaak om te help met toekomstige voortplanting vanaf sade in kommersiële kwekerye. Daar is opgemerk dat selfs in hierdie vroeë stadium van die projek, verskille in die seleksies duidelik is, en na verwagting sal lei tot beduidende veranderinge in kwekerypraktyke, blaredak-ontwikkeling en vrugpotensiaal. Nadat saailinge op grond van die seleksiekriteria geselekteer is, is hulle met ogies van 'Nadorcott LS' mandaryn en 'Midnight' Valencia geïnokuleer, om aanplanting in Maart 2023, as deel van twee nuwe boorde by Crocodile Valley Estate in Nelspruit, moontlik te maak.

#### 4.5.2 FINAL REPORT: Application of CTV infectious clones to combat HLB

Project 1160 (2016/17 – 2021/2) by R Bester, G Cook, JHJ Breytenbach, HJ Maree (CRI), JT Burger, D Aldrich (SU), WO Dawson (University of Florida, USA) and

##### Summary

The confirmed presence of both '*Candidatus Liberibacter asiaticus*' (CLas), and *Diaphorina citri* in East Africa, requires a proactive approach from the South African citrus industry to prepare for the eventual incursion of HLB. The aim of this project was to establish a suite of citrus tristeza virus (CTV) infectious clones with a range of silencing targets (payloads) that would form part of a management strategy to contain HLB and limit its impact. CTV infectious clones of genotype T36 were imported from collaborator Prof W.O. Dawson (Florida University) at the start of the project and protocols optimized to successfully infect citrus using the T36 clone. The T36 clone was converted into the local RB genotype. However, no systemic infection in *Nicotiana benthamiana* could be established. Plasmid DNA was sent for low coverage high-throughput sequencing and three mutations in critical open reading frames were identified that can influence virus infectivity and spread. Attempts were made to correct these mutations but were unsuccessful to date. The T36 clone was utilised in the meantime to screen different payloads. Four RNAi gene targets, *arginine kinase*, *abnormal wing disc*, *chitin synthase* and *ran GTPase*, in *Diaphorina citri* were identified from literature and homologues for *Trioza erytreae* was identified using HTS data generated from *T. erytreae* RNA. Three CTV clones with payloads were successfully assembled, two *T. erytreae* insect-specific (all four genes of *T. erytreae*) and one abnormal wing disc gene-specific (one gene of both *T. erytreae* and *D. citri*). Systemic CTV infection was observed in *N. benthamiana* plants, indicating that the CTV T36 clone can tolerate large exogenous insertions in the 3' terminal end. Small RNA (sRNA) sequencing was conducted on CTV positive *N. benthamiana* material. The virus-derived sRNA (vsiRNA) coverage across the CTV clone genomes was high. Hotspots in vsiRNA alignment depth were observed at two of CTV's silencing suppressors, providing detail on host plant-virus interaction. Insert-specific vsiRNAs were produced in planta, covering almost the complete silencing insert/payload. The production of insert-specific vsiRNAs provides some proof of concept for this vsiRNA delivery strategy. Inoculation into Mexican lime was attempted for the *T. erytreae* specific CTV clones to evaluate the effect in citrus. The efficacy of the CTV RNAi strategy will potentially be tested on a *Trioza* colony as part of project 1315.

Additionally, this project also includes the identification of CTV-induced stem pitting determinants. CTV infectious clones with different open reading frames deleted or replaced with open reading frames of different CTV variants were assembled and citrus plants infected. Once infected, established plants were visually evaluated for stem pitting and anatomically characterised using nano-CT scanning, biological staining, fluorescence microscopy and various electron microscopy applications. Plant responses to different stem pitting pressures were further assessed by untargeted profiling of secondary metabolites as well as quantitation of the stress-responsive phytohormones, abscisic acid, jasmonic acid and salicylic acid.

##### Opsomming

Die bevestigde teenwoordigheid van beide '*Candidatus Liberibacter asiaticus*' (CLas), en *Diaphorina citri* in Oos-Afrika, vereis 'n proaktiewe benadering van die Suid-Afrikaanse sitrusbedryf om voor te berei vir die uiteindelijke indringing van HLB. Die doel van hierdie projek was om 'n reeks van sitrus tristeza virus (CTV) infektiewe klone te vestig met 'n reeks stilmaaktekens (payloads) wat sal deel vorm van 'n bestuurstrategie om die impak van HLB te beperk. CTV-infektiewe klone van genotipe T36 is ingevoer vanaf medewerker, Prof W.O. Dawson (Florida Universiteit) aan die begin van die projek en protokolle is geoptimeer om sitrus suksesvol met die T36-kloon te besmet. Die T36-kloon is omgeskakel na die plaaslike RB genotipe. Geen sistemiese infeksie in *Nicotiana benthamiana* kon egter waargeneem word nie. Plasmied DNA is gestuur vir lae-dekking hoë-deurset-volgordebepaling en drie mutasies in kritiese leesrame is geïdentifiseer wat die infektiwiteit en verspreiding van virusse kan beïnvloed. Pogings is aangewend om hierdie mutasies reg te stel, maar was tot dusver onsuksesvol. Die T36-kloon is intussen gebruik om verskillende payloads te evalueer. Vier RNAi-geen-teikens, *arginienkinase*, *abnormale vlerkskyf*, *chitien-sintase* en *ran-GTPase*, in *D. citri* is uit literatuur geïdentifiseer en homoloë vir *Trioza erytreae* is geïdentifiseer deur gebruik te maak van HTS-data gegenereer vanaf *T. erytreae* RNA. Drie CTV klone met 'payloads' is suksesvol saamgestel, twee *T. erytreae*

insekspesifiek (al vier gene van *T. erytrae*) en een abnormale vlerkskyfgeenspesifiek (een geen van beide *T. erytrae* en *D. citri*). Sistemiese CTV infeksie is waargeneem in *N. benthamiana* plante, wat aandui dat die CTV T36 kloon groot invoegings in die 3' terminale punt kan verdra. Klein RNA (sRNA) volgordebepaling is uitgevoer op CTV positiewe *N. benthamiana* materiaal. Die virus afgeleide sRNA (vsiRNA) dekking oor die CTV kloon genome was hoog. Verhogings in vsiRNA-volgorde spesifieke diepte is waargeneem by twee van CTV se stilmaak onderdrukkers, wat besonderhede verskaf oor gasheerplant-virus-interaksie. Insetselspesifieke/'payload' vsiRNAs is in planta geproduseer, wat byna die volledige stilmaakinsetsel/'payload' dek. Die produksie van insetselspesifieke/'payload' vsiRNAs bied 'n mate van bewyse vir hierdie vsiRNA afleweringstrategie. Die *T. erytrae* spesifieke CTV klone is geinokuleer in Mexikaanse lemmetjie plante om die effek in sitrus ook te evalueer. Die doeltreffendheid van die CTV RNAi-strategie sal moontlik op 'n Trioza-kolonie getoets word as deel van projek 1315.

Daarbenewens sluit hierdie projek ook die identifikasie van CTV-geïnduseerde stamgleuf determinante in. CTV-kloonmutante met verskillende leesrame wat geskrap of vervang is met leesrame van verskillende CTV variante is saamgestel en sitrusplante geïnfekteer. Sodra infeksie gevestig was, is plante visueel geëvalueer vir stamgleuf en anatomies gekarakteriseer deur gebruik te maak van nano-CT-skandering, biologiese kleuring, fluoressensiemikroskopie en verskeie elektronmikroskopiëtoepassings. Plantreaksies op verskillende stamgleufdruk is verder geassesseer deur profilerings van sekondêre metaboliete sowel as kwantifisering van die stres-responsiewe fitohormone, absisiensuur, jasmoniese suur en salisielsuur.

## Introduction

The aim of this project is to establish a suite of CTV infectious clones with a range of 'payloads' that have the potential to slow the spread, or eradicate, HLB in Southern African orchards.

The confirmed presence of both '*Candidatus Liberibacter asiaticus*' (CLAs), the Huanglongbing (HLB) pathogen and its vectors *Diaphorina citri* and *Trioza erytrae* on the African continent, requires a proactive approach from the South African citrus industry to prepare for the eventual incursion of both CLAs and the Asian citrus psyllid (ACP). The use of RNA interference (RNAi) for pathogen or vector control has shown significant potential as an innovative management tool to be used in conjunction with the conventional array of pest control strategies. This mechanism in eukaryotes is highly conserved and sequence specific. The most critical factors impacting successful control is the identification of the appropriate gene targets and the efficient delivery of the RNAi. Field application requires a continuous supply of dsRNA/siRNA and the administration of RNAi can include feeding by means of artificial diet, natural diet, sprays and transgenic plants. The use of Citrus tristeza virus (CTV) as a vector to target both the pathogen and the vector is a uniquely specific approach due to the phloem limitation of both CTV and the pathogen, as well as the phloem feeding nature of the vector. This approach allows for a wider application to a range of citrus types and is a far quicker approach compared to the construction of transgenic plants. The technology to use CTV to administer RNAi targeting a number of ACP genes has been developed and was successfully demonstrated to significantly reduce and prevent psyllid progeny development in cage experiments (Hajeri et al. 2016). The proposal (extension) is to build on the resources developed thus far and to convert them to vectors to deliver various payloads.

'*Ca. Liberibacter africanus*' (CLaf), the African greening disease agent is currently controlled by management strategies that are largely reliant on insecticidal control of the vector, *T. erytrae*. The African greening disease is far less devastating than HLB, but the CLaf and CLAs bacteria of the respective diseases are sufficiently similar. CLaf can therefore initially be used as a model test organism. Similarly, conserved gene regions targeted for RNAi control of the vector, *T. erytrae*, as an endemic pest, can be used to test efficacy of clones developed in the study. However, strong collaboration with existing research groups will enable the testing of these clones to CLAs and ACP in other laboratories avoiding the risk of testing under quarantine conditions.

CRI projects 1056 and 1100, in addition to the various CTV field trial projects, have provided important information regarding the effect of various CTV strains on a variety of citrus types. This information was used to identify ideal CTV strains to use as vectors. It is important not to introduce foreign strains, but to ensure that locally characterised strain sequences are built into these clones to avoid introducing further disease pressures. This first objective is therefore to build the necessary infectious clones based on local strains.

The approach to use CTV as a vector to deliver specific 'payloads', is regarded as a one of the most realistic strategies to control the pathogen and/or the insect vector to curb the spread of HLB. This project is the first step in this longer-term strategy and aims to develop vectors constructed from indigenous CTV strains. This proof-of-concept study will determine the efficacy of these vectors to deliver their respective 'payloads' and the potential impact of these 'payloads' on the target organisms. The vectors will be evaluated in local contained trials for CLaf and *T. erythrae*. However, for the contained experiments targeting CLAs and ACP, sites still need to be identified and budgets are not reflected in this application. It is envisaged that, within the South African context, these vectors will eventually be introduced to propagation budwood as part of the cross-protection inoculum.

### Stated objectives

1. Construct and evaluate CTV infectious clones of different genotypes and combinations of genotypes.
  - a. Import CTV infectious clones from the collaborator (Prof WO Dawson, University of Florida).
  - b. Design and construct additional clones based on South African genotypes
  - c. Determine pathogenic determinants
2. Convert asymptomatic CTV infectious clones from Objective 1 into virus vectors.
  - a. Evaluate different genomic regions for payload insertion (reporter gene or small interfering (siRNA) construct).
3. Evaluate the tolerance of CTV vectors from Objective 2 to accommodate a range of payloads.
  - a. Identify a range of candidate payloads targeted towards *Diaphorina citri* (model *Trioza erythrae*) that can be used to eliminate or slow down the spread of HLB.
  - b. Construct a full-length CTV vector with a targeted payload towards the insect vector.
4. Evaluate optimized CTV vectors to deliver RNAi payloads.
  - a. Perform greenhouse trials to determine the level of small RNAs produced using the constructed RNAi clones.
  - b. Evaluate RNAi vectors that yield sufficient sRNAs for efficacy against the insect vectors in a follow up study.
5. Application of CTV infectious clones to study the CTV-Citrus pathosystem.
  - a. Construct CTV infectious clones by deleting or replacing the open reading frames (ORFs) of CTV thought to be implicated in stem pitting induction.
  - b. Infect citrus plant with infectious clones to investigate if severe pitting could be induced by exchanging the ORFs of a moderate-pitting infectious clone (T36) with those of a severe-pitting isolate of CTV (T3-KB).
  - c. Evaluate severity of stem pitting in inoculated plants.

### Materials and methods

1. Construct and evaluate CTV infectious clones of different genotypes and combinations of genotypes.

Infectious clones of genotype T36 were imported from the collaborator (Prof W.O. Dawson, Florida University). A strategy was designed to construct CTV infectious clones based on local strains of CTV. The Dawson set of clones were used as the backbone to construct a recombinant clone that contain the ORFs of the local genotype of CTV, RB. RB is a mild genotype in terms of stem pitting symptoms. A full-length clone of RB was constructed, and systemic spread was evaluated in *N. benthamiana*. The sequence of the full-length clone was validated with high throughput sequencing of the plasmid DNA. Three critical mutations were identified in the sequence and attempts were made to correct the mutations using restriction enzyme cloning.

2. Convert asymptomatic CTV infectious clones from Objective 1 into virus vectors.

A dual CTV infectious clone for expression of GFP and silencing of PDS was constructed to evaluate the delivery efficiency of the reporter gene insertion site. Two different sites were evaluated by using a GFP reporter gene between the minor Coat protein and the Coat protein ORFs and a PDS gene in the 3'UTR. The PDS reporter gene was subsequently replaced by different small interfering (siRNA) constructs to use as payloads to potentially slow down HLB.

3. Evaluate the tolerance of CTV vectors from Objective 2 to accommodate a range of payloads.

Literature was used to identify a range of candidate payloads that can be used to eliminate or slow down the spread of HLB. The payloads are targeted towards *Diaphorina citri* and the model *Trioza erytreae*. These payloads are potentially involved in gene silencing of metabolic pathways critical to the survival of the insect vector via RNA interference (RNAi). To obtain sequence information of the *Trioza erytreae* target genes, adult and nymph total RNA was sent for HTS. Four genes were identified from literature and the *Diaphorina citri* and *Trioza erytreae* sequences were obtained. Primers were designed to amplify the gene sequences and to clone them in different combinations into an intermediate vector. Restriction digest-based cloning was used to clone the constructs from the intermediate vector into the CTV T36 vector.

4. Evaluate optimized CTV vectors to deliver payloads.

Greenhouse trials were performed to determine the level of small RNA that can be produced for RNAi with the vectors constructed in Objective 3.

The production of small RNAs in this case virus-derived vsRNAs were determined by small RNA-Seq. The HTS data analyses allowed for quantitative and qualitative evaluation of the sRNAs produced.

In a follow up study (1315) the efficacy of these sRNAs in a RNAi strategy will be evaluated for efficacy against *T. erytreae*.

5. Application of CTV infectious clones to study the CTV-Citrus pathosystem.

The different CTV mutants of this study were generated by means of a restriction-free cloning approach, using the principles of QuikChange II site-directed mutagenesis. The full-length CTV clone (CTV-fl6) is too large to use directly with this protocol, as such we opted to utilise intermediate plasmid vectors. CTV-fl6 fragments of interest, carrying the open reading frames (p33, p18 and p13) thought to be involved in stem pitting induction in citrus (Tatineni and Dawson, 2012), were PCR-amplified and cloned into intermediate plasmid vectors (pGEM). The same approach was employed to both remove T36 open reading frames and insert open reading frames from a severe-pitting CTV strain (T3) into the corresponding positions in the mild-pitting CTV-fl6 fragments of interest. This was done to ultimately evaluate the effect of ORF replacements from CTV strains varying in pathogenicity. The modified CTV regions were then cloned back into the CTV-fl6 backbone by means of a cycled ligation assembly approach, to yield the various full-length mutants of this study. The same approach was applied to generate the four additional GFMS12 ORF-replacement clones, which serve as follow up work to results obtained in project 1100. The ORFs of interest for these clones are p33 and p23. Mexican lime and Duncan seedlings were infected with the different mutant clones and stem pitting evaluated nine months after the plants were pruned back. Stem pitting was evaluated visually as well as with CT scanning technology. Confocal- and scanning electron microscopy have also been evaluated for characterising virus localisation and stem tissue morphology in pitted areas of citrus stems.

Additionally, key secondary metabolites and phytohormone levels in test plants were quantified and characterised to glean a better understanding of the plant response to the various clone mutants. Mass spectrometry (LC/MS) was employed to generate metabolite profiles which can be compared between test groups to find any key differences between clone infections. The involvement of phytohormones such as salicylic acid, jasmonic acid and abscisic acid in mediating plant biotic stress responses were also evaluated.

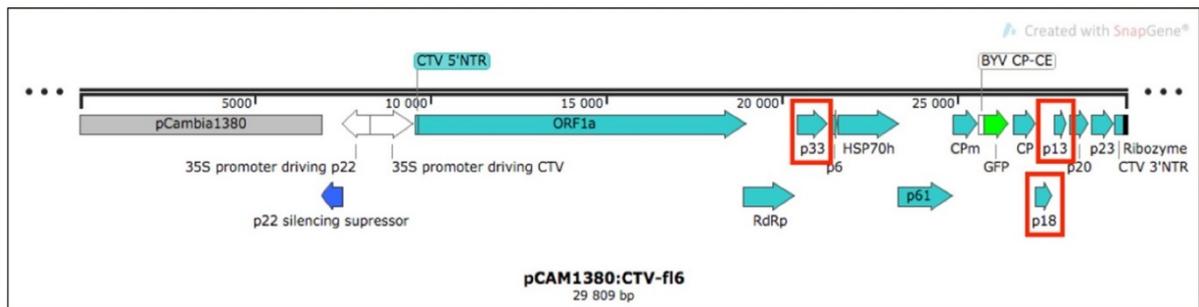
## Results and discussion

Objective	Achievement (March 2022)
1. Construct and evaluate CTV infectious clones of different genotypes and combinations of genotypes.	The construction of a full-length clone based on the CTV-RB genotype has been completed. Low coverage high-throughput sequencing of plasmid DNA identified three mutations that can prevent systemic spread.
2. Convert asymptomatic CTV infectious clones from Objective 1 into virus vectors.	A dual reporter infectious clone based on the T36 clone that expresses GFP and also contains a PDS silencing cassette proved to be an effective CTV-based vector as an expression and/or silencing vector.
3. Evaluate the tolerance of CTV vectors from Objective 2 to accommodate a range of payloads.	RNAi targets were identified from literature and homologues for <i>Trioza erytreae</i> have been identified using the HTS data generated from <i>Trioza erytreae</i> RNA. Three full-length CTV vectors with a targeted payload towards the insect vector were successfully assembled and systemic spread confirmed in <i>N. benthamiana</i> .
4. Evaluate optimized CTV vectors to deliver payloads.	Evaluation of vectors in <i>N. benthamiana</i> were performed using small RNA sequencing. The data provided proof of concept for this vsiRNA delivery strategy.
5. Application of CTV infectious clones to study the CTV-Citrus pathosystem.	<p>All 18 CTV infectious clone mutants proposed for the stem pitting trials of this study have been successfully constructed and proved to be replication competent in <i>N. benthamiana</i>. Additionally, these clones have been sent for low-coverage high-throughput sequencing to validate the plasmid sequence and have full sequences of the clones on record.</p> <p>All available test plants (Duncan and Mexican Lime) for the stem pitting trials have been bark-patch infected with the appropriate clone mutants and wild-type CTV sources. The infection status of all plants was confirmed, and plants were pruned back to allow for stem pitting characterizations. Stem pitting evaluations were performed nine months after plants were pruned back.</p>
Publications	1. Aldrich, D.J., Bester, R., Cook, G., Burger, J.T., and Maree, H.J. 2021. Evaluating the utility of high-resolution CT scanning technology to study citrus tristeza virus-induced stem pitting. <i>Journal of Citrus Pathology</i> . <a href="https://doi.org/10.1007/s12539-021-00093-1">iocv_journalcitruspathology 50093</a> .

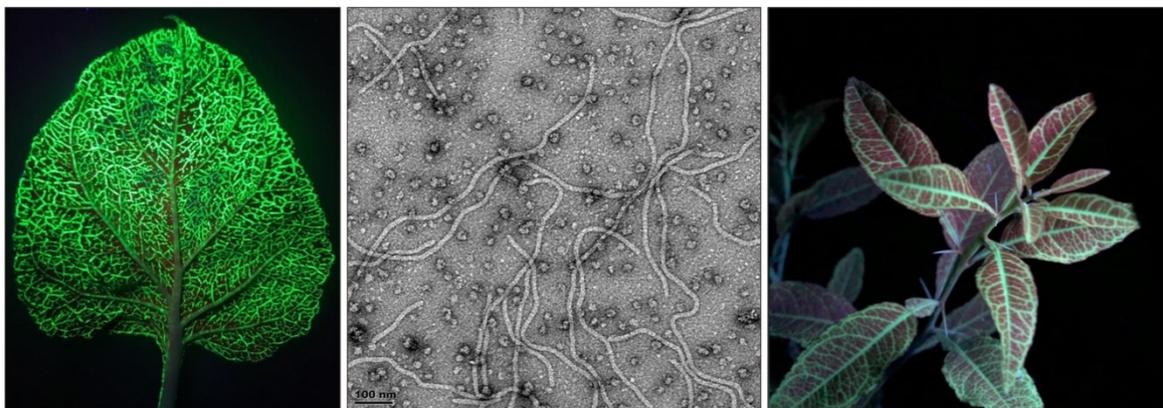
1. Construct and evaluate CTV infectious clones of different genotypes and combinations of genotypes.

The construction of a full-length clone based on the CTV-RB genotype was completed. However, no systemic infection in *N. benthamiana* could be observed after infiltrations. Plasmid DNA was sent for low coverage high-throughput sequencing to be able to evaluate the presence of potential mutations that can influence systemic spread. Three mutations in two critical open reading frames were identified. Multiple attempts were made to correct the mutations using a combination of approaches; however, they were unsuccessful. The construction

of these full-length clones is a very low probability event and to date a full proof approach was not established. It was decided to continue with the proof of principle using the CTV-fl6 (T36 clone) obtained from CREC for objectives 2 -5 (See figure 4.5.2.1 and 4.5.2.2).



**Figure 4.5.2.1.** Vector map of CTV infectious clone (CTV-fl6) tagged with GFP. ORFs targeted for deletion and replacement studies in Objective 5 indicated in red rectangles.



**Figure 4.5.2.2.** The use of the CTV-fl6 infectious clones f.l.t.r. GFP expression of CTV-fl6 in *Nicotiana benthamiana* leaf. Electron micrograph of partially purified CTV virions. GFP expression of CTV-fl6-GFP:PDS in Mexican Lime

## 2. Convert asymptomatic CTV infectious clones from Objective 1 into virus vectors.

This objective delivered a full-length CTV vector that contains a reporter construct that can be replaced by a targeted payload. To test the efficiency of the reporter gene insertion site, a dual CTV infectious clone was constructed harbouring GFP for expression and PDS for RNAi. The GFP:PDS CTV clone was evaluated in *N. benthamiana* and in citrus (See figure 3).



**Figure 4.5.2.3.** Phytoene Desaturase (PDS) silencing in Mexican lime plants induced by a CTV infectious clone harbouring a portion of the grapefruit PDS sequence.

3. Evaluate the tolerance of CTV vectors from Objective 2 to accommodate a range of payloads.

Four RNAi gene targets were identified from literature and homologues for *Trioza erytreae* were identified using HTS data generated from *Trioza erytreae* RNA. These four candidate genes included *arginine kinase*, *abnormal wing disc*, *chitin synthase* and *ran GTPase*. Insect- and gene-specific silencing inserts were constructed. A set of 24 intermediate plasmids was assembled, harbouring the silencing inserts in the 3' terminal end of the T36 CTV clone (CTVfl6). Assembly of the complete CTVfl6 VIGS vectors was attempted by replacing the 3' end of the original sequence with the newly engineered intermediate 3' end. Three full-length clones were successfully assembled, two *T. erytreae* insect-specific (all four genes of *T. erytreae*) and one abnormal wing disc gene-specific (one gene of both *T. erytreae* and *D. citri*). Systemic CTV VIGS vector infection was observed in 22% to 33% of *N. benthamiana* plants 5-6 weeks post-*Agrobacterium tumefaciens*-infiltration, indicating that CTVfl6 can tolerate large exogenous insertions in the 3' terminal end. Inoculation into Mexican lime was attempted for the *T. erytreae* specific CTV VIGS vectors. As soon as the citrus plants test positive for CTV, the efficacy of the CTV RNAi strategy will be tested on a *Trioza* colony as part of project 1315. In this project increased mortality, malformed phenotypes, decreased molting, extended body form, failure to moult, lower weight or reduction in offspring in an established *Trioza erytreae* colony will be monitored.

4. Evaluate optimized CTV vectors to deliver payloads.

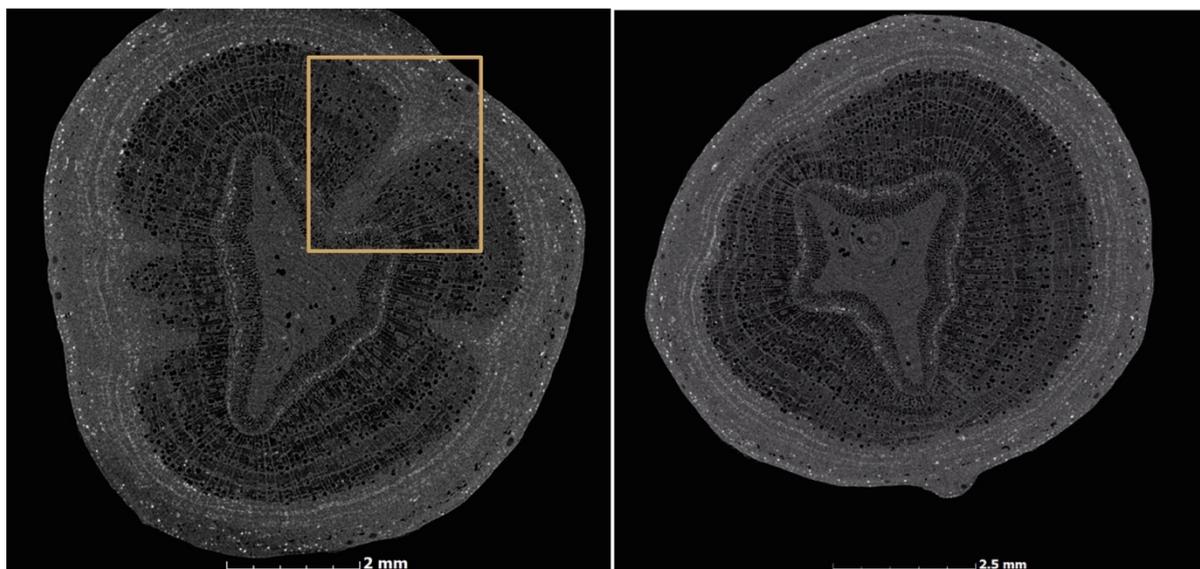
Inoculation into Mexican lime was attempted for the *T. erytreae* specific CTV VIGS vectors. As CTV was not yet detectable in Mexican lime at the time of sampling, small RNA (sRNA) sequencing was only conducted on CTV positive *N. benthamiana* material. The vsiRNA coverage across the CTV clone genomes was over >99%. Hotspots in vsiRNA alignment depth were observed at two of CTV's silencing suppressors, providing detail on host plant-virus interaction. Insert-specific vsiRNAs were produced in planta, covering >99.78% of the silencing insert. The production of insert-specific vsiRNAs provides some proof of concept for this vsiRNA delivery strategy.

5. Application of CTV infectious clones to study the CTV-Citrus pathosystem.

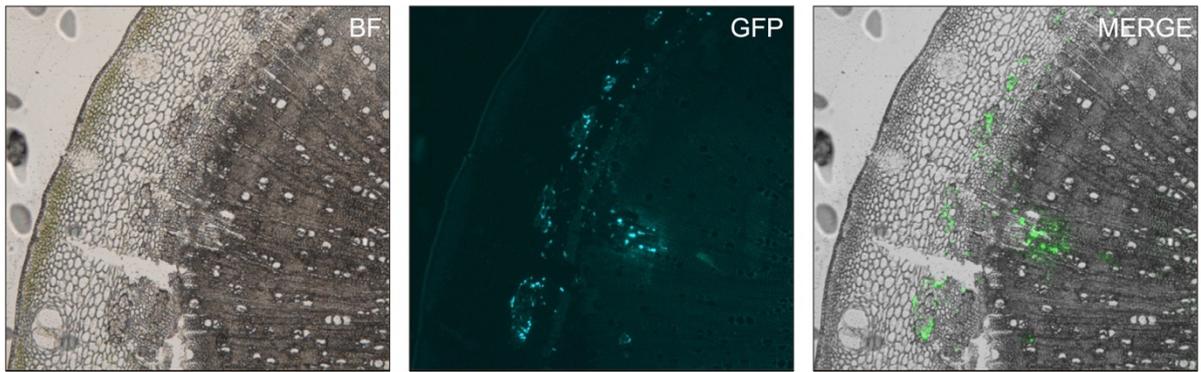
A panel of CTV clone mutants were generated by deleting or replacing the three open reading frames (ORFs) of CTV thought to be implicated in stem pitting induction, namely p33, p18 and p13. ORF deletion and replacement mutants were generated to investigate if severe pitting could be induced by exchanging the ORFs

of a moderate-pitting infectious clone (T36) with those of a severe-pitting isolate of CTV (T3-KB). The full complement of 18 CTV infectious clone mutants for the stem pitting trials of this study were successfully assembled and proven replication competent in *Nicotiana benthamiana*. These clones were also sent for low-coverage high-throughput sequencing (Central Analytical Facilities, Stellenbosch University) to validate plasmid sequences. All available test plants (Duncan and Mexican Lime) for stem pitting trials were bark-patch infected with the appropriate clone mutants and wild-type CTV sources. The infection status of all the bark-patch plants were confirmed with RT-PCR and Sanger sequencing. All plants were pruned back to allow for stem pitting characterizations. A mass spectrometry trial was conducted in the material that was cut back as a preliminary analysis in which untargeted metabolite profiling was employed in test plants via LC/MS to further characterize the plant response to different stem pitting pressures. This trial yielded a preliminary profile that can potentially be linked to early stem pitting formation. Phytohormone quantitation were also carried out in these trials to evaluate how they are mediated in plant biotic stress responses.

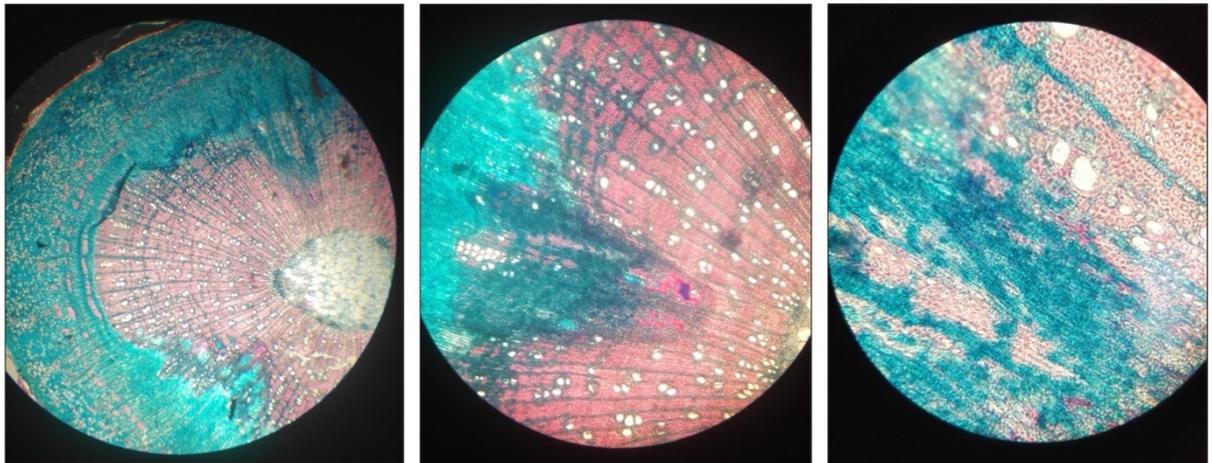
Several approaches were evaluated for characterizing stem pit morphology and virus localization in citrus material. Nano-CT scanning technology was employed to view stem pits in CTV strain T3-infected Star Ruby material with the bark layer still intact, yielding high-resolution images highlighting how the phloem and xylem tissues are impacted in pitted areas (See figure 4.5.2.4). Cryosectioning was also investigated for generating thin sections of the woody citrus material. Sections have been visualized under confocal microscopy to view the virus localization based on GFP expression. A light microscope with a GFP filter attached to it, for generating superimposed light/GFP photos as an alternative to confocal microscopy was also tested (See figure 4.5.2.5). The staining protocols for Safranin-O/Alcian Blue staining of the thin sections was also optimized to better characterize the cell types in pitted areas of the stem, based on lignification differences to the surrounding, normal tissue. The staining allowed clear identification of impacted tissue in citrus cross-sections under the light microscope (See figure 4.5.2.6). An ultra-microtome sectioning and scanning electron microscopy (EM Unit, CAF, Stellenbosch) trial was also conducted to determine if this approach can be used to confidently identify the cell types in pitted areas based on cell wall structures. Sectioning of the woody citrus material is proving difficult. However, these trials shed more light on the nature of the xylem tissue facilitating the aberrant CTV replication outside the normal ring of phloem-associated cells, seen in cases of severe pitting.



**Figure 4.5.2.4.** Nano-CT scans of pitted (left) and unpitted (right) T3 grapefruit citrus stems with several stem pits visible in the pitted (left) panel. A stem pit is highlighted in the gold rectangle.



**Figure 4.5.2.5.** Characterisation of virus localisation in citrus thin sections (cryosections) based on GFP expression using a microscope equipped with a GFP detector filter. The bright-field (BF) panel shows the light image of the thin section, the GFP panel shows the GFP signal visible using the GFP detector filter and the MERGE panel is a merged image of the two inputs (generated using ImageJ software)



**Figure 4.5.2.6.** Light microscope images of Safranin-O/Alcian Blue-8GX staining of pitted thin sections (cryosections) of a wild-type T3-infected greenhouse plant to visualise differences in lignification between pitted and non-pitted portions of the stem.

Stem pitting in plants inoculated with the range of CTV infectious clone mutants were visually evaluated nine months after the plants were pruned back. The presence of ORF p18 from T3-KB yielded the most significant increase in stem pitting severity in Mexican lime plants among the three ORFs investigated, with a range of stem pitting pressures induced across the panel of clones investigated (See figure 4.5.2.7). All leaf samples were processed for profiling of secondary metabolites via LC/MS and quantitation of stress-responsive phytohormones. Metabolite profiling was performed at the end of March and the results will form the departing point of project 1382.



**Figure 4.5.2.7.** Stem pitting severity in Mexican lime plants infected with the panel of hybrid clones containing open reading frames from the severe-pitting CTV isolate T3-KB as well as the unaltered CTV-fl6 and the wild-type CTV T3-KB. The test infection in each case is noted at the bottom of each panel. Panel 1 – Unaltered CTV infectious clone, Panels 2 to 6 - ORF hybrid clones, Panel 7 – Wild-type CTV T3-KB.

## Conclusion

This project aimed to establish a suite of citrus tristeza virus (CTV) infectious clones with silencing targets in *Diaphorina citri* and *Trioza erytreae* to form part of a management strategy to contain HLB and limit its impact. Significant progress was made in this proof of principle project. Protocols for the transmission of infectious clones once constructed were successfully optimised. Strategies for the assembly of infectious clones based on the local RB genotype (mild stem pitting) have been completed and clone constructions have been partially completed. A dual reporter infectious clone based on the CTV-fl6 (T36) clone that expresses GFP and contains a silencing cassette was evaluated in citrus demonstrating that CTV can be used as a silencing vector. While the construction of the RB clone is in progress, the proof of principle RNAi experiments targeting the insect vector were performed with the T36 clone. The full-length assembly of these clones are still problematic and remain a very low probability event despite various optimization attempts. However, three RNAi constructs were successfully cloned into the T36 backbone and two showed systemic infections in *N. benthamiana*. These clones are ready for evaluation in citrus which will form part of project 1315 together with the evaluation of the effect on *T. erytreae* when they feed on these infected plants.

All CTV infectious clone mutants for the stem pitting evaluation trials of this study were successfully assembled and proved to be replication competent in *N. benthamiana*. These clones were also sent for high-throughput sequencing to validate plasmid sequences. The clones were successfully infiltrated into Duncan and Mexican lime plants and CTV infection was confirmed. These plants were pruned back, and stem pitting characterizations were performed. Viral open reading frames implicated in induction of severe stem pitting could be identified. The LC/MS analysis trial yielded a preliminary profile that can potentially be linked to early stem pitting formation. Stem pits were also anatomically characterised using nano-CT scanning, biological staining, fluorescence microscopy and various electron microscopy applications to better understand the nature of the xylem and phloem tissues impacted in cases of severe pitting. The addition of biological plant data in response to differential stem pitting pressures provided a unique perspective into the changes that accompany stem pitting induction and provides valuable opportunities to further characterise these pathways in future studies.

## Future research

- Completion of the RB genotype infectious clone
- Build more CTV RNAi vectors

- Evaluate the effect of the RNAi vectors in citrus plants using small RNA sequencing
- Evaluate the effect of the RNAi vectors on *Trioza erytreae* (project 1315)
- Evaluate stem pitting response in plants infected with the different CTV clones at different timepoints using visual evaluations, RT-qPCR, Nano-CT scanning technology, staining protocols, mass spectrometry and HTS (project 1382)

## Technology transfer

52nd Congress of the Southern African Society for Plant Pathology, Pretoria, South Africa. 1-3 August 2022. Aldrich, D.J., Bester, R., Cook, G., Burger, J.T., and Maree, H.J. Characterisation of citrus tristeza virus-induced stem pitting: applying infectious clones to our advantage. (Oral)

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### 4.5.3 FINAL REPORT: Validation of LAMP diagnostics for in-field detection of HLB

Project 1265 (1 April 2021 – 31 March 2022) by Ronel Roberts (ARC-TSC) and Glynnis Cook (CRI)

## Summary

Citrus Huanglongbing (HLB), associated with '*Candidatus Liberibacter asiaticus*' (CLAs) has been detected on the African continent and poses an imminent threat to the South African citrus industry. It is therefore of great importance that available diagnostic assays be able to detect CLAs without false positive amplification of non-target organisms such as its close relative, '*Ca. L. africanus*' (CLaf) and its various subspecies, one of which is known to infect citrus, i.e. '*Ca. L. africanus* subsp. *clausenae*' (CLafCl). Loop-mediated isothermal amplification (LAMP) is a detection method which can be used in-field. A colour change indicates the presence of amplified target DNA. Various LAMP protocols have been published for the detection of CLAs, but *in silico* analyses as well as lab tests indicated that these are not optimal for the differentiation of CLAs and CLaf and would likely result in false positive reactions. Within the current study, LAMP primers were designed targeting the *nrdB* gene region of CLAs and CLaf. The LAMP CLAs and CLaf assays were shown to specifically amplify their intended targets, with no cross-amplification occurring with the CLAs assay with any of the other known citrus-infecting Liberibacters, whereas the CLaf assay did additionally amplify CLafCl bv. citrus. These LAMP assays were further shown to be as sensitive as the SYBR real-time PCR assay developed by CRI and the ARC targeting the same gene region, but less sensitive than published real-time PCR assays targeting the 16S gene region of citrus-infecting Liberibacters. Furthermore, a crude extraction method for field application was tested and initial results were promising, but the system requires further validation before implementation. LAMP assays, to separately amplify CLAs and CLaf, were therefore successfully developed and together with the crude extraction is a potential and useful in-field-testing system.

## Opsomming

Sitrus Huanglongbing (HLB), wat geassosieer word met '*Candidatus Liberibacter asiaticus*' (CLAs), is op die Afrika continent opgespoor en hou 'n bedreiging vir die Suid Afrikaanse sitrus bedryf in. Dit is dus belangrik dat beskikbare diagnostiese tegnieke CLAs identifiseer en nie vals-positiewe reaksies gee weens die teenwoordigheid van ander naverwante organismes soos '*Ca. L. africanus*' (CLaf) en sy subspecies, waarvan een geken is om sitrus te infekteer, naamlik '*Ca. L. africanus* subsp. *clausenae*' (CLafCl). 'Loop-mediated isothermal amplification', of LAMP, is 'n diagnostiese tegniek wat in die veld gebruik kan word om teiken organismes te identifiseer deur middel van 'n kleur reaksie wat geskied na die amplifisering van teiken DNS met inkubasie teen 'n spesifieke temperatuur. Verskeie LAMP protokolle is gepubliseer vir die opsporing van

CLas, maar *in silico* ontledings, asook laboratorium toetse het aangedui dat hierdie toetse nie optimaal is vir die onderskeid van CLaf en CLas nie, en kan moontlik tot vals positiewe reaksies lei. Binne die huidige studie is LAMP DNS voorlopers ontwerp wat die *nrdB* geen van CLas teiken en geen kruis-reaksie toon met enige ander sitrus Liberibakter spesies nie. Die CLaf spesifieke LAMP toets toon wel kruis-reaksie met die naverwante CLafCI bv. sitrus. Daar is verder gewys dat die sensitiwiteit van hierdie LAMP-toetse vergelyk met die SYBR-PKR toets wat deur CRI en die LNR ontwikkel is, en wat ook die *nrdB* geen teiken, maar sensitiwiteit was laer as die 'real-time PCR' toetse wat die 16S geen van Liberibakters teiken. Verder, is 'n kru-ekstraksie metode getoets en aanvanklike resultate was belowend, maar bevestigende toetse word benodig voor algemene gebruik. LAMP-toetse om CLas en CLaf afsonderlik optespoor is dus suksesvol ontwikkel en saam met die kru-ekstraksie is dit 'n potensieële waardevolle in-veld-toetsstelsel.

## Introduction

Citrus Huanglongbing (HLB), associated with the fastidious bacterium '*Candidatus Liberibacter asiaticus*' (CLas) (Garnier and Bové, 1983, Jagoueix *et al.*, 1994) poses an imminent threat to South African citrus production as both CLas and its vector, *Diaphorina citri* Kuwayama (Capoor *et al.*, 1967) are present on the African continent (Saponari *et al.*, 2010, Kalyebi *et al.*, 2015, Shimwela *et al.*, 2016, Ajene *et al.*, 2020). Currently, diagnostics of CLas involves the use of sensitive detection technologies such as real-time PCR which can only be performed by trained personnel in a well-equipped laboratory. Typically, confirming the presence of CLas from any given sample (insect vectors or plant material) can take up to two days, excluding shipping time to the laboratory. A rapid, in-field test would be beneficial for certain purposes, but should not be seen to replace the standard diagnostic tests. In instances such as cross-border surveys, in the absence of laboratory capabilities or in the absence of sample permits, such in-field diagnostics would be valuable. Such a test might also be beneficial in providing unconfirmed results during surveys to inform precautionary interventions and to assist in the prioritising of survey samples.

Loop-mediated isothermal amplification (LAMP) is a molecular detection tool which combines the sensitivity of molecular testing with the ease of performing rapid identification of target organisms from samples in-field. LAMP reactions are performed at a single temperature and a visible colour change indicates the presence of the target organism (Notomi *et al.*, 2000). The LAMP reaction can also be conducted on crude extracts, making this conducive for field use. Various LAMP protocols are reported for the detection of CLas (Okuda *et al.*, 2005, Malamud *et al.*, 2014, Keremane *et al.*, 2015, Ghosh *et al.*, 2016, Wu *et al.*, 2016, Qian *et al.*, 2017, Choi *et al.*, 2018). These assays target various gene regions of the CLas genome, including 16S rDNA (Ghosh *et al.*, 2016, Wu *et al.*, 2016), phage-related sequences (Keremane *et al.*, 2015, Qian *et al.*, 2017) as well as unassigned gene regions (Malamud *et al.*, 2014). A Liberibacter, closely related to CLas, is already present in South Africa, i.e. '*Ca. L. africanus*' (CLaf) and it will be important to determine whether these published assays are specific for CLas. Additionally, various CLaf-subspecies were described from indigenous Rutaceae hosts of the insect vector of CLaf, *Trioza erytreae* (McClellan and Oberholzer, 1965), in South Africa (Roberts *et al.*, 2015, Roberts and Pietersen 2017). One of these sub-species, '*Ca. L. africanus* subsp. *clausenae*' (CLafCI) was found to infect citrus in eastern Africa (Roberts *et al.*, 2017). It will be important to ensure that none of these sub-species are non-specifically amplified as *T. erytreae* may contain trace DNA of the various CLaf-subspecies from feeding on CLaf-subspecies-infected material (Moran 1968).

## Stated objectives

Objective A: Validate LAMP assays against a range of Liberibacter positive citrus samples

Objective B: Comparison of crude extraction methods to be used in field application

## Materials and methods

*Objective A: Validate LAMP assays against a range of Liberibacter positive citrus samples*

LAMP primer sets for Las detection, targeting the *rpJ* gene region (Okuda *et al.* 2005) and 16S rDNA (Ghosh *et al.* 2016) (Wu *et al.* 2016), were assessed *in silico* by BLAST analyses and assessment of multiple sequence

alignments of the relevant sequences. Primers were ordered for the CLas assay targeting the *rpIJ* gene which showed the most promise based on the in silico analysis.

Additionally, five primer sets each for CLas and CLaf were designed based on available *nrdB* sequences, with each primer set being composed of six primers (F3, B3, FIP, BIP, Loop F and Loop R). Each of the primer sets, as well as the published primer set were tested for specificity against CLaf, CLas, CLam and CLafCI bv. citrus, as well as healthy citrus material and a no template control. Reactions were set up in 10 µl reactions, according to the manufacturer's instructions, using WarmStart Colorimetric LAMP 2X Master Mix (Cat no: M1800S, NEB) and monitored over 120 minutes, viewing colour reactions at 30 minute intervals. The final protocol is described in Appendix A.

Once appropriate primer pairs were identified and specificity of the assays for the intended targets were established, the CLaf assay was validated against a number of CLaf positive citrus types. These reactions included DNA from CLaf positive and negative, 'Madam vinous' sweet orange, 'Rough' lemon, 'Mexican' lime, an unknown sweet orange and 'Carizo citrange' rootstock samples. The presence of CLaf was further confirmed by the CLaf-specific real-time PCR assay of Li *et al.*, (2006).

To evaluate the sensitivity of the LAMP reactions DNA dilution series were tested using the WarmStart fluorescent LAMP kit (Cat No E1700S, NEB). The dilutions were prepared as follow: Positive CLaf and CLas DNA controls were standardized to 100ng/ul and 5x serial dilutions prepared in Liberibacter negative citrus DNA extracts, also standardized to 100ng/ul. Each reaction contained a negative control, and a no template control. The reactions were set up as per protocol listed in Appendix A.

In comparison, the serially diluted samples were subject to the HRM assay as developed by CRI for the simultaneous detection of Laf, Las and Lam. This assay targets the same gene region (*nrdB*) as the LAMP assays. Reactions were set up using the same serial dilutions and negative control and also a no-template control.

To further determine the sensitivity of the LAMP assays in comparison to published assays, the dilution series were subjected to published real-time PCR assays targeting the 16S rDNA Liberibacter sequences using the primers published by Li *et al.* 2006 and Bao *et al.* 2019.

The LAMP assays were additionally validated against CLas and CLaf positive samples from *D. citri* and *T. erythrae* supplied by CRI. The samples are listed in Table 4.5.3.2. All samples were subjected to the optimized Laf and Las LAMP assays and results were verified by three different real-time PCR assays, i.e. LibUF, Laf-specific and Las-specific using modified Li *et al.* (2006) primers.

*Objective B: Comparison of crude extraction methods to be used in field application*

Three different crude extraction methods were assessed.

The first being published by Qian *et al.*, (2018) in which petioles are macerated in 0.5 M NaOH and then diluted 1:50 in water. The second is a protocol published by Drias *et al.*, (2019) in which samples are macerated in 0.5 M sodium acetate, and the third is a 'dip-stick' method described by Zhou *et al.*, (2017). For the latter method, samples were macerated in a lysis buffer (20 mM Tris, 25 mM NaCl, 2.5 mM EDTA, 0.05% SDS), a strip of Whatman filter paper is then dipped into the macerated solution which is then briefly transferred to a wash buffer (10 mM Tris, 0.1% Tween-20) and finally 'dipped' into the LAMP master mix. The LAMP reactions were set up as for the DNA extractions, with 1 µl of each of the crude extract templates, with the exception of the dipstick-method, which was added to the mix. Reactions were monitored over 90 min.

The crude extraction method of Qian *et al.*, (2018) was further assessed against different cultivars, i.e. 'Madam vinous' sweet orange, 'Rough lemon', 'Mexican' lime, a sweet orange field sample and 'Carrizo citrange'. Healthy controls were included for each citrus type. The crude extracts were added in 1µl and 2µl reactions to the CLaf LAMP assay.

## Results and discussion

*Objective A: Validate LAMP assays against a range of Liberibacter positive citrus samples*

For both CLaf and CLas assays, a primer pair from the designed *nrdB* primers, was identified which were able to detect the intended targets. Each primers pair was able to specifically give the desired colour reaction for their intended targets, whereas other non-targets, healthy and negative controls remained negative, with the exception of the CLaf primer set which gave a positive reaction with CLafCl. For the CLaf primer, the specificity was constant across the different citrus species assessed. Amplification of CLaf is optimal at 60°C and the CLas assay at 65°C. The published *rplJ* primer set gave a positive signal for all Liberibacters included in the reaction and was thus omitted from further validation. The *nrdB* primer sequences are listed in Table 4.5.3.1.

**Table 4.5.3.1.** Primer sequences for CLaf and CLas LAMP assays

Primer	CLaf	CLas
F3	CCACATTCCTTCAAAAA CCA	GACATAAAAAACGAAGAGATCTTGT
B3	TATTGTGGAAAGTCTC GGT	AGTGCATAGCCATACCTTC
FIP	TTCCCCATCATTTTCCA CTGGAATAAGATCACG CAAGAACTC	TGGGCTTTACAGTATACTCAA ACTCTGATCTGCGTCTTTTGTTC C
BIP	ATTGTAAAGCCCAGCT TGCTTGGAGAATTATTT AACATGTACCGA	TGGCTTTAGCGGTGATGGAAGCATTATCACTAGTCTCGGTCTT
LF	ACAAATGAACGTGATC AA	GCTCTCCATCCTTCACTACT
LB	TATTGGTAATAGAGGG AATTTC	GGACTTCTCGATACATGTTAAA

The sensitivity of both CLaf and CLas LAMP assays, using the WarmStart Fluorescence kit, were able to detect the respective target DNA up to 3-fold dilutions, with no subsequent fluorescence detected for the remaining dilutions and controls. The reactions were also fully developed after a 30 min incubation with purified DNA as the template. The LAMP sensitivity was comparable to the real-time SYBR PCR assay developed by CRI and ARC. The LAMP assays were, however, not as sensitive as the 16S rDNA-based real-time PCR assays as described by Li *et al.*, (2006) and Boa *et al.*, (2019). With these assays, both CLaf and CLas positive samples could be detected up to 5-fold dilutions. For CLaf, the 4 and 5 fold dilutions gave Ct values over 30 and a Ct value of 30 was obtained for the 5- fold dilution of CLas.

When using DNA extracted from psyllids with the LAMP assays, all positive samples gave a positive signal after 30 min whereas all negative samples remained negative after 60 min of incubation. Positive samples were all confirmed by the LibUF real-time PCR, which is combined with an internal control for psyllid DNA. All samples gave a positive Ct value for the internal control. The presence of either Las or Laf were also verified by the modified Li *et al* (2006) assay, which confirmed the identity of the Liberibacter present. This verifies that the LAMP assays remain specific for their intended targets when insect DNA is used and does not non-specifically react with insect DNA.

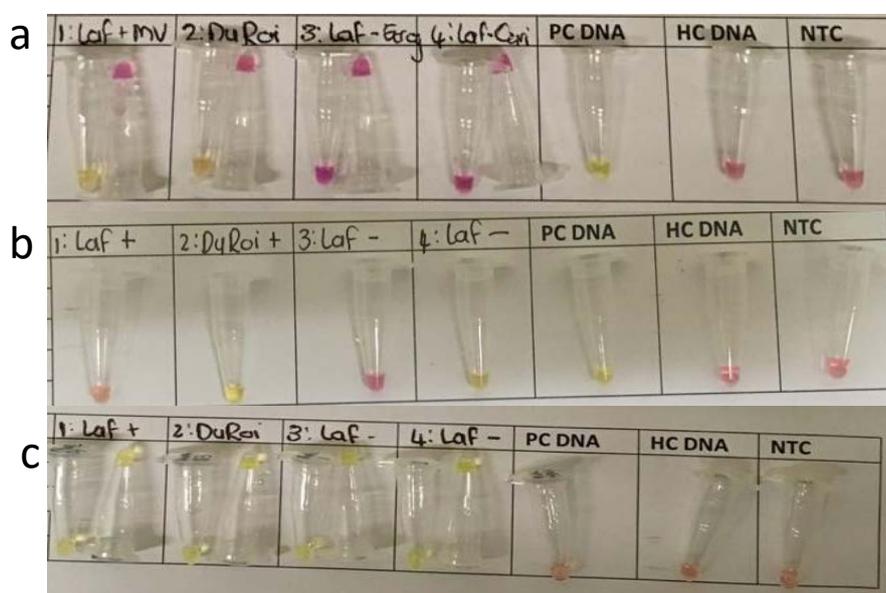
**Table 4.5.3.2.** Comparative results to validate the LAMP Liberibacter assays with real-time probe-based PCR assays using DNA extracted from either *Diaphorina citri* or *Trioza erytrae* DNA.

	DNA sample description (DNA number /psyllid species / Liberibacter originally identified)	LAMP CLas result	LAMP CLaf result	Ct value (CLas-probe PCR)	Ct value (CLaf-probe PCR)
1	D170216-1: D citri Las +	Pos	Neg	21.68	Neg
2	D170216-2: D citri Las -	Neg	Neg	Neg	Neg

3	D170216-3: D citri Las +	Pos	Neg	24.91	Neg
4	D170216-4: D citri Las -	Neg	Neg	Neg	Neg
5	D170216-8: D citri Las +	Pos	Neg	29.14	Neg
6	D170216-9: D citri Las -	Neg	Neg	Neg	Neg
7	D170216-10: D citri Las -	Neg	Neg	Neg	Neg
8	D211105-10: Trioza Survey- Laf +	Neg	Pos*	Neg	24.68
9	D211202-11: Trioza	Neg	Neg	Neg	Neg
10	D211202-12: Trioza	Neg	Neg	Neg	Neg
11	D211202-13: Trioza	Neg	Neg	Neg	Neg
12	D211105-14: Trioza Survey - Laf +	Neg	Pos	Neg	26.71

**Objective B: Comparison of crude extraction methods to be used in field application**

Of the three crude extractions assessed, the protocol of Qian *et al.*, 2018 gave the most promising results as indicated in figure 4.5.3.1 below.



**Figure 4.5.3.1.** Comparison of crude extractions tested for detection of CLaf within the LAMP assay: a) NaOH extraction of Qian *et al.*, (2018) after 90 min. Yellow indicates a positive colour reaction whereas pink is a negative/no reaction. For each sample, the first tube was diluted 1:50 with water, whereas the second tube per sample was undiluted, Samples are as follow: 1: CLaf positive madam vinous, 2: CLaf positive unknown sweet orange, 3 CLaf negative Etrog, 4: CLaf negative carizo citrange, a CLaf positive DNA control, CLaf negative DNA control and a water no template control b) 'dipstick' method of Zhou *et al.*, (2017) after 90 minutes incubation, c) sodium acetate method of Drias *et al.*, (2019) after only 1 minute of incubation.

As the protocol by Qian *et al.*, 2018 was the only extraction which did not render false positives, it was further assessed against a number of positive CLaf samples originating from different cultivars, including 'Madam vinous' sweet orange, 'Rough lemon', 'Mexican' lime, an unknown sweet orange and 'Carrizo citrange'. Liberibacter negative controls were included for each citrus type. One and 2 µl of extract were included in each reaction which was monitored over 60 min. A positive colour change was observed for positive 'Madam vinous', 'Mexican' lime and sweet orange, with their respective healthy controls remaining negative. No reactions were observed for CLaf positive 'Rough lemon' and 'Carizo citrange'. Positive reactions were also more readily distinguished from negative reactions after 60 min, indicating that, using crude extraction as template, a longer incubation time is required to clearly distinguish positive samples. Additionally, positive reactions were only observed when 1 µl of template was used in the reaction, with no colour change being observed for any of the

samples when 2 µl of sample was used. The reaction was repeated using DNA extracts from the same samples, validating the assay in different citrus types. The crude extraction protocol is provided in appendix A.

## Conclusions

Whilst not being as sensitive as the real-time PCR assays optimised for the detection of *Liberibacter* 16S rDNA, the LAMP assays designed within this study are capable of detecting their intended targets up to a 3-fold dilution. These assays have also been shown to be specific for their intended targets and CLaf could be detected in different citrus backgrounds.

The crude extraction of Qian et al., (2018) proved to be the most robust extraction method. This extraction is also easy to perform in a field setting as minimal equipment is required. It is however important to note that the assay should be incubated for a minimum of 60 minutes when using the crude extract as template to clearly resolve positive samples.

## Technology transfer

None.

## Future Research

None.

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## Appendix A:

### **WarmStart LAMP Kit (DNA & RNA) Protocol**

Reagent	1 X reaction (µl)	.....X
Warmstart LAMP MM (2X)	5	_____
LAMP primer mix	1	_____

Fluorescent Dye	0.1	_____
Mol grade water	2.9	_____
DNA extract	1	_____
<b>Totals</b>	<b>10ul</b>	_____

Run reaction at 60°C for CLaf and 65°C for cCLAs for 60min

**Qian et al, 2018 NaOH Crude extraction protocol**

Macerate petioles and midribs of 2 leaves showing symptoms in 3 ml of 0.5 M NaOH

Total time should not exceed 5 min of maceration and standing

Dilute 1:50 in water

LAMP setup:

<b>Reagent</b>	<b>1 X reaction</b>	.....X
Warmstart LAMP MM (2X)	5	_____
LAMP primer mix	1	_____
Mol grade water	2	_____
DNA extract	2	_____
<b>Totals</b>	<b>10ul</b>	_____

Run reaction at 60°C for CLaf and 65°C for CLAs for 60min

Primer Mix:

	Amount added to mix (µl)	Final Concentration (µM)
<b>FIP</b>	16	1.6
<b>BIP</b>	16	1.6
<b>F3</b>	2	0.2
<b>B3</b>	2	0.2
<b>LF</b>	4	0.4
<b>LB</b>	4	0.4
<b>H<sub>2</sub>O</b>	56	

**4.5.4 FINAL REPORT: Studies to improve seed production of rootstock trees**

Project 1264 (Apr 2020 – Mar 2022) by Johané Niemann and Paul Cronje (CRI)

**Summary**

Citrus rootstocks are a determining factor for the success of commercial citrus plantings. Propagation of rootstocks occurs mainly using seeds. As a result, high seed quantity and quality are important to ensure a continuous supply. No clear citriculture guidelines exist to ensure a consistent seed production of rootstock trees. This study aimed to obtain information on the phenology of the important rootstock cultivars used in South Africa to develop production guidelines. Trials were conducted at the Citrus Foundation Block (CFB) in 2020 and 2021 on Rough lemon, C-35, X639, Carrizo citrange, Swingle citrumelo, and MxT trees planted in 2015 and US-812 trees (planted in 2010). The average fruit diameter (mm) differed significantly between cultivars. SC has the largest fruit size and X639 and US-812 the smallest. The average seed number/fruit for SC (19 seeds/fruit) was higher than all cultivars except for CC (18 seeds/fruit), while US-812 had the lowest seed number (3 seeds/fruit). Similar trends were seen for the 2021 season, where C-35 (20/fruit) had the largest seed count but was not significantly different from SC. In most cases, the seed count does not differ greatly between green, colour break and full-colour sampling stages for each cultivar. Large fruit produced more mature seeds than small fruit. The average yield/tree varied amongst cultivars. RL had the highest yield (121.61 kg) followed by X639 (81.48 kg), CC (57.30 kg), SC (53.81 kg), MxT (34.09 kg) and C-35 (22.97 kg) for the first season. In 2021, a similar trend was seen, but there were fewer differences between cultivars. RL

again had the highest yield (83 kg) but not significantly higher than SC (78 kg), and C-35 had the lowest yield. The yield for each cultivar varied between seasons (2020 and 2021); some cultivars showed an increase, whereas a reduction was seen for others. X639 produced an average of 2516 (1<sup>st</sup> season) and 1997 (2<sup>nd</sup> season) fruit/tree, whereas all other cultivars had below 900 fruit/tree. CC had the highest millilitre viable seed (2613 ml), followed by X639 (2133 ml), RL (1450 ml), and < 1000 ml for SC, C-35 and MxT for 2020. In 2021, CC again had the highest seed production (4063 ml), followed by SC and all other cultivars lower than 1250 ml. The average seed count/fruit determined from the millilitre seed following the seed treatment process was 11/fruit for C-35 (not differing from CC and RL) and 5/fruit for X639, which was the lowest (first season). In 2021, CC (16/fruit), C-35 (15/fruit) and SC (13/fruit) were amongst the top three, with X639 again having the lowest seed number (3/fruit). Seed germination (in an incubator) was higher for fruit sampled at colour break than the green fruit, except for RL, which showed good germination at both stages for 2020. For full coloured fruit, RL and US-812 showed high germination >85%, and MxT had the lowest (<59%). In 2021, RL had the best germination compared to the other cultivars at the green stage. The germination % increased from green to full colour in most cases except for RL and C-35. The seedling germination in the greenhouse (temperature controlled) after 88d was >83% for all cultivars except MxT (72.8%). MxT seedlings were the tallest at 88d after sowing compared to the other rootstock seedlings. Overall, the % 'Off-type' seedlings was < 5.6% (for CC) and 0% for MxT. Exogenous GA<sub>3</sub> sprays (10, 15, 20 ppm) did not influence the fruit set % compared to untreated control in most cases (X639 had 2X percentage fruit set, 16.83%, at 20 ppm compared to the control). GA<sub>3</sub> did not affect the fruit yield. Nitrogen levels were below optimum over both seasons for most rootstock cultivars, except for CC and C-35 in 2020. The potassium levels were lower than optimum in 2020 for most cultivars except for CC and US-812. In 2021, the K levels remained below optimum for RL, X639, and MxT. Overall, the tree health of all cultivars was good, with no obvious signs of yellowing. In conclusion, these results provide valuable cultivar-specific information to assist in future planning for rootstocks farms, i.e., the number of trees needed per cultivar to ensure the seed supply is met to assist in HLB management when the turnaround time for commercial citrus trees are more rapid than it is currently. Therefore, yearly rootstock seed supplies need to be consistent, and cultivation practices on seed farms need to be adapted accordingly.

## Opsomming

Sitrusonderstamme is 'n bepalende faktor vir die sukses van kommersiële sitrusaanplantings. Voortplanting van onderstamme vind hoofsaaklik plaas deur sade, en 'n hoë hoeveelheid en kwaliteit saad is dus belangrik om voortdurende voorraad te verseker. Tans bestaan geen duidelike bestuurspraktykryglyn om sodoende 'n konsekwente saadproduksie van onderstambome te verseker nie. Die doel van hierdie studie was om inligting te verkry oor die fenologie van die belangrike onderstamkultivars wat tans in SA gebruik word om sodoende produksieriglyn te ontwikkel. Die proewe is by die Sitrus Grondvesblok (SGB) in 2020 en 2021 uitgevoer op Growweskil suurlemoen (GS), C-35, X639, Carrizo citrange, Swingle citrumelo en MxT bome, geplant in 2015 en US-812 bome (geplant in 2010). Die gemiddelde vrugdeursnee (mm) het betekenisvol tussen kultivars verskil. SC was die grootste en X639 en US-812 vrugte die kleinste. Die gemiddelde saadgetal/vrug vir SC (19 sade/vrug) was hoër as alle kultivars behalwe vir CC (18 sade/vrug), terwyl US-812 die laagste saadgetal (3 sade/vrug) gehad het. Soortgelyke neigings is gesien vir die 2021-seisoen, waar C-35 (20/vrug) die grootste saadtelling gehad het, maar nie beduidend verskil het van SC nie. In die meeste gevalle het die saadtelling nie tot 'n groot mate verskil tussen groen-, kleurbreek- en volkleurstadiums vir elke kultivar nie. Groot vrugte het meer volwasse sade as klein vrugte geproduseer. Die gemiddelde opbrengs/boom het verskil tussen kultivars. GS het die hoogste opbrengs (121.61 kg) gevolg deur X639 (81.48 kg), CC (57.30 kg), SC (53.81 kg), MxT (34.09 kg) en C-35 (22.97 kg) vir 2020. Gedurende 2021 is 'n soortgelyke tendens gesien, maar daar was minder verskille tussen kultivars. GS het weer die hoogste opbrengs (83 kg), maar nie betekenisvol hoër as SC (78 kg) gehad nie en C-35 het die laagste opbrengs. Die opbrengs vir elke kultivar het gewissel tussen seisoene (2020 en 2021), sommige kultivars het 'n toename getoon, terwyl 'n afname vir ander gesien is. X639 het gemiddeld 2516 (1ste seisoen) en 1997 (2de seisoen) vrugte/boom geproduseer, terwyl alle ander kultivars minder as 900 vrugte/boom gehad het. CC het die hoogste milliliter volwasse saad (2613 ml) geproduseer, gevolg deur X639 (2133 ml), GS (1450 ml), en < 1000 ml vir SC, C-35 en MxT vir 2020. In 2021 het CC weer die hoogste saad hoeveelheid gehad (4063 ml), gevolg deur SC, en alle ander kultivars laer as 1250 ml. Die gemiddelde saadtelling/vrug wat uit die milliliter saad na die saadbehandelingsproses bepaal is, was 11/vrug vir C-35 (nie verskil van CC en GS nie), en 5/vrug vir X639, wat die laagste was (1ste seisoen). In 2021 was CC (16/vrug), C-35 (15/vrug) en SC (13/vrug) onder die top drie, met X639 wat weer die laagste saadgetal

(3/vrug) gehad het. Saadontkieming (in 'n broeikas) was hoër vir vrugte wat by kleurbreek gemonster is in vergelyking met groen vrugte, behalwe vir GS wat goeie ontkieming in beide stadiums vir 2020 getoon het. Vir volkleur vrugte het GS en US-812 hoër ontkieming >85% getoon, en MxT het die laagste (<59%). In 2021 het GS die beste ontkieming gehad in vergelyking met die ander kultivars in die groenstadium. In die meeste gevalle het die ontkieming % toegeneem van groen na volkleur behalwe vir GS en C-35. Saadontkieming in die kweekhuis (temperatuurbeheer) na 88d was >83% vir alle kultivars behalwe MxT (72.8%). MxT saailinge was die hoogste op 88d na saai in vergelyking met die ander onderstamsaailinge. Algeheel was die % 'Af-tipe' saailinge < 5.6% (vir CC) en 0% vir MxT. Eksogene GA<sub>3</sub>-bespuitings (10, 15, 20 dpm) het in die meeste gevalle nie die vrugset % in vergelyking met onbehandelde kontrole beïnvloed nie (X639 het 2X persentasie vrugset, 16.83%, teen 20 dpm in vergelyking met die kontrole gehad). GA<sub>3</sub> het geen effek op die vrugopbrengs gehad nie. Daar was 'n neiging dat stikstofvlakke gedurende beide seisoene vir die meeste onderstamkultivars onder die optimale vlak was, behalwe vir CC en C-35 in 2020. Die kaliumvlakke was laer in 2020 as die optimale vir meeste kultivars, behalwe vir CC en US-812. In 2021 het die vlakke van K onder die optimale vir GS, X639 en MxT gebly. Oor die algemeen was die boomgesondheid van alle kultivars goed, sonder duidelike tekens van vergelyking. Ten slotte bied hierdie resultate waardevolle kultivar-spesifieke inligting om dus by te dra tot toekomstige boordaanplantings en beplanning van elke kultivar. Dit sal bydra tot die bestuur van HLB wanneer sitrusbome meer gereeld vervang sal moet word as wat tans die geval is. Daarom moet die jaarlikse voorsiening van onderstamsaad konsekwent wees, en die bestuurspraktyke van saadplase moet na gelang aangepas word.

## Introduction

As the name implies, Citrus rootstocks serve as the root system of the scion, grafted onto the rootstock, and therefore plays an important role in how the tree responds in different growing areas and conditions (Castle *et al.*, 1993). Seeds are the main propagation method of rootstocks, and, as mentioned by Tolley (2017), are the starting point of your citrus crop. The production of high quality and quantity seed to serve as a rootstock is a physical building block of the SA citrus industry.

A high demand in the previous 5-10 years has led to an undersupply from the CFB in certain seasons. This had a negative impact on income and a delay in supplying nurseries timeously with high-quality seed. Fruit production for seed is a neglected area of Citricultural research. Seed production for the industry will be more complex as increased rootstock options are made available and the requirement for seeds increase. The problem lies in the widely different citrus species used for seed with phenological and botanical differences, clearly illustrated in the leaves, branching habits and fruit. Furthermore, flower development and pollination are important processes that precede fruit and seed production. This is highly influenced by climate (Iglesias *et al.*, 2007; Lenz, 1969), an uncontrollable factor between seasons. Climatic events could affect total seed production differently between seasons, and so affect various rootstock cultivars differently.

To date, no clear Citriculture guidelines and rootstock specific species management actions exist to enable a consistent increase in seed production. Research mainly focuses on how the different rootstock combinations influence the scion's yield and tree growth, not the rootstock alone (Castle *et al.*, 2010; Stover *et al.*, 2004). In addition, the reaction of standard practises such as gibberellic acid, urea and girdling, used for flower and fruit set manipulation, is unknown. Furthermore, no information on the phenology of the various main seed cultivars exist in terms of flower formation and the need to allow vegetative development. The impact of nutrition on the fruit set of these cultivars is also unknown, and no nutrition norms exist to facilitate optimum production.

The current high demand for seed will most likely continue in the foreseeable future in the SA citrus industry. In addition, new rootstocks, higher density plantings and orchards being replaced at a higher interval could become a key part in HLB management to obtain sustainable production in orchards. Therefore, it is necessary to develop production systems to facilitate a constant supply of the fully required rootstock range.

The main aim of this study was to gain information on the phenology of the most important rootstock cultivars used in South Africa to develop production guidelines to maintain consistent seed production. The rootstocks evaluated included Rough lemon (*Citrus jambhiri*), C-35 citrange (*C. sinensis* x *Poncirus trifoliata*), X639 citrandarin (*Cleopatra mandarin* (*C. reticulata*) x *P. trifoliata*), Carrizo citrange (*C. sinensis* x *P. trifoliata*),

Swingle citrumelo (*C. paradisi* x *P. trifoliata*), MxT (Minneola (*C. paradisi* x *C. reticulata*) x *P. trifoliata*) and US-812: Sunki Mandarin (*C. reticulata*) x Benecke trifoliolate orange (*P. trifoliata*). The trials focused on establishing data on the yield, seed production, and germination of seeds at different maturity stages. One of the fruit set practices used on commercial citrus farms, Gibberellic acid (GA<sub>3</sub>), was evaluated for efficacy in alternate bearing cultivars. The project objectives are listed below.

### Stated objectives

- Objective 1: Compile a literature review of any available information on citrus rootstock seed production.
- Objective 2: Describe the bearing habit and main horticultural properties of the 7 main rootstock cultivars.
- Objective 3: Quantify the yield of fruit vs. seed for these cultivars
- Objective 4: Determine if noticeable differences exist in nutrition norms between these rootstocks.
- Objective 5: Test efficacy of GA<sub>3</sub> to enhance fruit set.
- Objective 6: Develop vegetative management guidelines to enable consistent bearing.

### Materials and methods

#### Trial site and Location

The trial site was located at the Citrus Foundation Block in Uitenhage, Eastern Cape Province, South Africa. The rootstock cultivars used in the respective trials included Carrizo citrange (CC), Swingle citrumelo (SC), Rough Lemon (RL), X639 citrandarin, Citrange 35 (C-35) and Mineola x trifoliolate hybrid (MxT) trees, all of which are grafted on CC and planted in 2015. Due to the limited amount of trees for some cultivars, an additional MxT orchard planted in 1997 grafted on RL was also used for the seed germination trial. In addition, US-812 (refer to in the industry as Sunki Benecke) trees grafted on CC and planted in 2010 was used for the germination trials and fruit growth measurements in the 2021 season. Similar orchard practices i.t.o. irrigation and cultural practices were followed for all experimental blocks. The experiment were carried out over two consecutive seasons, 2019/2020 and 2020/2021.

#### Seed quantification and germination at different maturity levels

Ten trees of uniform size, health, and crop-load within the same row were tagged as single tree replicates (n=10) (experimental units) for fruit samples at three different maturity stages based on rind colouration viz. green (G) and after colour-break (CB) during phase II of fruit development (Bain, 1958), and full colour (FC) (Phase III of fruit development). This was done for all rootstock cultivars except MxT, due to limited available trees. MxT grafted on RL rootstock was used instead. In Table 4.5.4.1, the days before the final harvest indicate each maturity stage for the respective cultivars and the differences in ripening patterns.

During the first season (2020) fruit were sampled only at the green (10 fruit/rep) and colourbreak (5 fruit.rep) stage from the selected trees. For 2021, fruit were sampled at all three stages, but the fruit nr/rep varied between cultivars due to limited available seeds to use for germination trials. Five fruit/rep were sampled at all three stages for SC, CC, MxT on RL, and RL and for X639 (10 fruit/rep) and SxB (20 fruit/rep). For C-35, 10 fruit at green and 5 fruit/rep at CB and FC stage.

**Table 4.5.4.1.** The number of days before harvest (dbh) that each cultivar was sampled at for seed extraction at the two respective maturity stages based on the rind colouration for the two respective seasons (2020 and 2021).

Cultivar	Season	Fruit maturity stage			
		Green (dbh)		Colour-break (dbh)	
C-35	2020	42		20	
	2021	34		16	
SC	2020	63		41	
	2021	48		32	
CC	2020	79		43	

	2021	90		70
RL	2020	92		56
	2021	77		45
US-812	2020	92		56
	2021	63		45
X639	2020	119		75
	2021	50		38
MxT grafted on RL	2020	104		36
	2021	72		34

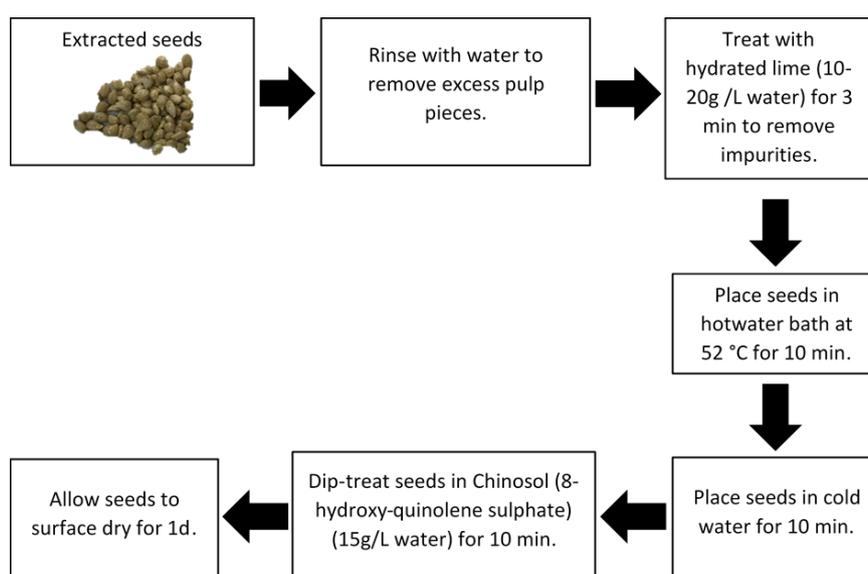
Seeds were extracted by cutting the rind, cutting through the pulp, along the diagonal section, and twisting the fruit to separate it. The fruit was then squeezed into a plastic strainer to remove all the seeds and rinsed with water to remove excess pulp pieces. The seeds were then separated into mature (fully developed seeds) and immature seeds (Figure 4.5.41), to quantify the viable seeds.



**Figure 4.5.4.15.** Fully developed (mature) (A) vs. immature/non-viable (B) Swingle citrumelo seeds.

A series of treatments need to be completed to allow for high seed germination, which is shown in the figure below (Fig. 4.5.4.2). On completion of the seed treatment, the air-dried seeds are soaked in water for 2h, where after a measuring spoon was used to place  $\pm 30$  ml seed per rep in a container lined with a paper towel. The container was closed with a small hole in the lid, to allow airflow, and placed in an oven at  $\pm 28$  °C for 20 days. The paper towel was wetted occasionally during the germination period. The germination percentage was determined on completion of the 20 d period using the following equation:

$$\text{Germination \%} = \frac{n \text{ germinated seeds}}{n \text{ total seeds (germinated+non germinated)}} \times \frac{100}{1} \quad [1]$$



**Figure 4.5.4.16.** Flow diagram of required steps after seed extraction to successfully germinate citrus seeds.

### Monthly fruit size measurements

The fruit development of CC, SC, MxT, C35, and X639 were followed from Dec. 2019 until harvest in 2020. Ten trees, uniform in health, size, and crop-load within a row, were selected per cultivar (single tree replicates). On each tree, 20 fruit were tagged with a self-tie label for continuous monthly fruit diameter, measured with an electronic fruit size measure and data logger (GÜSS Manufacturing (Pty) Ltd., Strand, South Africa). For the 2020/21 season, fruit were not tagged, but 20 random fruit were measured/tree each month. In addition, RL and US-812 trees were included. For the RL cultivar, fruit were tagged, to avoid measurements of different fruit sets on the tree.

### Fruit yield and fruit number at harvest

The yield (kg/tree) and the number of fruit per tree were determined from the same trees used for fruit measurements for the 2019/20 and 2020/21 season. All the fruit were harvested from each tree (number/tree) before placing the fruit in plastic crates. An electronic orchard scale was used to determine the kg per tree. According to the tree number for subsequent seed extraction, the crates were marked to determine each cultivar's seed number per tree.

### Seed quantification/tree at harvest

The seeds were extracted separately for each rep(tree)/cultivar for the following cultivars, CC, RL, X639, MxT, SC, and C35. For RL the fruit from two trees (i.e. tree 1+2 etc.) were pooled, due to the high fruit numbers. Seeds were extracted using a seed extraction machine consisting of a fruit shredder and a water separator for separating the pulp/juice from the seeds (for all steps and illustrations see: Commonsense Citrus page 146; Tolley, 2017). Seeds were collected, treated according to Figure 4.5.4.2, air-dried, and quantified and recorded as milliliter (ml) viable seed, as this is the standard quantification method used by the CFB. In addition, the average seed number per fruit after the seed extraction and treatment process was also calculated. From a sample of 20 mature seeds per cultivar, the average weight of a mature seed (g) was calculated before, measuring the total ml viable seed (kg). The number of seed was calculated as follows:

$$\text{Number of Seed} = \frac{\text{Weight of total viable seed (g)}}{\text{Average weight of a mature seed (g)}} \quad [\text{Eq. 1}]$$

This value was then divided by the fruit used in the seed extraction process to obtain the average seed per fruit. This was done for both seasons (2020 and 2021).

### Relationship between fruit size (large vs small) and seed quantity

At harvest during the first season (2020), for each cultivar, fruits of contrasting size (5 to 10 mm depending on the rootstock cultivar) were picked. From the experimental units, 10 large and 10 small fruit were sampled per tree (n=10). The seeds from the two fruit size classes were extracted and quantified, as previously described, to determine if there is a difference in seed number. Subsequently, the seeds from each replicate were pooled for subsequent germination trials (germination at full colour and in the greenhouse).

### Germination percentage at harvest: Incubator and greenhouse

The seeds extracted from large and small fruit were used to determine the germination percentage (%) at full colour (at standard commercial harvest) in the incubator and the greenhouse during the 2020 season. The same seed treatment procedure was followed as described earlier for germinating the seeds in the incubator. In addition, seeds were placed in water for 1h before sowing for the temperature-controlled greenhouse germination trial. Seedling plastic tubes were prepared as per standard CFB practice and the medium used was a mixture of 50% sand and peat respectively. A total of 35 seeds per cultivar rep (n=10) were planted (1 seed per tube (90 cm<sup>3</sup>)) except for US-812, where only 18 seeds per rep were planted due to limited available seeds. A small hole of 2.5 cm was made in the soil of each tube, where after the seed was inserted and covered with soil. The trays (tubes inserted into nursery trays) were watered and fertigated as per standard procedure by the CFB.

Seedlings were evaluated after 88 and 225 days. During the 88 day evaluation, the germination percentage was determined by recording any sign of germination, including seedlings that emerged but died thereafter. However, it was recorded as dead, at the 225 day evaluation. The number of seedlings per tray were recorded

as mono or multiple seedlings. The height of each seedling was recorded and for multiple seedlings, only the tallest seedling height was recorded. From the seedlings that germinated at the 88 day evaluation, the number of seedlings > 20 cm, seedlings < 20cm, dead seedlings, misshapen growth, and off-type seedlings were recorded and expressed as a percentage. At the 225 day evaluation, the seedling establishment (post-germination) was evaluated by determining if there was any new germination that occurred after 88 days and counting the number of seedlings that can be transplanted and used for grafting.

### **Efficacy of gibberellic acid (GA<sub>3</sub>) on fruit set percentage (%) and fruit yield**

The trial consisted of a randomized complete block design with six single tree replicates (n=6) per treatment for RL, X639, and C-35 respectively and five single tree replicates (n=5) for CC and SC. The treatments included an untreated control and 10, 15, and 20 ppm ProGibb 40% SG as a source of GA<sub>3</sub> was used. Due to a limited number of trees, only two MxT trees per treatment (n=2) were selected and only three treatments were applied (untreated control, 10 ppm and 20 ppm). In addition, there was a buffer tree between treatments to allow for drift during application.

ProGibb® 40% SG (Valent BioSciences Corporation, Libertyville, Illinois 60048, USA) containing 400 g·kg<sup>-1</sup> GA<sub>3</sub> active ingredient was applied as a foliar spray application using a backpack mist-blow sprayer (Stihl SR430; Andreas Stihl (Pty) Ltd, Pietermaritzburg, South Africa) at a rate of approximately 4.5 L spray mixture per tree. All the GA<sub>3</sub> treatments were applied with 18 ml-per 100 L<sup>-1</sup> water, non-ionic wetting agent Villa 51 (wetter). The three foliar GA<sub>3</sub> treatments, were applied at 10 mg·L<sup>-1</sup> (commercial control), 15 mg·L<sup>-1</sup> or 20 mg·L<sup>-1</sup>. GA<sub>3</sub> ideally should be applied at 80% petal drop to optimise fruit set; however, due to protracted flowering, the applications were made during 50% and 100% petal- drop to cover as many fruitlets as possible.

In order to determine the efficacy of GA<sub>3</sub> on the fruit set, ten 6-12 month old flowering shoots were tagged per replicate and the flower number was determined. Flowers were counted after the first GA<sub>3</sub> spray for all cultivars except for X639 and MXT, where the flowers were counted a day/two before the first GA<sub>3</sub> application. In December 2020, following physiological fruit drop, the number of fruitlets on the tagged branches were recorded and the fruit set % was determined:

$$fruit\ set\% = \frac{\#\ fruitlets}{\# number\ of\ flowers} \times \frac{100}{1}. \quad [Eq. 2]$$

The yield (kg fruit/tree) and fruit nr/tree were determined during commercial harvest in the 2021 season for the four respective GA<sub>3</sub> applied treatments on the different rootstock cultivars. Thereafter the fruit from the replicate trees were pooled for each treatment and an overall seed quantity (liter seed) were determined

### **Flower number and fruit set % for the 6 rootstock cultivars**

The ten trees tagged for yield and fruit measurements were used to determine the total flower nr and fruit set % of the different cultivars. Ten shoots in similar length and between 6-12 months old were tagged randomly per tree. All the flowers on the shoot were counted (green bud to full bloom/fruitlet stage) during Sept.-Oct. 2021 season. Following physiological fruit drop, the number of fruitlets present on each shoot were quantified on 30 Nov. 2021. The fruit set% for each cultivar was determined thereafter by using Eq. 2.

### **Tree volume**

The tree volume was calculated per tree for the trees used for the GA<sub>3</sub> trials. A messfix-S measuring stick was used to determine the height and radius of the canopy on each side of the tree, North, South, West and East. The tree volume was determined by the following equation:

$$V\ (volume,\ m^3) = r^2(\pi h - 1.046r)$$

r =canopy radius

h = height of fruit bearing canopy

### **Statistical analysis**

Statistical analyses were done through XLstat software. A one-way ANOVA for a completely randomized design was performed for **fruit size, fruit yield, fruit number/tree, germination at harvest (2020 season),**

**greenhouse germination, average seed number per fruit at colour break, viable seed count at harvest, flower count 2021/22 season and fruit set % 2021/22 season** to compare differences between cultivars. In the case where data was not normally distributed i.e. % dead seedlings a Kruskal-Wallis test for non-parametric data was done.

**Germination % at two seedling maturity stages.** A completely randomized split plot was performed to determine which cultivars show the best germination % at the respective maturity stages (88 and 225 days after planting).

**Fruit set %, fruit yield per tree, fruit nr per tree and average fruit/m<sup>3</sup> for GA<sub>3</sub> trials.** A one-way ANOVA for a randomised complete block design.

**Seed count vs fruit size.** A one-way ANOVA for a randomised complete block design, where each tree served as an experimental unit (block) (n=10) from which two fruit were sampled for the two contrasting treatments (large and small).

**Seed count at different maturity stages.** Data was analysed by means of a one-way ANOVA for a randomized complete block design, where each tree served as an experimental unit (block) (n=10) from which green and colour break fruit were sampled for the 2020 season. In 2021, the green, colour break and full colour was analyzed.

For all analysis the Fisher Least Significant Difference (LSD) tests were used for the Post Hoc-testing and significant differences were determined at  $p \leq 0.05$  (5% significant level).

## Results and discussion

Below is the literature review from Objective 1. Further results from the various trials done for the respective objectives, follow after the conclusion from the literature review.

### Literature Review: Seed production in Citrus Rootstock trees

#### 1. Introduction

In citriculture, rootstocks play a critical role in determining the success of how citrus is produced in different areas and conditions (Castle *et al.*, 1993). A rootstock functions as an anchor for the specific scion cultivar that is grafted onto the rootstock. In addition, it is also the root system of the tree, thereby being responsible for nutrient and water absorption and supply to the vegetative and reproductive development.

Each rootstock has one or more undesirable traits, i.e., being susceptible to *Phytophthora*, root rot, or Citrus Tristeza virus; however, there are individual characteristics that make a positive contribution to the performance of citrus trees (Castle, 2010). In general, citrus rootstocks have three purposes, one of which includes the reduction of juvenility of commercial cultivars. The scion cultivars are budded on rootstock seedlings, ensuring early fruiting and more regular and uniform trees instead of propagating the scion cultivars by means of seeds. Furthermore, rootstocks also aid in adaption to different soil conditions, resistance to diseases, and nematodes. In addition, the rootstock influences the horticultural performance of the scion, i.e. water relation, mineral nutrition uptake, and differences in fruit yield and internal quality (juice total soluble solids and acid percentage) (Castle *et al.*, 1993; Castle, 2010; Hardy, 2004).

Citrus rootstocks are mainly propagated by means of seeds. Commercial propagation's success is highly dependent on fruit with an adequate number of nucellar embryonic seeds (Spiegel-Roy and Goldschmidt, 1996). Nucellar embryony means that the seedlings which arise from the nucellus are uniform with an identical genotype to the mother plant. The seedlings' uniformity makes it ideal for rootstock propagation (Hartmann and Kester, 2011). In order to ensure a continuous supply of rootstock seeds to commercial citrus nurseries, production of a high number of fruit from each rootstock source tree is critical. Furthermore, each fruit must have a high number of viable seed with a good germination percentage.

In order for fruit development to occur, flower development, followed by pollination, fertilization, and subsequent seed development must take place (Kretdorn, 1986). The influence of climate in the process is critical, as it is the main uncontrollable factor that influences flower development (Davenport, 1990. Lenz, 1969; Goldschmidt *et al.*, 1985) and pollination (Iglesias *et al.*, 2007), which consequently influences fruit yield.

In citriculture, several cultural practices and crop manipulation techniques are implemented on trees to increase flower intensity and fruit set. This includes water stress, nitrogen application, girdling, mechanical pruning and exogenous gibberellic acid application (Furr *et al.*, 1947; Lovat *et al.*, 1988; Menino *et al.*, 2003; Schaffer *et al.*, 1985; Southwick and Davenport, 1986; Talon *et al.*, 1992; Mesejo *et al.*, 2020). This research is, however, focused on valuable commercial cultivars relevant to the fresh or juice industry. The wide range in genetic origins of rootstock brings into question the efficacy of these fruit set practices.

The principle tree physiology of various citrus rootstock trees is an unknown field, with research being focused on how the differences in yield, tree growth and fruit quality are related to different rootstocks, in combination with a scion (Bowman and Román, 1999; Castle *et al.*, 2010; Hussain *et al.*, 2013; Louzanda *et al.*, 2008; Roose, 2014; Stover *et al.*, 2004). In addition, it is also unknown what contribution each rootstock and scion have on the final tree characteristics. Therefore, different cultural practices and crop manipulation techniques might be required for the specific rootstock cultivar to enable the mother plants to flower and set fruit with an adequate number of seeds.

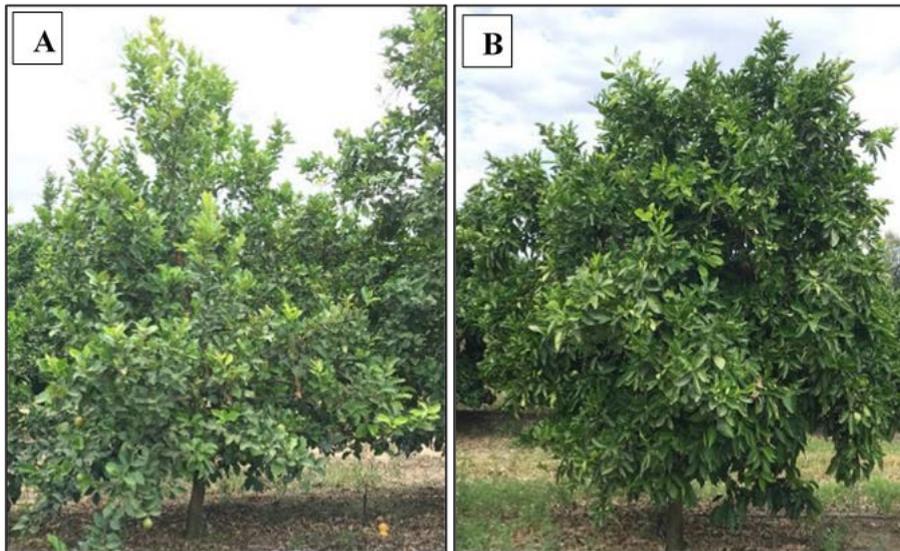
## 2. Citrus tree anatomy

### Shoots and leaves

The anatomy of *Citrus* is very well described by Schneider (1968) in “The Citrus Industry.” In brief, commercial citrus trees have a single trunk of cylindrical shape, with main branches diverging from the trunk approximately 60-120 cm above the ground. This branching habit results in the tree having a spherical shape (Fig. 4.5.4.3.). However, the tree density differs between cultivars, orange trees have a dense growth, where lemon trees have fewer and larger branches, resulting in a more open canopy (Schneider, 1968) (Fig. 4.5.4.4.).



**Figure 4.5.4.17.** A picture illustrating the branch formation of a defoliated Navel sweet orange tree (*Citrus sinensis*).



**Figure 4.5.4.18.** The difference in tree density/canopy structure between a Eureka lemon tree (A) compared to a Navel orange tree (B).

Shoot growth in *Citrus* has distinct growth flushes (Bevington and Castle, 1985). These growth flushes are influenced by climate, with 2-5 flushes occurring in subtropical regions and no defined flushes in the tropical regions due to growth continuing uninterruptedly. However, the flushes differ in growth habit, which consequently influences the tree growth pattern/architecture (Mendel, 1969). The spring flush is considered to be the most important flush, since it has both vegetative and reproductive (flowering) shoots (Goldschmidt *et al.*, 1985; Spiegel-Roy and Goldschmidt, 1996) (Fig. 4.5.4.5), whereas the subsequent flushes are generally vegetative with fewer, but longer vigorously growing shoots (Spiegel-Roy and Goldschmidt, 1996).

The composition of the spring flower bearing shoots range from:

- 1) Cymose inflorescence (flowers + aborted leaves).
- 2) Flowers, fully formed leaves and aborted leaves.
- 3) Leafy shoots with a terminal flower and few or more axillary flowers.
- 4) Sterile vegetative shoots.

Generally, flower bearing shoots have eight nodes in length. Leafy flower-bearing shoots and vegetative shoots have long internodes, with a triangular cross section, whereas shoots with aborted leaves have short internodes and might have fewer than eight internodes with a circular cross-section. There are also instances in lemons, where the vegetative nature of the flower-bearing shoots are modified to short and round, and appear like flower stalks where there is a solitary, terminal flower with the aborted leaves being inconspicuous (Schneider, 1968).

The new flush grows at a slight angle to the previous one (Fig. 4.5.4.6) and the growth flushes can also be distinguished by short, swollen internodes at the beginning and end of each flush. The young stems are green and tender with a prominent ridge, but become hard and round during secondary growth (Schneider, 1968).



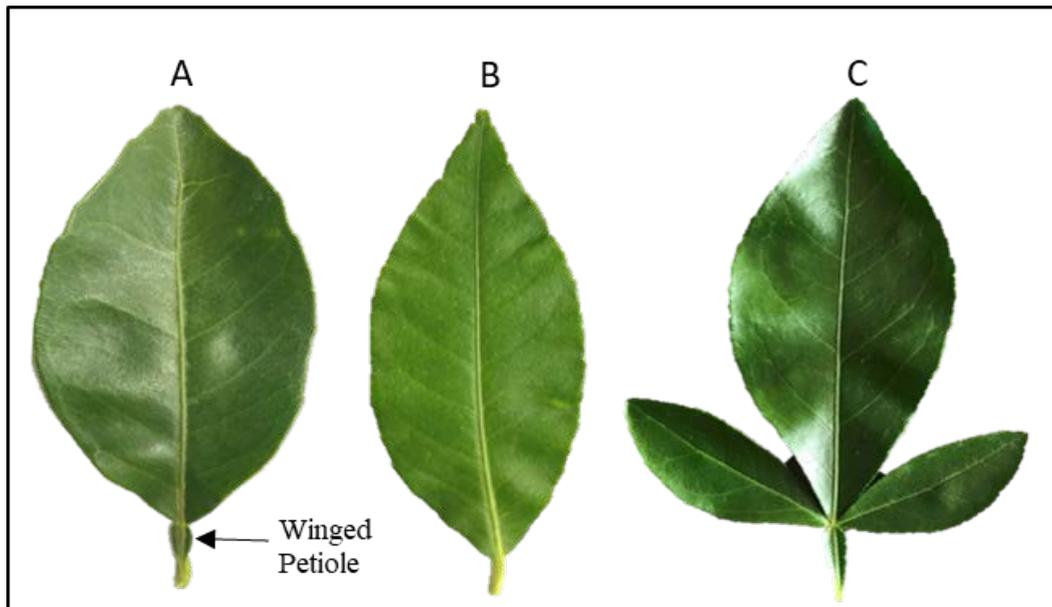
**Figure 4.5.4.19.** A drawing of spring flush of Shamouti orange (*C. sinensis*) with vegetative shoot (A), and reproductive shoots, leafy inflorescence (B) and leafless inflorescence (C). (Source: Spiegel-Roy and Goldschmidt, 1996).



**Figure 4.5.4.20.** Summer flush emerging in Palmer Navel orange (*C. sinensis*). Note that the new shoot develop at a slight angle to the older shoot.

### Leaves

Mature citrus leaves have a dark green upper surface, with a light yellow-green colouration on the lower surface. The leaf blades are oval to oblong in form and there is a prominent mid-vein that forms the leaf's vascular system, which becomes smaller towards the leaf tip. In terms of phyllotaxis the leaves of citrus trees are arranged spirally (left or right) around the stem, with the spiral direction alternating between the growth flushes. The citrus leaf of commercial scion cultivars is a unifoliate compound (Fig. 4.5.4.7A), and most of the citrus species have winged petioles, with the size of the wings varying amongst varieties. However, lemon leaves have no petiole wings (Fig. 4.5.4.7B). (Schneider, 1968). In addendum A, the leaves of the commercial important rootstocks in South Africa are shown. Trifoliate orange, Swingle citrumelo, and Carrizo citrange leaves are correctly described as trifoliate, where three leaflets are attached to a common axis (Fig. 4.5.4.7C).



**Figure 4.5.4.21.** Example of a unifoliate compound leaf with a winged petiole (A), a lemon leaf with no winged petiole (B) and a trifoliate leaf of Swingle citrumelo (C).

### Root system

The root system is considered as a separate biological entity in the tree, and rootstocks are used to function as the rooting system and therefore the effect of the root system can be manipulated by rootstock choices (Castle, 1978). The root system is an anchor of the tree and a source of water, minerals, and hormones to the above ground plant parts (Spiegel-Roy and Goldschmidt, 1996).

During germination, the primary root is the first organ to appear. The taproot that grows straight down when planted later becomes the primary root. Thereafter, secondary lateral roots form, consisting of large pioneer roots and bunches of fine fibrous roots (Schneider, 1968) (Fig. 4.5.4.8). The fibrous roots are located in the top part of the soil profile (shallow roots) and aids in rapidly absorbing nutrients and water from light rains. The deeper roots prevent extreme drought stresses and also absorb nutrients not absorbed by fibrous roots (Castle, 1978).



**Figure 4.5.4.22.** The root system of a 225 day old Carrizo (A) and Swingle citrumelo (B) rootstock seedling.

Root elongations occur in flushes (Schneider, 1968) and follow a cyclic growth pattern due to root growth alternating with shoot growth. In addition, soil temperature (< 13 °C negative) and soil water content (drought) also influence root growth negatively (Bevington and Castle, 1985).

With the emphasis on root growth, differences amongst rootstocks is an unknown field, with limited research on whether cultural practices, i.e. water and nutrient application, should be adapted. Bevington and Castle (1985) investigated the pattern of root growth of 'Valencia' orange [*Citrus sinensis* (L.) Osbeck.] trees on two rootstocks, Rough Lemon (*C. jambhiri* Lusch.) and Carrizo citrange [*Poncirus trifoliata* (L.) Raf. X *C. Sinensis*], and found no differences. In contrast, Morgan *et al.* (2007) proposed that irrigation depth and depth for fertilizer placement based on root distribution should be rootstock specific. They found that the fibrous root length density and root length was significantly larger and/longer for Swingle citrumelo (*C. paradisi* Macf. X *P. trifoliata*) compared to Carrizo citrange grafted on 'Hamlin' sweet orange [*C. sinensis* (L.) Osb.] within the 0-15 cm soil depth.

These observations are important, as cultivation practices that may be adapted in the management of various rootstock mother plants are involved.

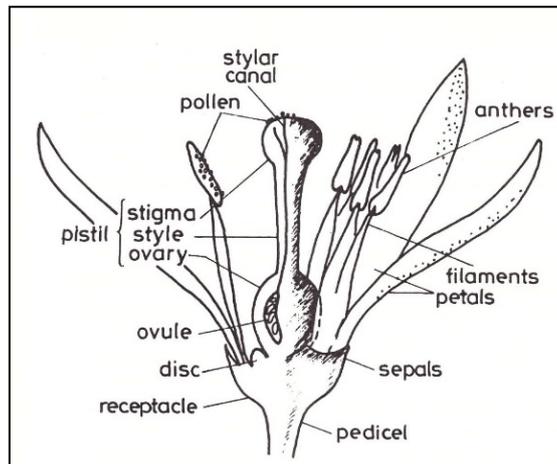
### **3. Citrus flowering and fruiting**

#### **3.1. Flowers**

Flowering is the start of the reproductive process, finally leading to fruit (Kinet, 1993). Citrus flower development had been explained and/reviewed in depth by researchers such as Schneider (1968), Davenport (1990) and Krajewski and Rabe (1995). In this review, only a brief description of the structure of a mature flower, different flowering shoots, and factors influencing flower development will be given.

The basic structure of a mature citrus flower is shown in Figure 4.5.4.9. The pedicel serves as a support to all the floral structures which broadens to form the receptacle at the base (Davenport, 1990). The calyx is a cuplike structure with five sepals on the rim of the cup (Fig. 4.5.4.10). The five petals, called the corolla, alternate with the sepals (Schneider, 1968). In addition, the petals are white of colour and have a thick, leathery appearance (Davenport, 1990). At the base of the ovary is a disc structure called the nectary due to the function of secreting watery nectar through the stomata. Furthermore, the anthers are yellow, 4-lobbed structures, containing cells within the lobes that produce pollen grains (Schneider, 1968).

The stigma is the floral organ that develops at the end of the style and is receptive to pollen grains after anthesis (flower fully opened) (Ortiz, 2002). On the stigmas surface are papillose hairs which secrete a sweet viscous fluid that aids in retention and germination of pollen grains that land on the stigma (Davenport, 1990; Schneider, 1968). There are also canals present on the stigma that pass through the style to the ovules. The ovary is located in the middle between the other structures. Within the ovary are ovules, where fertilization occurs, and the canals on the stigma pass through the style to the ovules (Davenport, 1990).



**Figure 4.5.4.23.** A drawing of an open citrus flower (Source: Spiegel-Roy and Goldschmidt, 1996).



**Figure 4.5.4.24.** An example of the calyx indicated by the arrows of a C-35 citrange flower at balloon stage (A), open flower (B) and after petal fall (C).

As mentioned previously, there are two types of flowering shoots that occur (Goldschmidt et al., 1985). These flowering shoots are referred to as inflorescences (leafy and leafless). A leafy inflorescence (mixed shoots) occurs on the new season's growth and consists of a terminal flower or many single axillary flowers with leaves present while the leafless inflorescence occurs on the previous season's growth, and is referred to as a generative shoot with one or more flowers with no leaves (Davenport, 1990; Davies and Albrigo, 1994). The different inflorescent types influence fruit set. Jahn (1973) found more fruit on generative shoots; however, the percentage of fruit set, taking into account the number of flowers, was higher for leafy inflorescence.

### 3.2. Pollination, fertilization and seed development

Pollination, fertilization and seed development are important processes in most citrus varieties for fruit development to occur (Ortiz, 2002). The pollination process starts when pollen grains come in contact with the stigma and germinates to form a pollen tube that moves through the style and ultimately into an ovule where two sperm nuclei are discharged. A zygote, the sexual embryo, is formed through fusion of the egg cell and one sperm nuclei. The other sperm cell combines with two polar nuclei and a triploid endosperm is formed, which serves as a nutrient source for the embryos that develop in the seed (Jackson and Futch, 1986).

The phenomenon of polyembryony exists in most of the citrus cultivars and describes the presence of multiple embryos within a seed (Koltunow *et al.*, 1996). Apart from the zygotic embryo (sexual fertilisation) present, nucellar embryos are present which are developed through the mitotic division of nucellus cells, therefore asexual development. Nucellar seedlings are identical to the seed parent (mother plant) (Frost and Soost,

1968). Most of the citrus cultivars used as rootstocks are polyembryonic. It is the nucellar embryony, and the vigor of the nucellar seedling, making it ideal for rootstock propagation to ensure uniformity and identical genetic material from the mother plant. In addition, the nucellar seedlings are free of viruses and other systemic pathogens (Hartman and Kester, 2011).

However, different types of pollination methods/processes vary between citrus cultivars (Frost and Soost, 1968).

- **Self-Pollination (Self-compatible flowers)**: Occurs when the flower has functional pollen and ovules and as the word describes, is pollinated by its own pollen produced by the anthers. The fruit will mostly contain seeds (Kahn and Choa, 2004).
- **Cross-Pollination (Self-incompatible flowers)**: The flowers contain functional pollen and ovules, but can only be pollinated by other flowers, i.e. pollen from another flower is transferred to the stigma of the pollinated flower. When these varieties are grown isolated, the fruit will be seedless (Kahn and Choa, 2004). Cross-pollination mainly occurs through insects, with honey bees being considered as the main natural cross-pollinator in citrus (Frost and Soost, 1968).
- **Parthenocarpic**: Flowers contain none to very few functional pollen and ovules. These flowers are not considered as a pollen source for other varieties. Parthenocarpic varieties are mostly seedless and include Navel oranges, Satsuma mandarin, Midnight and Delta Valencia oranges (Kahn and Choa, 2004).

These above-mentioned pollination types show that functional pollen and facilities of pollination are principle controlled factors during the pollination process (Frost and Soost, 1968). In addition, it was concluded by Mesejo *et al.* (2007) that the genotype is an important factor that influences the flower receptivity to pollen. They investigated factors such as pollen tube development, stigmatic receptivity, and ovule longevity, and how these factors influence seed set in various citrus species including, 'Clemenules' Clementine mandarin (*C. clementina* Hort. Ex Tanaka), 'Valencia' sweet orange, and 'Owari' Satsuma mandarin (*C. unshiu* Marcovich). Stigmatic receptivity influenced seed set in 'Clemenules' and 'Valencia', whereas shorter ovule longevity determined the seed set in 'Owari' Satsuma. However, pollen tube development was not a limiting factor for seed set in any of these citrus species.

Furthermore, the pollination process can also be influenced by climate, as suggested by Chelong and Sdoodee (2012). The suggestion is based on the pollen viability, pollen germination and pollen tube growth of 'Shogun' tangerine (*C. reticulata* Blanco) that differed amongst two climatically diverse areas. They classified high temperature, light intensity, low rainfall, and relative humidity (RH) as stress-inducing factors for pollination, and proposed that the climatic effect on fruit set requires further investigation.

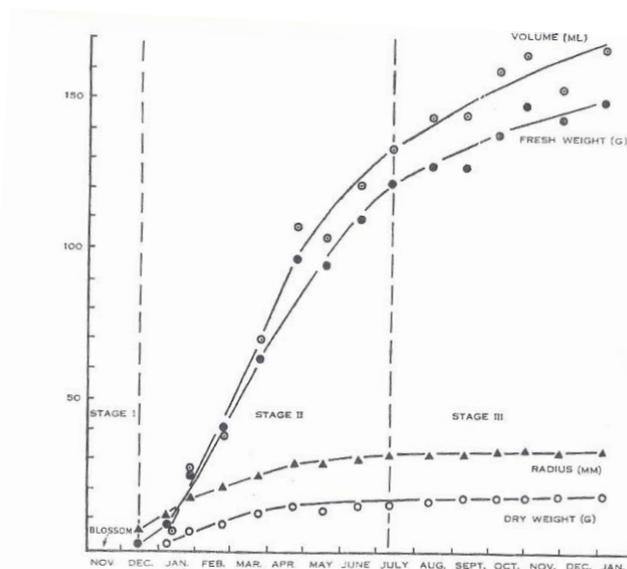
Rootstocks are propagated mainly through seeds. As a result, pollination and consequent seed development is important. However, the number of seeds per fruit could be inconsistent, with the seed number per fruit segment ranging from 1-4 or more (Hodgson, 1967). As mentioned earlier, the genetics origin of the tree and climate influences the pollination process. This means that each rootstock may have different flowers-types, which can either be self-compatible or self-incompatible. This will consequently influence seed development, depending on the orchard layout in relation to limited cross-pollination resources. In addition, the climatic effects between seasons can also be affecting pollination, resulting in inconsistent seed number and thereby limiting the supply seed for certain rootstocks that are high in demand.

### **3.3. Fruit set and development**

Fruit set is considered as the stage of the reproductive process where an ovary develops into a fruit that will continue with development until maturity (Guardiola, 1997). Fruit set occurs following the first physiological fruit drop after flowering, where the tree naturally thinned fruitlets according to the tree's bearing potential (Spiegel-Roy and Goldschmidt, 1996). Cultivar characteristics impacting on flowering intensity and physiological fruit drop, in addition to tree management and climate, are factors known to influence fruit set. (Guardiola, 1997).

During citrus fruit development, a typical sigmoidal curve (Bain, 1958) is seen with three distinct developmental phases (Fig. 4.5.4.11). In summary, **cell division** is the first phase: the cell number in the tissue of the developing fruit increased to form the tissue of the mature fruit. The fruit size consist mainly of the rind volume, with the albedo (white part of the citrus rind) constituting the most. The juice sacs also enlarge and fill half of the pulp segment. During phase two, **cell enlargement** occurs. This is the period of maximum fruit growth, with rapid changes in the morphology, anatomy and physiology of the fruit. The juice content increase, with an increase in the pulp segment. Rind colour development occurs, and internal quality changes i.e., total soluble solids ( $^{\circ}$ Brix) increase and acid decrease is evident (Bain, 1958).

The last phase, known as the **maturation phase** has less morphological, anatomical and physiological changes occurring. Acid content decreases further, and the rind colour attains the characteristic colour associated with the specific cultivar. The fruit size increases for the duration that the fruit remains on the tree (Bain, 1958).



**Figure 4.5.4.25.** Fruit growth of Valencia orange (*C. sinensis* (L.) Osbeck) with the three developmental stages distinguished on a calendar basis. (Source: Bain, 1958).

The patterns and key influencer of rootstock fruit set and development of rootstock trees are largely unknown, with current recommendation stemming from commercial relevant scion cultivars. In addition, the correlation between fruit size and seed number, if any, is also unknown, and would be of interest, especially if the number of set fruit should be adapted in order to yield fruit with a higher number of seeds.

#### 4. Cultural practices enhancing flower formation, fruit set and yield.

One of the major constraints in citrus cultivation or any crop cultivation is not having consistent yield from year to year. One possibility is the occurrence of alternate bearing in these rootstock cultivars i.e., one year the tree has a high yield, and the next year a low yield. Pollination and factors impacting on this process for each rootstock cultivar is unknown and could have an impact on sustainable fruit set. Furthermore, not every tree has the ability to easily set fruit, by either not producing enough flowers or high fruitlet abscission during the abscission period; however, growers use a few plant manipulation techniques to attain the desired yield. The first is based on the principle of removing certain tree organs e.g., pruning and fruit thinning to reduce resource dilution. The second technique includes girdling, where interfering with translocation between major tree organs modifies auxin and carbohydrate distribution (Goren *et al.*, 2004). Furthermore, irrigation and nutrition practices and gibberellic acid sprays have also shown some promising results (Lovat *et al.*, 1988; Menino *et al.*, 2003; Talon *et al.*, 1992).

**IRRIGATION.** Water stress of 'Frost Lisbon' lemon trees (*C. limon*) grafted on 'Troyer' citrange rootstocks (*C. sinensis* x *P. trifoliata*), resulted in a higher number of flowers per tree. The duration and higher severity of

water stress resulted in a higher flower intensity (Lovat *et al.*, 1988). Southwick and Davenport (1986) found a similar effect in 'Tahiti' lime (*C. latifolia* Tan.) (grafted on *C. macrophylla* Wester). However, the opposite was true for Satsuma mandarin on trifoliolate rootstock (*P. trifoliata*) where the percentage of flowering nodes per total node was higher for moderately stressed trees (3 days) as opposed to 7-10 day stress (severe) (Koshita and Takahara, 2004). In this trial, a higher concentration of gibberellic acid (GA<sub>3</sub>) was present in the leaves of trees that produced less flowers, implying an inhibiting effect of GA<sub>3</sub>. This emphasize that there are various interrelating factors influencing flowering in citrus.

**NITROGEN.** In the same study where Lovatt *et al.* (1988) investigated water stress, they found that urea application and moderate water stress resulted in a higher flower number per tree. In a study by Menino *et al.* (2003), where they investigated the effect of nitrogen application on the flower numbers of Lane Late orange trees (*C. sinensis* L. Osb.) grafted on *Carrizo citrange* rootstock, a higher flower number was present with nitrogen application. They also found that the flower yield correlated with the nitrogen concentration in the flowers.

**GIBBERELIC ACID (GA<sub>3</sub>).** Gibberellic acid is widely used in practice for fruit set with GA<sub>3</sub> being the most biochemical active and therefore commercially used in citrus. Talon *et al.* (1992) studied the effect of GA<sub>3</sub> application on fruit set of a Clementine and a Satsuma mandarin, known to set fruit parthenocarpically. GA<sub>3</sub> applications between anthesis and petal fall increased fruit set in clementine. For satsuma, no effect was seen, suggesting that clementine does not have adequate endogenous GA levels.

Talon *et al.* (1992) further investigated paclobutrazol's influence, which inhibits GA<sub>3</sub> biosynthesis, and found that fruit abscission was present in both. This led to the suggestion that a threshold GA<sub>3</sub> is required for fruit set (Talon *et al.* 1992). In a study where GA<sub>3</sub> was applied at petal-fall on 'Navelate' sweet orange, no effect was evident on the yield; however, when trees were girdled after GA<sub>3</sub> application, the yield increased with 5 mg.L<sup>-1</sup> giving the best results (Agusti *et al.*, 1982). This suggests that the reproductive system of a citrus tree depends on various interrelating factors (nutritional and hormonal) and not one sole factor.

**GIRDLING.** Girdling is a historical horticultural technique still used today to increase crop production. The science behind girdling is based on the interruption of phloem transport between the canopy and roots, consequently influencing the transport of photosynthates (Goren *et al.*, 2004). Schaffer *et al.* (1985) investigated the effect of girdling on non-alternating 'Shamouti' sweet orange and 'Murcott' (*C. reticulata* Hybrid), an alternate bearing cultivar. The final percentage of fruit set increased for 'Shamouti', with only a small, non-comparable effect on 'Murcott'.

The effect of girdling on fruitlet abscission was seen in a study by Rivas *et al.* (2006), where 'Fortune' mandarin ('Clementine' mandarin x 'Dancy' tangerine) and 'Clausellina' Satsuma mandarin both on Carrizo rootstock were girdled on the main scaffold branches. Both cultivars showed a delay in fruitlet abscission and enhancement in fruit set when compared to non-girdled trees. Furthermore, the timing of girdling is important. The yield of low bearing 'Fortune' increased when girdled at 15 days before anthesis, as opposed to when girdled at anthesis and several days after anthesis. However, for the high-bearing tree, girdling at 35 days after anthesis resulted in the highest yield. For 'Clausellina,' the highest yield was evident in trees which were girdled 40 days after anthesis.

In another study by Rivas *et al.* (2007), the response of girdling on fruit set appear to be dependent on the type of flowering shoot that is girdled. 'Loretina' mandarin (*C. reticulata* Blanco) on Carrizo rootstock and 'Nova' mandarin [(*C. reticulata* Hort. Ex Tan.) x (*C. paradisi* Macf. X *C. tangerine* Hort. Ex Tan.)] on *P. trifoliata* rootstock were trunk girdled at anthesis (60% of flowers open). Fruitlet abscission was delayed, irrespective of the shoot type. However, the fruit set increased for a leafy flowering shoot, while no effect was seen for the leafless flowering shoot.

Although girdling has these advantages, it is not practiced widely by every citrus grower due to the difficulty of determining the optimal time and environmental conditions for each cultivar to ensure the efficacy. There is also the uncertainty that exists of how girdling would affect the tree, with a possibility of severe damage by

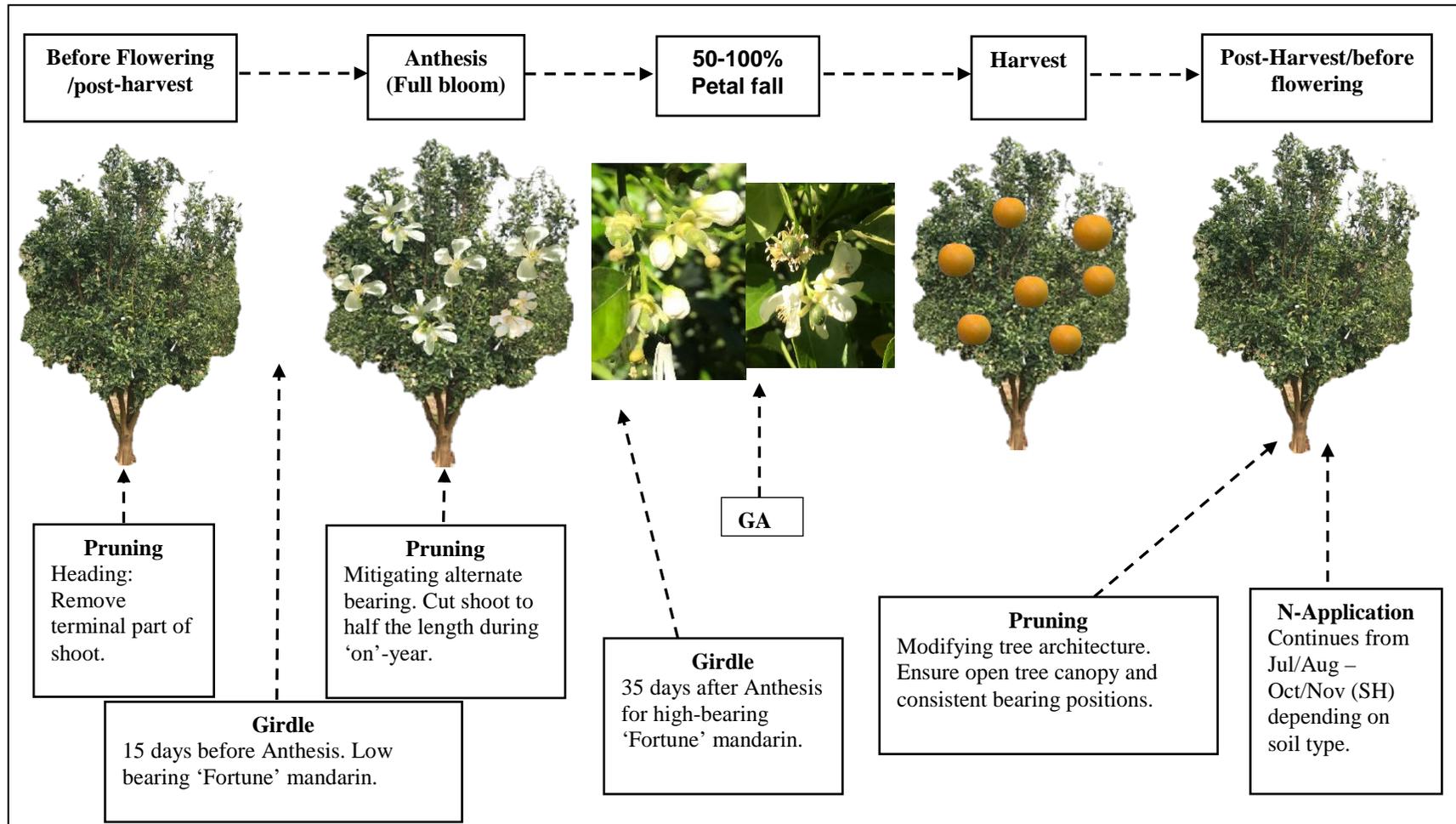
either single or repeated treatments (Goren *et al.*, 2004). This is a concern in colder production regions, where the recovery of vascular bundles are delayed, and can result in dieback.

**PRUNING.** Pruning is a critical part of orchard management, which is implemented to control vegetative growth and also to maintain a healthy, productive citrus tree. However, the response of pruning is different for each tree, since variety, tree age, fruiting habit, vigor, growing conditions, and production practices are all contributable factors (Tucker *et al.*, 1994). Tucker *et al.* (1994) and Fake (2012) have documented good guidelines and pruning techniques important for citrus. According to Tucker *et al.* (1994), the main types of pruning in citriculture are a heading cut (where the terminal part of the shoot is removed) to break apical dominance and thereby allowing lateral bud break and thinning (removal of the complete branch, to open the tree canopy).

Pruning removes vegetative growth, thereby reducing potential bearing positions for the following season's growth and the sink demand and consequently affecting the yield. This was seen in an experiment done on 'Orlando tangelo trees (*C. paradisi* Macf. x *C. reticulata* Blanco) where, irrespective of the type of pruning cut (Gable-top, at 30° angle or flat top, no angle), the yield was reduced compared to the control. Trees were pruned in March [Northern Hemisphere (NH)] and harvested in December (NH) of the same year. The yield from the consecutive years were not determined, which is unfortunate, as the type of pruning could possibly have had a positive long-term effect on the yield (Morales and Davies, 2000).

Pruning is also implemented on alternate bearing trees as a manipulation technique. Mesejo *et al.* (2020) proved that mechanical pruning, where the shoot length was cut to half of the length during flowering in the on-year (start of trial), it reduces alternate bearing in 'Nadorcott' mandarin trees. Consequently, the cumulative yield increased during the 4-year period compared to the control trees. Pruning was done yearly during the period.

A summary/illustration of when the various plant manipulation techniques or cultural practices can be applied in order to enhance the flower quantity and ensure fruit set can be seen in Figure 4.5.4.12.



**Figure 4.5.4.26.** A summary of the timing for plant manipulation techniques and cultural practices to be applied to enhance flower intensity and fruit set/yield

## 5. Conclusion

From experimental work done at the Citrus Foundation Block in South Africa, it is evident that there are distinct differences in the horticultural properties amongst the various rootstock cultivars i.e., yield/tree, seed no./tree, germination %, multiple seedlings, thereby making it evident that individual management guidelines are needed. A lot of interrelating factors come into play to produce seeds for propagation; therefore, it is necessary to build up a database to understand each rootstock cultivar and their cultural and climatic requirements to ensure a consistent, viable seed supply.

### **Results and discussion: Objectives 2-6:**

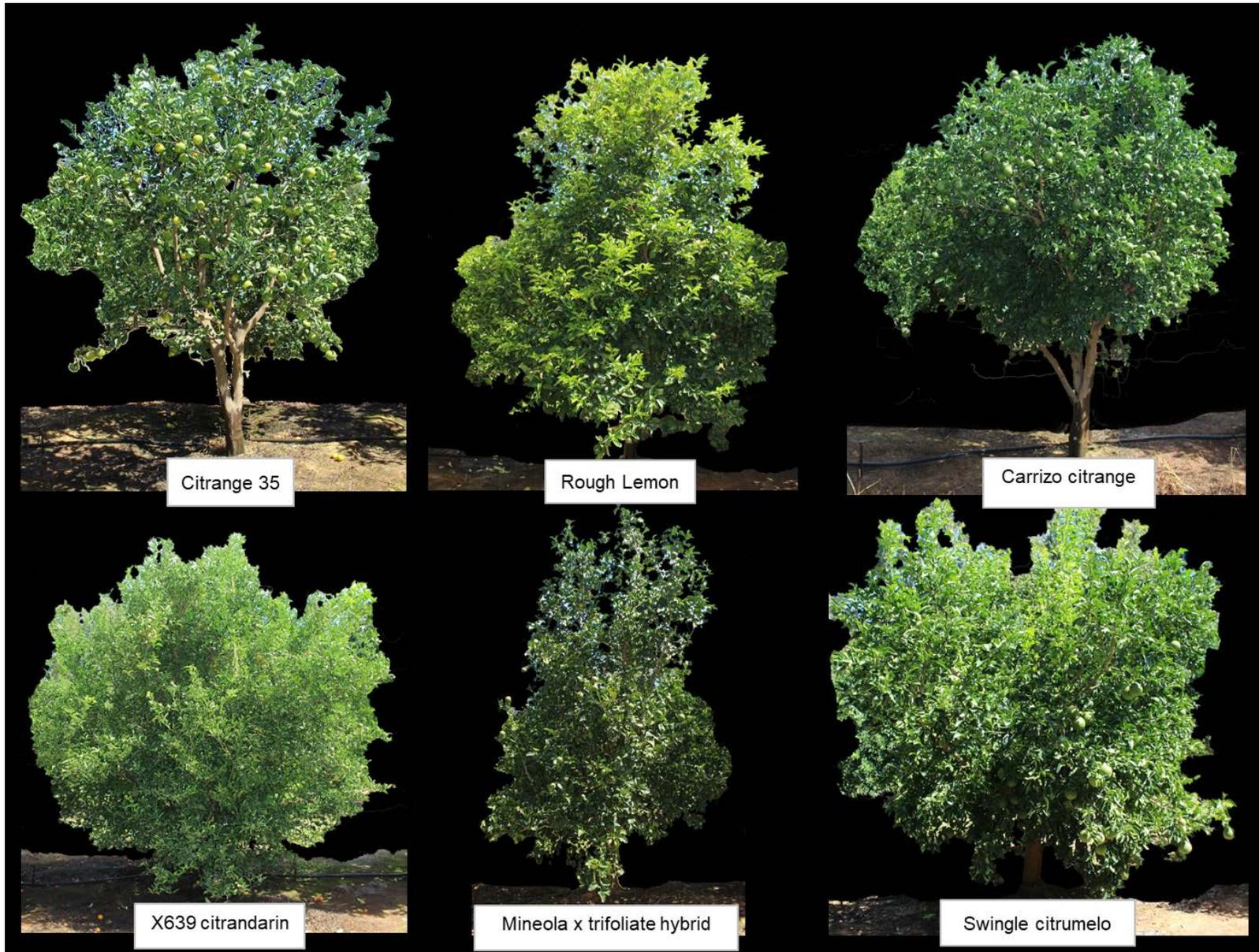
#### **Objective 2 and 3**

The tree growth pattern of the six commercial rootstock cultivars that were investigated during this trial are shown in Figure 4.5.4.13 below. C-35 and CC have very similar tree growth, whereas SC has a more dense canopy. The tree growth of RL trees is typical of the commercial lemon trees, with a more open tree canopy as opposed to SC, for example. MxT has a very upright growth habit, with less foliage on the lower part of the branches. It also has a very vigorous growth (Table 4.5.4.2) with X639 on the other hand, having a unique canopy structure with a bushy appearance and a lot of branches that extend from the framework. In Table 4.5.4.2, the differences in tree vigour are summarised, as well as the different leaf-types that were observed. Furthermore, the timing of maturity of when the different cultivars are harvested are also indicated.

In addendum A, Fig. 1-7 shows the different rootstock trees at harvest, the leaves, seeds, as well as a photo of their flower and fruit at harvest. The fruit development and rind colouration throughout the season is shown in Addendum B, Fig. 1-7. Furthermore, in Addendum C, Table 1 serves as summary highlighting data from the respective trials and differences amongst the rootstock cultivars during the 2020 season.

**Table 4.5.4.2.** A summary of horticultural properties of 7 important rootstocks used commercially in South Africa. The trees used to describe the horticultural properties are located at the Citrus Foundation Block in Uitenhage, Eastern Cape. All trees were planted in 2015 except for US-812, which was planted in 2010.

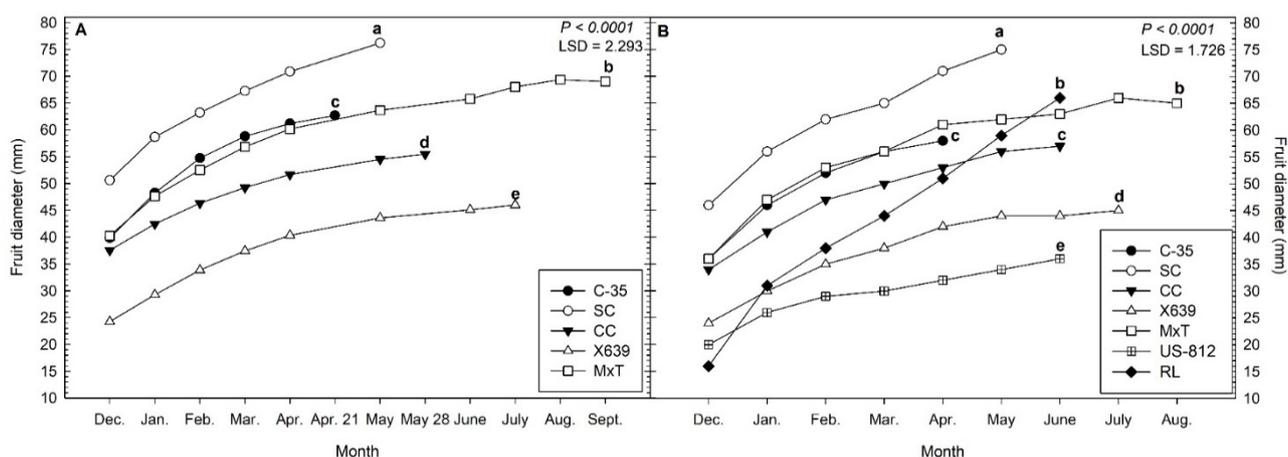
	<b>Rough Lemon</b>	<b>Mineola x trifoliolate hybrid (MxT)</b>	<b>Carrizo citrange</b>	<b>Swingle citrumelo</b>	<b>Citrango 35 (C-35)</b>	<b>X639 citrandarin</b>	<b>US-812 (Sunki x Benecke)</b>
<b>Tree vigour</b>	Vigorous	Vigorous	Intermediate	Intermediate	Intermediate	Intermediate	Vigorous
<b>Type of leaves</b>	Unifoliolate with no petiole wings.	High presence of unifoliolate (winged petioles) compared to trifoliolate leaves.	High presence of trifoliolate leaves, and low % of unifoliolate (winged petiole)	High presence of trifoliolate leaves, and low % of unifoliolate (winged petiole)	High presence of trifoliolate leaves, and low % of unifoliolate (winged petiole)	Trifoliolate and unifoliolate (winged petiole) leaves	High presence of unifoliolate (winged petiole) and low % of trifoliolate leaves.
<b>Fruit maturation period (2019/2020)</b>	Mid (Mid-June)	Late (Early September)	Early (End of May)	Early (Mid-May)	Early (Mid-April)	Mid (Early- July)	Mid (Mid-June)



**Figure 4.5.4.27.** Photos illustrating the differences in tree growth of the 6 rootstocks grafted on CC. All trees were planted in 2015.

**Fruit growth pattern and size.** The fruit growth patterns from December until harvest for the different rootstock cultivars (2019/20 & 2020/21 season) followed the typical characteristic curve of citrus fruit (Bain, 1958). The average fruit diameters of the cultivars measured at harvest differed significantly from each other (Fig. 4.5.4.14). The largest fruit diameter was recorded for SC (76 mm), followed by MxT (69 mm), C-35 (63 mm), CC (55 mm), and X639, resulting in the smallest fruit (46 mm) for the first season (2019/20) (Fig. 4.5.4.14A). During the second season, RL and US-812 fruit were also measured. Again SC had the larger fruit diameter (75 mm) followed by RL, MxT, C-35, CC, X639 and US-812 having the smallest average fruit diameter (36 mm). RL and MxT had similar fruit diameters, and C-35 and CC also did not differ significantly for the second season (Fig. 4.5.4.14B).

The graph also indicates the month of maturity of the respective cultivars during the respective seasons, represented by the last data point of each line.



**Figure 4.5.4.28.** The fruit growth pattern of five commercially important rootstocks in South Africa during the 2019/20 season (A). In B, the seven important rootstocks are shown for the 2020/21 season. Different letters denote significant differences in final fruit size between cultivars at harvest for each season at 5% significant level. Values reported are the means of 10 single tree replicates per treatment (20 fruit per tree).

**Seed quantity at three maturity stages.** There was no difference in the number of mature seeds for each cultivar at the two maturity stages, except for X639 (Table 3) for the first season. However, the difference of 2 seeds/fruit is not of commercial importance, and can be ascribed to possible variations between fruit.

During the second season, there was significant differences in viable seed count between the different sampling stages for RL, X639, CC and US-812 (Table 4.5.4.3). However this difference can primarily be ascribed to variation between fruit as the difference is not big, except for RL, where an average of 8 seeds/fruit was seen at the green stage and 16 and 13/fruit at the CB and FC stage. In addition, the immature seed count for the RL, decreased from 5/fruit at the green stage, to 1/fruit for the CB and FC sampling stage. It may be possible that some seeds has not reached maturity and that the embryos was still expanding.

This again indicates that the number of seeds that each cultivar are capable of developing is already established during the green stage (Phase II of fruit development).

**Table 4.5.4.3.** The average mature seed number per fruit for each cultivar extracted at green, colour-break, and full colour rind fruit stage. Seeds were quantified directly after hand extraction. Means are the values of 10 single tree replicates per cultivar.

Season	Colour stage	Rootstock Cultivars													
		RL		X639		CC		SC		MxT on RL		C-35		US-812	
		Viable seed	Immature seed	Viable seed	Immature seed	Viable seed	Immature seed	Viable seed	Immature seed	Viable seed	Immature seed	Viable seed	Immature seed	Viable seed	Immature seed
2020	Green <sup>z</sup>	17 <sup>NS</sup>	- <sup>x</sup>	8 b	- <sup>x</sup>	18 <sup>NS</sup>	- <sup>x</sup>	18 <sup>NS</sup>	- <sup>x</sup>	17 <sup>NS</sup>	- <sup>x</sup>	17 <sup>NS</sup>	- <sup>x</sup>	4 <sup>NS</sup>	- <sup>x</sup>
	Colourbreak <sup>y</sup>	16	-	10 a	-	18	-	19	-	16	-	17	-	3	-
	<i>P-Value</i>	0.793		0.000		0.765		0.274		0.241		0.787		0.106	
	<i>LSD</i>	3.368		0.86		2.28		2.721		2.269		3.623		1.021	
2021	Green <sup>w</sup>	9 c	4 a	8 b	7 b	13 b	11 <sup>NS</sup>	18 <sup>NS</sup>	8 b	15 <sup>NS</sup>	4 <sup>NS</sup>	21 <sup>NS</sup>	9 <sup>NS</sup>	3.5 ab	1 b
	Colourbreak <sup>y</sup>	16 a	1 b	10 a	8 ab	16 a	11	18	9 b	16	5	20	9	2.9 b	1 b
	Full colour <sup>v</sup>	13 b	1 b	9 ab	9 a	17 a	12	17	13 a	16 <sup>w</sup>	5 <sup>w</sup>	20	10	3.9 a	2 a
	<i>P-Value</i>	<0.0001	<0.0001	0.015	0.011	0.049	0.811	0.639	0.0001	0.708	0.105	0.806	0.571	0.010	0.003
	<i>LSD</i>	2.794	1.148	1.349	1.357	2.796	1.974	2.011	1.932	4.089	1.759	2.231	2.175	0.611	0.454

<sup>NS</sup> Non significant difference between means within a column ( $P > 0.05$ ). Means was separated using Fishers LSD.

<sup>z</sup> Means are the value of a 10 fruit.

<sup>y</sup> Means are the value of a 5 fruit sample.

<sup>x</sup> No data recorded.

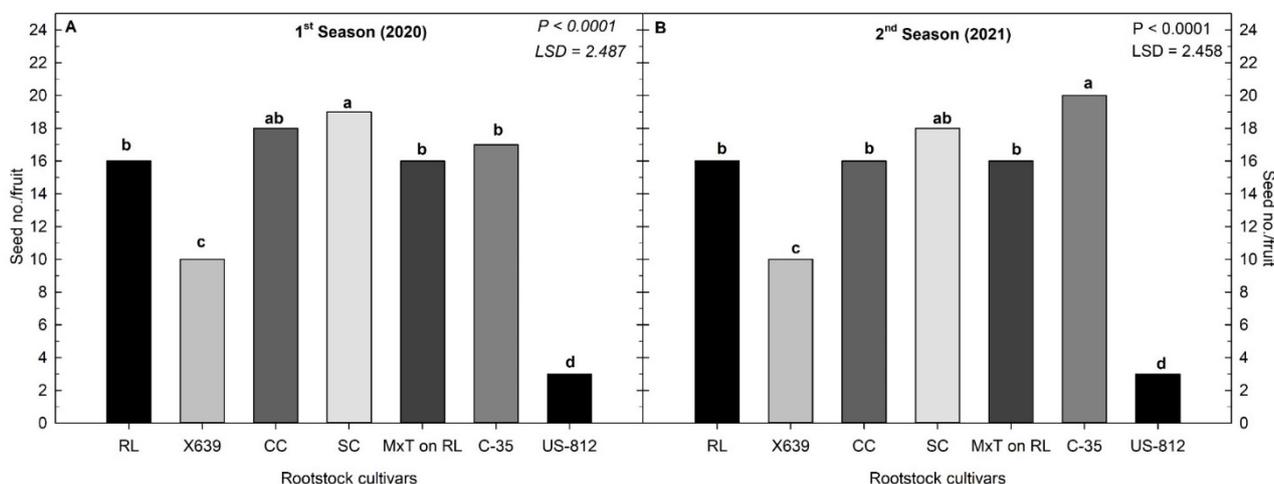
<sup>w</sup> Means are the value of 5 fruit/rep (n=10) except for X639 and C-35 (10 fruit) and US-812 (20 fruit).

<sup>v</sup> Means are the value of 5 fruit/rep (n=10) except for X639 (10 fruit) and US-812 (20 fruit).

**Seed number at colour break stage.** For the 2020 season, an average of 19 mature (viable) seeds/fruit were extracted for SC, which was significantly higher than the other cultivars, except CC, which had 18 seeds/fruit (Fig. 4.5.4.15A). RL, MxT grafted on RL and C-35 did not differ significantly from CC. Furthermore, the lowest seed number/fruit was recorded for X639 (10/fruit) and US-812 (3/fruit) respectively for the first season. A similar trend was seen for the 2021 season, the only difference was C-35 which had the highest seed count (20/fruit), however not significantly higher than SC (Fig. 4.5.4.15B). These differences in seed number between cultivars is most likely related to fruit size which also differs between cultivars (Fig. 4.5.4.14), as also previously reported by De Carvalho *et al.* (2021), the number of seeds was different depending on rootstock variety and fruit size. In the same study, they also found that the seed number was dependant on the season (De Carvalho *et al.*, 2021). During the current trial, were the seed number per fruit for the respective cultivars relatively constant between seasons, which is a good measure to use to ensure the seed supply to the industry is met. It must however be taken into account, that the seed number may vary between seasons.

The average immature seed count at colour break was also compared between the cultivars. CC had the highest number of immature seeds (11) and RL and US-812 had 1/fruit, which was significantly lower than all other cultivars. C-35(9), SC (9) and X639(8) did not differ from each other and MxT on RL had 5 seeds/fruit and differed significantly from the other cultivars ( $P < 0.0001$ ,  $LSD = 1.236$ ). These results indicate that each cultivar has a potential number of seeds that develop, but the percentage of seeds that has embryo development/expansion and becomes mature, varies amongst cultivars. For example, from the data from the 2022 season, 94% of the seed in RL, will develop to mature seed (embryo expansion), while for CC, only 59% of the seed on average will become mature. Koltunow *et al.* (1995) found that only 2-8% of the ovules developed into mature seed for 'Valencia' fruit. Although 'Valencia' is a scion cultivar, may this be the case for rootstock fruit also.

These seed counts for the different cultivars may differ between seasons. Androde-Rodriguez *et al.* (2004) found for *C. volkameriana* that the total number of seeds and full seeds (seeds where embryo sacs are filled with embryos) differed between seasons.



**Figure 4.5.4.29.** The average seed number per fruit for seven rootstock cultivars at colour break stage for the first (A) and second season (B). The data expressed are the means of ten single tree replicates (5 fruit/rep for all cultivars except for X639 (10 fruit) and US-812 (20 fruit)). Different letters indicate a significant difference between cultivars at  $P < 0.05$  for each season.

**Relationship between fruit size i.e., large vs small and seed quantity.** Fruit size significantly influenced the number of mature and immature seed per fruit for all cultivars (Table 4.5.4.4). A higher average seed number was found in large fruit compared to small fruit. A difference of 8 seeds/fruit was recorded for C-35 and RL, followed by 6 seeds for MxT, 5 for CC, and 2 for X639. Only a 1 seed/fruit difference for SC and US-812 was recorded. This concurs with Bisi *et al.* (2020), who found that the average number of seeds per fruit for a rootstock cultivar were positively associated with fruit size, however, they only investigated US-812, while the rest were other rootstock cultivars not included in this trial.

Agusti and Primo-Millo (2020) made a statement based on the findings from Bermejo *et al.* (2015) that the presence of seeds increases fruit size because ovaries containing fertilized ovules produce higher levels of hormones. Endogenous synthesized GA, which is known to be present in seeds, was reported to be higher in mature ovaries (Bermejo, 2015; Ben-Cheikh, 1997). Based on a model proposed by Stander (2018), GA<sub>3</sub> causes carbohydrate allocation towards developing sinks. This means that the higher levels of GA<sub>3</sub> will cause carbohydrate allocation of the fruit in this case, contributing to the fruit growth and size, as it is a critical factor for fruit enlargement (Goldschmidt and Monselise, 1977; Goldschmidt, 1999).

During the seed extraction process, when the fruit was cut along the cross-section, it was observed that SC has a very thick rind compared to the rest and possibly due to its grapefruit parentage (*C. paradisi* x *P. trifoliata*). The difference of 15 mm between the large and small size fruit could therefore mainly be due to the thick rind and not the pulp section where the seeds are. Unfortunately, the pulp diameter was not measured during this trial. In contrast for the US-812 there was not a lot of variation in fruit size, as was observed for the other cultivars, and the difference in fruit diameter between large and small was only 4mm.

Furthermore, this data indicates that the small fruit of each cultivar also has an adequate number of seeds available, since it was observed that the size distribution is spread out for all cultivars except US-812 during the growing season. This indicates that every fruit does have the potential to contribute to seed supply.

**Table 4.5.4.4.** The difference in average viable/mature and non-viable seed number per fruit for large and small size fruit. Values are the means of 10 single tree replicates from which 10 fruit per size category were sampled per rep for each of the respective rootstock cultivars. Mean separation was done by means of Fishers LSD. All the trees were grafted onto CC rootstock and planted in 2015, except for US-812 (planted 2010).

Cultivar	Size category	Fruit Diameter (mm)	Viable/Mature seed number/fruit	Immature/Non-viable seed number/fruit
RL	Large	79 a <sup>z</sup>	18 a	0.9 a
	Small	59 b	10 b	0.4 b
	<i>P-value</i>	<0.0001	<0.0001	0.015
	<i>LSD</i>	1.829	1.93	0.424
X639	Large	51 a	7 a	10 a
	Small	41 b	5 b	6 b
	<i>P-value</i>	<0.0001	0.005	0.001
	<i>LSD</i>	1.206	0.912	1.523
CC	Large	60 a	17 a	11 a
	Small	50 b	12 b	8 b
	<i>P-value</i>	<0.0001	0.000	0.037
	<i>LSD</i>	1.109	1.644	2.049
SC	Large	84 a	15 a	16 a
	Small	69 b	14 b	11 b
	<i>P-value</i>	< 0.0001	0.048	0.000
	<i>LSD</i>	1.586	1.341	1.991
MxT	Large	79 a	12 a	5 a
	Small	64 b	8 b	2 b
	<i>P-value</i>	<0.0001	0.004	0.003
	<i>LSD</i>	2.253	2.456	1.832
C-35	Large	66 a	23 a	14 a
	Small	54 b	15 b	7 b
	<i>P-value</i>	< 0.0001	0.000	0.001
	<i>LSD</i>	1.791	3.093	2.818
US-812	Large	44 a	5 a	3 a
	Small	40 b	4 b	2 b
	<i>P-value</i>	<0.0001	0.001	0.018
	<i>LSD</i>	1.708	0.888	0.752

<sup>z</sup> Different letters within a column for each cultivar denotes significant differences at  $P < 0.05$ .

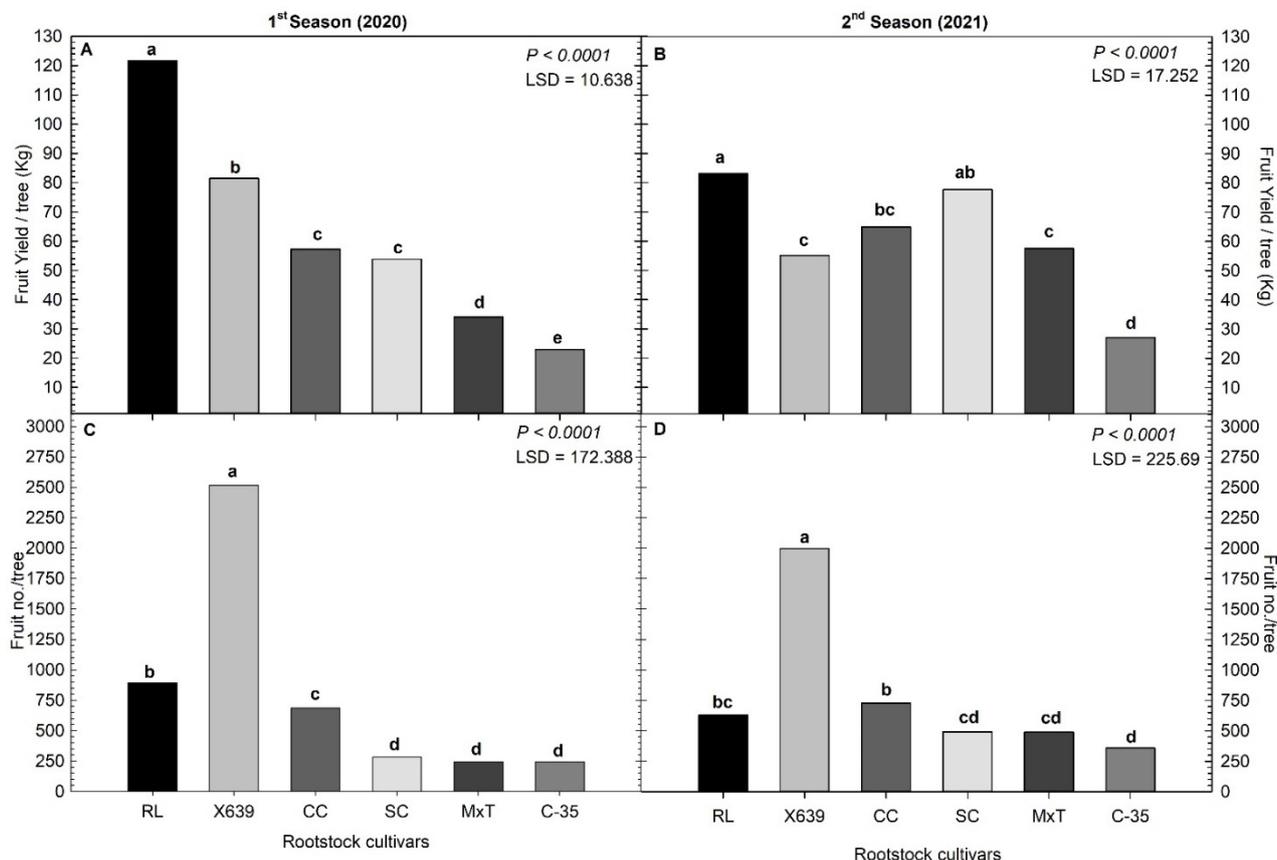
**Average fruit yield and fruit number per tree.** During the first season (2020) RL trees produced the highest fruit yield (121.61 kg) followed by X639 (81.48 kg), CC (57.30 kg), SC (53.81 kg), MxT (34.09 kg) and C-35 (22.97 kg) (Fig. 4.5.4.16A). The yield between cultivars differed significantly, except for CC and SC. For the 2021 season, there was less cultivars that differed significantly from each other. RL had the highest yield (83 kg), but it was not significantly different from SC (78 kg). C-35 again had the lowest significant average yield (27 kg) (Fig. 4.5.4.16B). Further did CC, X639 and MxT not differ significantly from each other, and no difference was evident for CC and SC (Fig. 4.5.4.16B).

Overall, when looking at the graph for the two season, some cultivars had a decrease in yield, whilst some showed an increase during the second season. This variation in yield between seasons, is also an important contributor in seed supply, and therefore a third seasons data for the yield especially will be recorded, to determine if there is an alternate bearing pattern evident for some cultivars.

The highest fruit number per tree was recorded for X639, which produced around 2516 fruit per tree (Fig. 4.5.4.16C) (first season). Since X639 had the lowest average seed per fruit, one can argue that the tree needs to produce a high number of fruit to assure that the seed demand is met as compared to the other cultivars.

The second largest fruit number/tree was recorded for RL (891) and the third largest for CC (687). The lowest fruit numbers per tree were recorded for SC (285), MxT (245), and C-35 (244) respectively.

For the 2021 season, X639 had 1997 fruit/tree which was significantly higher than all other cultivars (Fig. 4.5.4.16D) and CC had the second highest fruit number, but not significantly higher than RL. Although C-35 had the lowest fruit number, was is not significantly lower than SC and MxT.



**Figure 4.5.4.30.** Average fruit yield per tree (A&B) and fruit number per tree (C&D) of the respective rootstocks during the 2019/2020 and 2020/2021 season respectively. The means are a replicate of 10 trees per cultivar (n=10) and 9 trees for MxT (n=9). Different letter on each graph denotes significant differences between rootstock cultivars at 5% significant level.

**Total viable seed production and seed no./fruit.** The seeds were quantified following the entire seed extraction (mechanical) process as is standard practice for the CFB. The seeds were also sorted and graded according to CFB standard and therefore represent the commercial quantity available for nurseries. It should therefore be noted that this data, where the seed no./tree is given, will differ from the previous data where seeds were quantified directly after hand seed extraction, because some seeds get lost in the process of mechanical seed extraction and seed treatment, as well as during the sorting/grading process, where dirty seeds (brown lesions) gets discarded.

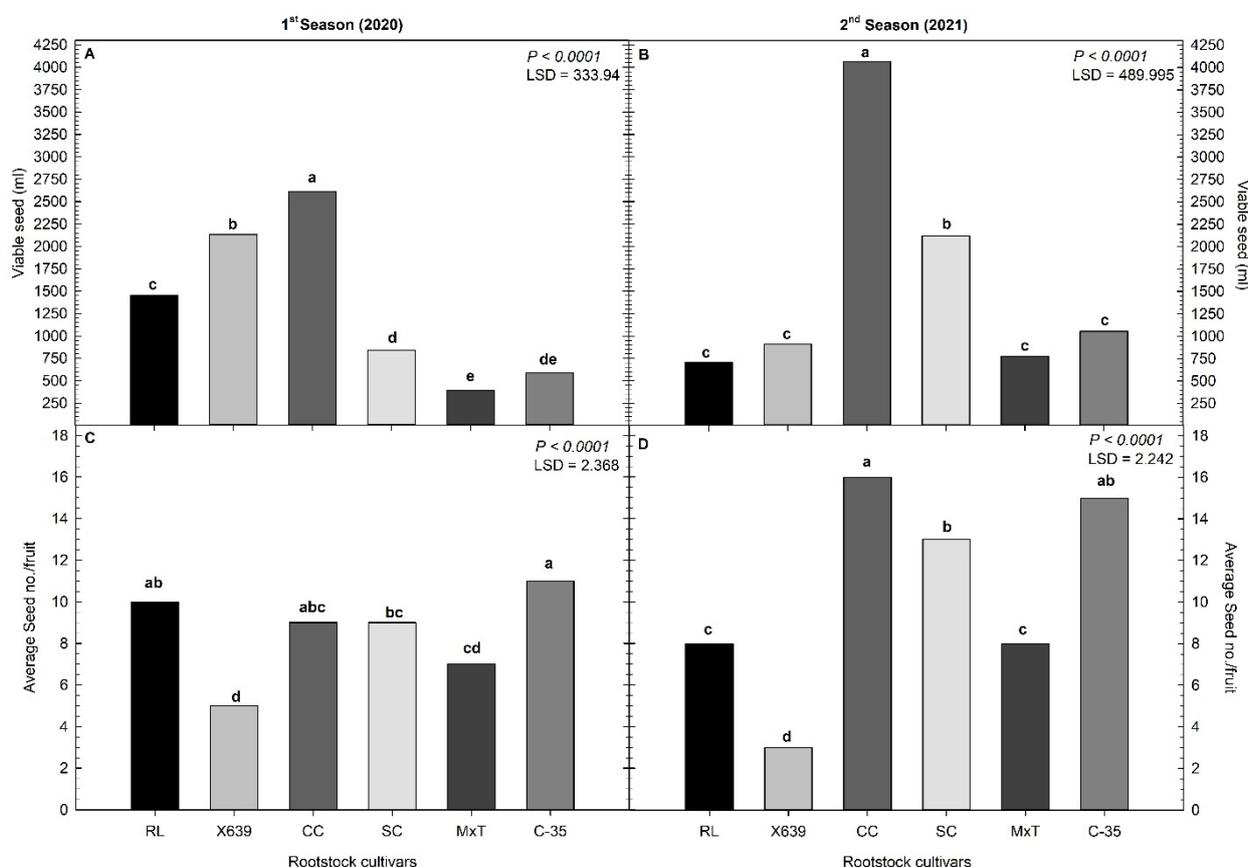
Carrizo citrange produced the highest viable seed (expressed in millimeters (ml) as in commercial seed trade), 2613 ml, while X639 produced 2133 ml, followed by 1450 ml for RL. The lowest viable seeds were found for SC (840 ml), C-35 (590 ml) and MxT (392 ml) respectively (Fig. 4.5.4.17A) for the first season (2020). Results from the 2021 season showed that CC again had the highest seed quantity (4063 ml), followed by SC (2121 ml). No significant differences was seen between RL, X639, MxT and C-35, with all four having the lowest significant millimeter seed.

In the book, Rootstocks for Florida citrus (Castle *et al.*, 1993), there is a table indicating the amount of seeds for the selection of rootstocks/quart. A quart equals a value of 1.13L (1130ml). According to Castle *et al.* (1993) there are approximately 2600 seeds/1130 ml for CC, 6000 seeds/1130 ml for RL and 3500 seeds/1130 ml for

SC. This difference in seed per volume is an indication of the size of the respective seeds. In the current project it was observed that CC had the largest seed, and RL the smallest, with the rest of the cultivars falling in between this range, which concur with Castle *et al.* (1993).

The average number of seeds per fruit from the volume (ml) of viable seed and seeds used in the seed extraction were also determined for both seasons. The average seed no./fruit ranged from 11 (C-35) to 5 seeds per fruit (X639) in the first season (Fig. 4.5.4.17C). There was however, no significant difference between C-35, RL and CC. For the 2021 season, CC (16/fruit) had the highest seed no./fruit, but not significantly higher than C-35 (15/fruit), which was again not significantly higher than SC (13). A similar pattern was seen as in the 2020 season, with X639 having on average 3 seed/fruit, which is the lowest value, following the dry and grading process (Fig. 4.5.4.17 D).

This important commercial data can be used as forecast planning by the CFB in predicting the seed yield for the season, and also to aid in future orchard plantings. One could argue that for cultivars that produce a lower number of seeds, more hectares of that cultivar should be cultivated to meet the seed demand by the industry.



**Figure 4.5.4.31.** The average viable seed in millilitre (A-B) and the average seed count per fruit (C-D) for the respective cultivars during the first (2020) and second (2021) season respectively. The data presented are the means of ten trees (n=10) per cultivar except for MxT (n=9) and RL (n=8). These are seeds that were extracted, treated and air dried. The average seed number per fruit were calculated by the total weight of viable seed (g) divided by the average mass of a viable seed (g), and also the (ml seed/15 ml)\*# seed in 15 ml. The average value from the two equations were then divided by the number of fruit used in seed extraction. Different letters on each graph depict significant differences between cultivars at 5% significant level.

**Seed germination percentage at green and colour-break.** The seed germination percentage shown in Figure 4.5.4.18A, represents the germination in an incubator for 20 days at  $\pm 28^{\circ}\text{C}$  from fruit sampled at the green and colour-break stage respectively. The data from green and colour-break can be compared within a cultivar at the two respective stages, and also between cultivars, since there was interaction between colour stage and cultivar.

The germination percentage was higher when fruit were sampled at colour-break compared to green fruit for all cultivars except for RL, which showed a high germination percentage at both stages (78 & 73% respectively). In addition, US-812 also showed good germination at the green stage (64%) compared to the other cultivars harvested at the green stage. Overall, MxT showed the lowest germination percentage at the two stages (green and colour-break), although not significantly different from SC at the green stage (Fig. 4.5.4.18A).

For the fruit harvested when a full rind colour developed, RL had a significantly higher germination percentage (95%) than all other cultivars shown in Figure 4.5.4.18B except for US-812 (86%). In general, MxT seeds do not have a very high germination percentage (<59%) with the rest having a good germination percentage above > 65%.

For the second season, there was a significant interaction evident between cultivar and colour stage, which means that the combination of the two factors influences the germination percentage. A similar trend as for 2020 for most of the rootstock cultivars was seen where the germination percentage increased from colourbreak to full colour. However, for C-35, the germination percentage decreased significantly from green to colourbreak and full colour. This resulted in C-35 having the lowest germination percentage at harvest for the 2021 season.

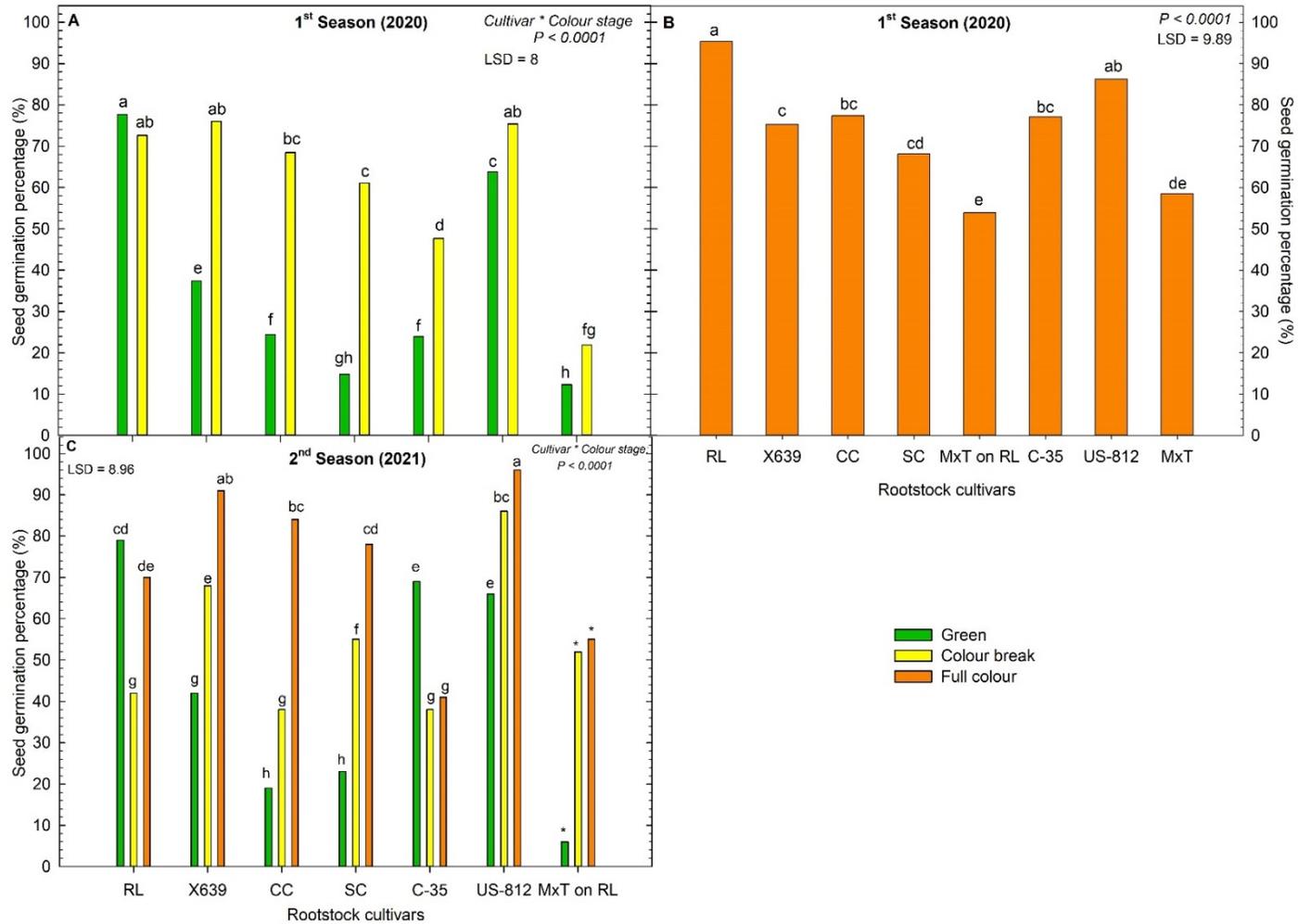
Overall, US-812 seeds from full coloured fruit had the best germination (96%), but not significantly different from X639 (91%). Seeds from green RL fruit had the best germination (79%) compared to the other cultivars at the green stage, which was followed by C-35 (69%) and US-812 (66%). Contradicting to this, was CC and SC which only had a germination percentage of 19 and 23% respectively (Fig. 4.5.4.18C). The MxT on RL germination was not analyzed statistically due to sampling that was not done from the same trees, and is therefore only indicated on the graph to show a trend.

Temperature are one of the factors that influences germination. In a study done by Rouse and Sherrod (1996), they found that the optimum soil temperature for most citrus rootstock type seeds to germinate ranged between 28.8 – 30.8 °C for CC, RL, SC, C-35 and X639 which was some of the cultivars they included in their study. The germination range was between 5-28 days. It may be required, that for MxT which showed overall a low germination percentage at 20 days, that either a higher temperature is required, or a longer germination period.

Chilembwe *et al.* (1992), showed that seed weight or size, did not affect the mean days to seedling emergence and emergence of the first seed for CC and SC investigated in the study. This means that although some seed sizes were not identical, the germination percentage differences seen especially with C-35 during the second season, where there was a decrease from green to full colour, are related to the fruit from which the seed were extracted and not the rind colour. De Carvalho *et al.* (2021), proved in their study, that rind colour change in the peel is not the best indicator of seed maturity, and that only when the fruit has an increase in the sensitivity for abscission, then the seed are ready to germinate, i.e. physiological ready. Therefore, the fruit sampled may contained seeds that was not physiological ready.

Viable seeds are present at an early fruit growth stage already; however, it would not be advised to harvest at such an early stage, not only due to the low germination percentage. It is possible that some percentage of seeds need to complete the maturation proses. In terms of a practical observation harvesting at maturity help seed recovery as the juice sacs of most cultivars was not being completely loose at this early green stage. Fruit for the green stage were sampled during the second week in March 2020. As a result, the seed extraction by hand for the later maturing cultivars, as can be seen in the fruit growth figure (Fig. 4.5.4.14), was difficult. In addition, Table 4.5.4.1 indicates the days before harvest for each cultivar, which is also an indication of how mature the fruit was as each stage. This will also not be ideal for machine extraction. It would be best to harvest fruit when the juice sacs are loose from colour-break onwards.

If there are green fruit present on a tree at harvest, due to a second set or delay in coloration, but the amount of green fruit does not justify a second harvest, one can argue that the fruit can be harvested green, as there will be adequate germination occurring in the seeds from green fruit. Removal of all out of season fruit as soon as possible will aid flower development for the subsequent season.



**Figure 4.5.4.32.** The average germination percentage of seeds placed in an incubator for 20 days at 28°C at two different fruit maturity stages (A) and for fruit harvested at full colour (B) during the first season. In figure C, the germination % of the three maturity stages during the 2021 season is shown. Different letters indicate significant differences between cultivars at the different maturity stages on each graph. Significant differences were determined at  $P < 0.05$ . \* Note that MxT on RL for the 2021 season has no statistical analysis and is only shown to indicate the trend.

**Seed germination percentage in the greenhouse.** The germination percentage were determined in the greenhouse 88 days after sowing for all rootstock cultivars (Fig. 4.5.4.19A). MxT again had the lowest germination (72.8%), whereas all other cultivars had a germination percentage above 83%. Bisi *et al.* (2020) investigated seedling characteristics of different citrus hybrid rootstocks, with SC and US-812 included. They found that US-812 and SC had 96 and 98% germination, respectively. There are however different optimum soil temperatures for various rootstock cultivars that will affect germination and the days of seedling germination also varies between different rootstock cultivars (Rouse and Sherrod, 1996). It is important to determine the optimum soil temperature for each rootstock in order to ensure the highest germination percentage.

The emerged seedlings were also classified as single or multiple seedlings (Fig. 4.5.4.19 B&C), which was determined by dividing the number of trays with single seedlings, by the number of total trays that had seedlings which emerged. X639 (79%) and C-35 (78%) had a significantly higher percentage of single seedlings, compared to the other cultivars where the percentage was 65% and lower (Fig. 4.5.4.19B). This in turn led to these two cultivars having the lowest multiple seedling percentage (Fig. 4.5.4.19C). Overall, the multiple seedling percentage was not higher than 55%, with US-812 having the highest percentage (55%), but not significantly higher than RL (47%) and MxT (47%). These results for US-812 and SC were in line with a study done by Bisi *et al.* (2020), who found that US-812 produced 58% of multiple seedlings and SC, 24%. However, the rate of polyembryony may differ between seasons and seeds on the same tree. De Carvalho and Silva (2013), proposed that polyembryony are related to influences on the environment within the seed.

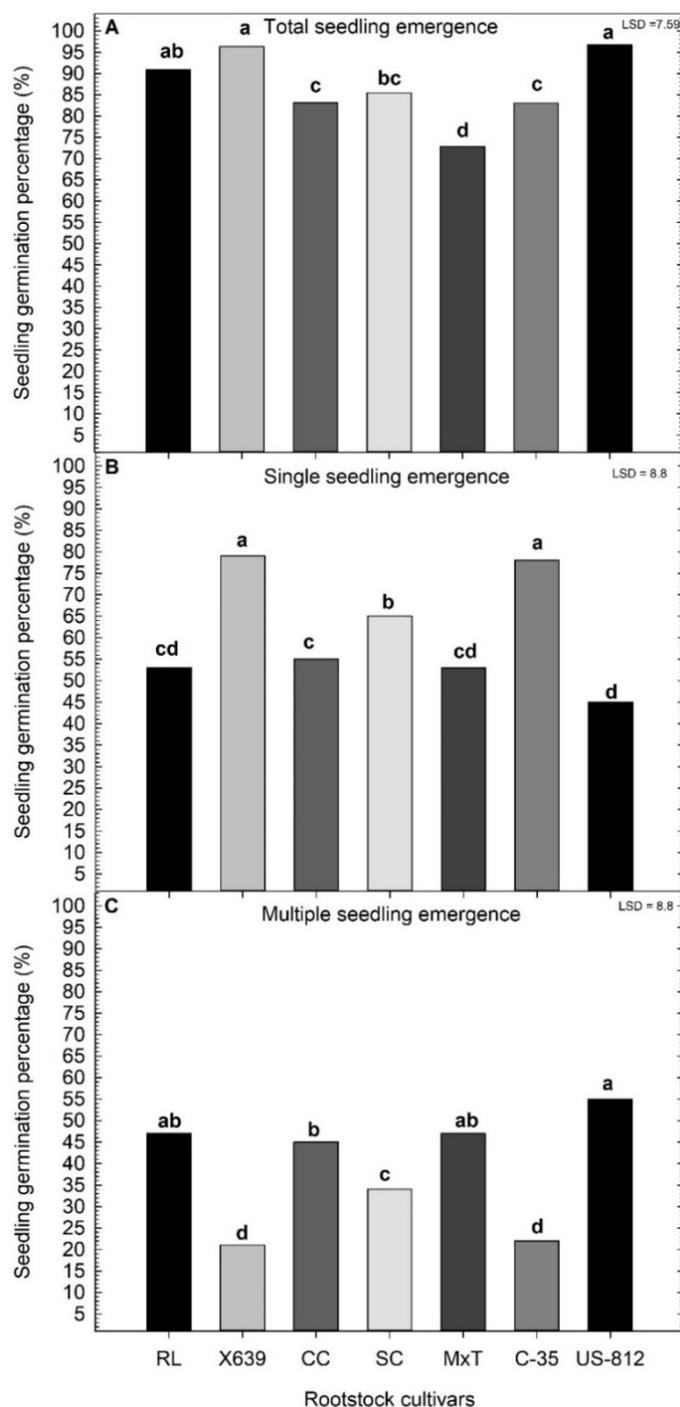
We also determined for each cultivar the percentage multiple and single seedlings, which is a break-up of the germination percentage from Fig. 4.5.4.19A. These values differ from the results in figure 19B-C, because it is worked out per cultivar and the sum of the two values adds up to the germination percentage for the respective cultivar. The data only compares differences in seedling type within each cultivar. Three cultivars, X639, SC, and C-35 had a significantly higher number of single seedlings than multiple seedlings, while the remaining cultivars, RL, CC, MxT, and US-812 did not have a huge difference in seedling type, and it was almost a ratio of 1:1 (Table 4.5.4.5).

In a study done by Anderson *et al.* (1991) they found that when two seedlings was produced from a SC seed, the smaller one of the two was more frequently zygotic (off-type). The zygotic seedlings therefore needs to be discarded. The emergence of multiple seedlings per tray, should be monitored carefully to remove smaller seedlings, and to ensure optimal root growth of the true-to-type seedling in the seedling tray. Removal of the extra seedlings per tray should be done at an early stage, before the roots tangle up in the seedling tray, which will make separation difficult at a later stage and possibly damage roots. This can therefore be more labour intensive in the nursery for rootstocks which produces a high amount of multiple seedlings (i.e. RL, CC, MxT and US-812 (Table 4.5.4.5) to rogue out possible zygotic seedlings.

**Table 4.5.4.5.** The percentage of multiple and single seedlings per cultivar based on germination percentage at 88d evaluation in the greenhouse during the 2020 season. The seedling type is a breakup of the germination percentage. Values are the means of 35 seeds per replicate (n=10), except for US-812, where only 18 seeds/rep were sowed.

Seedling type	Rootstock Cultivar						
	RL	X639	CC	SC	MxT	C-35	US-812
Single seedling %	48 a <sup>z</sup>	76 a	45 a	56 a	38 a	65 a	54 a
Multiple seedling %	43 a	21 b	38 a	29 b	35 a	18 b	43 a
<i>P</i> -value	0.442	<0.0001	0.267	0.001	0.487	< 0.0001	0.198
LSD-value	14.5%	7.1%	13.1%	11.6%	10.3%	9%	17.1%

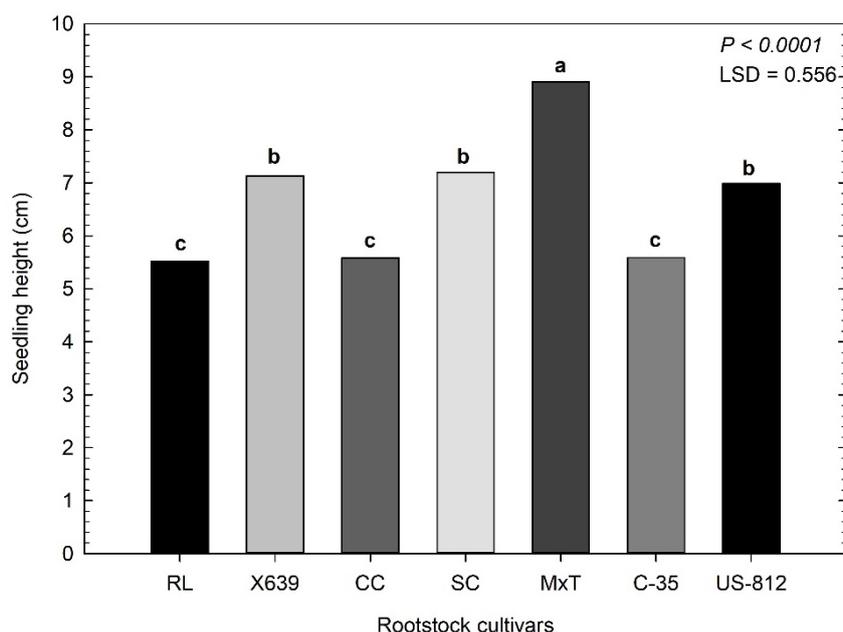
<sup>z</sup> Different letters within a column, indicates significant differences between treatment means at  $P < 0.05$ .



**Figure 4.5.4.19.** The total average germination percentage of seedlings that emerged following 88d after sowing in a temperature-controlled greenhouse (A). Values are the means of 35 seeds per replicate (n=10), except for US-812, where only 18 seeds/rep were sowed. The growth medium is a 50/50 mix of sand and peat. B and C are breakups of the % single and multiple seedlings that emerged at 88d visual assessment in the greenhouse. These percentages were determined by the amount of single seedlings divided by only the # of total seedlings that germinated. Different letters in each graph indicate a significant difference between cultivars at  $P < 0.0001$ .

**Seedling height (mm) at 88 days assessment in the greenhouse.** The seedling height was determined by measuring the stem from the soil level to the growing tip. On average, the MxT seedlings reached a height of 8.91 cm after 88 days in the soil, which was significantly higher than all the other cultivars (Fig. 4.5.4.20). SC (7.20 cm), X639 (7.13 cm) and US-812 (6.99 cm) were the second tallest seedlings, and did not differ significantly from each other. The smallest seedling height was RL (5.52 cm), CC (5.58 cm) and C-35 (5.59 cm) respectively. This gives an indication of the vigour of the different rootstocks, with MxT being a more vigorous grower compared to RL, CC, and C-35. In addition, this is an important commercial aspect as the

transplant date, i.e. when a seedling reaches 20 cm and gets transplanted into a larger pot, will be at an earlier stage for MxT compared to the other cultivars and thereby save propagation time.



**Figure 4.5.4.33.** The average seedling height of each cultivar at 88 days visual evaluation in the temperature-controlled greenhouse. Data reported are the means of ten replicates (35 seeds/rep) for all cultivars, except for US-812, which had 18 seeds/rep sowed. Different letters denote a significant difference between cultivars at  $P < 0.05$ .

**Seedling evaluation at 225 days in the greenhouse (Table 4.5.4.6).** The data are presented as percentages based on the number of germinated seedlings, as determined at 88 days for each cultivar. The parameter in the first column, seedling height  $>20$  cm, indicates the percentage of seedlings that can be transplanted to larger planting pots, for grafting. MxT had the highest percentage of seedlings that can be transplanted (93.2%) and was significantly higher than SC, C-35, CC and RL (59.7%), with the latter being significantly lower than the rest (Table 4.5.4.6). This reflects the seedling height data measured at 88 days, where MxT on average had the highest, and RL the shortest seedling height. This possibly represents the growth vigour of the various rootstock cultivars.

This qualitative parameter, seedling height  $<20$  cm, indicates that a longer period will be required for RL seedlings (24%  $< 20$  cm), before transplanting as opposed to the other cultivars, except for C-35 (17.2%  $< 20$ cm), which did not differ significantly from RL.

Furthermore, with regards to the other three parameters (dead, off-type or irregular/stunted growth) used to characterize the seedlings at 225 days, no significant differences were found between cultivars for dead seedlings recorded and irregular/stunted growth. The percentage of 'off-type' seedlings was overall very low, ranging from 5.6% (CC) -0% (MxT) (Table 4.5.4.6). The percentage of 'off-type' US-812 seedlings was in accordance with Bisi *et al.* (2020). These low percentages of off-type and irregular/stunted growth of seedlings is a very good trait (Nucellar polyembryony) that ensures the commercial success of rootstocks, being able to continue propagation by means of seeds (Bisi *et al.*, 2020). However, the 'off-type' seedlings were only rogued out based on morphological traits (different leaves and visual appearance) and no Isozymic identification was done were multiple seedlings arise from a single seed. In a previous study by Anderson *et al.* (1991), they found that zygotic seedlings are more frequently found where 2 or more seedlings arise from one seed. Therefore, the percentage of 'off-type' seedlings may be more for each cultivar if not only rogued out on visual appearance.

**Table 4.5.4.6.** Visual assessment of seedling characteristics after 225 days in the greenhouse. The data represents the percentage of seedlings that can be transferred for grafting (height >20 cm) based on the germination percentage/number of seedlings at the 88 day assessment. Data expressed are the means of ten replicates per cultivar (n=10) with 35 seeds/rep for all except for US-812 (18 seeds/rep). Mean separation was done by means of Fishers LSD test at  $P < 0.05$ .

Cultivar	Seedling height > 20 cm (%)	Seedling height < 20 cm (%)	Dead seedling (%)	Off-type seedling (%)	Irregular growth/stunted (%)
RL	59.7 d <sup>z</sup>	24.3 a	15.1 <sup>NS</sup>	0.6 bc	1.1 <sup>NS</sup>
X639	82.3 ab	1.5 c	14.9	0.3 c	1.4
CC	65.2 cd	11.1 b	18.2	5.6 a	0
SC	75.2 bc	13 b	10.2	1.6 abc	0
MxT	93.2 a	2.3 c	4.5	0.0 c	0
C-35	69.2 bcd	17.2 ab	7.7	4.8 ab	1.9
US-812	81.2 ab	2.8 c	12.6	3.5 abc	1.8
<i>P-value</i>	$< 0.0001$	$< 0.0001$	0.237	$< 0.0001$	0.116

<sup>NS</sup> Non significant differences between treatments at 5% significant level.

<sup>z</sup> Different letters within a column indicates significant differences between means.

#### **Flower number and fruit set percentage for the 2021/2022 season**

The highest average flower numbers were recorded for RL (18.7 flowers/shoot) and CC (16.5), followed by X639, C-35, SC and MxT (7.6) (Table 4.5.4.7) during the 2021 flowering season. The cultivar with the highest fruitset percentage was C-35, with 17.1 %, however, it did not differ significantly from RL (13.6%) (Table 4.5.4.7). Based on these data, some rootstocks does produce a higher number of flowers, but also sets a higher percentage of fruitlets, i.e. RL, CC and C-35. These cultivars sets on average between 1-2 fruit/shoot, whereas the others ranged below 1/shoot, from the average of 10 shoots/tree.

What is however important to remember is, that there are various factors on orchard level that influences flowering i.e. temperature, light, and soil water (Krajewski and Rabe, 1995). Climate does vary between seasons, therefore flower number for each cultivar is seasonally, and more seasons should be included to determine an overall average per cultivar. This was only done to indicate how the flower number varies between the rootstock cultivars.

**Table 4.5.4.7.** The average flower number per shoot and fruit set percentage which was determined during the 2021 flowering season for the different rootstock cultivars. Values reported are the means of 10 shoots per tree (n=10).

<b>Cultivar</b>	<b>Flower nr./shoot</b>	<b>Fruitset (%)</b>
RL	18.7 a	13.6 ab
X639	11.6 b	7.5 c
CC	16.5 a	10.9 bc
SC	9.4 bc	5.5 c
MxT	7.6 c	6.2 c
C-35	10.9 b	17.1 a
<i>P-value</i>	<i>&lt;0.0001</i>	<i>0.001</i>
<i>LSD</i>	<i>2.51</i>	<i>5.69</i>

**Objective 4:**

Individual Leaf analysis from each cultivar were done for 2020 and 2021 seasons of the 7 respective rootstocks, SC, RL, MxT, CC, C-35, US-812 and X639 (Table 8). No nutritional norms exist for rootstock cultivars probably due to the low number of rootstock orchards requiring nutritional advice. However, the general nutritional norms used in citriculture (Raath, 2021) are supplied in the table to allow comparison and to identify noticeable differences that exist between the rootstocks. Different colour shades in the table indicate whether the values are below or above the standard optimum for each element for citrus. Overall, tree health of all cultivars was good, with no various symptoms of yellowing associated with deficiencies.

Nitrogen (N) levels were below optimum levels over both seasons for most of the rootstock cultivars, except for CC and C-35, in the 2020 season. Phosphorus (P), calcium (Ca) and magnesium (Mg) show no extreme deviations from the norms over the two seasons for all cultivars. Potassium (K) levels were lower than optimum in 2020 for the majority of cultivars, except for CC and US-812. In 2021, K levels remained below optimum for RL, X639 and MxT.

**Table 4.5.4.8.** Leaf analysis of the 7 different rootstocks from the 2020 and 2021 season. Samples were taken from 25 trees according to industry guidelines on the Eastern and Western side of the tree. Leaves were analysed by Labserve.

Season	Cultivar	N %	P %	K %	Ca %	Mg %	Na mg/kg	Mn mg/kg	Fe mg/kg	Cu mg/kg	Zn mg/kg	B mg/kg	S %	Mo µg/kg
2020	RL	2.09	0.12	0.46	6.04	0.49	<100	400	204	358	82	163	0.34	2515
	X639	2.36	0.11	0.62	4.56	0.33	<100	328	217	334	29	130	0.31	5043
	CC	2.65	0.13	0.75	6.12	0.49	129	401	271	577	56	135	0.38	6408
	SC	2.09	0.09	0.49	5.81	0.42	105	346	279	448	73	190	0.32	6761
	MxT	2.33	0.13	0.60	5.62	0.48	<100	310	272	406	42	108	0.30	6475
	C-35	2.80	0.12	0.68	4.31	0.52	<100	458	221	374	70	120	0.28	1129
	US-812	2.08	0.11	0.82	5.02	0.31	147	137	160	177	33	145	0.25	167
2021	RL	1.50	0.12	0.58	4.23	0.39	137	167	162	149	55	85	0.30	<100
	X639	1.93	0.12	0.66	4.27	0.34	243	234	186	183	49	103	0.29	780
	CC	2.26	0.14	0.76	4.68	0.39	113	144	150	169	53	92	0.38	107
	SC	2.24	0.11	0.71	4.06	0.37	206	116	169	195	29	94	0.27	<100
	MxT	2.14	0.15	0.57	5.25	0.45	111	175	290	143	39	85	0.40	233
	C-35	2.18	0.14	0.74	4.14	0.47	163	209	148	141	82	87	0.27	<100
	US-812	1.93	0.13	0.76	4.76	0.41	172	178	152	195	37	121	0.28	<100
	Norms	2.4-2.60	0.11-0.14	0.70-1.10	3.5-6.0	0.35-0.50	<1600	40-150	-	5-20	25-100	75-200	0.20-0.30	100-1000

## Objective 5

To test the efficacy of GA<sub>3</sub> application on fruit set, ProGibb 40% were applied on RL, X639, CC, SC and C-35; however, these trials were not performed in-depth for MxT due to limited available trees, and so the data for MxT, which will be discussed in the text was not statistically analyzed. Flowers were counted following the first GA<sub>3</sub> (50% petal fall) except for X639, where it was counted before the foliar spray. Very small flowering buds were excluded, since GA<sub>3</sub> would not have an effect on it, because the flower must be open for GA<sub>3</sub> to have an effect.

Overall, no significant differences in fruit-set percentage were observed between the untreated control and the three respective GA<sub>3</sub> concentrations, 10 mg·L<sup>-1</sup>·GA<sub>3</sub>, 15 mg·L<sup>-1</sup>·GA<sub>3</sub>, 20 mg·L<sup>-1</sup>·GA<sub>3</sub> (10, 15 and 20 ppm) applied. However, for X639, the 20 ppm applied resulted in almost double the fruit set % compared to the untreated control and the 10 and 15 ppm. It should be noted that X639 and RL have various flowering periods and fruit sets, making it difficult to apply GA<sub>3</sub> at the right time. For C-35, there was approximately 75% petal fall when flowers were counted. Some fruitlets were present on the tagged branches where the stylar-end was already off, possibly indicating that the fruit was already after the stage for GA<sub>3</sub> to have an effect on the fruitlet, and that the fruitlet set naturally.

The untreated control trees for RL, SC, C-35, and CC showed a high fruit set. It can therefore be argued that the external application of GA<sub>3</sub> is not required, and that the endogenous GA<sub>3</sub>, present in the seeds (Bermejo, 2015; Ben-Cheikh, 1997), is sufficient to ensure fruit set. In addition, fruitlet abscission is dependent on the variety, climate and flower intensity of the tree. Varieties containing seeds also have a higher fruit set ability (Agusti and Primo-Millo, 2020). For MxT, the fruit set percentage ranged from 11.5 (Control), to 8.5% and 7% for 10 and 20 ppm respectively (data not indicated on table).

There was no significant difference in the fruit yield recorded between treatments for any of the rootstock cultivars (Table 9). Due to these differences not being significant, the trend for differences in yield between treatments can be ascribed to variation between trees rather than a treatment effect. Further was no treatment differences evident with regards to the fruit number per tree, except for SC, where the 10 ppm had higher fruit number than 20 ppm. Further was the yield recorded for MxT (data not indicated on table), 61.5, 51.39, and 58.17 Kg for control, 10 and 20 ppm respectively. The fruit number for the control was 483, in comparison to 388 for 10 ppm and 434 for 20 ppm. Overall the trend indicated for the control to have higher values, therefor showing no effect of exogenous GA<sub>3</sub> application. However, no GA<sub>3</sub> applied treatments differed from the control indicating that differences is possibly related to variation between the trees.

The average fruit number per m<sup>3</sup> (tree volume) was also determined for the different treatments. There was no significant differences evident, again indicating no treatment effect of exogenous GA<sub>3</sub> application on the five different rootstock trees (Table 4.5.4.10).

Overall, based on the results from the 2021 season, the application of GA<sub>3</sub> did not have a significant effect compared to the control, and that fruit set is not a limiting factor, but rather the effect of alternate bearing that might cause some trees to vary in yield from season to season.

In Table 4.5.4.11, the seed volume of viable and cracked seed is shown for four respective GA<sub>3</sub> applied treatments on the different rootstocks. The data is shown to determine if exogenous applied GA<sub>3</sub> might have had an effect on the seed development. The data is not statistically analyzed because the fruit from each replicate were pooled for each treatment and was not extracted separately. The only large differences was seen for CC where 20 ppm had almost double the amount of seeds, as compared to the other treatments. For SC, the 10 ppm however had very little seed, only 480 ml, compared to the other treatments who had higher than 2000ml seed on average per tree. This is not necessarily related to a treatment effect, since for all other rootstocks the seed quantity was relative constant, and variation between fruit in seed number might be the main factor. Further, the CC and SC also had a higher volume of cracked seeds, which is especially very common for SC (Table 4.5.4.11).

**Table 4.5.4.9.** Average fruit set percentage, yield and fruit number/tree of the rootstock cultivars following GA<sub>3</sub> application at 50% and 100% petal fall during the 2020/2021 season. Ten shoots (6-12 month old) per tree were tagged to quantify the flower number. Fruitlets were counted in December 2020, following physiological fruit drop, where after the fruit set percentage was determined. The yield and fruit nr/tree were determined during the 2021 harvest season.

Treatment	RL <sup>z</sup>			X639 <sup>z</sup>			CC <sup>y</sup>			SC <sup>y</sup>			C-35 <sup>z</sup>		
	Fruit set %	Yield (Kg)	Fruit #/tree	Fruit set %	Yield (Kg)	Fruit #/tree	Fruit set %	Yield (Kg)	Fruit #/tree	Fruit set %	Yield (Kg)	Fruit #/tree	Fruit set %	Yield (Kg)	Fruit #/tree
Untreated control	40.8 <sup>NS</sup>	85.31 <sup>NS</sup>	651 <sup>NS</sup>	8.33b <sup>v</sup>	29.87 <sup>NS</sup>	834 <sup>NS</sup>	43.20 <sup>NS</sup>	39.01 <sup>NS</sup>	462 <sup>NS</sup>	16.40 <sup>NS</sup>	105.49 <sup>NS</sup>	668ab	42.50 <sup>NS</sup>	42.82 <sup>NS</sup>	410 <sup>NS</sup>
10 mg-L-1 .GA <sup>3</sup>	42.33	72.96	550	6.00 b	28	734	30.80	39.33	443	17.60	125.84	802a	47.17	47.14	462
15 mg-L-1 .GA <sup>3</sup>	54.33	77.22	654	8.50 b	30.54	798	22.80	40.26	458	17.00	104.76	680ab	41.67	43.11	440
20 mg-L-1 .GA <sup>3</sup>	55.33	77.98	628	16.83 <sup>a</sup>	29.58	854	37.60	45.03	504	17.00	90.26	536b	43.50	44.18	434
<i>P-value</i>	0.173	0.690	0.544	0.004	0.966	0.778	0.144	0.840	0.928	0.993	0.099	0.015	0.946	0.975	0.971
<i>LSD</i>	20.59	21.96	170.69	5.54	10.95	262.97	18.40	16.34	208.50	8.86	27.837	146.27	20.92	22.32	231.98

<sup>NS</sup> Denotes non-significant difference at 5% significant level.

<sup>z</sup> Values reported per treatment are the means of 10 shoots per tree of six single tree replicates (n=6).

<sup>y</sup> Values reported per treatment are the means of 10 shoots per tree five single tree replicates (n=5).

<sup>x</sup> No statistical analysis were performed due to a limited tree nr. Data reported are the mean of two single tree replicates per treatment.

<sup>w</sup> Data not recorded

<sup>v</sup> Different letters within in column denotes significant differences between treatments at 5% significant level. Mean separation was done by means of Fishers LSD.

**Table 4.5.4.10.** The average fruit number per m<sup>3</sup> of the tree volume for the different GA<sub>3</sub> applied treatments during the 2020/2021 flowering season on the 5 different rootstock cultivars. Values reported are the means of 6 single tree replicates for all cultivars, except for SC, which had 5 single trees per replicate.

Treatment	Rootstock cultivars				
	RL	X639	CC	SC	C-35
Untreated control	119 <sup>NS</sup>	94 <sup>NS</sup>	146 <sup>NS</sup>	79 <sup>NS</sup>	69 <sup>NS</sup>
10 mg-L-1 .GA <sup>3</sup>	122	96	143	93	66
15 mg-L-1 .GA <sup>3</sup>	141	91	141	87	67

**Table 4.5.4.11.** The average seed quantity in millilitre recorded for the different GA<sub>3</sub> concentration treatments that was applied during the 2020/2021 flowering season. Fruit were harvested at commercial maturity and the seeds were extracted from the fruit to be quantified. No statistical analysis was performed since fruit were pooled for each treatment and seeds were not extracted separately per rep.

Cultivar	Type of seed	Treatment			
		Untreated control	10mg·L <sup>-1</sup> .GA <sup>3</sup>	15 mg·L <sup>-1</sup> .GA <sup>3</sup>	20 mg·L <sup>-1</sup> .GA <sup>3</sup>
RL	Viable (ml)	692	633	467	642
	Cracked (ml)	33	37	38	28
X639	Viable (ml)	592	450	633	625
	Cracked (ml)	37	50	50	78
CC	Viable (ml)	3152	3170	2760	6280
	Cracked (ml)	213	192	211	501
SC	Viable (ml)	3340	480	2076	2451
	Cracked (ml)	792	1080	651	757
MxT <sup>z</sup>	Viable (ml)	1125	775	-	725
	Cracked (ml)	-	-	-	-
C-35	Viable (ml)	1592	1783	1792	1458
	Cracked (ml)	0	0	0	0

<sup>z</sup> There were limited trees available, and therefore no 15 mg·L<sup>-1</sup> GA<sup>3</sup> treatment was applied.

<sup>y</sup> Cracked seed quantity was negligible.

<b>20 mg·L<sup>-1</sup> .GA<sup>3</sup></b>	132	107	148	74	77
<b><i>P-value</i></b>	<i>0.667</i>	<i>0.736</i>	<i>0.998</i>	<i>0.271</i>	<i>0.922</i>
<b>LSD-value</b>	41.563	31.54	83.846	21.638	36.734

<sup>NS</sup> indicates nonsignificant difference between treatments within a column at 5% significant level.

## Objective 6

This objective was not carried out experimentally due to the limited number of available trees to perform all the trials on. In addition, the 10 data trees of the various cultivars used to determine the yield over various seasons have been pruned to have a more uniform tree canopy, where water shoots were removed, as well as shoots where no branching on the lower side of the branch was present. This was not done for MxT, since it was harvested at a very late stage in the season, and the flowering initiation already started. The main goal for this objective was to prune the trees in such a manner to prevent it from influencing the yield of the following season to a great extent. It was also important to prune the trees from an early stage to prevent the trees from reaching heights in the future that will not only affect the harvesting procedure, but also the general orchard management, i.e. spraying the trees.

## Conclusion

Although this study only shows data from two seasons, it serves as a sound basis to build on to develop different management strategies, ensuring consistent fruit set, yield and seed count per fruit. It is also evident from the data that the seeds are viable, and the germination percentage is higher than 50% for all cultivars. However, since it is known that there are various aspects responsible for the production of a high number of viable seeds (climate), more seasons should be evaluated to build on this data to gain more insight.

The average seed number per fruit, yield per tree, and average fruit count per tree is useful data that can aid in the forecast of the average seed production per cultivar and also to plan the size (hectare) of future plantings for each cultivar to meet the increasing demand of rootstock supply for commercial plantings. For cultivars that produce less milliliters of viable seeds, i.e. RL, SC, C-35, X639 and MxT, more hectares should be planted, or at a higher density to ensure an adequate seed supply.

The fruit diameter at harvest and average seed number per fruit remained relatively constant over the two seasons, for each cultivar. Further, was there some cultivars that showed a reduction in yield for the second season (RL, X639), whereas for others there was a slight increase (CC, SC, MxT). It was evident that there were variation in yield between the trees for each cultivar in the second season. It may be possible that alternate bearing is evident for some cultivars, therefore, a third season's data on yield will be conducted for 2022. This third season's data, will be valuable in order to have a 3-year average of yield/tree for each rootstock cultivar, to aid in forecast planning.

Successful and high percentage of seed germination is an important process that determines the success of rootstock propagation. The germination percentage varied between seasons for each cultivar. Therefore, germination is likely dependent on the seed itself and its potential to germinate as seeds of the same size some did germinate while other did not. What would be of interest is to determine the germination percentage of the seed, extracted per fruit, and not a pooled sample.

Overall in most cases, it is advised that fruit should be harvested at least when colour-break is evident, but full colour is better to ensure the best germination percentage possible of all seeds. MxT seeds showed an overall low germination percentage compared to the other cultivars. This can be altered by sowing more seeds to meet the supply amount for MxT rootstocks. As previously mentioned, seeds from fruit of rootstock trees are polyembryonic, however, not all embryos germinate and produce multiple seedlings (Primo-Millo and Agusti, 2020). The advantage of multiple seedlings/seed when true-to-type, is that nurseries are able to plant out seedlings into separate trays, thereby having a higher number of available seedlings to use i.e. US-812 showed 53 % of multiple seedlings that germinated. However, it is not always very accurate to only separate seedlings based on morphology, as zygotic seedlings ('off-type') may be present and may be missed. Therefore, extra caution should be taken when multiple seedlings that emerged are separated to make sure it is true-to-type and that the roots does not interfere with the other seedlings. This may be more time consuming.

All rootstocks investigated during the trial (RL, X639, CC, SC, C-35, US-812, and MxT) are sufficient to propagate by means of seeds. To summarise, these results provide valuable cultivar-specific information to assist in future planning for rootstock farms to ensure the seed supply is met to assist in HLB management,

when the turnaround time for trees will be more rapid than it is currently. Therefore, yearly seed supply needs to be consistent.

This remains a very important research field and is of high importance to ensure the consistency of seed production in citrus rootstocks, especially in South Africa. As the saying goes, “only time will tell”.

### **Future research**

Germination of seeds per fruit would be of interest to see how much seed per fruit has the potential to germinate. The yield per tree for the different rootstock cultivars, should also be recorded, to build a data basis over various seasons, as climate differs between seasons, and the alternate bearing factor may come into play.

### **Technology transfer**

Talk or presentation at the future planned CRI Research symposium.

A possible paper/ article on the horticultural differences between rootstock cultivars used in South Africa.

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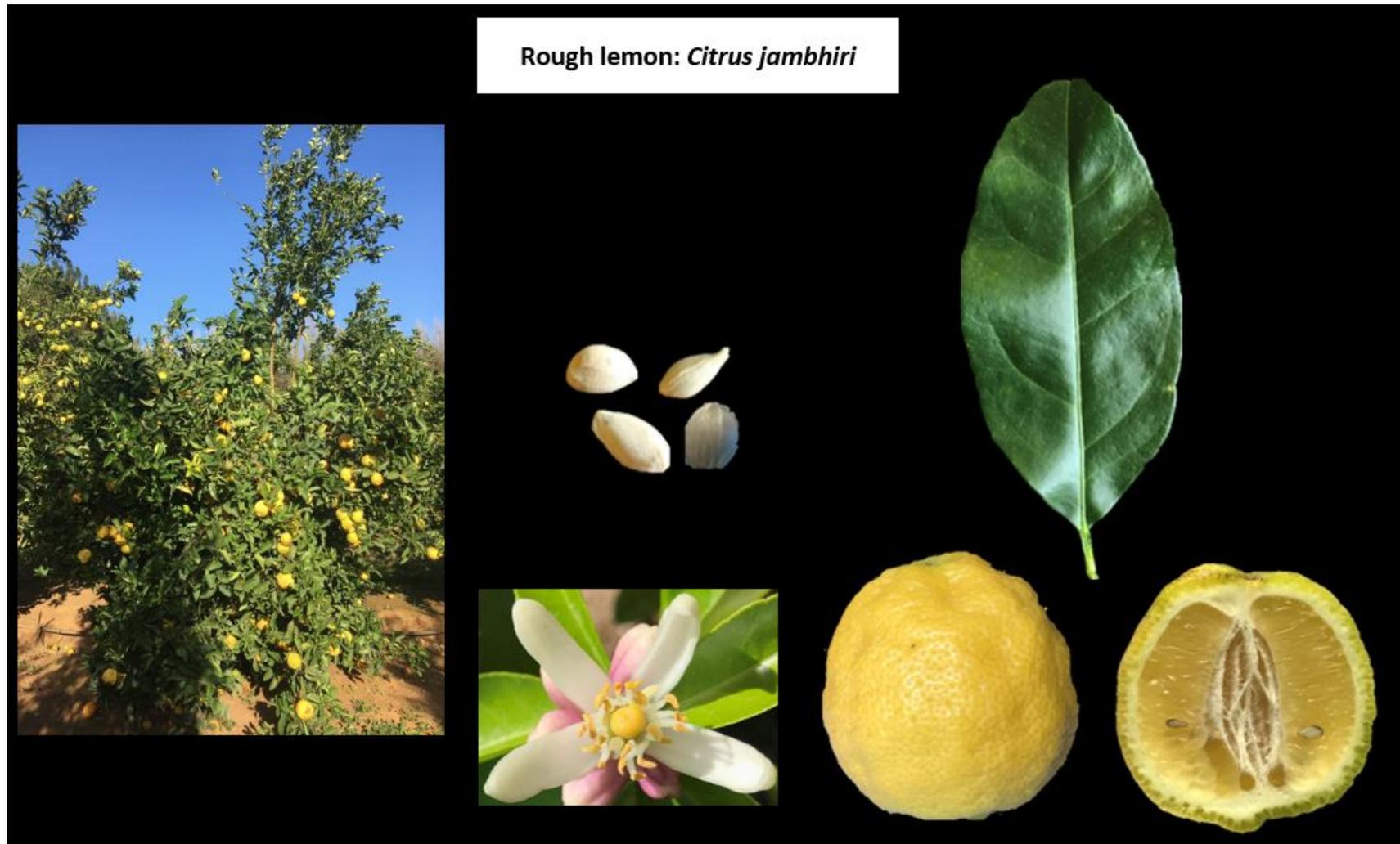
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Addendum A



**Figure 1.** An illustration of tree growth, flower at full bloom, seeds, fruit appearance at harvest, and mono-foliolate leaf (no wings at the petiole) of a Rough lemon rootstock.

*\*Note: The photos are not 100% according to scale and merely serve as an illustration.*

X639 citrandarin: *Cleopatra mandarin (Citrus reticulata) x Poncirus trifoliata*



**Figure 2.** An illustration of the tree growth, flower at full bloom, seeds, fruit appearance at harvest, and the tri-foliolate leaf of a X639 citrandarin.

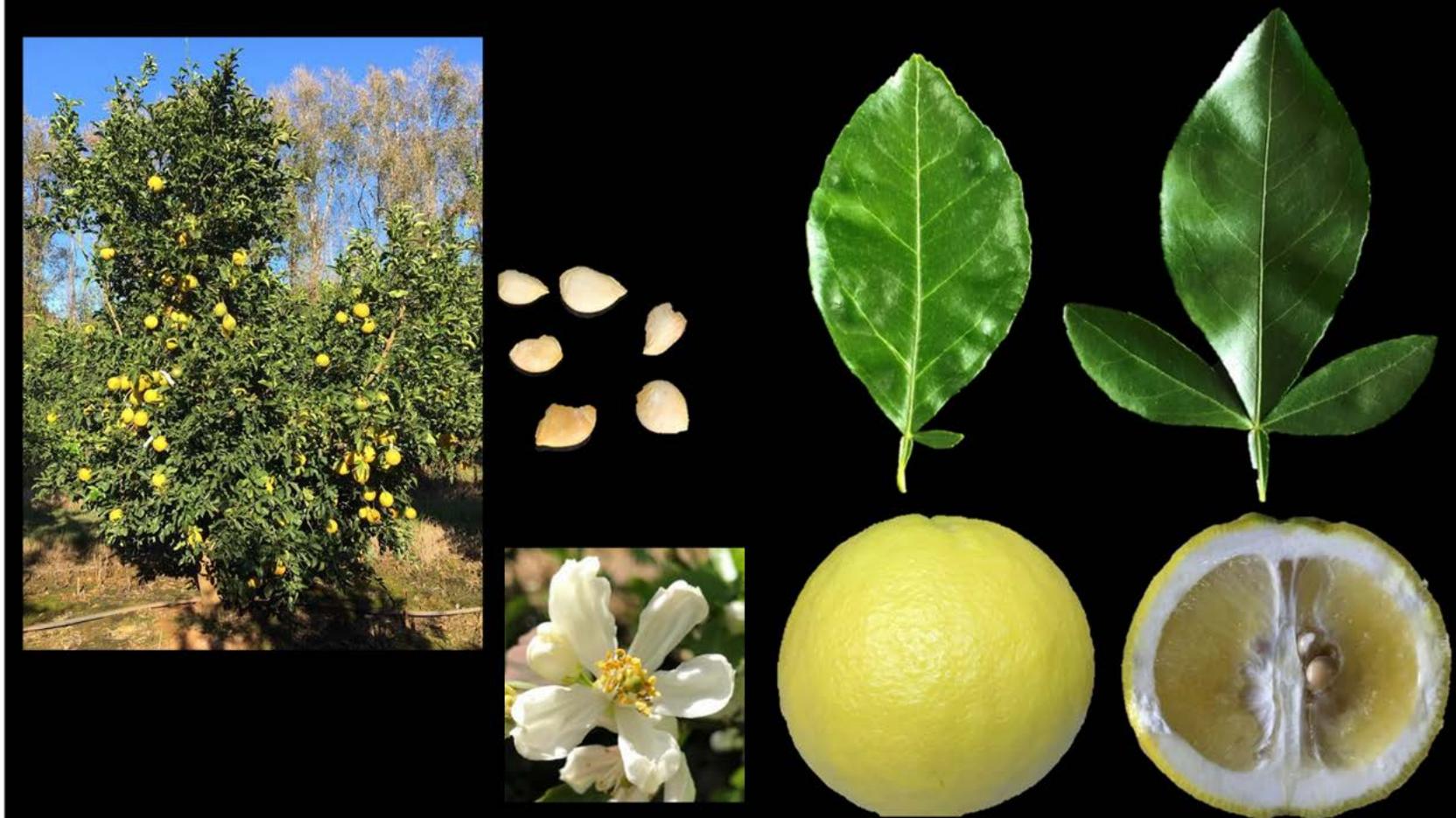
*\*Note: The photos are not 100% according to scale and merely serve as an illustration.*

Carrizo citrange: *Citrus sinensis* x *Poncirus trifoliata*



**Figure 3.** An illustration of the tree growth, flower at full bloom, seeds, fruit appearance at harvest, and tri-foliolate leaf of a Carrizo citrange rootstock.  
*\*Note: The photos are not 100% according to scale and merely serve as an illustration.*

Swingle citrumelo: *Citrus paradisi* x *Poncirus trifoliata*



**Figure 4.** An illustration of the tree growth, flower at full bloom, seeds, fruit appearance at harvest, and tri-foliolate leaf of a Swingle citrumelo rootstock.  
*\*Note: The photos are not 100% according to scale and merely serve as an illustration.*

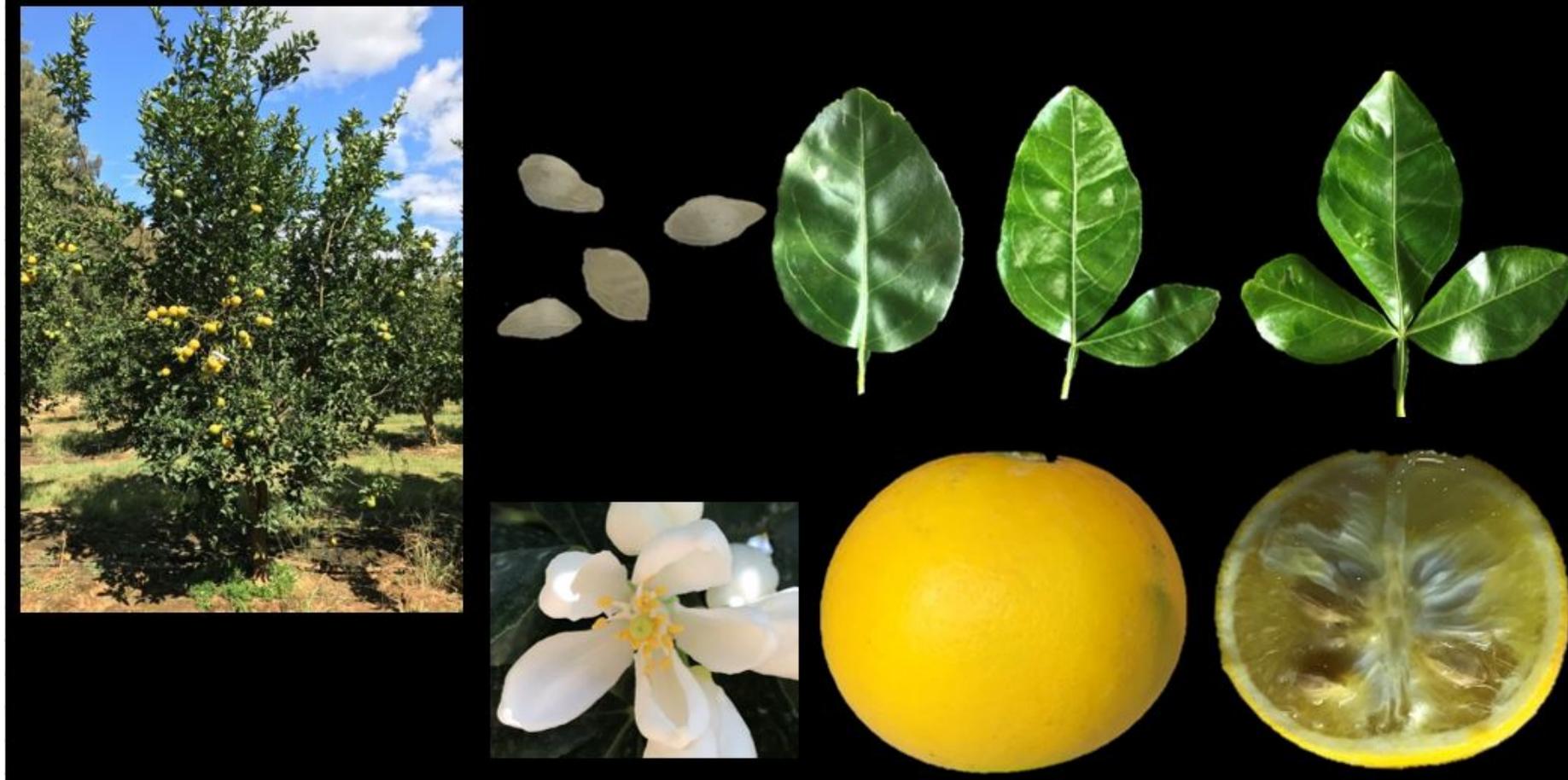
MxT : Minneola (*Citrus paradisi* x *Citrus reticulata*) x *Poncirus trifoliata*



**Figure 5.** An illustration of the tree growth, flower at full bloom, seeds, fruit appearance at harvest, and tri-foliolate leaf of a MxT rootstock.

*\*Note: The photos are not 100% according to scale and merely serve as an illustration.*

**C-35 citrange: *Citrus sinensis* x *Poncirus trifoliata***



**Figure 6.** An illustration of the tree growth, flower at full bloom, seeds, fruit appearance at harvest, and tri-foliolate leaf of a C-35 rootstock.  
*\*Note: The photos are not 100% according to scale and merely serve as an illustration.*

US-812: Sunki Mandarin (*Citrus reticulata*) x Benecke trifoliolate orange (*Poncirus trifoliata*)



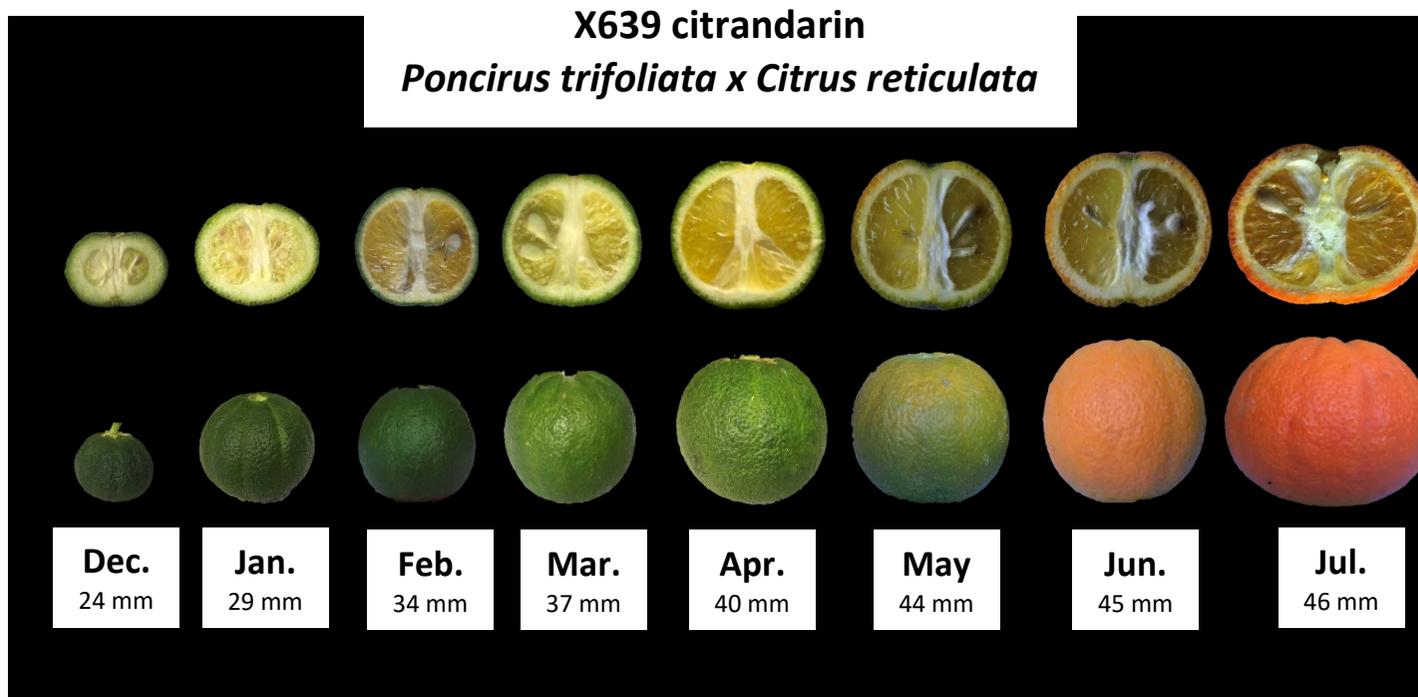
**Figure 7.** An illustration of the tree growth, seeds, fruit appearance at harvest, and the tri-foliolate leaf of US-812.

*\*Note: The photos are not 100% according to scale and merely serve as an illustration.*

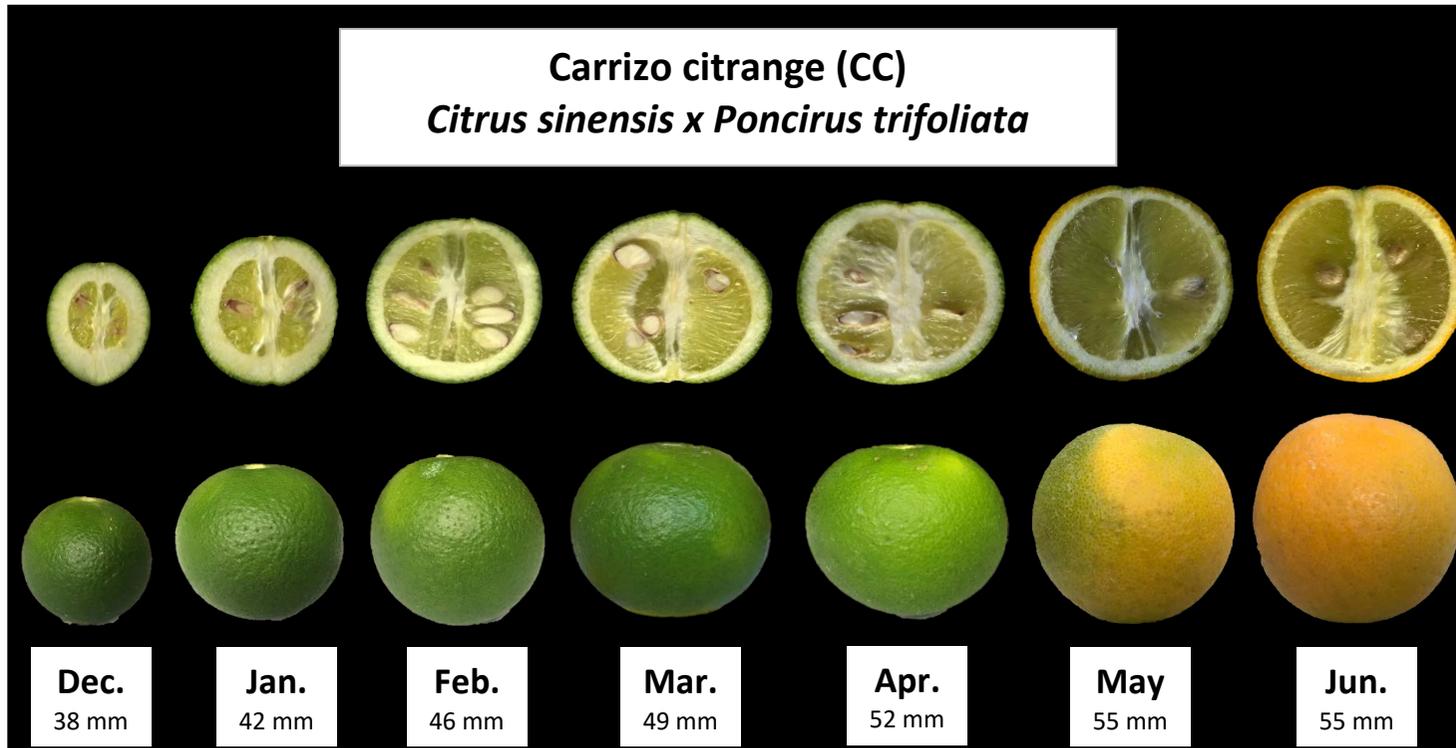
Addendum B



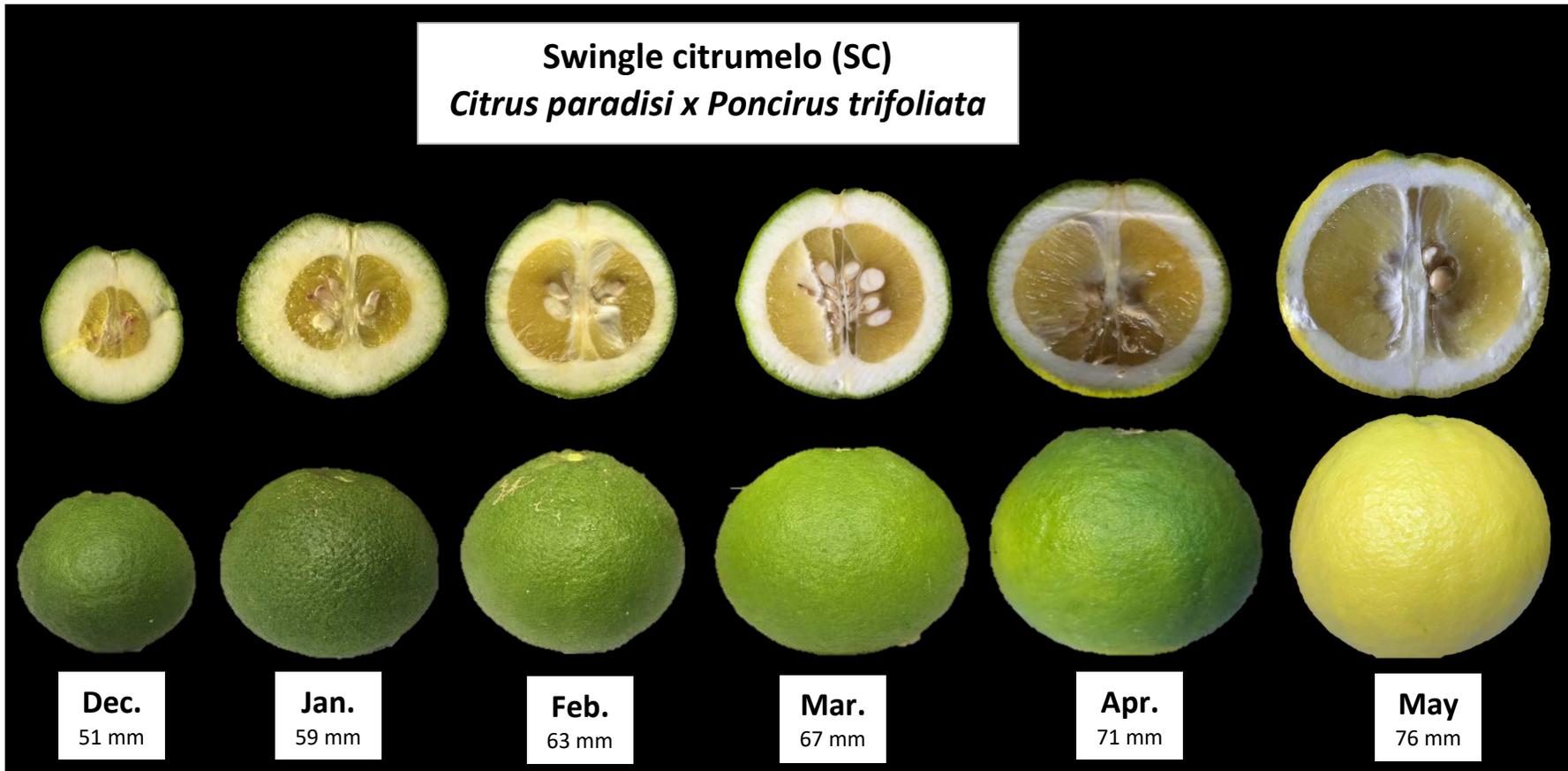
**Figure 1.** Fruit development from Dec. to Harvest for C-35. Note the photos of the fruit is a percentage of the fruit diameter shown in the photo.



**Figure 2.** Fruit development from Dec. to Harvest for X639. Note the photos of the fruit is a percentage of the fruit diameter shown in the photo.



**Figure 3.** Fruit development from Dec. to Harvest for CC. Note the photos of the fruit is a percentage of the fruit diameter shown in the photo.

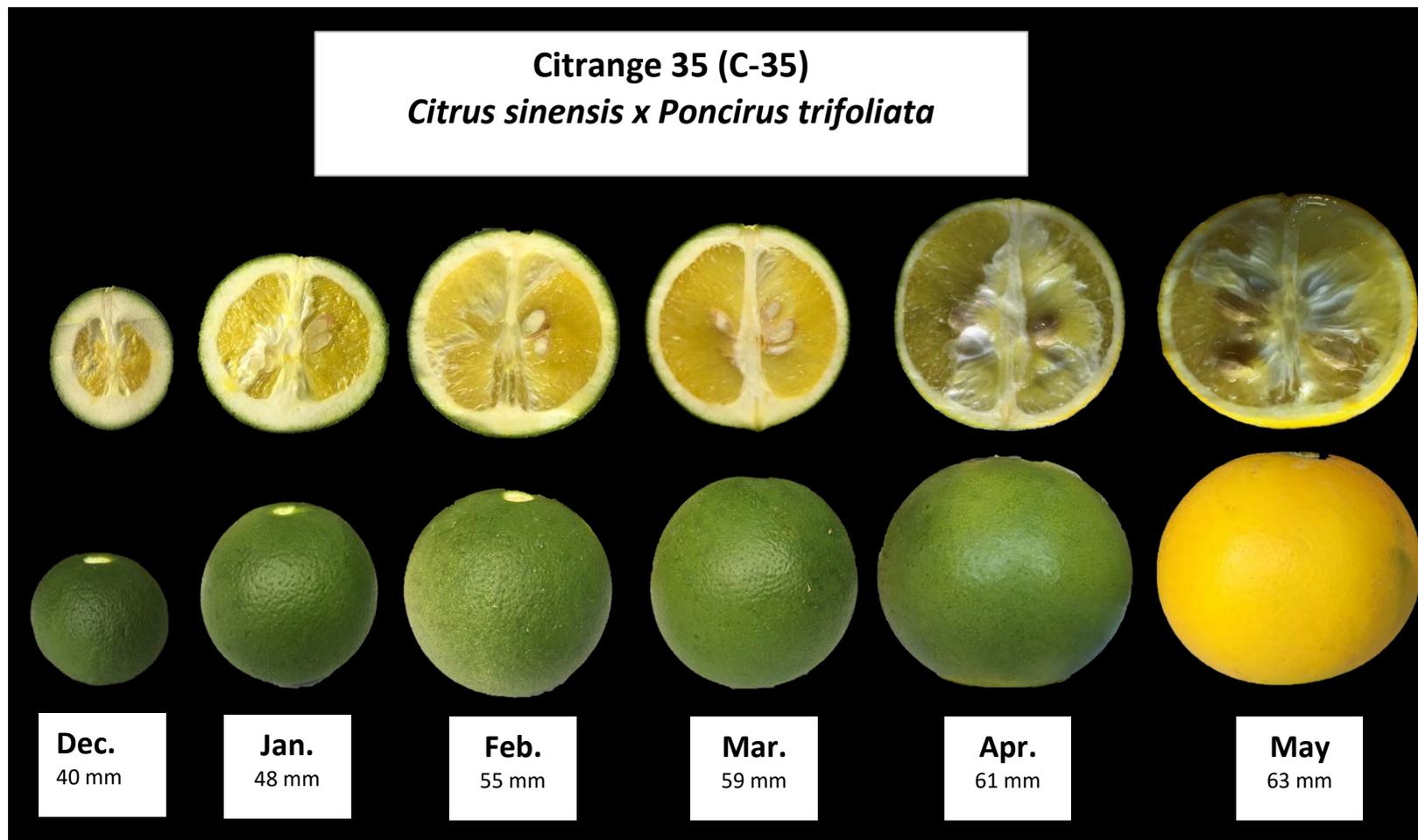


**Figure 4.** Fruit development from Dec. to Harvest for SC. Note the photos of the fruit is a percentage of the fruit diameter shown in the photo.

**Mineola x trifoliata hybrid (MxT)**  
*(Citrus paradisi x C. reticulata) x Poncirus trifoliata*



**Figure 5.** Fruit development from Dec. to Harvest for MxT. Note the photos of the fruit is a percentage of the fruit diameter shown in the photo.



**Figure 6.** Fruit development from Dec. to Harvest for C-35. Note the photos of the fruit is a percentage of the fruit diameter shown in the photo.

**US-812 (Sunki x Benecke)**  
*Citrus reticulata x Poncirus trifoliata*



**Figure 7.** Fruit development from Dec. to Harvest for US-812. Note the photos of the fruit is a percentage of the fruit diameter shown in the photo.

### Addendum C

**Table 1.** A summary of important horticultural properties of 7 important rootstocks used commercially in South Africa. The trees used to describe the horticultural properties are located at the Citrus Foundation Block in Uitenhage, Eastern Cape. All trees were planted in 2015 except for US-812, which was planted in 2010. The data presented here are an average of trials conducted during the 2019/2020 and 2020/2021 season.

	<b>Rough Lemon</b> <i>C. jambhiri</i>	<b>Mineola x trifoliolate hybrid (MxT)</b> <i>(C. paradisi x C. reticulata) x P. trifoliolata</i>	<b>Carrizo citrange</b> <i>C. sinensis x P. trifoliolata</i>	<b>Swingle citrumelo</b> <i>C. paradisi x P. trifoliolata</i>	<b>Citrango 35 (C-35)</b> <i>C. sinensis x P. trifoliolata</i>	<b>X639 citrandarin</b> <i>P. trifoliolata x C. reticulata</i>	<b>US-812 (Sunki x Benecke)</b> <i>C. reticulata x P. trifoliolata</i>
<b>Tree vigor</b>	Vigorous	Vigorous	Intermediate	Intermediate	Intermediate	Intermediate	Vigorous
<b>Type of leaves</b>	Unifoliolate with no petiole wings.	High presence of unifoliolate (winged petioles) compared to trifoliolate leaves.	High presence of trifoliolate leaves, and low % of unifoliolate (winged petiole)	High presence of trifoliolate leaves, and low % of unifoliolate (winged petiole)	High presence of trifoliolate leaves, and low % of unifoliolate (winged petiole)	Trifoliolate and unifoliolate (winged petiole) leaves	High presence of unifoliolate (winged petiole) and low % of trifoliolate leaves.
<b>Fruit size</b>	68 mm	67mm	56 mm	76 mm	61 mm	46 mm	39 mm
<b>Average seed no./fruit quantified at colour-break stage before seed treatment process</b>	16	16	17	19	19	10	3
<b>Average Yield/tree (kg/tree)</b>	102.5 Kg/tree	46 Kg/tree	61 Kg/tree	66 Kg/tree	25 Kg/tree	68 Kg/tree	n/a
<b>Average fruit number/tree.</b>	761	367	707	388	301	2256.6	n/a.

	<b>Rough Lemon</b> <i>C. jambhiri</i>	<b>Mineola x trifoliata hybrid (MxT)</b> <i>(C. paradisi x C. reticulata) x P. trifoliata</i>	<b>Carrizo citrange</b> <i>C. sinensis x P. trifoliata</i>	<b>Swingle citrumelo</b> <i>C. paradisi x P. trifoliata</i>	<b>Citrange 35 (C-35)</b> <i>C. sinensis x P. trifoliata</i>	<b>X639 citrandarin</b> <i>P. trifoliata x C. reticulata</i>	<b>US-812 (Sunki x Benecke)</b> <i>C. reticulata x P. trifoliata</i>
<b>Viable seed in ml following seed extraction and grading.</b>	1080 ml	584 ml	3338 ml	1480 ml	823 ml	3045 ml	n/a
<b>Average Seediness/fruit after seed extraction and grading</b>	8	7	13	10	13	4	n/a
<b>Seed germination in oven (26-28 °C)</b>	83%	59%	81%	73%	59%	83%	91%
<b>Seed germination in greenhouse (30-40 °C) at 88d after sowed</b>	91%	73%	83%	85%	83%	96%	97%
<b>Multiple seedling emergence at 88d after sowing in greenhouse</b>	47%	47%	45%	34%	22%	21%	55%
<b>Average seedling height at 88d in greenhouse visual assessment.</b>	5.5 cm	8.9 cm	5.6 cm	7.2 cm	5.6 cm	7.1 cm	7.0 cm
<b>Average % 'off-type' seedlings from total seeds germinated at 88d</b>	0.6%	0%	5.6%	1.6%	4.8%	0.3%	3.5%
<b>Maturation period (2019/2020)</b>	Mid (Mid-June)	Late (Early September)	Early (End of May)	Early (Mid-May)	Early (Mid-April)	Mid (Early- July)	Mid (Mid-June)

#### 4.5.5 PROGRESS REPORT: New systemic insecticides for citrus

Project 1148 (2016/7-2022/3) by T G Grout, P R Stephen (CRI), S M Faris and P Nderitu (*icipe*)

##### Summary

In order to prepare for the arrival of *Diaphorina citri* in South Africa, we need to find more systemic insecticides that can be used frequently in nurseries and for non-bearing trees. The brown citrus aphid *Aphis citricidus* was previously used as an indicator pest for screening systemic insecticides on potted lemon trees. The registered imidacloprid drench and recently registered acephate (Spectra Stem) stem treatment were used as standards and resulted in all aphids dropping off the leaves within 7 days. Two dosages of sulfoxaflor as a drench gave the same result after 7 days, although the mortality rate was slower. Results from an unregistered product, used in some countries as a soil drench for vegetables, were disappointing and a higher dosage will be required. Another unregistered systemic insecticide, used on citrus in the USA, gave promising results as a soil drench. A field trial against cotton aphid on young citrus was attempted but populations were sporadic and results very variable. Focus then switched to developing a culture of *D. citri* on potted citrus plants in an insect-proof structure in SE Kenya in collaboration with *ICIPE* so that the same chemicals could be evaluated against *D. citri* on potted plants. However, populations of *D. citri* on citrus in the region have remained low and the numbers in the culture have been inadequate for trials, despite the addition of *Murraya koenigii* plants to the culture. P. Nderitu did conduct a survey of the citrus production areas in Kenya in search of *D. citri* and possible HLB infections in hot regions. Of the 18 sites visited, *D. citri* was only recovered from Lungalunga in SE Kenya close to the Tanzania border.

##### Opsomming

Om voor te berei vir die koms van *Diaphorina citri* in Suid-Afrika, moet ons meer sistemiese insekdoders vind wat gereeld in kwekerye en vir nie-draende bome gebruik kan word. Die bruin sitrus plantluis, *Aphis citricidus*, is voorheen as 'n indikatorplaag gebruik vir die sifting van sistemiese insekdoders op gepotte suurlemoenbome. Die geregistreerde imidacloprid deurdrenkbehandeling, en onlangs geregistreerde asefaat (Spectra Stem) stambehandeling, is as standaard gebruik en het daartoe gelei dat alle plantluis binne 7 dae van die blare afgeval het. Twee dosisse sulfoxaflor as 'n deurdrenkbehandeling het dieselfde resultaat na 7 dae gegee, alhoewel die mortaliteitsyfer stadiger was. Resultate van 'n ongeregisteerde produk, wat in sommige lande as 'n gronddeurdrenking vir groente gebruik word, was teleurstellend en 'n hoër dosis sal vereis word. Nog 'n ongeregisteerde sistemiese produk wat op sitrus in die VSA gebruik is, het belowende resultate as 'n gronddeurdrenking gegee. 'n Veldproef teen katoen plantluis op jong sitrus is probeer, maar populasies was sporadies en resultate baie wisselvallig. Fokus het toe oorgeskakel na die teel van 'n populasie van *D. citri* op gepotte sitrusplante in 'n insekbestande struktuur in SO Kenia, in samewerking met *ICIPE* sodat dieselfde chemikalieë teen *D. citri* op potplante geëvalueer kan word. Populasies van *D. citri* op sitrus in die streek het egter laag gebly en die getalle in die teelpopulasie was onvoldoende vir proewe, ten spyte van die toevoeging van *Murraya koenigii*-plante tot die teelpopulasie. P. Nderitu het 'n opname van die sitrusproduksiegebiede in Kenia gedoen, op soek na *D. citri* en moontlike HLB-infeksies in warm streke. Van die 18 terreine wat besoek is, is *D. citri* net vanaf Lungalunga in SO Kenia, naby die Tanzanië-grens, gevind.

#### 4.5.6 PROGRESS REPORT: Evaluation of new University of Florida (UF) rootstocks

Project 1246 (2019/20 – 2022/3) by PJR Cronje, J Niemann and J Joubert (CRI)

##### Summary

The UF/CREC rootstock program has been breeding rootstocks for 30+ years to address various problems in this production area. In the recent past and due to the massive natural screening as the HLB epidemic spread in Florida, some potential tolerant/resistant rootstocks, with commercial potential, were identified (rescue trees). After successfully importing the seeds of 20 new selections from this breeding program it was successfully submitted to DALRDD/Plant Health for pathogen screening in 2021. Thereafter the seeds were delivered to Du Roi nursery at Letsitele for germination to develop seedlings. At this stage, each rootstock selection was assessed to select uniform seedlings. For each selection, a standard photo ID chart of distinguishing characteristics i.e., leaf types, growth pattern and root development were made to assist in

future propagation from seeds in commercial nurseries. It was observed, that even at this early stage of the project, differences in the selections are evident and are expected to result in significant changes in nursery practices, canopy development and fruiting potential. After selecting seedlings based on the selection criteria, they were budded with 'Nadorcott LS' mandarin and 'Midnight' Valencia to allow planting in March 2023 as part of two new orchards at Crocodile Valley Estate in Nelspruit.

## Opsomming

Die UF/CREC teel en ontwikkel al vir die laaste 30+ jaar onderstamme om verskillende probleme in hierdie area aan te spreek. As gevolg van die massiewe natuurlike sifting wat plaasgevind het soos die HLB-epidemie in Florida versprei het, is enkele potensiële verdraagsame/weerstandige onderstamme geïdentifiseer (reddingsbome). Ná saad van 20 onderstam seleksies ingevoer is, is die sade aan DALRDD/Plant Gesondheid vir patogeen-ondersoek gelewer en daarna vrygestel. Die saad is aan Du Roi kwekery in Letsitele verskaf vir ontkieming en saailing vestiging. Hierna is in 'n poging om vir elke seleksie 'n standaard tipe te identifiseer vir latere plantvermeerdering, 'n foto ID kaart gemaak wat identifiseerbare aspekte soos blaartipes, groeiwyse en wortelontwikkeling insluit. In hierdie vroeë stadium van die projek kan alreeds groot verskille tussen die seleksies gesien word wat vermoedelik 'n impak sal maak op kwekerypraktyke, boomontwikkeling en potensiaal om vrugte te produseer. Die geselekteerde saailinge is met 'Nadorcott LS' mandaryn en 'Midnight' Valencia geokuleer om gereed te wees vir plant in Maart 2023 as deel van twee nuwe boorde op Crocodile Valley Estate in Nelspruit.

### 4.5.7 PROGRESS REPORT: Distinguishing *Diaphorina* spp. and *Trioza* spp. from other psylloids likely to be caught on yellow traps

Project 1255 (2021/2 – 2022/3) by Evans Mauda, Glynnis Cook, Aruna Manrakhan, Hano Maree (CRI), Rachelle Bester (CRI/SU), Rochelle de Bruyn, Leani Serfontein (CRI), Daniel Burckhardt (Naturhistorisches Museum, Switzerland), Tim Grout (CRI)

## Summary

The identification of *Diaphorina* species in South Africa and other parts of the world is difficult. Two species (*Diaphorina punctulata* and *Diaphorina zebrana*) have been found to feed on citrus as adults, however, these are not considered vectors of African greening (Catling and Atkinson 1974). Taxonomy of psylloids in the Afrotropical region has been largely ignored. There are currently no morphological keys for identification of these species. Collected specimens from the field surveys are being sorted, recorded and identified to the closest possible genus level and where possible to species level using an unpublished taxonomic key prepared by Daniel Burckhardt. Specimen collection is continuing in and around citrus environments in Limpopo, Mpumalanga, and Eastern Cape provinces. Indigenous plants were confirmed as hosts of psylloids with the presence of eggs, immature and adult *Diaphorina* species collected. Indigenous *Trioza* sp. and *Diaphorina* sp. including a *D. citri* lookalike have been sent for DNA barcoding and sequencing.

*Diaphorina* specimens collected from 2020 to 2021 were mostly obtained from sticky traps placed for biosecurity surveys. Specimens were removed from traps and DNA was extracted. PCR amplification for DNA barcoding and identification purposes using universal primers were unsuccessful. However, primers designed for specific amplification of *D. citri*, but targeting a different region of the mitochondrial Cytochrome C oxidase sub unit I (COI) to the previous primers, were able to amplify most unknown *Diaphorina* specimens and nucleotide sequences were generated for 36 specimens. These *Diaphorina* specimens all showed closer sequence identity to *Diaphorina lycii* rather than to *D. citri* or *Diaphorina communis* sequences available on GenBank. A conserved region was found in a sequence alignment allowing the design of a forward primer, better suited to *Diaphorina* and triozids, to be used with the universal reverse primer allowing for the generation of sequence data in the general COI bar-code region. Using the new assay, sequences for *Diaphorina virgata* (6) and morphologically different looking *Diaphorina punctulata* (15) specimens were obtained. A bar-code sequence for a *Diaphorina* specimen, collected in Colchester, Eastern Cape and morphologically a lookalike to *D. citri*, showed that the specimen was not *D. citri*, but an indigenous lookalike occurring on the indigenous tree *Harpephyllum caffrum* commonly known as African Plum tree.

Sixteen trioqid specimens, mostly collected from sticky traps were morphologically identified as either *Trioza erytrae* or *Trioza litseae* or *Trioza* sp. (*litseae*-group) and were bar coded using Sanger sequencing. Nucleotide sequence diversity was observed between these specimens and sequences broadly grouped into 5 clusters. Thus far, '*Candidatus* Liberibacter africanus' (CLaf) was detected in specimens in two clusters. A unique Liberibacter sp. was identified in a trioqid sample from a trap in Knysna and further characterisation and plant host identification of this Liberibacter is required. The trioqid barcode of this sample grouped in a cluster with a specimen from East London.

## Opsomming

Die identifisering van *Diaphorina*-spesies in Suid-Afrika en ander dele van die wêreld is moeilik. Daar is gevind dat twee spesies (*Diaphorina punctulata* en *Diaphorina zebrana*) as volwassenes op sitrus voed, maar word egter nie as vektore van Afrika-vergroening beskou nie (Catling en Atkinson 1974). Taksonomie van psilloïede in die Afro-tropiese streek is grootliks geïgnoreer. Daar is tans geen morfologiese sleutels vir die identifikasie van hierdie spesies nie. Versamelde monsters van die veld-opnames word gesorteer, aangeteken en tot die naaste moontlike genusvlak, en waar moontlik tot spesievlak, geïdentifiseer met behulp van 'n ongepubliseerde taksonomiese sleutel wat deur Daniel Burckhardt opgestel is. Monsterversameling gaan voort in en om sitrus-omgewings in Limpopo, Mpumalanga en Oos-Kaap provinsies. Inheemse plante is as gashere van psilloïede bevestig, met die teenwoordigheid van eiers, onvolwasse en volwasse *Diaphorina* spesies wat versamel is. Inheemse *Trioza* sp. en *Diaphorina* sp., insluitend 'n monster wat soos *D. citri* lyk, is vir DNS-strepieskodering en -volgordebepaling gestuur.

*Diaphorina*-monsters wat vanaf 2020 tot 2021 versamel is, is meestal vanaf kleeflokvalle verkry wat vir biosekuriteitsopnames geplaas is. Monsters is uit lokvalle verwyder en DNS is geëkstraheer. PCR-amplifikasie vir DNS-strepieskodering en identifikasiedoeleindes met behulp van universele inleiers, was onsuksesvol. Inleiers wat vir spesifieke amplifikasie van *D. citri* ontwerp is, maar wat 'n ander area van die mitokondriale Sitokroom C-oksidas sub-eenheid I (COI) as die vorige inleiers geteiken het, was egter in staat om die meeste onbekende *Diaphorina*-monsters te amplifiseer, en nukleotiedvolgordes is vir 36 monsters gegeneer. Hierdie *Diaphorina*-monsters het almal 'n nader volgorde-identiteit aan *Diaphorina lycii* getoon, as aan *D. citri*- of *Diaphorina communis*-volgordes, beskikbaar op GenBank. 'n Gekonserveerde area is in 'n volgordebelyning gevind wat die ontwerp van 'n voorwaartse inleier moontlik maak, beter geskik vir *Diaphorina* en triosiede, wat gebruik kan word met die universele omgekeerde inleier, wat die generering van volgordedata in die algemene COI-strepieskode-gebied moontlik maak. Deur die nuwe toets te gebruik, is volgordes vir *Diaphorina virgata* (6) en *Diaphorina punctulata* (15) monsters wat morfologies anders lyk, verkry. 'n Strepieskode-volgorde vir 'n *Diaphorina*-monster, versamel in Colchester, Oos-Kaap, en morfologies soortgelyk aan *D. citri*, het getoon dat die monster nie *D. citri* was nie, maar 'n inheemse spesie wat soortgelyk lyk en wat op die inheemse boom *Harpephyllum caffrum*, algemeen bekend as Afrika Pruimboom, voorkom.

Sestien triosied-monsters, meestal vanaf kleeflokvalle versamel, is morfologies as óf *Trioza erytrae* óf *Trioza litseae* óf *Trioza* sp. (*litseae*-groep) geïdentifiseer, en is strepieskodes met behulp van Sanger-volgordebepaling gegee. Nukleotiedvolgorde diversiteit is tussen hierdie monsters waargeneem en volgordes is breedweg in 5 groepe gegroepeer. Tot dusver is '*Candidatus* Liberibacter africanus' (CLaf) in monsters in twee groepe opgespoor. 'n Unieke Liberibacter sp. is in 'n triosiedmonster vanaf 'n lokval in Knysna geïdentifiseer en verdere karakterisering en plantgasheer-identifikasie van hierdie Liberibacter word vereis. Die triosied strepieskode van hierdie monster het in 'n groep met 'n monster vanaf Oos-Londen gegroepeer.

### 4.5.8 PROGRESS REPORT: Development of novel monitoring and control tools for citrus psyllids Project 1315 (2021/22 – 2022/23) by C W Weldon, K Krüger (UP) and A Manrakhan (CRI)

## Summary

The South African citrus industry is currently faced with the potential introduction of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), and *Candidatus* Liberibacter asiaticus (Las) which it vectors. Las can also be vectored by the indigenous citrus psyllid pest, *Trioza erytrae* (Del Guercio) (Hemiptera: Triozidae). Techniques used for detection and surveillance of these pests are ineffective at low pest density,

require skilled labour and are time consuming, so better methods are needed. Another approach to protect South African citrus from Las is to investigate alternative approaches for suppression of the vectors. One approach for controlling citrus psyllids is to use a gene silencing method called RNA interference which has been developed in CRI project 1160. The aim of this project is to develop novel monitoring and control tools for citrus psyllids in South Africa. Our objectives are to (A) evaluate odour-based monitoring tools for psyllids, (B) develop and evaluate a vision-based system for identification of *T. erytrae* and *D. citri* on traps, and (C) test the efficacy of CTV RNAi constructs to suppress psyllids. Not much progress has been made regarding Objective A due to delays in delivery of the needed consumables and heavy rainfall when fieldwork was planned. In contrast, we have made considerable progress on Objective B. To date, 64 yellow sticky traps have been inspected for psyllids, photographed, and the images annotated and sent to our collaborators in Florida to develop and train artificial intelligence algorithms that identify psyllids. The system is producing promising results for *T. erytrae* identification but more sticky trap samples are being collected to improve accuracy and the ability to detect *Diaphorina citri* and *Diaphorina* species native to South Africa. Objective C has not yet started because we are negotiating access to facilities with biosecurity accreditation.

## Opsomming

Die Suid-Afrikaanse sitrusbedryf word tans gekonfronteer met die potensiële inkomsvan die Asiatiese sitrusbladvlooi, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), en *Candidatus Liberibacter asiaticus* (Las) waarvoor dit die vektor is. Las kan ook deur die inheemse sitrusbladvlooiplaag, *Trioza erytrae* (Del Guercio) (Hemiptera: Triozidae) oorgedra word. Tegnieke wat gebruik word vir die opspoor en waarneem van hierdie plaag, is ondoeltreffend teen lae plaagdigtheid, vereis geskoolde arbeid, en is tydrowend, dus word beter metodes benodig. Nog 'n benadering om Suid-Afrikaanse sitrus teen Las te beskerm, is om alternatiewe benaderings vir die onderdrukking van die vektore te ondersoek. Een benadering vir die beheer van sitrusbladvlooi, is om 'n geen "silencing" metode, genaamd RNS-inmenging te gebruik wat in CRI-projek 1160 ontwikkel is. Die doel van hierdie projek is om nuwe moniterings- en beheerhulpmiddele vir sitrusbladvlooi in Suid-Afrika te ontwikkel. Ons doelwitte is om (A) reuk-gebaseerde moniteringshulpmiddele vir bladvlooi te evalueer, (B) 'n visie-gebaseerde stelsel te ontwikkel en te evalueer vir identifikasie van *T. erytrae* en *D. citri* op lokvalle, en (C) die doeltreffendheid van CTV RNAi-modelle te toets om bladvlooi te onderdruk. Daar is nie veel vordering gemaak met betrekking tot Doelwit A nie, as gevolg van vertraging in die aflewering van die benodigde verbruiksgoedere, en swaar reënval toe veldwerk beplan is. Daarteenoor het ons aansienlike vordering gemaak met Doelwit B. Tot op hede is 64 geel kleeflokvalle vir bladvlooi geïnspekteer, gefotografeer en die beelde is geannoteer en aan ons medewerkers in Florida gestuur om kunsmatige intelligensie-algoritmes te ontwikkel en op te lei wat bladvlooi identifiseer. Die stelsel lewer belowende resultate vir *T. erytrae*-identifikasie, maar meer kleeflokvalmonsters word ingesamel om akkuraatheid en die vermoë om *Diaphorina citri* en *Diaphorina* spesies, inheems aan Suid-Afrika, op te spoor, te verbeter. Doelwit C het nog nie begin nie omdat ons toegang tot fasiliteite met biosekuriteit akkreditering onderhandel.

### 4.5.9 PROGRESS REPORT: Determination of genome diversity of citrus infecting '*Candidatus Liberibacter africanus*' species and subspecies

Project 1322 (2021/22 – 2022/23) by R Bester, G Cook (CRI), R Roberts (ARC), G Pietersen (Pathosolutions), CRI-Biosecurity team, P Fourie and HJ Maree (CRI)

## Summary

Detection and identification of *Liberibacter* species is critical to the HLB Action plan and requires continuous evaluation to ensure the accuracy of the detection assays. In recent years, several indigenous *Liberibacter* species and subspecies have been identified that complicates these assays and if not updated, can lead to false positive or negative reports.

This project aims to construct a *Liberibacter* genome reference database that would support the development of accurate detection and identification assays. To achieve this aim *Liberibacter* infected samples from different geographical regions were subjected to high-throughput sequencing. *Liberibacter* genomes will be assembled and current detection assays evaluated to ensure detection specificity. If needed current assays will be updated or new assays will be designed and evaluated for efficacy in detecting specific *Liberibacter* species.

To date samples from previous surveys conducted from 2016-2021 were screened with the existing detection assays to identify appropriate material for high-throughput sequencing (HTS). The DNA of 20 samples (4 CLaf Cls: 2 Uganda, 1 Kenya, 1 Knysna and 16 CLafs: 1 Uganda, 15 SA) were sent for HTS on the Ion Torrent platform. A CLaf genome was generated for each of the 20 samples using a reference read mapping strategy. Success of genome construction was dependent on *Liberibacter* concentration and a genome coverage range of 15%-99% was obtained. More data were generated for samples with low genome coverage. The constructed genomes are currently being evaluated for diversity with a specific focus on the regions in the genome used for the current detection assays. Other regions will be evaluated for the design/optimisation of a new validation assay.

## Opsomming

Opsporing en identifikasie van *Liberibacter* spesies is van kritieke belang vir die HLB-Aksieplan en vereis deurlopende evaluering om die akkuraatheid van die opsporingtoetse te verseker. In onlangse jare is verskeie inheemse *liberibacter* spesies en subspecies geïdentifiseer wat hierdie toetse bemoeilik en as dit nie opgedateer word nie, kan dit lei tot vals positiewe of negatiewe resultate. Hierdie projek het ten doel om 'n *Liberibacter*-genoomverwysingsdatabasis op te stel wat die ontwikkeling van akkurate opsporing en identifikasietoetse sal ondersteun.

Om hierdie doel te bereik, was *Liberibacter*-geïnfekteerde monsters van verskillende geografiese streke aan hoë-deurset-volgordebepaling onderwerp. *Liberibacter*-genome sal saamgestel word en huidige opsporingstoetse sal geëvalueer word om spesifisiteit van opsporing te verseker. Indien nodig sal huidige toetse opgedateer word of nuwe toetse sal ontwerp en geëvalueer word vir doeltreffendheid in die opsporing van spesifieke *liberibacter* spesies. Tot op datum is monsters van vorige opnames wat vanaf 2016-2021 gedoen is, met die bestaande opsporingstoetse gekeur om toepaslike materiaal vir hoë-deurset-volgordebepaling (HTS) te identifiseer. Die DNS van 20 monsters (4 CLaf Cl's: 2 Uganda, 1 Kenia, 1 Knysna en 16 CLafs: 1 Uganda, 15 SA) is gestuur vir HTS op die Ion Torrent-platform. 'n CLaf-genoom is vir elk van die 20 monsters gegenereer deur 'n verwysingslees-karteringstrategie te gebruik. Sukses van genoomkonstruksie was afhanklik van *liberibacter* konsentrasie en 'n genoom dekking van 15%-99% is verkry. Meer data is gegenereer vir die monsters met lae genoomdekking. Die gekonstrueerde genome word tans vir diversiteit geëvalueer met 'n spesifieke fokus op die streke in die genoom wat vir die huidige opsporingstoetse gebruik word. Ander streke sal geëvalueer word vir die ontwerp/optimisering van 'n nuwe valideringstoets.

### 4.5.10 PROGRESS REPORT: Evaluation of the influence of CTV infection on '*Candidatus Liberibacter africanus*' titre

Project 1346 (2021/22 – 2022/23) by R Bester, G Cook, JHJ Breytenbach and HJ Maree (CRI)

## Summary

Citrus greening or Huanglongbing (HLB), is a devastating disease of citrus that resulted in major economic losses in many countries. Three species of '*Candidatus Liberibacter*' are associated with citrus greening and include '*Candidatus Liberibacter asiaticus*' (CLAs), '*Candidatus Liberibacter americanus*' (CLam) and '*Candidatus Liberibacter africanus*' (CLaf). Citrus greening disease in South Africa is associated with CLaf and has a less devastating disease expression. Citrus tristeza virus (CTV), another pathogen of citrus has also caused major tree losses resulting in a severe decline in production. Some citrus-producing countries, including South Africa, have reduced the negative effects of CTV by applying a cross-protection management strategy. Plant material is inoculated with mild-strain sources of CTV to decrease the damaging effects of secondary CTV infections. As a result of the South African citrus improvement scheme, the majority of citrus plants in South Africa are infected with mild genotypes of CTV. Therefore, most CLaf-infected trees in South Africa are also infected with CTV. In 2019 it was reported that mild CTV genotypes can limit the multiplication and spread of CLAs. Co-infections of CTV and CLaf was also previously investigated and it was reported that specific CTV isolates reduced the incidence of CLaf symptomatic trees. The CTV protective effect against CLaf or CLAs can potentially vary depending on the strain of CLAs, CLaf and CTV as well as the variety of citrus. In this study, the aim is to investigate the effect of CTV infections on the concentration of a CLaf infection. Two CTV genotypes will be used to establish seedlings with CTV infections with different severity to measure the

effect on a secondary CLaf infection. As a measure to characterize the interaction between the citrus host and CLaf, seedlings will also be 1) co-inoculated with CTV and CLaf, 2) first inoculated with CLaf and then inoculated with CTV, 3) only infected with CTV and 4) only infected with CLaf. The concentration of both CLaf and CTV will be determined using qPCR assays. The study aims to identify either a neutral, synergistic or cross-protective effect between CLaf and specific CTV genotypes. Two hundred *C. sinensis* cv. 'Madam Vinous', sweet orange seedlings were established in the greenhouse. Sweet orange was selected due to the susceptibility to both CTV and CLaf, however the growth observed to date were very poor and plants could not be inoculated with CTV or CLaf. Plants will be inoculated as soon as sufficient growth occurred. In the meantime the different qPCR assays for pathogen quantitation are being optimised.

## Opsomming

Sitrusvergroening of huanglongbing (HLB), is 'n verwoestende siekte van sitrus wat groot ekonomiese verliese in baie lande tot gevolg gehad het. Drie spesies '*Candidatus Liberibacter*' word met sitrusvergroening geassosieer en sluit in '*Candidatus Liberibacter asiaticus*' (CLas), '*Candidatus Liberibacter americanus*' (CLam) en '*Candidatus Liberibacter africanus*' (CLaf). Sitrusvergroeningsiekte in Suid-Afrika word met CLaf geassosieer en het 'n minder skadelike siekte-uitdrukking. Sitrus tristeza virus (CTV), 'n ander patoogeen van sitrus, het ook groot boomverliese veroorsaak wat 'n ernstige afname in produksie tot gevolg gehad het. Sommige sitrusproduserende lande, insluitend Suid-Afrika, het die negatiewe uitwerking van CTV verminder deur 'n kruisbeskermingbestuurstrategie toe te pas. Plantmateriaal word met ligte simptome verhoorsaakende bronne van CTV geënt om die skadelike effekte van sekondêre CTV-infeksies te verminder. As gevolg van die Suid-Afrikaanse sitrusverbeteringskema is die meerderheid sitrusplante in Suid-Afrika met ligte simptome verhoorsaakende genotipes van CTV besmet. Daarom is die meeste CLaf-besmette bome in Suid-Afrika ook met CTV besmet. In 2019 is berig dat ligte CTV-genotipes die vermenigvuldiging en verspreiding van CLas kan beperk. Gelyktydige infeksies van CTV en CLaf is ook voorheen ondersoek en daar is gerapporteer dat spesifieke CTV-isolate die voorkoms van simptome verhoorsaakende CLaf bome verminder het. Die CTV-beskermerende effek teen CLaf of CLas kan moontlik wissel na gelang van die variant van CLas, CLaf en CTV sowel as die tipe sitrus. In hierdie studie is die doel om die effek van CTV-infeksies op die konsentrasie van 'n CLaf-infeksie te ondersoek. Twee CTV genotipes sal gebruik word om saailinge met CTV infeksies met verskillende erns te vestig om die effek op 'n sekondêre CLaf infeksie te meet. As 'n maatstaf om die interaksie tussen die sitrusgasheer en CLaf te karakteriseer, sal saailinge ook 1) gelyktydig met CTV en CLaf geïnfekteer word, 2) eers met CLaf geïnfekteer word en dan met CTV geïnfekteer word, 3) slegs met CTV geïnfekteer word en 4) slegs geïnfekteer word met CLaf. Die konsentrasie van beide CLaf en CTV sal met behulp van qPCR-toetse bepaal word. Die studie het ten doel om 'n neutrale, sinergistiese of kruisbeskermerende effek tussen CLaf en spesifieke CTV genotipes te identifiseer. Tweehonderd *C. sinensis* cv. 'Madam Vinous', lemoen saailinge is in die kweekhuis gevestig. Lemoen is geselekteer as gevolg van die vatbaarheid vir beide CTV en CLaf, maar die groei wat tot dusver waargeneem is was baie swak en plante kon nie met CTV of CLaf geënt word nie. Plante sal ingeënt word sodra voldoende groei plaasgevind het. Intussen word die verskillende qPCR-toetse vir patoogeen-kwantifisering geoptimeer.

### 4.6 CRI Diagnostic Centre (Elaine Basson, Charmaine Olivier, Aubrey Metane, Samuel Ndlovu, Nozipho Shabangu and Jan van Niekerk)

Analysis	Citrus nurseries	Commercial samples	Other crops	Research samples
<b>Nematode:Roots</b>	37	2047	57	1302
<b>Nematode:Soil</b>	31	779	194	1291
<b>Phytophthora</b>	7459 <sup>1</sup>	1662	673	326
<b>Water spore trap</b>	261	0	32	0
<b>Black spot identification (PCR)</b>	0	489	0	13
<b>Black spot benzimidazole resistance</b>	0	290	0	0
<b>Postharvest Sensitivity</b>	0	13	0	77
<b>Fruit &amp; Foliar identification</b>	0	72	16	62
<b>Soil dilution plating</b>	0	460	60	0

<b>Biosecurity: ACP traps</b>	0	0	0	4249
<b>GTD: Greening PCR</b>	0	0	0	386
<b>Entomology: Mealybug PCR</b>	0	220	0	5
<b>SUB-TOTAL</b>	<b>7788</b>	<b>6032</b>	<b>1032</b>	<b>7711</b>

<sup>1</sup> Total samples received for citrus nurseries – includes quarterly samples, re-tests and non-certified nurseries

### Citrus Certified Nurseries

It is compulsory for all citrus nurseries participating in the Citrus Improvement Scheme to send samples for *Phytophthora* analysis on a quarterly basis. The irrigation water must also be tested for *Phytophthora* by making use of the spore trap method. In total, 6223<sup>2</sup> nursery samples were received by the diagnostic centre for *Phytophthora* analyses. Of these samples, 5.13% tested positive. In addition to soil and water samples, nurseries are required to send root samples once a year to test for the presence of *Tylenchulus semipenetrans*. For the nematode root samples, 0% tested positive and for the nematode soil samples 0% tested positive.

Footnote:

<sup>2</sup> Sample number and the percentage positive are only for certified nurseries and only for the quarterly samples received.

### Commercial samples

Samples were received from the following citrus growing areas: Botswana, Eastern Cape, Kwazulu-Natal, Limpopo, Mpumalanga, Northern Cape, North West, Swaziland, Western Cape and Zimbabwe. Most of the samples received from citrus growers were analysed for *Phytophthora* species including *P. nicotianae* and *P. citrophthora*, and the citrus nematode, *T. semipenetrans*. Forty five percent of the 2047 samples analysed for citrus nematode females had counts above the threshold value of 1000 females per 10g of roots, and nematicide treatments were recommended. Sixty four percent of the 1662 samples analysed for *Phytophthora* tested positive.

### Other crops

Nematode counts were done on soil or root samples of Avocado, Bean, Litchi, Macadamia, Pecan, Potting Mix, Sugarcane and Turfgrass. Nematodes found present on these crops included: *Helicotylenchus*, *Hemicycliophora*, *Hoplolaimus*, *Meloidogyne*, *Mesocriconema*, *Paratrichodorus*, *Paratylenchus*, *Pratylenchus*, *Rotylenchus*, *Rotylenchulus*, *Scutellonema* and *Xiphinema*. *Phytophthora* and *Pythium* analyses were done on Avocado, Bean, Blueberries, Cucumber, Ginger, Kiwi, Macadamia, Pecan, Potting Mix, Sugarcane, and Vegetables. The diagnostic centre analysed 82 soil samples from avocado nurseries for the presence of *Phytophthora cinnamomi*.

### Research samples

Nematode and *Phytophthora* analysis were done on 2919 samples from experimental trials and extension samples. The Diagnostic Centre assisted in trials to identify possible citrus black spot lesions using PCR protocols on 13 fruit samples. As part of Biosecurity 4249 traps were read for the detection of Asian Citrus Psyllid, with 386 samples tested for the detection of African Greening and Huanglongbing.

### CRI Diagnostiese Sentrum

<b>Ontleding</b>	<b>Sitrus kwekerye</b>	<b>Kommersiële monsters</b>	<b>Ander gewasse</b>	<b>Navorsings-monsters</b>
Aalwurms: Wortels	37	2047	57	1302
Aalwurms: Grond	31	779	194	1291
<i>Phytophthora</i>	7459 <sup>1</sup>	1662	673	326
Water spoorlokval	261	0	32	0
Swartvlek (PKR)	0	489	0	13
Swartvlek benzimidazole bestandheid	0	290	0	0

Na-oes sensitiviteitstoetse	0	13	0	77
Vrug-en blaar identifikasie	0	72	16	62
Grondverduunning	0	460	60	0
Biosekuriteit: ACP lokvalle	0	0	0	4249
Entoordraagbare siektes: Vergroening PKR	0	0	0	386
Entomologie: Witluis PKR	0	220	0	5
<b>TOTAAL</b>	<b>7788</b>	<b>6032</b>	<b>1032</b>	<b>7711</b>

<sup>1</sup> Totale hoeveelheid monsters ontvang van gesertifiseerde kwekerye – sluit in kwartaal monsters, hertoets monsters en nie-gesertifiseerde kwekerye

## Sitrus Gesertifiseerde Kwekerye

Dit is verpligtend vir al die sitruskwekerye wat aan die Sitrus Verbeteringskema deelneem om kwartaalmonsters vir *Phytophthora* te laat ontleed. Die besproeiingswater moet ook deur middel van die spoorlokval metode vir *Phytophthora* getoets word. In totaal 6223<sup>2</sup> monsters is deur die diagnostiese sentrum vir *Phytophthora* ontleding ontvang, waarvan 5.13% positief getoets het. Benewens die water en grondmonsters, moet kwekerye een keer per jaar 'n wortelmonster instuur om vir die teenwoordigheid van *Tylenchulus semipenetrans* te toets. Van die wortel- en grondmonsters wat ontvang is, het 0.0% positief getoets vir die teenwoordigheid van *T. semipenetrans*.

Voetnota:

<sup>2</sup> Monster hoeveelheid en die persentasie positief is net vir gesertifiseerde kwekerye en slegs vir die kwartaal monsters ontvang.

## Kommersiële monsters

Monsters is uit die volgende sitrusverbouingsareas ontvang: Botswana, Oos-Kaap, KwaZulu-Natal, Limpopo, Mpumalanga, Noord-Kaap, Noord-Wes, Swaziland, Wes-Kaap en Zimbabwe. Die meeste van die monsters wat van sitrusprodusente ontvang is, is vir *Phytophthora nicotianae* en die sitrusaalwurm, *Tylenchulus semipenetrans*, ontleed. Vyf-en-veertig persent van die 2047 aalwurmmonsters wat ontleed is, het tellings hoër as die drempelwaarde van 1000 wyfies per 10g wortels gehad. Aalwurmdoderbehandelings is in daardie gevalle aanbeveel. Vier-en-sestig persent van die 1662 monsters wat vir *Phytophthora* ontleed is het positief getoets.

## Ander Gewasse

Aalwurmtellings is op grond- of wortelmonsters van Avokado, Boontjies, Litchi, Makadamia, Pekan, Potmingsel, Suikerriet en Turfgras. Aalwurms teenwoordig gevind op hierdie gewasse sluit in: *Helicotylenchus*, *Hemicyclophora*, *Hoplolaimus*, *Meloidogyne*, *Mesocriconema*, *Paratrichodorus*, *Paratylenchus*, *Pratylenchus*, *Rotylenchus*, *Rotylenchulus*, *Scutellonema* en *Xiphinema*. Avokado, Boontjies, Bloubessies, Komkommer, Gemmier, Kiwi, Makadamia, Pekan, Potmingsel, Suikerriet, en Groente monsters is vir *Phytophthora* en *Pythium* ontleed. Die diagnostiese sentrum het 82 monsters vanaf avokado kwekerye ontvang om vir *Phytophthora cinnamomi* te ontleed.

## Navorsingsmonsters

Aalwurm en *Phytophthora* ontledings is op 2919 monsters afkomstig uit navorsingsprojekte gedoen. Die Diagnostiese Sentrum het ook hulp verleen aan navorsingsprojekte in die identifikasie van moontlike sitrus swartvlek letsels deur middel van PKR op 13 vrug monsters. As deel van Biosekuriteit, is 4249 lokvalle gelees vir die monitering van "Asian Citrus Psyllid", met 386 monsters molekulêr getoets vir die bespeuring van Afrika vergroening en Huanglongbing.

## 5 PORTFOLIO: CITRICULTURE

### 5.1 PORTFOLIO SUMMARY

By Paul Cronje (Portfolio Manager: Citriculture, CRI)

The projects in the Citriculture portfolio are identified and planned to address specific producers' needs which impact bearing an adequate volume of quality fruit for the export market. This includes projects on irrigation, nutrition, crop manipulation, rind condition, cold chain, cultivar evaluation and rootstock development, in all of which important progress has been made. In the completed adaptive nutrition project, the use of foliar analysis to make in-season adjustments to N, P and K fertilisation programmes has limited to no value. This is due to a lack of short-term changes observed in leaf nutrient concentration in response to under- and over-fertilisation and increments thereof. Furthermore, it was shown that when leaf analysis results are interpreted, a level just above the minimum norm should be regarded as optimal, i.e., not the middle to high range between the minimum and maximum norms. Results from the cold chain research projects have been employed to improve ventilation in the A15C carton and containers across all packaging configurations enabling more homogenous temperature profiles during export. Cultivar and rootstock evaluation and research have identified the increased risk of oleocellosis under shade netting and more so for trees grafted on Rough Lemon. In addition, a renewed focus on rootstock evaluation has been implemented and a semi-commercial planting of new rootstocks is being established to illustrate the potential of alternative rootstock cultivars. Evaluation of all citrus types in all the production areas also yields actionable information for producers to plan for future orchard replacements. Projects on rind disorders such as chilling injury and oleocellosis, which lead to a marked reduction in export quality, have already resulted in improved recommended practices on the farm and packhouse level. The portfolio has expanded the scope and depth of research projects to increase the ability to supply timely recommendations to producers, packhouses and exporters.

### PORTEFEULJE OPSOMMING

Die projekte in die *Citriculture* portefeulje word geïdentifiseer en beplan om produsente se spesifieke behoeftes aan te spreek wat 'n impak op die dra van 'n voldoende volume kwaliteit vrugte vir die uitvoermark het. Dit sluit in projekte oor besproeiing, voeding, gewasmanipulasie, skiltoestand, koue-ketting, kultivar-evaluering en onderstam-ontwikkeling, wat almal belangrike vordering gemaak het. In die voltooië aanpasbare voedingsprojek, is getoon dat die gebruik van blaar-ontledings om in-seisoen aanpassings aan N-, P- en K-bemestingsprogramme te maak, beperkte tot geen waarde het nie. Dit is as gevolg van 'n gebrek aan korttermyn veranderinge wat in blaarvoedingstofkonsentrasie waargeneem is, in reaksie op beide onder- en oorbemesting, sowel as inkremente daarvan. Verder is aangetoon dat wanneer blaar-ontledingsresultate geïnterpreteer word, 'n vlak net bokant die minimum norm as optimaal beskou moet word, dit wil sê, nie die middel tot hoë reeks tussen die minimum en maksimum norme nie. Resultate van die koue-ketting navorsingsprojekte is aangewend om ventilasie in die A15C-karton en houers oor alle verpakkingskonfigurasies te verbeter, wat meer homogene temperatuurprofiële tydens uitvoer moontlik maak. Kultivar- en onderstam-evaluering en -navorsing het die verhoogde risiko van oleosellose onder skadunet geïdentifiseer, en meer nog vir bome wat op *Rough Lemon* geokuleer is. Boonop is 'n hernude fokus op onderstam-evaluering geplaas namate semi-kommersiële aanplanting van nuwe onderstamme gevestig word om die potensiaal van alternatiewe onderstamkultivars te illustreer. Evaluering van alle sitrustipes in al die produksiegebiede lewer ook bruikbare inligting vir produsente om te beplan vir toekomstige boordvervangings. Projekte op skil-afwykings soos koue-skade en oleosellose, wat tot 'n merkbare afname in uitvoer kwaliteit lei, het reeds tot verbeterde aanbevole praktyke op plaas- en pakhuisvlak gelei. Die portefeulje het die omvang en diepte van navorsingsprojekte uitgebrei om die vermoë te verbeter om tydige en relevante aanbevelings aan produsente, pakhuisse en uitvoerders te verskaf.

### 5.2 PROGRAMME: RIND CONDITION AND COLD CHAIN

Programme coordinator: Paul Cronje (CRI)

#### 5.2.1 Programme summary

The rind condition and cold chain program aim to directly address relevant factors negating fruit rind quality leading to physiological disorders in the orchards to the cold chain. The rind condition and cold chain research program has been expanded over the two seasons to increase the capacity and scope of projects focusing on the cold chain. This is important to develop technology and practices to ensure uniform application of the required temperatures during export. In six projects, aspects ranging from the inland supply chain to pallets and carton design have been implemented and collected. This information has already fed into optimising the container cooling processes via carton ventilation improvements and container loading practices. Incidence of chilling injury will be the major rind disorder affecting the SA citrus industry due to the 2°C and below shipping temperature in nearly 60% of our markets. Ongoing results have highlighted the inherent susceptibility due to cultivar and production region. Currently, there are no reliable alternatives to thiabendazole and wax application in the packhouse to reduce chilling injury symptoms. Oleocellosis is a well-known rind disorder but accounts for an alarmingly high percentage of packhouse rejections. In this new project, the potential negative effect of shade netting and rootstock was also quantified. The results will be expanded on in the current season to develop guidelines to reduce the incidence of these disorders.

### **Program-opsomming**

Die skiltoestand- en koue-kettingprogram het ten doel om relevante faktore, wat vrugskilkwiteit negatief beïnvloed, en tot fisiologiese afwykings in die boorde na die koue-ketting lei, direk aan te spreek. Die skiltoestand- en koue-kettingnavorsingsprogram is oor die twee seisoene uitgebrei om die kapasiteit en omvang van veral projekte wat op die koue-ketting fokus, te vergroot. Dit is belangrik om tegnologie en praktyke te ontwikkel om eenvormige toepassing van die vereiste temperature tydens uitvoer te verseker. In ses projekte is aspekte, wat wissel van die binnelandse voorsieningsketting tot palette en karton-ontwerp, geïmplementeer en ingesamel. Hierdie inligting is reeds bygewerk om die houerverkoelingsprosesse te optimaliseer deur kartonventilasie verbeterings en houerlaaipraktyke. Die voorkoms van koue-skade sal die grootste skil-afwyking wees wat die SA sitrusbedryf raak as gevolg van die 2°C en laer verskepingstemperatuur in byna 60% van ons markte. Deurlopende resultate het die inherente vatbaarheid as gevolg van kultivar en produksiestreek beklemtoon. Tans is daar geen betroubare alternatiewe vir thiabendazole en wakstoediening in die pakhuis om koue-skadesimptome te verminder nie. Oleosellose is 'n bekende skil-afwyking, maar is verantwoordelik vir 'n kommerwekkende hoë persentasie pakhuis-afkeurings. In hierdie nuwe projek word die potensiële negatiewe effek van skadunet en onderstam ook gekwantifiseer op die voorkoms van oleocellosis. Die resultate sal in die huidige seisoen uitgebrei word om riglyne te ontwikkel om die voorkoms van hierdie afwykings te verminder.

#### **5.2.2 PROGRESS REPORT: Investigation of factors contributing toward the non-conformance of in-transit citrus container shipments to cold protocol markets**

Project 1240 (PHI 4-11) (2019/2 – 2021/22) by P Cronje, T Berry (CRI) and L Gebers-Goedhals and G Khumalo (SU-Logistics)

### **Summary**

This study investigates factors that may contribute to the non-conformance of in-transit cold sterilisation protocols of citrus shipments due to deviations occurring before the sea leg commences (landside failure to initiate the protocol) or during the voyage (water failure and/or quality failure). The citrus industry is concerned that there is a high risk for the commercial failure of shipments resulting from inadequate temperature management, causing chilling and freeze injury. Two main avenues of investigation were followed during the project: firstly, a detailed literature review of the project problems statements and objectives; secondly, in situ accessing the Durban port's logistical situation and directly engaging with exporters and freight forward personnel. Preparation of consignments (pallets) and monitoring during the precooling and loading are critical to reducing failures of initiating the process or a failure in terms of fruit quality loss. Using independent temperature data loggers is essential to monitoring the process. A cellular data logger gives an added benefit to gaining information on an hourly basis of the cold chain while the consignment is still within cellular reception. This new technology has been well received in the citrus industry. Exporters use it to monitor and reduce temperature independently from the officially shipping line data probes to identify inefficiencies and problem

areas in their cold chain. The recent growth of export volumes to the Far Eastern markets and the increase in the incidence of cold sterilisation failures have highlighted the urgent importance of improving current handling protocols. "Failure" of a cold sterilisation protocol is a broad term which does not indicate cold treatment failure, but incidences of these failures must be reduced. This project identified options to include in best practise by the citrus industry to reduce problematic situations arising in the critical new markets.

## Opsomming

Hierdie studie ondersoek faktore wat kan bydra tot die nie-nakoming van *in-transito* koue-sterilisasiëprotokolle van sitrusverskepinge as gevolg van afwykings wat plaasvind voordat die see-been begin (mislukking aan land om die protokol te inisieer) of tydens die vaart (water mislukking en/of kwaliteit mislukking). Die sitrusbedryf is bekommerd dat daar 'n hoë risiko is vir die kommersiële mislukking van verskepinge as gevolg van onvoldoende temperatuurbestuur, wat koue- en vriesskade veroorsaak. Twee hoof bane van ondersoek is tydens die projek gevolg: eerstens, 'n gedetailleerde literatuuroorsig van die projekprobleemstellings en -doelwitte; tweedens, *in situ* toegang tot die Durban-hawe se logistieke situasie en regstreekse skakeling met uitvoerders en vragpersoneel. Voorbereiding van besendings (palette) en monitering tydens die voorverkoeling en laai is van kritieke belang om mislukkinge om die proses te inisieer, of mislukking in terme van die verlies aan vrugkwaliteit, te verminder. Die gebruik van onafhanklike temperatuur dataversamelaars (*loggers*) is noodsaaklik om die proses te monitor. 'n Sellulêre dataversamelaar bied 'n bykomende voordeel om inligting op 'n uurlikse basis van die koue-ketting in te vorder, terwyl die besending nog binne sellulêre ontvangs is. Hierdie nuwe tegnologie is goed ontvang in die sitrusbedryf. Uitvoerders gebruik dit om temperatuur onafhanklik van die amptelike skeepsredery-datasensors te monitor en te verlaag, om ondoeltreffendhede en probleem-areas in hul koue-ketting te identifiseer. Die onlangse groei in uitvoervolumes na die Verre Oosterse markte en die toename in die voorkoms van koue-sterilisasië mislukkinge, het die dringende belang om die huidige hanteringsprotokolle te verbeter, beklemtoon. "Mislukking" van 'n koue-sterilisasiëprotokol is 'n breë term wat nie koue-behandeling mislukking aandui nie, maar die voorkoms van hierdie mislukkinge moet verminder word. Hierdie projek het opsies geïdentifiseer om in beste praktyk deur die sitrusbedryf ingesluit te word om problematiese situasies wat in die kritieke nuwe markte ontstaan, te verminder.

### 5.2.3 PROGRESS REPORT: An investigation into aspects affecting chilling injury of citrus

Project 1247 (2019/20-2021/22) by P Cronje and J North (CRI)

## Summary

Citrus fruit are exported to countries demanding a cold disinfestation protocol. This project aimed to identify and quantify factors contributing to the resulting chilling injury (CI) and develop postharvest options to reduce the incidence. In various experiments stretching over two seasons and encompassing all citrus production areas of SA, the impact of cultivar, maturity, rootstock, and shade netting were evaluated for impact on CI. In all instances, fruit were stored at  $-0.6^{\circ}\text{C}$  for 32 days to simulate the shipments. Results indicate that the production areas do contribute to susceptibility, indicating that cultivars from certain regions need additional focus on postharvest packhouse treatments and an optimal cold chain to reduce the CI. No consistent pattern could be found in fruit maturity, e.g., immature and overmature influence the CI. Rootstocks play an undocumented but significant role in determining fruit susceptible to CI. These novel results will need to focus on a new research project. However, shade netting did not negatively impact the CI of the cultivars evaluated, indicating that this is a viable technology to increase the export quality of citrus. The postharvest wax application remains the primary management tool in the postharvest environment to reduce CI, and the effective application should be the focus of all packhouse managers. Without adequate wax application CI could not be addressed in the commercial export market. CI will be a commercial reality for the SA citrus industry in the future. By assessing the impact of various factors in the project, a significant contribution to reducing CI incidence was made. This information is already incorporated by producers, exporters and packhouses in their recommendations and practices and has successfully continued to reduce CI.

## Opsomming

'n Groot gedeelte sitrusvrugte word uitgevoer na lande wat 'n koue-sterilisasiesprotokol vereis. Hierdie projek het ten doel gehad om faktore te identifiseer en te kwantifiseer wat bydra tot die gevolglike koue-skade (CI), en na-oes opsies te ontwikkel om die voorkoms te verminder. In verskeie eksperimente wat oor twee seisoene gestrek het en alle sitrusproduksiegebiede van SA ingesluit het, is die impak van kultivar, rypheid, onderstam en skadunet geëvalueer vir impak op CI. In alle gevalle is vrugte vir 32 dae by  $-0.6^{\circ}\text{C}$  gestoor om die verskepings te simuleer. Resultate dui daarop dat die produksiegebiede wel tot vatbaarheid bydra, wat daarop dui dat kultivars uit sekere streke addisionele fokus op na-oes pakhuisbehandelings benodig, asook 'n optimale koue-ketting ten einde die CI te verminder. Geen konsekwente patroon kon in vrugrypheid gevind word nie, bv. groen én oorryp beïnvloed die CI. Onderstamme speel 'n nog ongedokumenteerde maar beduidende rol in die bepaling van vrugvatbaarheid vir CI. Hierdie nuwe resultate sal die fokus van 'n nuwe navorsingsprojek moet wees. Skadunet het egter nie 'n negatiewe impak op CI van die kultivars wat geëvalueer is, gehad nie, wat daarop dui dat dit 'n lewensvatbare tegnologie is om die uitvoerkwaliteit van sitrus te verhoog. Die na-oes wastoediening bly die primêre bestuurshulpmiddel in die na-oes omgewing om CI te verminder, en die doeltreffende toediening behoort die fokus van alle pakhuisbestuurders te wees. Sonder voldoende wastoediening kon CI nie in die kommersiële uitvoermark aangespreek word nie. CI sal vorentoe 'n kommersiële werklikheid vir die SA sitrusbedryf wees. Deur die impak van verskeie faktore in hierdie projek te evalueer, is 'n beduidende bydra tot die vermindering in die voorkoms van CI gemaak. Hierdie inligting word reeds deur produsente, uitvoerders en pakhuse in hul aanbevelings en praktyke geïnkorporeer, en verminder CI nog steeds suksesvol.

#### 5.2.4 **PROGRESS REPORT: Optimise 2,4-D applications and investigate alternatives for calyx retention**

Project 1300 (2019/21 – 2023/4) by P Cronje, W du Plooy (CRI) S Mostert (US) and F Alferez (UF)

##### **Summary**

The synthetic auxin 2,4-D (2,4-dichlorophenoxy acetic acid) is a plant growth regulator that has been widely used in citriculture to retard calyx abscission. Abscission is an active developmental process in a specific zone, the abscission zone. In general, it is accepted that the increase in ethylene levels during abscission increases the cells' sensitivity to ethylene and induces the synthesis of cell wall hydrolytic enzymes. Therefore, retention of the calyx enhances the fruit's market value and is thought to reduce fungal decay. The 2,4-D sodium salt is applied to the fruit in a dip treatment of 500 ppm. Even though no alternative synthetic auxin worked, more recently developed compounds need to be investigated as possible alternatives due to the restrictions on 2,4-D usage in certain export markets. Furthermore, the re-evaluation of the role of 2,4-D in the modern packline and decay control strategies needs to be clarified. The project's objective was to compile complete literature on the topic, screen products for efficacy and determine the impact of 2,4-D on decay incidence in current best packline practices. During the first season, it became clear that there is more to this aspect than only manipulating the ethylene production to reduce the abscission process. It is evident that abscission is not the only process involved in calyx retention and it should rather be described as "calyx condition" consisting of two processes that influence this condition, viz. abscission and dehydration. In some instances, green buttons abscise; in contrast, completely dehydrated calyx do not abscise. A primary observation is that dehydration is the major problem in calyx conditions rather than abscission. This hypothesis will be further evaluated. Moreover, a lack of decay associated with abscission was noticeable and will be further evaluated.

##### **Opsomming**

Die sintetiese oksien 2,4-D (2,4-dichloorfenoksie asynsuur) is 'n plantgroeireguleerder wat algemeen tot sitrus toegedien word om kelk-ent absissie te vertraag. Absissie is 'n energie-verbruikende proses wat in 'n spesifieke area, genaamd die absissiesone, plaasvind. Dit word oor die algemeen aanvaar dat 'n toename in etileenvlakke tydens absissie die selle se sensitiwiteit daarvoor verhoog, asook die produksie van hidrolitiese ensieme vermeerder. Kelk-ent retensie verhoog die vrug se markwaarde en vermoedelik verminder die kans vir swaminfeksie. Die 2,4-D natriumsout word na-oes as 'n waterbadbehandeling van 500 dpm toegedien. Al het geen ander sintetiese oksien dieselfde effektiwiteit as 2,4-D gehad nie, moet onlangs ontwikkelde produkte ondersoek word as moontlike alternatiewe, aangaande die streng beperkings rondom 2,4-D in

menigte uitvoermarkte. Verder moet die rol van 2,4-D in die moderne paklyn herevalueer word, sowel as die rol in bederfbeheerstrategieë uitgeklaar word. Die doel van hierdie projek was om 'n volledige literatuuroorsig saam te stel rakende die onderwerp, evaluering van produkte vir effektiwiteit, en bepaling van 2,4-D se invloed op na-oes bederf gevalle. Tydens die eerste seisoen het dit waarneembaar geraak dat daar meer aan die absissieproses is as slegs etileen manipulasie. Dit is duidelik dat absissie nie die enigste proses is wat kelk-ent retensie beïnvloed nie. Dit moet eerder beskou word as “kelk-ent kondisie” wat deur twee prosesse bepaal word, naamlik absissie en dehidrasie. In sommige gevalle sal groen kelk-ente uitval terwyl gedehidreerde kelk-ente nie afsnoer nie. 'n Voorlopige waarneming dui daarop dat dehidrasie 'n groter probleem in kelk-ent kondisies kan speel as net absissie. Hierdie hipotese sal verder ondersoek word. 'n Gebrek aan bederf assosiasie met absissie is ook opgemerk en sal verder evalueer word.

#### 5.2.5 **PROGRESS REPORT: Modelling citrus inland supply chains for improved handling practices** Project 1309 (Apr 2021 - Apr 2024) by T Berry (CRI) and S Bulterman (SU, MEng)

##### **Summary**

The South African citrus logistical supply chain is extraordinarily complex, with production areas throughout the country, diverse transit corridors and unique infrastructure at each port. The Conditions and durations within the inland supply chain significantly impact both fruit quality and the fruit's susceptibility to quality-related disorders during shipping. However, the supply chain has, to date, not been characterised in detail and the industry has little knowledge of what the actual impact of the supply chain is. The aim of this project was thus to create a comprehensive mapping of the South African citrus supply chain and the associated timelines. At the same time, we explore the underlying infrastructure and associated capacity that facilitates the flow of fruit. The first year of this project has primarily been dedicated to collecting data from multiple sources and then merging this information into a single master database. An important finding of this study is that our industry's data is fragmented (across multiple isolated systems), and access is becoming increasingly and often unnecessarily restricted. Insights into this issue will be included in the final report. Other data collected included temperature and humidity exposure data for fruit under various transit corridors. The data was processed using several data science techniques (modelling and cleaning methods), describing all citrus pallet movements between production areas, cold stores, and shipping destinations. Currently, the database is being analysed to evaluate industry-wide capacity limitations (e.g. cold stores and ports) and efficiency losses, as well as to estimate fruit quality loss with respect to dwell periods. This information provides a robust understanding of the supply chain's current conditions and delivers information to equip the industry to deal with challenges in the coming years.

##### **Opsomming**

Die Suid-Afrikaanse sitrus logistieke voorsieningsketting is buitengewoon kompleks, met produksiegebiede regdeur die land, diverse *transito*-korridors en unieke infrastruktuur by elke hawe. Die toestande en tydsverloop binne die binnelandse voorsieningsketting het 'n beduidende impak op beide vrugkwaliteit en vrugvatbaarheid vir kwaliteit-verwante afwykings tydens verskeping. Die voorsieningsketting is egter tot op hede nie in detail gekarakteriseer nie, en die bedryf het min kennis van wat die werklike impak van die voorsieningsketting is. Die doel van hierdie projek was dus om 'n omvattende kartering van die Suid-Afrikaanse sitrusvoorsieningsketting en die gepaardgaande tydlyne te skep. Terselfdertyd ondersoek ons die onderliggende infrastruktuur en gepaardgaande kapasiteit wat die vloei van vrugte vergemaklik. Die eerste jaar van hierdie projek is hoofsaaklik gewy aan die insameling van data uit verskeie bronne, en dan die samevoeging van hierdie inligting in 'n enkele hoofdatabasis. 'n Belangrike bevinding van hierdie studie is dat ons bedryf se data gefragmenteer is (oor veelvuldige geïsoleerde stelsels), en toegang word toenemend, en dikwels onnodig, beperk. Insigte oor hierdie kwessie sal in die finale verslag ingesluit word. Ander data wat ingesamel is, sluit temperatuur- en humiditeitsblootstellingdata vir vrugte onder verskeie *transito*-korridors in. Die data is verwerk met behulp van verskeie datawetenskaptegnieke (modellering en skoonmaakmetodes), wat gelei het tot 'n beskrywing van alle sitruspalletbewegings tussen produksiegebiede, koelkamers en verskepingsbestemmings. Tans word die databasis ontleed om bedryfswye kapasiteitsbeperkings (bv. koelkamers en hawens) en doeltreffendheidsverliese te evalueer, asook om kwaliteitsverliese met betrekking

tot verblyfperiodes te skat. Hierdie inligting verskaf 'n robuuste begrip van die voorsieningsketting se huidige toestande en verskaf inligting om die bedryf toe te rus om uitdagings in die komende jare te hanteer.

#### 5.2.6 **PROGRESS REPORT: Investigating the relationship between creep testing of corrugated cartons and current industry test methods**

Project 1310 (Apr 2021 - Apr 2023) by J Jones (WITS), T Berry (CRI) and R Stambuli (WITS, MSc)

##### **Summary**

Traditionally, the South African packaging industry has predicted the required carton strength for industry use based on the load that bottom cartons must support. This value is then significantly adjusted by applying safety factors, which are believed to account for various application unknowns. The corrugated board box compression strength must then meet this adjusted value and is determined by performing box compression tests (BCT). These application unknowns can range from varying temperature and humidity conditions during transportation, corrugated carton manufacturing, damage, rough handling, impacts, as well as incorrect palletising. The aim of this project is to determine whether the creep behaviour of a corrugated carton can be related to the tests currently used by the South African corrugated board industry to characterise and predict the behaviour of a carton over its lifetime, i.e. from packing till arrival at destination. This project forms part of the push to improve the design methodology of corrugated cartons for export applications and thus reduce the safety factors associated with the cartons and subsequently reduce costs. The first phase of this project has been targeted at developing a mechanical creep tester. The tester needed to meet several criteria, which include (i) low cost, to increase its accessibility to the industry; (ii) safety, to accommodate the large forces being applied to the cartons and (iii) versatility, to ensure the tester can function at any conditions or locations (e.g. testing inside cold rooms). The design went through several iterations and was then rigorously evaluated using tools such as finite element analysis. Currently, five creep testers are now in the final stages of manufacturing, and the project will then immediately move into the test and evaluation phase.

##### **Opsomming**

Die Suid-Afrikaanse verpakkingsbedryf het tradisioneel die vereiste kartonsterkte vir gebruik in die bedryf, voorspel op grond van die vraag wat onderste kartonne moet dra. Hierdie waarde word dan aansienlik aangepas deur veiligheidsfaktore toe te pas, wat vermoedelik vir verskeie onbekende toepassings verantwoordelik is. Die riefelkartonboks druksterkte moet dan aan hierdie aangepaste waarde voldoen en word bepaal deur boksdruktoetse (BCT) uit te voer. Hierdie toepassing onbekendes kan wissel van wisselende temperatuur- en humiditeittoestande tydens vervoer; kartonvervaardiging, skade, rowwe hantering, impakte, asook verkeerde palletisering. Die doel van hierdie projek is om te bepaal of die verwringing van 'n karton in verband gebring kan word met die toetse wat tans deur die Suid-Afrikaanse kartonbedryf gebruik word om die gedrag van 'n karton oor sy leeftyd, d.w.s. vanaf verpakking tot aankoms by bestemming, te karakteriseer en te voorspel. Hierdie projek vorm deel van die dryfveer om die ontwerpmetodologie van geriffelde kartonne vir uitvoertoepassings te verbeter, en sodoende die veiligheidsfaktore wat met die kartonne geassosieer word te verminder, en gevolglik koste te verminder. Die eerste fase van hierdie projek is gemik op die ontwikkeling van 'n meganiese kruiptoetsers. Die toetsers moes aan verskeie kriteria voldoen, insluitend (i) lae koste, om sy toeganklikheid tot die bedryf te verhoog; (ii) veiligheid, om die groot kragte wat op die kartonne toegepas word te akkommodeer en (iii) veelsydigheid, om te verseker dat die toetsers by enige toestande of plekke kan funksioneer (bv. toetse binne koelkamers). Die ontwerp is herhalend getoets en is toe streng met behulp van instrumente soos presisie element-analise geëvalueer. Tans is vyf kruiptoetsers nou in die finale stadia van vervaardiging, en die projek sal dan dadelik in die toets- en evalueringsfase inbeweeg.

#### 5.2.7 **PROGRESS REPORT: Designing integrated packaging systems for enhanced cold treatments**

Project 1311 (Apr 2021 - Apr 2024) by T Berry, P Cronje (CRI) and Sung-Hee Chung (SU, MSc)

##### **Summary**

The South African citrus industry is reliant on in-transit cold temperature treatments during shipping. Refrigerated freight containers (RFC) are one of the main methods used to export fresh produce and thus represent a critical cold chain unit-operation. However, cooling performance during refrigerated container shipping is dependent on the: (i) container cooling capacity, (ii) the geometrical layout of the packaged fruit (e.g. loading pattern and presence of void plugs) and (iii) the porosity of the packaged pallets, which is the most controllable factor in the process. There is currently a significant knowledge gap regarding the optimal porosity of cartons and how to quantify packaging porosity. The project thus aims to determine optimal pressure-loss-coefficient (PLC) benchmarks for citrus packaging, develop an airflow resistance circuitry model to estimate the PLC across packaging systems, and develop CRI baseline ventilated carton design recommendations for use by the citrus industry. To date, the project has developed a circuitry model, which provided important insights into how to characterise PLC within a pallet and is being used to explore desirable ventilation designs in cartons. The study further evaluated the various packaging systems (cartons and pallet bases) with respect to their PLC. The respective findings were then applied to the development of a new ventilation design (S2) for the A15C carton, which increases pallet porosity by five times. Commercial container cooling trials further showed that the new A15C-S2 significantly reduced the incidence of hot spots during shipping, allowing for warmer set-points when shipping fruit packed in this carton. The next phase of this project will explore the potential design of a high porosity Opentop carton that has an equivalent PLC to the new A15C-S2 carton.

## Opsomming

Die Suid-Afrikaanse sitrusbedryf is op *in transit* koue-temperatuurbehandelings tydens verskeping aangewese. Verkoelde vraghouders (RFC) is een van die vernaamste metodes wat gebruik word om vars produkte uit te voer, en verteenwoordig dus 'n kritieke koue-ketting eenheid-operasie. Verkoelingsprestasie tydens verkoelde houerverskeping is egter afhanklik van die: (i) houerverkoelingskapasiteit, (ii) die geometriese uitleg van die verpakte vrugte (bv. laaipatroon en teenwoordigheid van oop vloerareas) en (iii) die porositeit van die verpakte palette, wat die mees beheerbare faktor in die proses is. Daar is tans 'n beduidende gebrek aan kennis met betrekking tot die optimale porositeit van kartonne en hoe om verpakkingsporositeit te kwantifiseer. Die projek het dus ten doel om optimale druk-verlies-koëffisiënt (PLC) maatstawwe vir sitrusverpakking te bepaal, 'n lugvloeiweerstandstroombaanmodel te ontwikkel om die PLC oor verpakkingsstelsels te skat, en CRI-basisvlak geventileerde kartonontwerpaanbevelings vir gebruik deur die sitrusbedryf te ontwikkel. Tot op datum het die projek 'n stroombaanmodel ontwikkel wat belangrike insigte verskaf het oor hoe om PLC binne 'n palet te karakteriseer, en word gebruik om gewenste ventilasie-ontwerpe in kartonne te ondersoek. Die studie het die verskillende verpakkingsstelsels (kartonne en paletbassis) verder met betrekking tot hul PLC geëvalueer. Die onderskeie bevindings is toe op die ontwikkeling van 'n nuwe ventilasie-ontwerp (S2) vir die A15C-karton toegepas, wat die porositeit van die palet met vyf keer verhoog. Kommersiële houerverkoelingproewe het verder getoon dat die nuwe A15C-S2 die voorkoms van warmer areas tydens versending aansienlik verminder het, wat die gebruik van warmer stelpunte moontlik maak wanneer vrugte wat in hierdie karton verpak is, verskeep word. Die volgende fase van hierdie projek sal die potensiële ontwerp van 'n hoë porositeit *vertoon*-karton ondersoek, wat 'n PLC gelykstaande aan die nuwe A15C-S2-karton het.

### 5.2.8 PROGRESS REPORT: Optimisation of pallet base designs for the citrus industry

Project 1312 (Apr 2021 - Apr 2023) by B Wessels (SU), T Berry (CRI) and M Rust (SU, MSc)

#### Summary

The aim of this project was to improve the current pallet designs used for citrus export in terms of airflow and strength characteristics. The project started by interviewing farmers, exporters and pallet manufacturers to determine their perception of problems with the current pallet designs. These interviews and a literature survey concluded that there was very little empirical data available related to the strength of pallets and most pallet designs were derived from a trial-and-error process. Six existing pallet designs were tested for strength performance to obtain a baseline data set. An existing pallet testing rig that can test various aspects of pallet strength, including impact and bending strength, was utilised for the tests. Results from these tests assisted with the identification of weaknesses in each of the pallet designs. The information was used to create

improved pallet designs, which were subsequently manufactured and are currently undergoing testing. New designs also incorporated improved slat spacing to enhance airflow and cooling. After talks with the pallet manufacturers and farmers, it was also noted that the nomenclature for naming pallets was somewhat ambiguous and a new naming convention has been proposed.

## Opsomming

Die doel van hierdie projek was om pallet-ontwerpe wat vir sitrusuitvoere gebruik word, in terme van lugvloei en sterkte-eienskappe, te verbeter. Die projek het begin deur onderhoude met sitrusboere, uitvoerders en palletvervaardigers te voer om hulle persepsie van probleme met huidige pallet-ontwerpe te bepaal. Vanuit die onderhoude en 'n literatuurstudie, is die gevolgtrekking gemaak dat daar weinig empiriese data oor die sterkte van pallette beskikbaar is, en dat meeste pallet-ontwerpe die uitvloeisel van die praktiese ervaring van palletvervaardigers oor tyd was. Ses bestaande pallet-ontwerpe is vir sterkte-eienskappe getoets om 'n basisvlak datastel te verkry. 'n Bestaande pallettoetstoestel wat verskeie sterkte-eienskappe van pallette kan bepaal, insluitende buig- en impaksterkte, is gebruik. Resultate van hierdie toetse is gebruik om swak punte in elk van die huidige pallet-ontwerpe te identifiseer. Verbeterde ontwerpe is ontwikkel wat reeds vervaardig is en huidiglik getoets word. Die ontwerpe neem ook dwarsplank-spasiëring in ag, wat sal help met verbeterde lugvloei en verkoeling. Die onderhoude met palletvervaardigers en sitrusboere het aangedui dat die benaming van pallette dikwels dubbelsinnig of niksseggend is, en dus word 'n nuwe benamings-konvensie ook voorgestel.

### 5.2.9 PROGRESS REPORT: Enhancing phytosanitary cold treatment capabilities of reefer refrigerated containers during citrus exports

Project 1313 (Apr 2021 - Apr 2024) by T Berry, P Cronje (CRI) and J van Zyl (SU, MSc student)

## Summary

Refrigerated containers loaded with citrus are prone to an undesirable temperature gradient between the front and back of the container, as well as from occasional hot-spots developing during the shipping process. These temperature 'off-sets' require lower set-points, which increase the risk of chilling injury in fruit closest to the cold delivery air. The aim of this project was to develop a computational fluid dynamics (CFD) model of a container loaded with citrus and, through numerical and experimental methods, explore the cooling rates, uniformity, hot-spot development and determine optimal packaging ventilation benchmarks. The project started by performing a high-resolution experiment on a reefer container. This cooling and airflow data was then used to validate a CFD model. Past work has made use of CFD predictions to infer research directions. However, with this new, fully validated model, we now have a much clearer understanding of the cooling processes occurring inside the container. Furthermore, using the experimental and numerical data, we have conclusive evidence showing that air sensors can take the place of pulp sensors if positioned between the fruit inside the carton. Furthermore, we have illustrated optimal monitoring positions in the containers, which can be used to motivate for more efficient market access cold treatment protocols. All these findings are currently in the process of being published in an international journal. In parallel to this work, we also developed the T-floor, a container loading aid device. A large-scale commercial trial showed that the T-floor significantly reduced the incidence of hot-spots and is particularly effective when combined with the new A15C-S2 carton. For the next phase of this project, we will use a combination of numerical and experimental work to explore alternative packaging systems, loading configurations and cold chain breaks.

## Opsomming

Verkoelde houers met sitrus gelaai, is geneig tot 'n ongewenste temperatuurgradiënt tussen die voor- en agterkant van die houer, sowel as warmer areas wat by geleentheid ontstaan, tydens die verskepingproses. Hierdie temperatuurvariasies vereis laer stelpunte, wat die risiko van koue-skade in vrugte naaste aan areas waar die lewering van die koue lug geskied, verhoog. Die doel van hierdie projek was om 'n berekeningsvloeiëstofdinamika (CFD) model van 'n houer, gelaai met sitrus, te ontwikkel en, deur numeriese en eksperimentele metodes, die verkoelingstempo's, eenvormigheid, en die ontwikkeling van temperatuurvariasie (*hot spots*) te ondersoek, en optimale verpakkingsventilasie-maatstawwe te bepaal. Die

projek het begin deur 'n hoë-resolusie-eksperiment op 'n koelhouer uit te voer. Hierdie verkoelings- en lugvloedata is dan gebruik om 'n CFD-model te valideer. Vorige werk het gebruik gemaak van CFD-voorspellings om die rigting van verdere navorsing te bepaal. Met hierdie nuwe, volledig gevalideerde model, het ons egter 'n baie duideliker begrip van die verkoelingsprosesse wat binne die houer plaasvind. Deur die eksperimentele en numeriese data te gebruik, het ons voldoende bewyse wat toon dat lugsensors die plek van pulpsensors kan inneem indien dit tussen die vrugte in die karton geplaas word. Ons het verder optimale moniteringsposisies in die houers geïllustreer, wat gebruik kan word om vir meer doeltreffende marktoegang koue-behandelingsprotokolle te motiveer. Al hierdie bevindinge is tans in die proses om in 'n internasionale joernaal gepubliseer te word. Parallel met hierdie werk, het ons ook die T-vloer ontwikkel, 'n hulpmiddel om lugvloei in houers te verbeter. 'n Grootse kommersiële proef het getoon dat die T-vloer die voorkoms van warm areas aansienlik verminder het, en is veral effektief wanneer dit in kombinasie met die nuwe A15C-S2-karton gebruik word. Vir die volgende fase van hierdie projek sal ons 'n kombinasie van numeriese en eksperimentele werk gebruik om alternatiewe verpakkingstelsels, laaikonfigurasies en koue-ketting onderbrekings te ondersoek.

### 5.3 **PROGRAMME: PRODUCTION AND QUALITY**

Programme coordinator: Pieter Raath (CRI)

#### 5.3.1 **Programme summary**

The focus of the Production and Quality Programme is to generate technology and practices that maintain profitable and viable citrus production, ensuring that the South African Industry remains competitive and continues to prosper. In the face of various challenges, i.e., drought, escalating fertiliser prices and future risk of Huanglongbing (HLB), nine trials (five running and four initiated) were being conducted during the 2021/22 season. Of these three are managed by the ARC, Department of Soil Science (Stellenbosch University) and Department of Plant and Soils Sciences (University Pretoria), respectively.

Two projects were completed in the past year. The results generated provide valuable cultivar-specific information to assist in future planning for rootstocks farms to assist in HLB management when the turnaround time for commercial citrus trees are more rapid than it is currently. The other completed trial provides valuable new insights regarding the interpretation of leaf nutrient analysis and fertilisation management of both mandarins and Valencias. All three of the projects focus on improvement of tree water use efficiency, with novel concepts like restricting tree root growth, and therefore vegetative growth, to reduce water use; clarifying the differences in tree water use efficiency of different irrigation approaches and system; and establishing the extent to which mandarins can be stressed at different phenological stages without a reduction in fruit quality/production.

The newly initiated projects all tie in to improve the producer's profitability and the Industry's competitiveness, i.e., to develop technology that will facilitate and improve the accuracy of decision-making re thinning applications and crop estimations; to enhance young tree performance to advance full production and maintain tree performance; to set Industry benchmarks for nutrient use efficiency whereby producers can evaluate and improve their fertiliser use; and to investigate the use of cover crops as a supplementary practice to reduce irrigation water requirements, chemical weed control and nitrogen fertilisation requirements.

#### **Program-opsomming**

Die fokus van die Produksie en Kwaliteit Navorsingsprogram is om tegnologie en praktyke te voorsien wat produsente se winsgewendheid en lewensvatbaarheid verseker, asook die Suid-Afrikaanse Bedryf kompetender en vooruitstrewend laat bly. In die lig van verskeie uitdagings, te wete, waterskaarste, stygende kunsmispryse and 'n moontlike bedreiging van Huanglongbing (HLB), is nege projekte (vyf lopend en vier nuut) in die 2021/22 seisoen deurgevoer. Hiervan word drie bestuur deur die LNR, Departement Grondkunde (Stellenbosch Universiteit) en die Departement Plantwetenskappe en Grondkunde (Universiteit Pretoria) onderskeidelik.

Twee projekte is afgehandel in die afgelope jaar. Die resultate verskaf kultivarspesifieke inligting wat help met toekomstige beplanning deur verskaffers van onderstamme sodat HLB bestuur in die toekoms effektief gedoen kan word wanneer die vervanging van kommersiële bome vinniger sal moet geskied as waaraan die Bedryf gewoond is. Die ander afgehandelde projek het waardevolle nuwe insigte verskaf t.o.v. interpretasie van blaarontledings en bemesting van mandaryne en Valencias.

Die drie lopende projekte fokus op die verbetering van water gebruikseffektiwiteit, waar interessante aspekte ondersoek word, soos die moontlike vermindering van waterverbruik deur die beperking van vegetatiewe groei deur wortelgroei te beperk; verskille in bome se watergebruik te kwantifiseer waar verskillende besproeiingsbenaderings en -sisteme gevolg word; en duidelikheid oor die mate wat mandaryn bome blootgestel kan word aan watertekorte in verskillende fenologiese stadiums sonder dat vruggehalte en produksie benadeel word.

Die nuwe projekte skakel almal in by die doel om produsente se winsgewendheid en die Industrie se kompetendheid te bevorder, naamlik om tegnologie te ontwikkel wat besluitneming rondom vruguitdunning, asook oesskattings te vergemaklik en meer akkuraat te maak; om jong bome se prestasie te bevorder, volproduksie te vervoeg en optimale boomprestasie te verleng; om verwysingswaardes daar te stel vir kunsmisgebruikseffektiwiteit in die bedryf sodat produsente hul kunsmisverbruik kan evalueer en verbeter, en om die gebruik van dekgewasse te ondersoek waardeur verbruik van besproeiingswater, chemiese onkruidodders en stikstofbemesting vermindering kan word.

### 5.3.2 FINAL REPORT: Studies to improve seed production of rootstock trees

Project 1264 (Apr 2020 – Mar 2022) by J Niemann and P Cronje (CRI)

#### Summary

Citrus rootstocks are a determining factor for the success of commercial citrus plantings. Propagation of rootstocks occurs mainly using seeds. As a result, high seed quantity and quality are important to ensure a continuous supply. No clear citriculture guidelines exist to ensure a consistent seed production of rootstock trees. This study aimed to obtain information on the phenology of the important rootstock cultivars used in South Africa to develop production guidelines. Trials were conducted at the Citrus Foundation Block (CFB) in 2020 and 2021 on Rough lemon, C-35, X639, Carrizo citrange, Swingle citrumelo, and MxT trees planted in 2015 and US-812 trees (planted in 2010). The average fruit diameter (mm) differed significantly between cultivars. SC has the largest fruit size and X639 and US-812 the smallest. The average seed number/fruit for SC (19 seeds/fruit) was higher than all cultivars except for CC (18 seeds/fruit), while US-812 had the lowest seed number (3 seeds/fruit). Similar trends were seen for the 2021 season, where C-35 (20/fruit) had the largest seed count but was not significantly different from SC. In most cases, the seed count does not differ greatly between green, colour break and full-colour sampling stages for each cultivar. Large fruit produced more mature seeds than small fruit. The average yield/tree varied amongst cultivars. RL had the highest yield (121.61 kg) followed by X639 (81.48 kg), CC (57.30 kg), SC (53.81 kg), MxT (34.09 kg) and C-35 (22.97 kg) for the first season. In 2021, a similar trend was seen, but there were fewer differences between cultivars. RL again had the highest yield (83 kg) but not significantly higher than SC (78 kg), and C-35 had the lowest yield. The yield for each cultivar varied between seasons (2020 and 2021); some cultivars showed an increase, whereas a reduction was seen for others. X639 produced an average of 2516 (1<sup>st</sup> season) and 1997 (2<sup>nd</sup> season) fruit/tree, whereas all other cultivars had below 900 fruit/tree. CC had the highest millilitre viable seed (2613 ml), followed by X639 (2133 ml), RL (1450 ml), and < 1000 ml for SC, C-35 and MxT for 2020. In 2021, CC again had the highest seed production (4063 ml), followed by SC and all other cultivars lower than 1250 ml. The average seed count/fruit determined from the millilitre seed following the seed treatment process was 11/fruit for C-35 (not differing from CC and RL) and 5/fruit for X639, which was the lowest (first season). In 2021, CC (16/fruit), C-35 (15/fruit) and SC (13/fruit) were amongst the top three, with X639 again having the lowest seed number (3/fruit). Seed germination (in an incubator) was higher for fruit sampled at colour break than the green fruit, except for RL, which showed good germination at both stages for 2020. For full coloured fruit, RL and US-812 showed high germination >85%, and MxT had the lowest (<59%). In 2021, RL had the best germination compared to the other cultivars at the green stage. The germination % increased from green to full colour in most cases except for RL and C-35. The seedling germination in the greenhouse (temperature

controlled) after 88d was >83% for all cultivars except MxT (72.8%). MxT seedlings were the tallest at 88d after sowing compared to the other rootstock seedlings. Overall, the % 'Off-type' seedlings was < 5.6% (for CC) and 0% for MxT. Exogenous GA<sub>3</sub> sprays (10, 15, 20 ppm) did not influence the fruit set % compared to untreated control in most cases (X639 had 2X percentage fruit set, 16.83%, at 20 ppm compared to the control). GA<sub>3</sub> did not affect the fruit yield. Nitrogen levels were below optimum over both seasons for most rootstock cultivars, except for CC and C-35 in 2020. The potassium levels were lower than optimum in 2020 for most cultivars except for CC and US-812. In 2021, the K levels remained below optimum for RL, X639, and MxT. Overall, the tree health of all cultivars was good, with no obvious signs of yellowing. In conclusion, these results provide valuable cultivar-specific information to assist in future planning for rootstocks farms, i.e., the number of trees needed per cultivar to ensure the seed supply is met to assist in HLB management when the turnaround time for commercial citrus trees are more rapid than it is currently. Therefore, yearly rootstock seed supplies need to be consistent, and cultivation practices on seed farms need to be adapted accordingly.

## Opsomming

Sitrusonderstamme is 'n bepalende faktor vir die sukses van kommersiële sitrusaanplantings. Voortplanting van onderstamme vind hoofsaaklik plaas deur sade, en 'n hoë hoeveelheid en kwaliteit saad is dus belangrik om voortdurende voorraad te verseker. Tans bestaan geen duidelike bestuurspraktykryglyne om sodoende 'n konsekwente saadproduksie van onderstambome te verseker nie. Die doel van hierdie studie was om inligting te verkry oor die fenologie van die belangrike onderstamkultivars wat tans in SA gebruik word om sodoende produksieriglyne te ontwikkel. Die proewe is by die Sitrus Grondvesblok (SGB) in 2020 en 2021 uitgevoer op Growweskil suurlemoen (GS), C-35, X639, Carrizo citrange, Swingle citrumelo en MxT bome, geplant in 2015 en US-812 bome (geplant in 2010). Die gemiddelde vrugdeursnee (mm) het betekenisvol tussen kultivars verskil. SC was die grootste en X639 en US-812 vrugte die kleinste. Die gemiddelde saadgetal/vrug vir SC (19 sade/vrug) was hoër as alle kultivars behalwe vir CC (18 sade/vrug), terwyl US-812 die laagste saadgetal (3 sade/vrug) gehad het. Soortgelyke neigings is gesien vir die 2021-seisoen, waar C-35 (20/vrug) die grootste saadtelling gehad het, maar nie beduidend verskil het van SC nie. In die meeste gevalle het die saadtelling nie tot 'n groot mate verskil tussen groen-, kleurbreek- en volkleurstadiums vir elke kultivar nie. Groot vrugte het meer volwasse sade as klein vrugte geproduseer. Die gemiddelde opbrengs/boom het verskil tussen kultivars. GS het die hoogste opbrengs (121.61 kg) gevolg deur X639 (81.48 kg), CC (57.30 kg), SC (53.81 kg), MxT (34.09 kg) en C-35 (22.97 kg) vir 2020. Gedurende 2021 is 'n soortgelyke tendens gesien, maar daar was minder verskille tussen kultivars. GS het weer die hoogste opbrengs (83 kg), maar nie betekenisvol hoër as SC (78 kg) gehad nie en C-35 het die laagste opbrengs. Die opbrengs vir elke kultivar het gewissel tussen seisoene (2020 en 2021), sommige kultivars het 'n toename getoon, terwyl 'n afname vir ander gesien is. X639 het gemiddeld 2516 (1ste seisoen) en 1997 (2de seisoen) vrugte/boom geproduseer, terwyl alle ander kultivars minder as 900 vrugte/boom gehad het. CC het die hoogste milliliter volwasse saad (2613 ml) geproduseer, gevolg deur X639 (2133 ml), GS (1450 ml), en < 1000 ml vir SC, C-35 en MxT vir 2020. In 2021 het CC weer die hoogste saad hoeveelheid gehad (4063 ml), gevolg deur SC, en alle ander kultivars laer as 1250 ml. Die gemiddelde saadtelling/vrug wat uit die milliliter saad na die saadbehandelingsproses bepaal is, was 11/vrug vir C-35 (nie verskil van CC en GS nie), en 5/vrug vir X639, wat die laagste was (1ste seisoen). In 2021 was CC (16/vrug), C-35 (15/vrug) en SC (13/vrug) onder die top drie, met X639 wat weer die laagste saadgetal (3/vrug) gehad het. Saadontkieming (in 'n broeikas) was hoër vir vrugte wat by kleurbreek gemonster is in vergelyking met groen vrugte, behalwe vir GS wat goeie ontkieming in beide stadiums vir 2020 getoon het. Vir volkleur vrugte het GS en US-812 hoër ontkieming >85% getoon, en MxT het die laagste (<59%). In 2021 het GS die beste ontkieming gehad in vergelyking met die ander kultivars in die groenstadium. In die meeste gevalle het die ontkieming % toegeneem van groen na volkleur behalwe vir GS en C-35. Saadontkieming in die kweekhuis (temperatuurbeheer) na 88d was >83% vir alle kultivars behalwe MxT (72.8%). MxT saailinge was die hoogste op 88d na saai in vergelyking met die ander onderstamsaailinge. Algeheel was die % 'Af-tipe' saailinge < 5.6% (vir CC) en 0% vir MxT. Eksogene GA<sub>3</sub>-bespuitings (10, 15, 20 dpm) het in die meeste gevalle nie die vrugset % in vergelyking met onbehandelde kontrole beïnvloed nie (X639 het 2X persentasie vrugset, 16.83%, teen 20 dpm in vergelyking met die kontrole gehad). GA<sub>3</sub> het geen effek op die vrugopbrengs gehad nie. Daar was 'n neiging dat stikstofvlakke gedurende beide seisoene vir die meeste onderstamkultivars onder die optimale vlak was, behalwe vir CC en C-35 in 2020. Die kaliumvlakke was laer in 2020 as die optimale vir meeste kultivars, behalwe vir CC en US-812. In 2021 het die vlakke van K onder die optimale vir GS, X639 en MxT gebly. Oor die algemeen was die boomgesondheid van alle kultivars goed, sonder duidelike

tekens van vergeling. Ten slotte bied hierdie resultate waardevolle kultivar-spesifieke inligting om dus by te dra tot toekomstige boordaanplantings en beplanning van elke kultivar. Dit sal bydra tot die bestuur van HLB wanneer sitrusbome meer gereeld vervang sal moet word as wat tans die geval is. Daarom moet die jaarlikse voorsiening van onderstamsaad konsekwent wees, en die bestuurspraktyke van saadplase moet na gelang aangepas word.

## Introduction

As the name implies, Citrus rootstocks serve as the root system of the scion, grafted onto the rootstock, and therefore plays an important role in how the tree responds in different growing areas and conditions (Castle *et al.*, 1993). Seeds are the main propagation method of rootstocks, and, as mentioned by Tolley (2017), are the starting point of your citrus crop. The production of high-quality and quantity seed to serve as a rootstock is a physical building block of the SA citrus industry.

High demand in the previous 5-10 years has led to an undersupply from the CFB in certain seasons. This had a negative impact on income and a delay in supplying nurseries timeously with high-quality seeds. Fruit production for seed is a neglected area of Citricultural research. Seed production for the industry will be more complex as increased rootstock options are made available and the requirement for seeds increases. The problem lies in the widely different citrus species used for seed with phenological and botanical differences, clearly illustrated in the leaves, branching habits and fruit. Furthermore, flower development and pollination are important processes that precede fruit and seed production. This is highly influenced by climate (Iglesias *et al.*, 2007; Lenz, 1969), an uncontrollable factor between seasons. Climatic events could affect total seed production differently between seasons, and so affect various rootstock cultivars differently.

To date, no clear Citriculture guidelines and rootstock specific species management actions exist to enable a consistent increase in seed production. Research mainly focuses on how the different rootstock combinations influence the scion's yield and tree growth, not the rootstock alone (Castle *et al.*, 2010; Stover *et al.*, 2004). In addition, the reaction of standard practices such as gibberellic acid, urea and girdling, used for flower and fruit set manipulation, is unknown. Furthermore, no information on the phenology of the various main seed cultivars exist regarding flower formation and the need to allow vegetative development. The impact of nutrition on the fruit set of these cultivars is also unknown, and no nutrition norms exist to facilitate optimum production.

The current high demand for seed will most likely continue in the foreseeable future in the SA citrus industry. In addition, new rootstocks, higher density plantings and orchards being replaced at a higher interval could become a key part in HLB management to obtain sustainable production in orchards. Therefore, it is necessary to develop production systems to facilitate a constant supply of the fully required rootstock range.

The main aim of this study was to gain information on the phenology of the most important rootstock cultivars used in South Africa to develop production guidelines to maintain consistent seed production. The rootstocks evaluated included Rough lemon (*Citrus jambhiri*), C-35 citrange (*C. sinensis* x *Poncirus trifoliata*), X639 citrandarin (*Cleopatra mandarin* (*C. reticulata*) x *P. trifoliata*), Carrizo citrange (*C. sinensis* x *P. trifoliata*), Swingle citrumelo (*C. paradisi* x *P. trifoliata*), MxT (Minneola (*C. paradisi* x *C. reticulata*) x *P. trifoliata*) and US-812: US Mandarin (*C. reticulata*) x *Benecke trifoliata orange* (*P. trifoliata*). The trials focused on establishing data on the yield, seed production, and germination of seeds at different maturity stages. One of the fruit set practices used on commercial citrus farms, Gibberellic acid (GA<sub>3</sub>), was evaluated for efficacy in alternate bearing cultivars. The project objectives are listed below.

## Stated objectives

- Objective 1: Compile a literature review of any available information on citrus rootstock seed production.
- Objective 2: Describe the bearing habit and main horticultural properties of the 7 main rootstock cultivars.
- Objective 3: Quantify the yield of fruit vs. seed for these cultivars
- Objective 4: Determine if noticeable differences exist in nutrition norms between these rootstocks.
- Objective 5: Test efficacy of GA<sub>3</sub> to enhance fruit set.
- Objective 6: Develop vegetative management guidelines to enable consistent bearing.

## Materials and methods

### Trial site and Location

The trial site was located at the Citrus Foundation Block in Uitenhage, Eastern Cape Province, South Africa. The rootstock cultivars used in the respective trials included Carrizo citrange (CC), Swingle citrumelo (SC), Rough Lemon (RL), X639 citrandarin, Citrange 35 (C-35) and Mineola x trifoliolate hybrid (MxT) trees, all of which are grafted on CC and planted in 2015. Due to the limited amount of trees for some cultivars, an additional MxT orchard planted in 1997 grafted on RL was also used for the seed germination trial. In addition, US-812 (refer to in the industry as US Benecke) trees grafted on CC and planted in 2010 was used for the germination trials and fruit growth measurements in the 2021 season. Similar orchard practices i.t.o. irrigation and cultural practices were followed for all experimental blocks. The experiment were carried out over two consecutive seasons, 2019/2020 and 2020/2021.

### Seed quantification and germination at different maturity levels

Ten trees of uniform size, health, and crop load within the same row were tagged as single tree replicates (n=10) (experimental units) for fruit samples at three different maturity stages based on rind colouration viz. green (G) and after colour-break (CB) during phase II of fruit development (Bain, 1958), and full colour (FC) (Phase III of fruit development). This was done for all rootstock cultivars except MxT, due to limited available trees. MxT grafted on RL rootstock was used instead. In Table 5.3.2.1, the days before the final harvest indicate each maturity stage for the respective cultivars and the differences in ripening patterns.

During the first season (2020) fruit were sampled only at the green (10 fruit/rep) and colourbreak (5 fruit.rep) stage from the selected trees. For 2021, fruit were sampled at all three stages, but the fruit nr/rep varied between cultivars due to limited available seeds to use for germination trials. Five fruit/rep were sampled at all three stages for SC, CC, MxT on RL, and RL and for X639 (10 fruit/rep) and SxB (20 fruit/rep). For C-35, 10 fruit at green and 5 fruit/rep at CB and FC stage.

**Table 5.3.2.1.** The number of days before harvest (dbh) that each cultivar was sampled at for seed extraction at the two respective maturity stages based on the rind colouration for the two respective seasons (2020 and 2021).

Cultivar	Season	Fruit maturity stage			
		Green (dbh)		Colour-break (dbh)	
C-35	2020	42		20	
	2021	34		16	
SC	2020	63		41	
	2021	48		32	
CC	2020	79		43	
	2021	90		70	
RL	2020	92		56	
	2021	77		45	
US-812	2020	92		56	
	2021	63		45	
X639	2020	119		75	
	2021	50		38	
MxT grafted on RL	2020	104		36	
	2021	72		34	

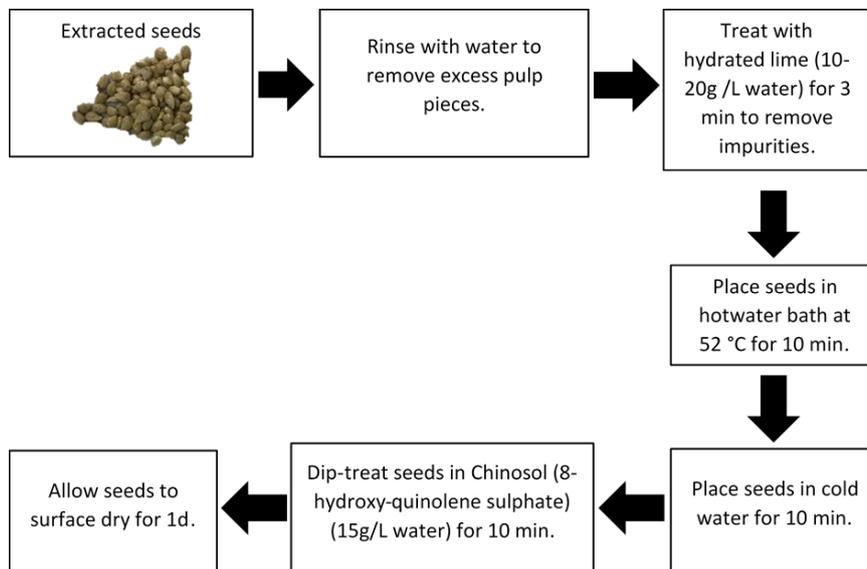
Seeds were extracted by cutting the rind, cutting through the pulp, along the diagonal section, and twisting the fruit to separate it. The fruit was then squeezed into a plastic strainer to remove all the seeds and rinsed with water to remove excess pulp pieces. The seeds were then separated into mature (fully developed seeds) and immature seeds (Figure 5.3.2.1), to quantify the viable seeds.



**Figure 5.3.2.34.** Fully developed (mature) (A) vs. immature/non-viable (B) Swingle citrumelo seeds.

A series of treatments need to be completed to allow for high seed germination, which is shown in the figure below (Fig. 5.3.2.2). On completion of the seed treatment, the air-dried seeds are soaked in water for 2h, where after a measuring spoon was used to place  $\pm 30$  ml seed per rep in a container lined with a paper towel. The container was closed with a small hole in the lid, to allow airflow, and placed in an oven at  $\pm 28$  °C for 20 days. The paper towel was wetted occasionally during the germination period. The germination percentage was determined on completion of the 20 d period using the following equation:

$$\text{Germination \%} = \frac{n \text{ germinated seeds}}{n \text{ total seeds (germinated+non germinated)}} \times \frac{100}{1} \quad [1]$$



**Figure 5.3.2.35.** Flow diagram of required steps after seed extraction to successfully germinate citrus seeds.

### Monthly fruit size measurements

The fruit development of CC, SC, MxT, C35, and X639 were followed from Dec. 2019 until harvest in 2020. Ten trees, uniform in health, size, and crop-load within a row, were selected per cultivar (single tree replicates). On each tree, 20 fruit were tagged with a self-tie label for continuous monthly fruit diameter, measured with an electronic fruit size measure and data logger (GÜSS Manufacturing (Pty) Ltd., Strand, South Africa). For the 2020/21 season, fruit were not tagged, but 20 random fruit were measured/tree each month. In addition, RL and US-812 trees was included. For the RL cultivar, fruit were tagged to avoid measurements of different fruit sets on the tree.

### Fruit yield and fruit number at harvest

The yield (kg/tree) and the number of fruit per tree were determined from the same trees used for fruit measurements for the 2019/20 and 2020/21 season. All the fruit were harvested from each tree (number/tree) before placing the fruit in plastic crates. An electronic orchard scale was used to determine the kg per tree. According to the tree number for subsequent seed extraction, the crates were marked to determine each cultivar's seed number per tree.

### Seed quantification/tree at harvest

The seeds were extracted separately for each rep(tree)/cultivar for the following cultivars, CC, RL, X639, MxT, SC, and C35. For RL the fruit from two trees (i.e. tree 1+2 etc.) were pooled, due to the high fruit numbers. Seeds were extracted using a seed extraction machine consisting of a fruit shredder and a water separator for separating the pulp/juice from the seeds (for all steps and illustrations, see: Commonsense Citrus page 146; Tolley, 2017). Seeds were collected, treated according to Figure 5.3.2.2, air-dried, and quantified and recorded as milliliter (ml) viable seed, as this is the standard quantification method used by the CFB. In addition, the average seed number per fruit after the seed extraction and treatment process was also calculated. From a sample of 20 mature seeds per cultivar, the average weight of mature seed (g) was calculated before, measuring the total ml viable seed (kg). The number of seeds was calculated as follows:

$$\text{Number of Seed} = \frac{\text{Weight of total viable seed (g)}}{\text{Average weight of a mature seed (g)}} \quad [\text{Eq. 1}]$$

This value was then divided by the fruit used in the seed extraction process to obtain the average seed per fruit. This was done for both seasons (2020 and 2021).

### Relationship between fruit size (large vs small) and seed quantity

At harvest during the first season (2020), fruits of contrasting size (5 to 10 mm depending on the rootstock cultivar) were picked for each cultivar. From the experimental units, 10 large and 10 small fruit were sampled per tree (n=10). The seeds from the two fruit size classes were extracted and quantified, as previously described, to determine if there is a difference in seed number. Subsequently, the seeds from each replicate were pooled for subsequent germination trials (germination at full colour and in the greenhouse).

### Germination percentage at harvest: Incubator and greenhouse

The seeds extracted from large and small fruit were used to determine the germination percentage (%) at full colour (at standard commercial harvest) in the incubator and the greenhouse during the 2020 season. The same seed treatment procedure was followed as described earlier for germinating the seeds in the incubator. In addition, seeds were placed in water for 1h before sowing for the temperature-controlled greenhouse germination trial. Seedling plastic tubes were prepared as per standard CFB practice and the medium used was a mixture of 50% sand and peat respectively. A total of 35 seeds per cultivar rep (n=10) were planted (1 seed per tube (90 cm<sup>3</sup>)) except for US-812, where only 18 seeds per rep were planted due to limited available seeds. A small hole of 2.5 cm was made in the soil of each tube, where after the seed was inserted and covered with soil. The trays (tubes inserted into nursery trays) were watered and fertigated as per standard procedure by the CFB.

Seedlings were evaluated after 88 and 225 days. During the 88 day evaluation, the germination percentage was determined by recording any sign of germination, including seedlings that emerged but died thereafter. However, it was recorded as dead, at the 225 day evaluation. The number of seedlings per tray were recorded as mono or multiple seedlings. The height of each seedling was recorded and for multiple seedlings, only the tallest seedling height was recorded. From the seedlings that germinated at the 88 day evaluation, the number of seedlings > 20 cm, seedlings < 20cm, dead seedlings, misshapen growth, and off-type seedlings were recorded and expressed as a percentage. At the 225 day evaluation, the seedling establishment (post-germination) was evaluated by determining if any new germination occurred after 88 days and counting the number of seedlings that can be transplanted and used for grafting.

### Efficacy of gibberellic acid (GA<sub>3</sub>) on fruit set percentage (%) and fruit yield

The trial consisted of a randomized complete block design with six single tree replicates (n=6) per treatment for RL, X639, and C-35, respectively and five single tree replicates (n=5) for CC and SC. The treatments included an untreated control and 10, 15, and 20 ppm ProGibb 40% SG as a source of GA<sub>3</sub> was used. Due to a limited number of trees, only two MxT trees per treatment (n=2) were selected and only three treatments were applied (untreated control, 10 ppm and 20 ppm). In addition, there was a buffer tree between treatments to allow for drift during application.

ProGibb® 40% SG (Valent BioSciences Corporation, Libertyville, Illinois 60048, USA) containing 400 g·kg<sup>-1</sup> GA<sub>3</sub> active ingredient was applied as a foliar spray application using a backpack mist-blow sprayer (Stihl SR430; Andreas Stihl (Pty) Ltd, Pietermaritzburg, South Africa) at a rate of approximately 4.5 L spray mixture per tree. All the GA<sub>3</sub> treatments were applied with 18 ml·per 100 L<sup>-1</sup> water, non-ionic wetting agent Villa 51 (wetter). The three foliar GA<sub>3</sub> treatments, were applied at 10 mg·L<sup>-1</sup> (commercial control), 15 mg·L<sup>-1</sup> or 20 mg·L<sup>-1</sup>. GA<sub>3</sub> ideally should be applied at 80% petal drop to optimise fruit set; however, due to protracted flowering, the applications were made during 50% and 100% petal- drop to cover as many fruitlets as possible.

In order to determine the efficacy of GA<sub>3</sub> on the fruit set, ten 6-12 month old flowering shoots were tagged per replicate and the flower number was determined. Flowers were counted after the first GA<sub>3</sub> spray for all cultivars except for X639 and MXT, where the flowers were counted a day/two before the first GA<sub>3</sub> application. In December 2020, following physiological fruit drop, the number of fruitlets on the tagged branches were recorded and the fruit set % was determined:

$$fruit\ set\% = \frac{\#\ fruitlets}{\# number\ of\ flowers} \times \frac{100}{1}. \quad [Eq. 2]$$

The yield (kg fruit/tree) and fruit nr/tree were determined during commercial harvest in the 2021 season for the four respective GA<sub>3</sub> applied treatments on the different rootstock cultivars. Thereafter the fruit from the replicate trees were pooled for each treatment and an overall seed quantity (liter seed) were determined

#### **Flower number and fruit set % for the 6 rootstock cultivars**

The ten trees tagged for yield and fruit measurements were used to determine the total flower nr and fruit set % of the different cultivars. Ten shoots in similar length and between 6-12 months old were tagged randomly per tree. All the flowers on the shoot were counted (green bud to full bloom/fruitlet stage) during Sept.-Oct. 2021 season. Following physiological fruit drop, the number of fruitlets present on each shoot were quantified on 30 Nov. 2021. The fruit set% for each cultivar was determined thereafter by using Eq. 2.

#### **Tree volume**

The tree volume was calculated per tree for the trees used for the GA<sub>3</sub> trials. A messfix-S measuring stick was used to determine the height and radius of the canopy on each side of the tree, North, South, West and East. The tree volume was determined by the following equation:

$$V\ (volume, m^3) = r^2(\pi h - 1.046r)$$

r =canopy radius

h = height of fruit bearing canopy

#### **Statistical analysis**

Statistical analyses were done through XLstat software. A one-way ANOVA for a completely randomized design was performed for **fruit size, fruit yield, fruit number/tree, germination at harvest (2020 season), greenhouse germination, average seed number per fruit at colour break, viable seed count at harvest, flower count 2021/22 season and fruit set % 2021/22 season** to compare differences between cultivars. In the case where data was not normally distributed i.e. % dead seedlings a Kruskal-Wallis test for non-parametric data was done.

**Germination % at two seedling maturity stages.** A completely randomized split plot was performed to determine which cultivars show the best germination % at the respective maturity stages (88 and 225 days after planting).

**Fruit set %, fruit yield per tree, fruit nr per tree and average fruit/m<sup>3</sup> for GA<sub>3</sub> trials.** A one-way ANOVA for a randomised complete block design.

**Seed count vs fruit size.** A one-way ANOVA for a randomised complete block design, where each tree served as an experimental unit (block) (n=10) from which two fruit were sampled for the two contrasting treatments (large and small).

**Seed count at different maturity stages.** Data was analysed by means of a one-way ANOVA for a randomized complete block design, where each tree served as an experimental unit (block) (n=10) from which green and colour break fruit were sampled for the 2020 season. In 2021, the green, colour break and full colour was analyzed.

For all analysis the Fisher Least Significant Difference (LSD) tests were used for the Post Hoc-testing and significant differences were determined at  $p \leq 0.05$  (5% significant level).

## Results and discussion

Below is the literature review from Objective 1. Further results from the various trials done for the respective objectives, follow after the conclusion from the literature review.

### Literature Review: Seed production in Citrus Rootstock trees

#### 4. Introduction

In citriculture, rootstocks play a critical role in determining the success of how citrus is produced in different areas and conditions (Castle *et al.*, 1993). A rootstock is an anchor for the specific scion cultivar grafted onto the rootstock. In addition, it is also the tree's root system, thereby being responsible for nutrient and water absorption and supply to the vegetative and reproductive development.

Each rootstock has one or more undesirable traits, i.e., being susceptible to *Phytophthora*, root rot, or Citrus Tristeza virus; however, there are individual characteristics that make a positive contribution to the performance of citrus trees (Castle, 2010). In general, citrus rootstocks have three purposes, one of which includes the reduction of juvenility of commercial cultivars. The scion cultivars are budded on rootstock seedlings, ensuring early fruiting and more regular and uniform trees instead of propagating the scion cultivars by means of seeds. Furthermore, rootstocks also aid in adaptation to different soil conditions, resistance to diseases, and nematodes. In addition, the rootstock influences the horticultural performance of the scion, i.e. water relation, mineral nutrition uptake, and differences in fruit yield and internal quality (juice total soluble solids and acid percentage) (Castle *et al.*, 1993; Castle, 2010; Hardy, 2004).

Citrus rootstocks are mainly propagated by means of seeds. Commercial propagation's success is highly dependent on fruit with an adequate number of nucellar embryonic seeds (Spiegel-Roy and Goldschmidt, 1996). Nucellar embryony means that the seedlings which arise from the nucellus are uniform with an identical genotype to the mother plant. The seedlings' uniformity makes it ideal for rootstock propagation (Hartmann and Kester, 2011). In order to ensure a continuous supply of rootstock seeds to commercial citrus nurseries, production of a high number of fruit from each rootstock source tree is critical. Furthermore, each fruit must have a high number of viable seed with a good germination percentage.

For fruit development to occur, flower development, followed by pollination, fertilization, and subsequent seed development must occur (Kretdorn, 1986). The influence of climate in the process is critical, as it is the main uncontrollable factor that influences flower development (Davenport, 1990. Lenz, 1969; Goldschmidt *et al.*, 1985) and pollination (Iglesias *et al.*, 2007), which consequently influences fruit yield.

In citriculture, several cultural practices and crop manipulation techniques are implemented on trees to increase flower intensity and fruit set. This includes water stress, nitrogen application, girdling, mechanical pruning, and exogenous gibberellic acid application (Furr *et al.*, 1947; Lovat *et al.*, 1988; Menino *et al.*, 2003; Schaffer *et al.*, 1985; Southwick and Davenport, 1986; Talon *et al.*, 1992; Mesejo *et al.*, 2020). However, this research focuses on valuable commercial cultivars relevant to the fresh or juice industry. The wide range in genetic origins of rootstock brings into question the efficacy of these fruit set practices.

The principal tree physiology of various citrus rootstock trees is an unknown field, with research being focused on how the differences in yield, tree growth and fruit quality are related to different rootstocks, in combination

with a scion (Bowman and Román, 1999; Castle *et al.*, 2010; Hussain *et al.*, 2013; Louzanda *et al.*, 2008; Roose, 2014; Stover *et al.*, 2004). In addition, it is also unknown what contribution each rootstock and scion have on the final tree characteristics. Therefore, different cultural practices and crop manipulation techniques might be required for the specific rootstock cultivar to enable the mother plants to flower and set fruit with an adequate number of seeds.

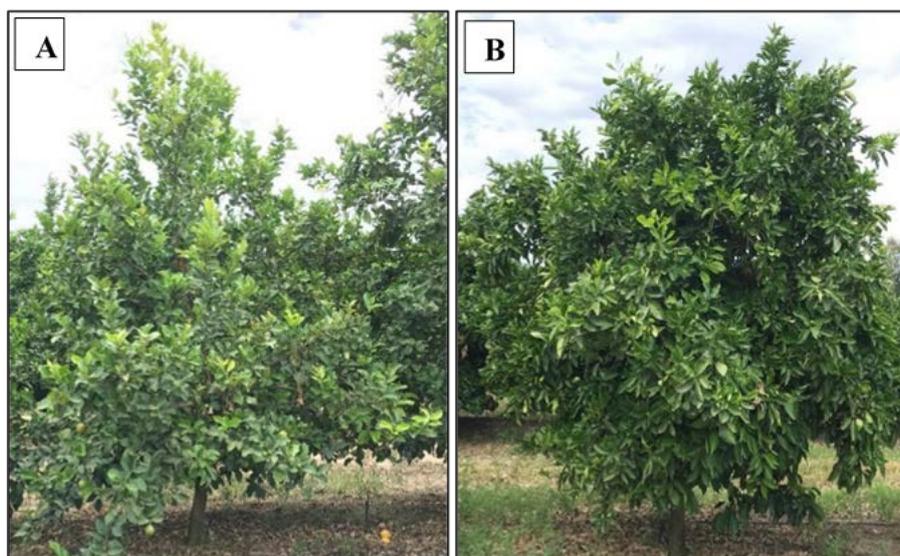
## 5. Citrus tree anatomy

### Shoots and leaves

The anatomy of *Citrus* is very well described by Schneider (1968) in “The Citrus Industry.” In brief, commercial citrus trees have a single trunk of cylindrical shape, with main branches diverging from the trunk approximately 60-120 cm above the ground. This branching habit results in the tree having a spherical shape (Fig. 5.3.2.3.). However, the tree density differs between cultivars, orange trees have a dense growth, where lemon trees have fewer and larger branches, resulting in a more open canopy (Schneider, 1968) (Fig. 5.3.2.4.).



**Figure 5.3.2.36.** A picture is illustrating the branch formation of a defoliated Navel sweet orange tree (*Citrus sinensis*).



**Figure 5.3.2.37.** The difference in tree density/canopy structure between a Eureka lemon tree (A) compared to a Navel orange tree (B).

Shoot growth in *Citrus* has distinct growth flushes (Bevington and Castle, 1985). These growth flushes are influenced by climate, with 2-5 flushes occurring in subtropical regions and no defined flushes in the tropical regions due to growth continuing uninterruptedly. However, the flushes differ in growth habit, which consequently influences the tree growth pattern/architecture (Mendel, 1969). The spring flush is considered to be the most important flush, since it has both vegetative and reproductive (flowering) shoots (Goldschmidt *et al.*, 1985; Spiegel-Roy and Goldschmidt, 1996) (Fig. 5.3.2.5), whereas the subsequent flushes are generally vegetative with fewer, but longer vigorously growing shoots (Spiegel-Roy and Goldschmidt, 1996).

The composition of the spring flower bearing shoots range from:

- 1) Cymose inflorescence (flowers + aborted leaves).
- 2) Flowers, fully formed leaves, and aborted leaves.
- 3) Leafy shoots with a terminal flower and few or more axillary flowers.
- 4) Sterile vegetative shoots.

Generally, flower bearing shoots have eight nodes in length. Leafy flower-bearing shoots and vegetative shoots have long internodes, with a triangular cross-section, whereas shoots with aborted leaves have short internodes and might have fewer than eight internodes with a circular cross-section. There are also instances in lemons, where the vegetative nature of the flower-bearing shoots are modified to short and round, and appear like flower stalks where there is a solitary, terminal flower with the aborted leaves being inconspicuous (Schneider, 1968).

The new flush grows at a slight angle to the previous one (Fig. 5.3.2.6) and the growth flushes can also be distinguished by short, swollen internodes at the beginning and end of each flush. The young stems are green and tender with a prominent ridge, but become hard and round during secondary growth (Schneider, 1968).



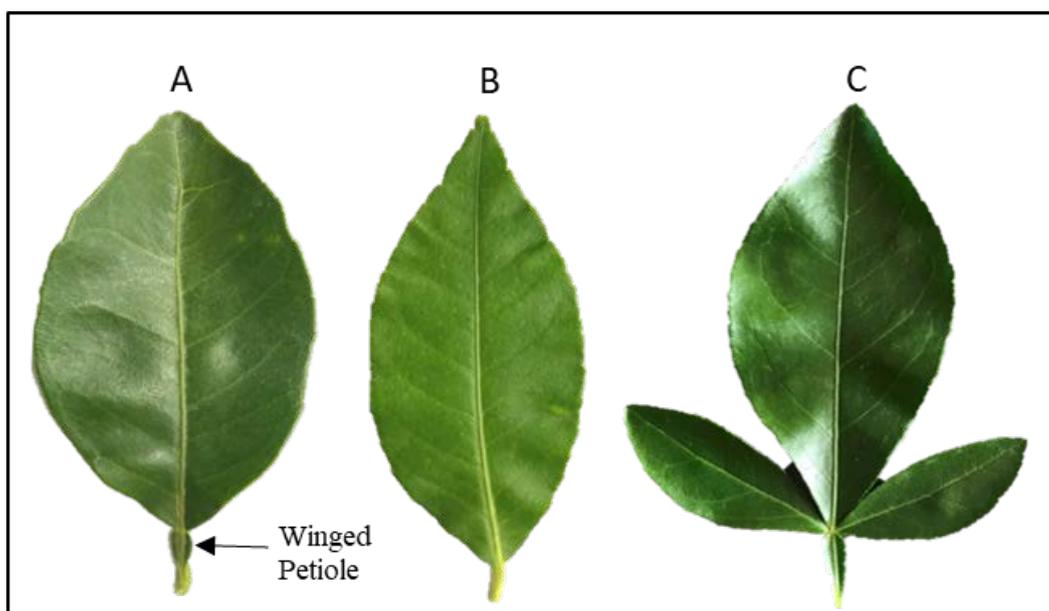
**Figure 5.3.2.38.** A drawing of spring flush of Shamouti orange (*C. sinensis*) with the vegetative shoot (A), and reproductive shoots, leafy inflorescence (B) and leafless inflorescence (C). (Source: Spiegel-Roy and Goldschmidt, 1996).



**Figure 5.3.2.39.** Summer flush emerging in Palmer Navel orange (*C. sinensis*). Note that the new shoot develops at a slight angle to the older shoot.

### Leaves

Mature citrus leaves have a dark green upper surface, with a light yellow-green colouration on the lower surface. The leaf blades are oval to oblong in form and there is a prominent mid-vein that forms the leaf's vascular system, which becomes smaller towards the leaf tip. Regarding phyllotaxis, the leaves of citrus trees are arranged spirally (left or right) around the stem, with the spiral direction alternating between the growth flushes. The citrus leaf of commercial scion cultivars is a unifoliate compound (Fig. 5.3.2.7A), and most of the citrus species have winged petioles, with the size of the wings varying amongst varieties. However, lemon leaves have no petiole wings (Fig. 5.3.2.7B). (Schneider, 1968). In addendum A, the leaves of the commercial important rootstocks in South Africa are shown. Trifoliate orange, Swingle citrumelo, and Carrizo citrange leaves are correctly described as trifoliate, where three leaflets are attached to a common axis (Fig. 5.3.2.7C).



**Figure 5.3.2.40.** Example of a unifoliate compound leaf with a winged petiole (A), a lemon leaf with no winged petiole (B) and a trifoliate leaf of Swingle citrumelo (C).

## Root system

The root system is considered as a separate biological entity in the tree, and rootstocks are used to function as the rooting system and therefore, the effect of the root system can be manipulated by rootstock choices (Castle, 1978). The root system is an anchor of the tree and a source of water, minerals, and hormones to the above-ground plant parts (Spiegel-Roy and Goldschmidt, 1996).

During germination, the primary root is the first organ to appear. The taproot that grows straight down when planted later becomes the primary root. Thereafter, secondary lateral roots form, consisting of large pioneer roots and bunches of fine fibrous roots (Schneider, 1968) (Fig. 5.3.2.8). The fibrous roots are located in the top part of the soil profile (shallow roots) and aids in rapidly absorbing nutrients and water from light rains. The deeper roots prevent extreme drought stresses and absorb nutrients not absorbed by fibrous roots (Castle, 1978).



**Figure 5.3.2.41.** The root system of a 225-day old Carrizo (A) and Swingle citrumelo (B) rootstock seedling.

Root elongations occur in flushes (Schneider, 1968) and follow a cyclic growth pattern due to root growth alternating with shoot growth. In addition, soil temperature ( $< 13^{\circ}\text{C}$  negative) and soil water content (drought) also influence root growth negatively (Bevington and Castle, 1985).

With the emphasis on root growth, differences amongst rootstocks are an unknown field, with limited research on whether cultural practices, i.e. water and nutrient application, should be adapted. Bevington and Castle (1985) investigated the pattern of root growth of 'Valencia' orange [*Citrus sinensis* (L.) Osbeck.] trees on two rootstocks, Rough Lemon (*C. jambhiri* Lusch.) and Carrizo citrange [*Poncirus trifoliata* (L.) Raf. X *C. Sinensis*], and found no differences. In contrast, Morgan *et al.* (2007) proposed that irrigation depth and depth for fertilizer placement based on root distribution should be rootstock specific. They found that the fibrous root length density and root length was significantly larger and longer for Swingle citrumelo (*C. paradisi* Macf. X *P. trifoliata*) compared to Carrizo citrange grafted on 'Hamlin' sweet orange [*C. sinensis* (L.) Osb.] within the 0-15 cm soil depth.

These observations are important, as cultivation practices that may be adapted to manage various rootstock mother plants are involved.

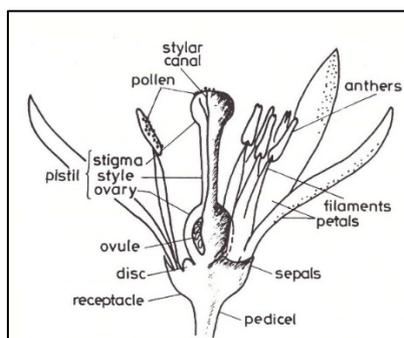
## 6. Citrus flowering and fruiting

## 5.1. Flowers

Flowering is the start of the reproductive process, finally leading to fruit (Kinet, 1993). Citrus flower development had been explained and/reviewed in depth by researchers such as Schneider (1968), Davenport (1990) and Krajewski and Rabe (1995). In this review, only a brief description of the structure of a mature flower, different flowering shoots, and factors influencing flower development will be given.

The basic structure of a mature citrus flower is shown in Figure 5.3.2.9. The pedicel supports all the floral structures, broadening to form the receptacle at the base (Davenport, 1990). The calyx is a cuplike structure with five sepals on the rim of the cup (Fig. 5.3.2.10). The five petals, called the corolla, alternate with the sepals (Schneider, 1968). In addition, the petals are white of colour and have a thick, leathery appearance (Davenport, 1990). At the base of the ovary is a disc structure called the nectary due to the function of secreting watery nectar through the stomata. Furthermore, the anthers are yellow, 4-lobbed structures, containing cells within the lobes that produce pollen grains (Schneider, 1968).

The stigma is the floral organ that develops at the end of the style and is receptive to pollen grains after anthesis (flower fully opened) (Ortiz, 2002). On the stigma's surface are papillose hairs which secrete a sweet viscous fluid that aids in the retention and germination of pollen grains that land on the stigma (Davenport, 1990; Schneider, 1968). There are also canals present on the stigma that pass through the style to the ovules. The ovary is located in the middle between the other structures. Within the ovary are ovules, where fertilization occurs, and the canals on the stigma pass through the style to the ovules (Davenport, 1990).



**Figure 5.3.2.42.** A drawing of an open citrus flower (Source: Spiegel-Roy and Goldschmidt, 1996).



**Figure 5.3.2.43.** An example of the calyx is indicated by the arrows of a C-35 citrange flower at balloon stage (A), open flower (B) and after petal fall (C).

As mentioned previously, there are two types of flowering shoots that occur (Goldschmidt et al., 1985). These flowering shoots are referred to as inflorescences (leafy and leafless). A leafy inflorescence (mixed shoots)

occurs on the new season's growth and consists of a terminal flower or many single axillary flowers with leaves present while the leafless inflorescence occurs on the previous season's growth, and is referred to as a generative shoot with one or more flowers with no leaves (Davenport, 1990; Davies and Albrigo, 1994). The different inflorescent types influence fruit set. Jahn (1973) found more fruit on generative shoots; however, the percentage of fruit set, taking into account the number of flowers, was higher for leafy inflorescence.

### 3.2 Pollination, fertilization and seed development

Pollination, fertilization and seed development are important processes in most citrus varieties for fruit development to occur (Ortiz, 2002). The pollination process starts when pollen grains come in contact with the stigma and germinates to form a pollen tube that moves through the style and ultimately into an ovule where two sperm nuclei are discharged. A sexual embryo zygote is formed through the fusion of the egg cell and one sperm nuclei. The other sperm cell combines with two polar nuclei and a triploid endosperm is formed, which serves as a nutrient source for the embryos that develop in the seed (Jackson and Futch, 1986).

The phenomenon of polyembryony exists in most of citrus cultivars and describes the presence of multiple embryos within a seed (Koltunow *et al.*, 1996). Apart from the zygotic embryo (sexual fertilisation) present, nucellar embryos are present, developed through the mitotic division of nucellus cells, therefore asexual development. Nucellar seedlings are identical to the seed parent (mother plant) (Frost and Soost, 1968). Most of the citrus cultivars used as rootstocks are polyembryonic. It is the nucellar embryony, and the vigor of the nucellar seedling, making it ideal for rootstock propagation to ensure uniformity and identical genetic material from the mother plant. In addition, the nucellar seedlings are free of viruses and other systemic pathogens (Hartman and Kester, 2011).

However, different types of pollination methods/processes vary between citrus cultivars (Frost and Soost, 1968).

- **Self-Pollination (Self-compatible flowers):** Occurs when the flower has functional pollen and ovules and as the word describes, is pollinated by its own pollen produced by the anthers. The fruit will mostly contain seeds (Kahn and Choa, 2004).
- **Cross-Pollination (Self-incompatible flowers):** The flowers contain functional pollen and ovules, but can only be pollinated by other flowers, i.e. pollen from another flower is transferred to the stigma of the pollinated flower. The fruit will be seedless when these varieties are grown isolated (Kahn and Choa, 2004). Cross-pollination mainly occurs through insects, with honey bees being considered as the main natural cross-pollinator in citrus (Frost and Soost, 1968).
- **Parthenocarpic:** Flowers contain none to very few functional pollen and ovules. These flowers are not considered as a pollen source for other varieties. Parthenocarpic varieties are mostly seedless and include Navel oranges, Satsuma mandarin, Midknight and Delta Valencia oranges (Kahn and Choa, 2004).

These above-mentioned pollination types show that functional pollen and facilities of pollination are principle controlled factors during the pollination process (Frost and Soost, 1968). In addition, it was concluded by Mesejo *et al.* (2007) that the genotype is an important factor that influences the flower receptivity to pollen. They investigated factors such as pollen tube development, stigmatic receptivity, and ovule longevity, and how these factors influence seed set in various citrus species including, 'Clemenules' Clementine mandarin (*C. clementina* Hort. Ex Tanaka), 'Valencia' sweet orange, and 'Owari' Satsuma mandarin (*C. unshiu* Marcovich). Stigmatic receptivity influenced seed set in 'Clemenules' and 'Valencia', whereas shorter ovule longevity determined the seed set in 'Owari' Satsuma. However, pollen tube development was not a limiting factor for seed set in any of these citrus species.

Furthermore, the pollination process can also be influenced by climate, as suggested by Chelong and Sdoodee (2012). The suggestion is based on the pollen viability, pollen germination and pollen tube growth of 'Shogun' tangerine (*C. reticulata* Blanco) that differed between two climatically diverse regions. They classified high temperature, light intensity, low rainfall, and relative humidity (RH) as stress-inducing factors for pollination, and proposed that the climatic effect on fruit set requires further investigation.

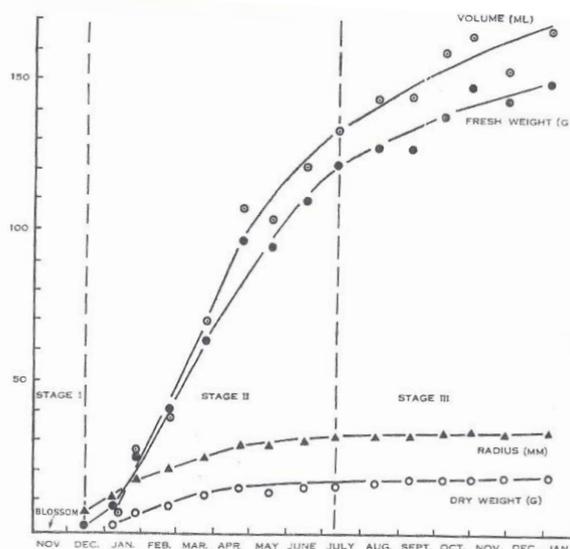
Rootstocks are propagated mainly through seeds. As a result, pollination and consequent seed development is important. However, the number of seeds per fruit could be inconsistent, with the seed number per fruit segment ranging from 1-4 or more (Hodgson, 1967). As mentioned earlier, the tree's genetics and climate influence the pollination process. This means that each rootstock may have a different flowers-types, which can either be self-compatible or self-incompatible. This will consequently influence seed development, depending on the orchard layout in relation to limited cross-pollination resources. In addition, the climatic effects between seasons could also be affecting pollination, resulting in inconsistent seed number and thereby limiting the supply of seed for certain rootstocks that are high in demand.

### 3.3 Fruit set and development

Fruit set is considered as the stage of the reproductive process where an ovary develops into a fruit that will continue with development until maturity (Guardiola, 1997). Fruit set occurs following the first physiological fruit drop after flowering, where the tree naturally thinned fruitlets according to the tree's bearing potential (Spiegel-Roy and Goldschmidt, 1996). Cultivar characteristics impacting on flowering intensity and physiological fruit drop, in addition to tree management and climate, are factors known to influence fruit set. (Guardiola, 1997).

A typical sigmoidal curve (Bain, 1958) is seen during citrus fruit development with three distinct developmental phases (Fig. 5.3.2.11). In summary, **cell division** is the first phase: the cell number in the tissue of the developing fruit increased to form the tissue of the mature fruit. The fruit size consists mainly of the rind volume, with the albedo (white part of the citrus rind) constituting the most. The juice sacs also enlarge and fill half of the pulp segment. During phase two, **cell enlargement** occurs. This is the period of maximum fruit growth, with rapid changes in the fruit's morphology, anatomy and physiology. The juice content increase, with an increase in the pulp segment. Rind colour development occurs, and internal quality changes i.e., total soluble solids ( $^{\circ}$ Brix) increase and acid decrease is evident (Bain, 1958).

The last phase, known as the **maturation phase** has less morphological, anatomical and physiological changes occurring. Acid content decreases further, and the rind colour attains the characteristic colour associated with the specific cultivar. The fruit size increases for the duration that the fruit remains on the tree (Bain, 1958).



**Figure 5.3.2.44.** Fruit growth of Valencia orange (*C. sinensis* (L.) Osbeck) with the three developmental stages distinguished on a calendar basis. (Source: Bain, 1958).

The patterns and key influencer of rootstock fruit set and development of rootstock trees are largely unknown, with current recommendation stemming from commercial relevant scion cultivars. In addition, the correlation

between fruit size and seed number, if any, is also unknown, and would be of interest, especially if the number of set fruit should be adapted in order to yield fruit with a higher number of seeds.

## 6. Cultural practices enhancing flower formation, fruit set and yield.

One of the major constraints in citrus cultivation or any crop cultivation is not having consistent yield from year to year. One possibility is the occurrence of alternate bearing in these rootstock cultivars i.e., one year the tree has a high yield, and the next year a low yield. Pollination and factors impacting on this process for each rootstock cultivar is unknown and could have an impact on sustainable fruit set. Furthermore, not every tree has the ability to easily set fruit, by either not producing enough flowers or high fruitlet abscission during the abscission period; however, growers use a few plant manipulation techniques to attain the desired yield. The first is based on the principle of removing certain tree organs e.g., pruning and fruit thinning to reduce resource dilution. The second technique includes girdling, where interfering with translocation between major tree organs modifies auxin and carbohydrate distribution (Goren *et al.*, 2004). Furthermore, irrigation and nutrition practices and gibberellic acid sprays have also shown some promising results (Lovat *et al.*, 1988; Menino *et al.*, 2003; Talon *et al.*, 1992).

**IRRIGATION.** Water stress of 'Frost Lisbon' lemon trees (*C. limon*) grafted on 'Troyer' citrange rootstocks (*C. sinensis* x *P. trifoliata*), resulted in a higher number of flowers per tree. The duration and higher severity of water stress resulted in a higher flower intensity (Lovat *et al.*, 1988). Southwick and Davenport (1986) found a similar effect in 'Tahiti' lime (*C. latifolia* Tan.) (grafted on *C. macrophylla* Wester). However, the opposite was true for Satsuma mandarin on trifoliolate rootstock (*P. trifoliata*) where the percentage of flowering nodes per total node was higher for moderately stressed trees (3 days) as opposed to 7-10 day stress (severe) (Koshita and Takahara, 2004). In this trial, a higher concentration of gibberellic acid (GA<sub>3</sub>) was present in the leaves of trees that produced less flowers, implying an inhibiting effect of GA<sub>3</sub>. This emphasizes that there are various interrelating factors influencing flowering in citrus.

**NITROGEN.** In the same study where Lovatt *et al.* (1988) investigated water stress, they found that urea application and moderate water stress resulted in a higher flower number per tree. In a study by Menino *et al.* (2003), where they investigated the effect of nitrogen application on the flower numbers of Lane Late orange trees (*C. sinensis* L. Osb.) grafted on Carrizo citrange rootstock, a higher flower number was present with nitrogen application. They also found that the flower yield correlated with the nitrogen concentration in the flowers.

**GIBBERELIC ACID (GA<sub>3</sub>).** Gibberellic acid is widely used in practice for fruit set with GA<sub>3</sub> being the most biochemical active and therefore commercially used in citrus. Talon *et al.* (1992) studied the effect of GA<sub>3</sub> application on fruit set of a Clementine and a Satsuma mandarin, known to set fruit parthenocarpically. GA<sub>3</sub> applications between anthesis and petal fall increased fruit set in the clementine. For satsuma, no effect was seen, suggesting that clementine does not have adequate endogenous GA levels.

Talon *et al.* (1992) further investigated paclobutrazol's influence, which inhibits GA<sub>3</sub> biosynthesis, and found that fruit abscission was present in both. This led to the suggestion that a threshold GA<sub>3</sub> is required for fruit set (Talon *et al.* 1992). In a study where GA<sub>3</sub> was applied at petal-fall on 'Navelate' sweet orange, no effect was evident on the yield; however, when trees were girdled after GA<sub>3</sub> application, the yield increased with 5 mg.L<sup>-1</sup> giving the best results (Agusti *et al.*, 1982). This suggests that the reproductive system of a citrus tree depends on various interrelating factors (nutritional and hormonal) and not one sole factor.

**GIRDLING.** Girdling is a historical horticultural technique still used today to increase crop production. The science behind girdling is based on the interruption of phloem transport between the canopy and roots, consequently influencing the transport of photosynthates (Goren *et al.*, 2004). Schaffer *et al.* (1985) investigated the effect of girdling on non-alternating 'Shamouti' sweet orange and 'Murcott' (*C. reticulata* Hybrid), an alternate bearing cultivar. The final percentage of fruit set increased for 'Shamouti', with only a small, non-comparable effect on 'Murcott'.

The effect of girdling on fruitlet abscission was seen in a study by Rivas et al. (2006), where 'Fortune' mandarin ('Clementine' mandarin x 'Dancy' tangerine) and 'Clausellina' Satsuma mandarin both on Carrizo rootstock were girdled on the main scaffold branches. Both cultivars showed a delay in fruitlet abscission and enhancement in fruit set when compared to non-girdled trees. Furthermore, the timing of girdling is important. The yield of low bearing 'Fortune' increased when girdled at 15 days before anthesis, as opposed to when girdled at anthesis and several days after anthesis. However, for the high-bearing tree, girdling at 35 days after anthesis resulted in the highest yield. For 'Clausellina,' the highest yield was evident in trees which were girdled 40 days after anthesis.

In another study by Rivas *et al.* (2007), the response of girdling on fruit set appear to be dependent on the type of flowering shoot that is girdled. 'Loretina' mandarin (*C. reticulata* Blanco) on Carrizo rootstock and 'Nova' mandarin [(*C. reticulata* Hort. Ex Tan.) x (*C. paradisi* Macf. X *C. tangerine* Hort. Ex Tan.)] on *P. trifoliata* rootstock were trunk girdled at anthesis (60% of flowers open). Fruitlet abscission was delayed, irrespective of the shoot type. However, the fruit set increased for a leafy flowering shoot, while no effect was seen for the leafless flowering shoot.

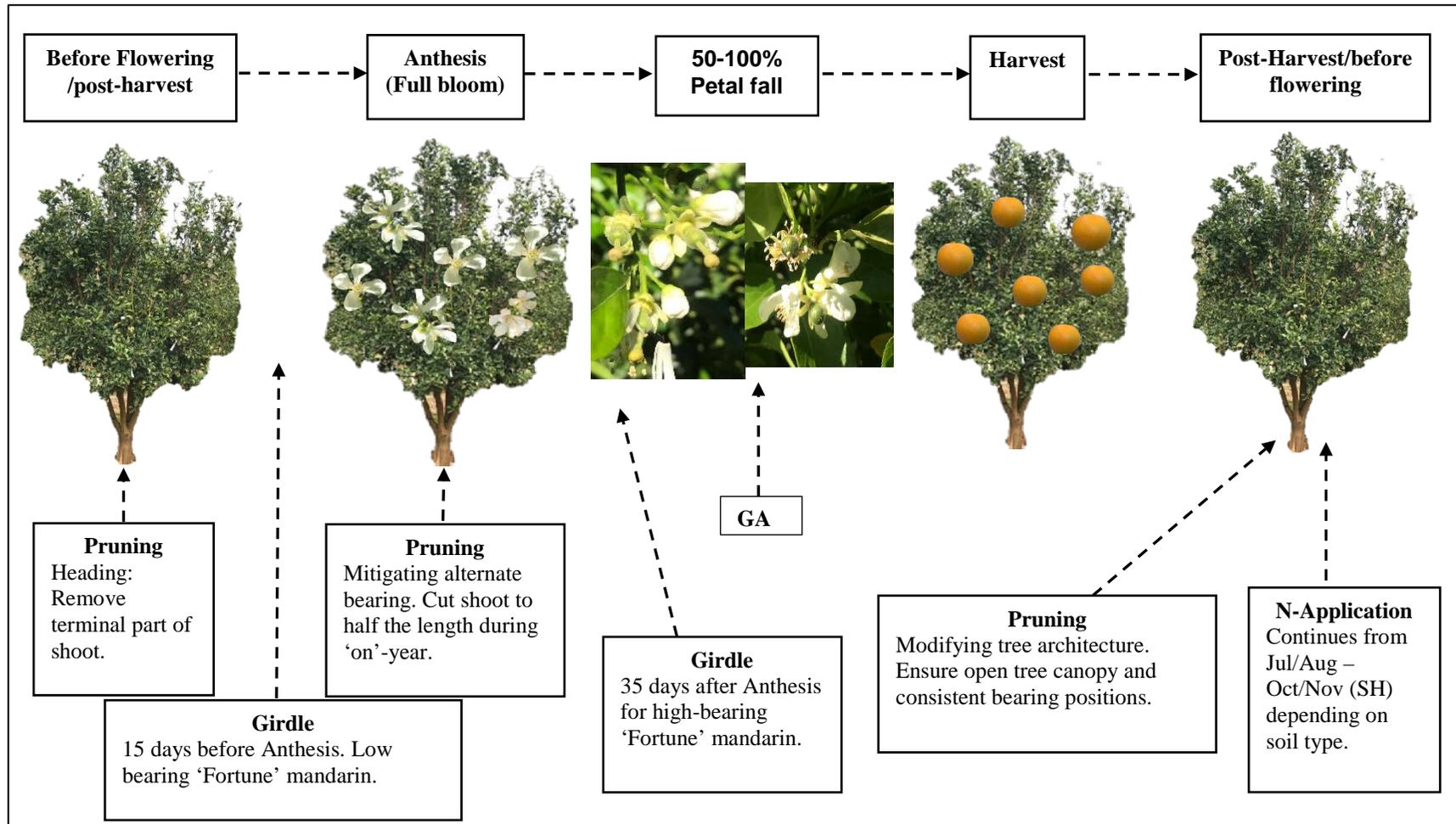
Although girdling has these advantages, it is not practiced widely by every citrus grower due to the difficulty of determining the optimal time and environmental conditions for each cultivar to ensure the efficacy. There is also the uncertainty that exists of how girdling would affect the tree, with a possibility of severe damage by either single or repeated treatments (Goren *et al.*, 2004). This is a concern in colder production regions, where the recovery of vascular bundles are delayed and can result in dieback.

**PRUNING.** Pruning is a critical part of orchard management, which is implemented to control vegetative growth and also to maintain a healthy, productive citrus tree. However, the response of pruning is different for each tree, since variety, tree age, fruiting habit, vigor, growing conditions, and production practices are all contributable factors (Tucker *et al.*, 1994). Tucker *et al.* (1994) and Fake (2012) have documented good guidelines and pruning techniques important for citrus. According to Tucker *et al.* (1994), the main types of pruning in citriculture are a heading cut (where the terminal part of the shoot is removed) to break apical dominance and thereby allowing lateral bud break and thinning (removal of the complete branch, to open the tree canopy).

Pruning removes vegetative growth, thereby reducing potential bearing positions for the following season's growth and the sink demand and consequently affecting the yield. This was seen in an experiment done on 'Orlando tangelo trees (*C. paradisi* Macf. x *C. reticulata* Blanco) where, irrespective of the type of pruning cut (Gable-top, at 30° angle or flat top, no angle), the yield was reduced compared to the control. Trees were pruned in March [Northern Hemisphere (NH)] and harvested in December (NH) of the same year. The yield from the consecutive years were not determined, which is unfortunate, as the type of pruning could possibly have had a positive long-term effect on the yield (Morales and Davies, 2000).

Pruning is also implemented on alternate bearing trees as a manipulation technique. Mesejo et al. (2020) proved that mechanical pruning, where the shoot length was cut to half of the length during flowering in the on-year (start of trial), it reduces alternate bearing in 'Nadorcott' mandarin trees. Consequently, the cumulative yield increased during the 4-year period compared to the control trees. Pruning was done yearly during the period.

A summary/illustration of when the various plant manipulation techniques or cultural practices can be applied in order to enhance the flower quantity and ensure fruit set can be seen in Figure 5.3.2.12.



**Figure 5.3.2.45.** A summary of the timing for plant manipulation techniques and cultural practices to be applied to enhance flower intensity and fruit set/yield

## 7. Conclusion

From experimental work done at the Citrus Foundation Block in South Africa, it is evident that there are distinct differences in the horticultural properties amongst the various rootstock cultivars i.e., yield/tree, seed no./tree, germination %, multiple seedlings, thereby making it evident that individual management guidelines are needed. A lot of interrelating factors come into play to produce seeds for propagation; therefore, it is necessary to build up a database to understand each rootstock cultivar and their cultural and climatic requirements to ensure a consistent, viable seed supply.

### **Results and discussion: Objective 2-6:**

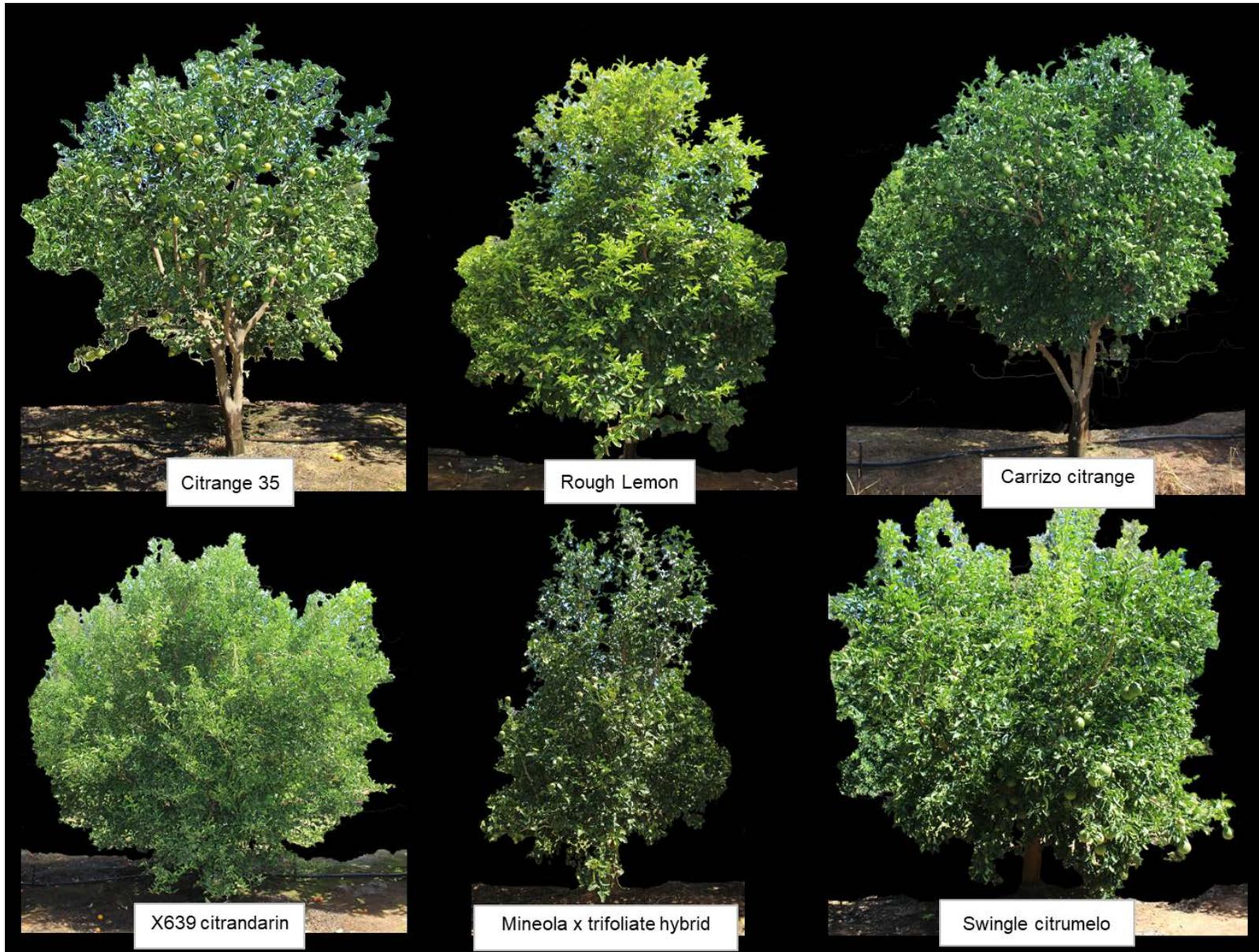
#### **Objective 2 and 3**

The tree growth pattern of the six commercial rootstock cultivars that were investigated during this trial are shown in Figure 5.3.2.13 below. C-35 and CC have very similar tree growth, whereas SC has a more dense canopy. The tree growth of RL trees is typical of the commercial lemon trees, with a more open tree canopy as opposed to SC, for example. MxT has a very upright growth habit, with less foliage on the lower part of the branches. It also has a very vigorous growth (Table 5.3.2.2) with X639 on the other hand, having a unique canopy structure with a bushy appearance and a lot of branches that extend from the framework. In Table 5.3.2.2, the differences in tree vigour are summarised, as well as the different leaf-types that were observed. Furthermore, the timing of maturity of when the different cultivars are harvested are also indicated.

In addendum A, Fig. 1-7 shows the different rootstock trees at harvest, the leaves, seeds, as well as a photo of their flower and fruit at harvest. The fruit development and rind colouration throughout the season is shown in Addendum B, Fig. 1-7. Furthermore, in Addendum C, Table 1 serves as a summary highlighting data from the respective trials and differences amongst the rootstock cultivars during the 2020 season.

**Table 5.3.2.2.** A summary of horticultural properties of 7 important rootstocks used commercially in South Africa. The trees used to describe the horticultural properties are located at the Citrus Foundation Block in Uitenhage, Eastern Cape. All trees were planted in 2015 except for US-812, which was planted in 2010.

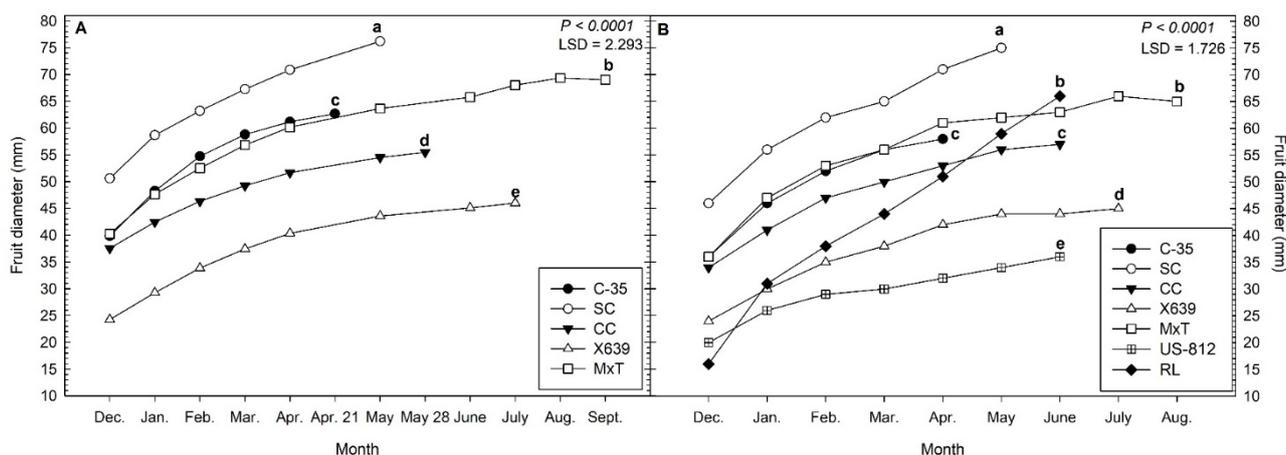
	Rough Lemon	Mineola x trifoliolate hybrid (MxT)	Carrizo citrange	Swingle citrumelo	Citrango 35 (C-35)	X639 citrandarin	US-812 (US x Benecke)
Tree vigour	Vigorous	Vigorous	Intermediate	Intermediate	Intermediate	Intermediate	Vigorous
Type of leaves	Unifoliolate with no petiole wings.	High presence of unifoliolate (winged petioles) compared to trifoliolate leaves.	High presence of trifoliolate leaves, and low % of unifoliolate (winged petiole)	High presence of trifoliolate leaves, and low % of unifoliolate (winged petiole)	High presence of trifoliolate leaves, and low % of unifoliolate (winged petiole)	Trifoliolate and unifoliolate (winged petiole) leaves	High presence of unifoliolate (winged petiole) and low % of trifoliolate leaves.
Fruit maturation period (2019/2020)	Mid (Mid-June)	Late (Early September)	Early (End of May)	Early (Mid-May)	Early (Mid-April)	Mid (Early- July)	Mid (Mid-June)



**Figure 5.3.2.46.** Photos illustrating the differences in tree growth of the 6 rootstocks grafted on CC. All trees were planted in 2015.

**Fruit growth pattern and size.** The fruit growth patterns from December until harvest for the different rootstock cultivars (2019/20 & 2020/21 season) followed the typical characteristic curve of citrus fruit (Bain, 1958). The average fruit diameters of the cultivars measured at harvest differed significantly from each other (Fig. 5.3.2.14). The largest fruit diameter was recorded for SC (76 mm), followed by MxT (69 mm), C-35 (63 mm), CC (55 mm), and X639, resulting in the smallest fruit (46 mm) for the first season (2019/20) (Fig. 5.3.2.14A). During the second season, RL and US-812 fruit were also measured. Again SC had the larger fruit diameter (75 mm) followed by RL, MxT, C-35, CC, X639 and US-812 having the smallest average fruit diameter (36 mm). RL and MxT had similar fruit diameters, and C-35 and CC also did not differ significantly for the second season (Fig. 5.3.2.14B).

The graph also indicates the month of maturity of the respective cultivars during the respective seasons, represented by the last data point of each line.



**Figure 5.3.2.47.** The fruit growth pattern of five commercially important rootstocks in South Africa during the 2019/20 season (A). In B, the seven important rootstocks are shown for the 2020/21 season. Different letters denote significant differences in final fruit size between cultivars at harvest for each season at 5% significant level. Values reported are the means of 10 single tree replicates per treatment (20 fruit per tree).

**Seed quantity at three maturity stages.** There was no difference in the number of mature seeds for each cultivar at the two maturity stages, except for X639 (Table 3) for the first season. However, the difference of 2 seeds/fruit is not of commercial importance and can be ascribed to possible variations between fruit.

During the second season, there was significant differences in viable seed count between the different sampling stages for RL, X639, CC and US-812 (Table 5.3.2.3). However this difference can primarily be ascribed to variation between fruit as the difference is not big, except for RL, where an average of 8 seeds/fruit was seen at the green stage and 16 and 13/fruit at the CB and FC stage. In addition, the immature seed count for the RL, decreased from 5/fruit at the green stage, to 1/fruit for the CB and FC sampling stage. It may be possible that some seeds has not reached maturity and that the embryos were still expanding.

This again indicates that the number of seeds each cultivar can develop is already established during the green stage (Phase II of fruit development).

**Table 5.3.2.3.** The average mature seed number per fruit for each cultivar extracted at green, colour-break, and full colour rind fruit stage. Seeds were quantified directly after hand extraction. Means are the values of 10 single tree replicates per cultivar.

Season	Colour stage	Rootstock Cultivars													
		RL		X639		CC		SC		MxT on RL		C-35		US-812	
		Viable seed	Immature seed	Viable seed	Immature seed	Viable seed	Immature seed	Viable seed	Immature seed	Viable seed	Immature seed	Viable seed	Immature seed	Viable seed	Immature seed
2020	Green <sup>z</sup>	17 <sup>NS</sup>	- <sup>x</sup>	8 b	- <sup>x</sup>	18 <sup>NS</sup>	- <sup>x</sup>	18 <sup>NS</sup>	- <sup>x</sup>	17 <sup>NS</sup>	- <sup>x</sup>	17 <sup>NS</sup>	- <sup>x</sup>	4 <sup>NS</sup>	- <sup>x</sup>
	Colourbreak <sup>y</sup>	16	-	10 a	-	18	-	19	-	16	-	17	-	3	-
	<i>P-Value</i>	0.793		0.000		0.765		0.274		0.241		0.787		0.106	
	<i>LSD</i>	3.368		0.86		2.28		2.721		2.269		3.623		1.021	
2021	Green <sup>w</sup>	9 c	4 a	8 b	7 b	13 b	11 <sup>NS</sup>	18 <sup>NS</sup>	8 b	15 <sup>NS</sup>	4 <sup>NS</sup>	21 <sup>NS</sup>	9 <sup>NS</sup>	3.5 ab	1 b
	Colourbreak <sup>y</sup>	16 a	1 b	10 a	8 ab	16 a	11	18	9 b	16	5	20	9	2.9 b	1 b
	Full colour <sup>v</sup>	13 b	1 b	9 ab	9 a	17 a	12	17	13 a	16 <sup>w</sup>	5 <sup>w</sup>	20	10	3.9 a	2 a
	<i>P-Value</i>	<0.0001	<0.0001	0.015	0.011	0.049	0.811	0.639	0.0001	0.708	0.105	0.806	0.571	0.010	0.003
	<i>LSD</i>	2.794	1.148	1.349	1.357	2.796	1.974	2.011	1.932	4.089	1.759	2.231	2.175	0.611	0.454

<sup>NS</sup> Non significant difference between means within a column ( $P > 0.05$ ). Means was separated using Fishers LSD.

<sup>z</sup> Means are the value of a 10 fruit.

<sup>y</sup> Means are the value of a 5 fruit sample.

<sup>x</sup> No data recorded.

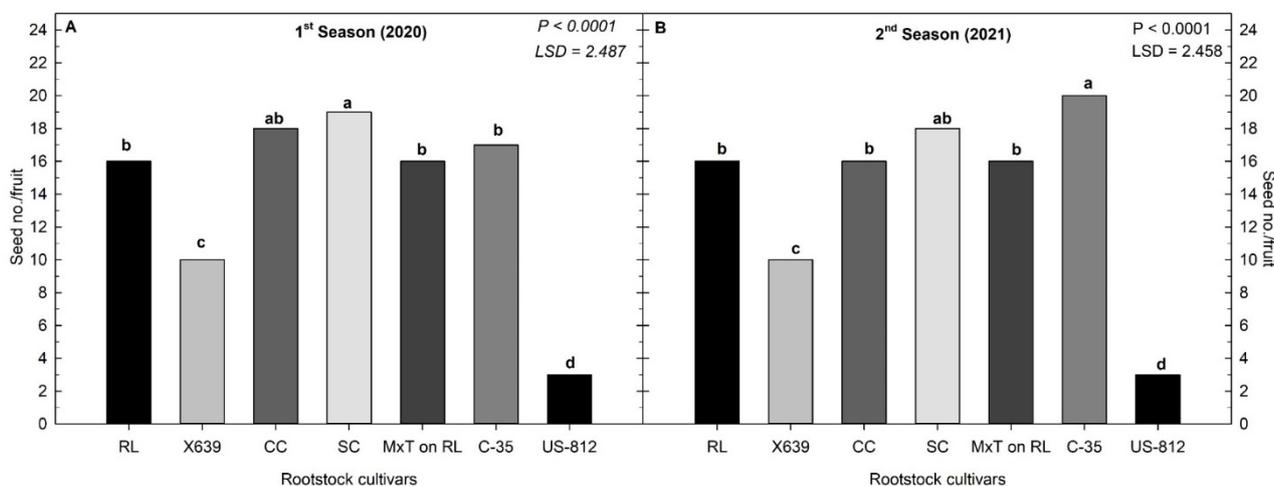
<sup>w</sup> Means are the value of 5 fruit/rep (n=10) except for X639 and C-35 (10 fruit) and US-812 (20 fruit).

<sup>v</sup> Means are the value of 5 fruit/rep (n=10) except for X639 (10 fruit) and US-812 (20 fruit).

**Seed number at colour break stage.** For the 2020 season, an average of 19 mature (viable) seeds/fruit were extracted for SC, which was significantly higher than the other cultivars, except CC, which had 18 seeds/fruit (Fig. 5.3.2.15A). RL, MxT grafted on RL and C-35 did not differ significantly from CC. Furthermore, the lowest seed number/fruit was recorded for X639 (10/fruit) and US-812 (3/fruit), respectively for the first season. A similar trend was seen for the 2021 season, the only difference was C-35 which had the highest seed count (20/fruit), however not significantly higher than SC (Fig. 5.3.2.15B). These differences in seed number between cultivars is most likely related to fruit size which also differs between cultivars (Fig. 5.3.2.14), as also previously reported by De Carvalho *et al.* (2021), the number of seeds was different depending on rootstock variety and fruit size. In the same study, they also found that the seed number was dependent on the season (De Carvalho *et al.*, 2021). During the current trial, were the seed number per fruit for the respective cultivars relatively constant between seasons, which is a good measure to use to ensure the seed supply to the industry is met. It must however be taken into account, that the seed number may vary between seasons.

The average immature seed count at colour break was also compared between the cultivars. CC had the highest number of immature seeds (11) and RL and US-812 had 1/fruit, which was significantly lower than all other cultivars. C-35(9), SC (9) and X639(8) did not differ from each other and MxT on RL had 5 seeds/fruit and differed significantly from the other cultivars ( $P < 0.0001$ ,  $LSD = 1.236$ ). These results indicate that each cultivar has a potential number of seeds that develop, but the percentage of seeds that has embryo development/expansion and becomes mature, varies amongst cultivars. For example, from the data from the 2022 season, 94% of the seed in RL will develop to mature (embryo expansion), while for CC, only 59% of the seed will become mature on average. Koltunow *et al.* (1995) found that only 2-8% of the ovules developed into mature seed for 'Valencia' fruit. Although 'Valencia' is a scion cultivar, this may also be the case for rootstock fruit.

These seed counts for the different cultivars may differ between seasons. Androde-Rodriguez *et al.* (2004) found for *C. volkameriana* that the total number of seeds and full seeds (seeds where embryo sacs are filled with embryos) differed between seasons.



**Figure 5.3.2.48.** The average seed number per fruit for seven rootstock cultivars at colour break stage for the first (A) and second season (B). The data expressed are the means of ten single tree replicates (5 fruit/rep for all cultivars except for X639 (10 fruit) and US-812 (20 fruit)). Different letters indicate a significant difference between cultivars at  $P < 0.05$  for each season.

**Relationship between fruit - large vs small and seed quantity.** Fruit size significantly influenced the number of mature and immature seed per fruit for all cultivars (Table 5.3.2.4). A higher average seed number was found in large fruit compared to small fruit. A difference of 8 seeds/fruit was recorded for C-35 and RL, followed by 6 seeds for MxT, 5 for CC, and 2 for X639. Only a 1 seed/fruit difference for SC and US-812 was recorded. This concurs with Bisi *et al.* (2020), who found that the average number of seeds per fruit for a rootstock cultivar were positively associated with fruit size, however, they only investigated US-812, while the rest were other rootstock cultivars not included in this trial.

Agusti and Primo-Millo (2020) made a statement based on Bermejo et al. (2015) that seeds increase fruit size because ovaries containing fertilized ovules produce higher levels of hormones. Endogenous synthesized GA, which is known to be present in seeds, was reported to be higher in mature ovaries (Bermejo, 2015; Ben-Cheikh, 1997). Based on a model proposed by Stander (2018), GA<sub>3</sub> causes carbohydrate allocation towards developing sinks. This means that the higher levels of GA<sub>3</sub> will cause carbohydrate allocation of the fruit in this case, contributing to the fruit growth and size, as it is a critical factor for fruit enlargement (Goldschmidt and Monselise, 1977; Goldschmidt, 1999).

During the seed extraction process, when the fruit was cut along the cross-section, it was observed that SC has a very thick rind compared to the rest and possibly due to its grapefruit parentage (*C. paradisi* x *P. trifoliata*). The difference of 15 mm between the large and small fruit could therefore be mainly due to the thick rind and not the pulp section where the seeds are. Unfortunately, the pulp diameter was not measured during this trial. In contrast, for the US-812 there was not a lot of variation in fruit size, as was observed for the other cultivars, and the difference in fruit diameter between large and small was only 4mm.

Furthermore, this data indicates that the small fruit of each cultivar also has an adequate number of seeds available since the size distribution is spread out for all cultivars except US-812 during the growing season. This indicates that every fruit does have the potential to contribute to the seed supply.

**Table 5.3.2.4.** The difference in average viable/mature and non-viable seed number per fruit for large and small size fruit. Values are the means of 10 single tree replicates from which 10 fruit per size category were sampled per rep for each of the respective rootstock cultivars. Mean separation was done by means of Fishers LSD. All the trees were grafted onto CC rootstock and planted in 2015, except for US-812 (planted 2010).

Cultivar	Size category	Fruit Diameter (mm)	Viable/Mature seed number/fruit	Immature/Non-viable seed number/fruit
RL	Large	79 a <sup>z</sup>	18 a	0.9 a
	Small	59 b	10 b	0.4 b
	<i>P-value</i>	<0.0001	<0.0001	0.015
	<i>LSD</i>	1.829	1.93	0.424
X639	Large	51 a	7 a	10 a
	Small	41 b	5 b	6 b
	<i>P-value</i>	<0.0001	0.005	0.001
	<i>LSD</i>	1.206	0.912	1.523
CC	Large	60 a	17 a	11 a
	Small	50 b	12 b	8 b
	<i>P-value</i>	<0.0001	0.000	0.037
	<i>LSD</i>	1.109	1.644	2.049
SC	Large	84 a	15 a	16 a
	Small	69 b	14 b	11 b
	<i>P-value</i>	< 0.0001	0.048	0.000
	<i>LSD</i>	1.586	1.341	1.991
MxT	Large	79 a	12 a	5 a
	Small	64 b	8 b	2 b
	<i>P-value</i>	<0.0001	0.004	0.003
	<i>LSD</i>	2.253	2.456	1.832
C-35	Large	66 a	23 a	14 a
	Small	54 b	15 b	7 b
	<i>P-value</i>	< 0.0001	0.000	0.001
	<i>LSD</i>	1.791	3.093	2.818
US-812	Large	44 a	5 a	3 a
	Small	40 b	4 b	2 b
	<i>P-value</i>	<0.0001	0.001	0.018
	<i>LSD</i>	1.708	0.888	0.752

<sup>z</sup> Different letters within a column for each cultivar denotes significant differences at  $P < 0.05$ .

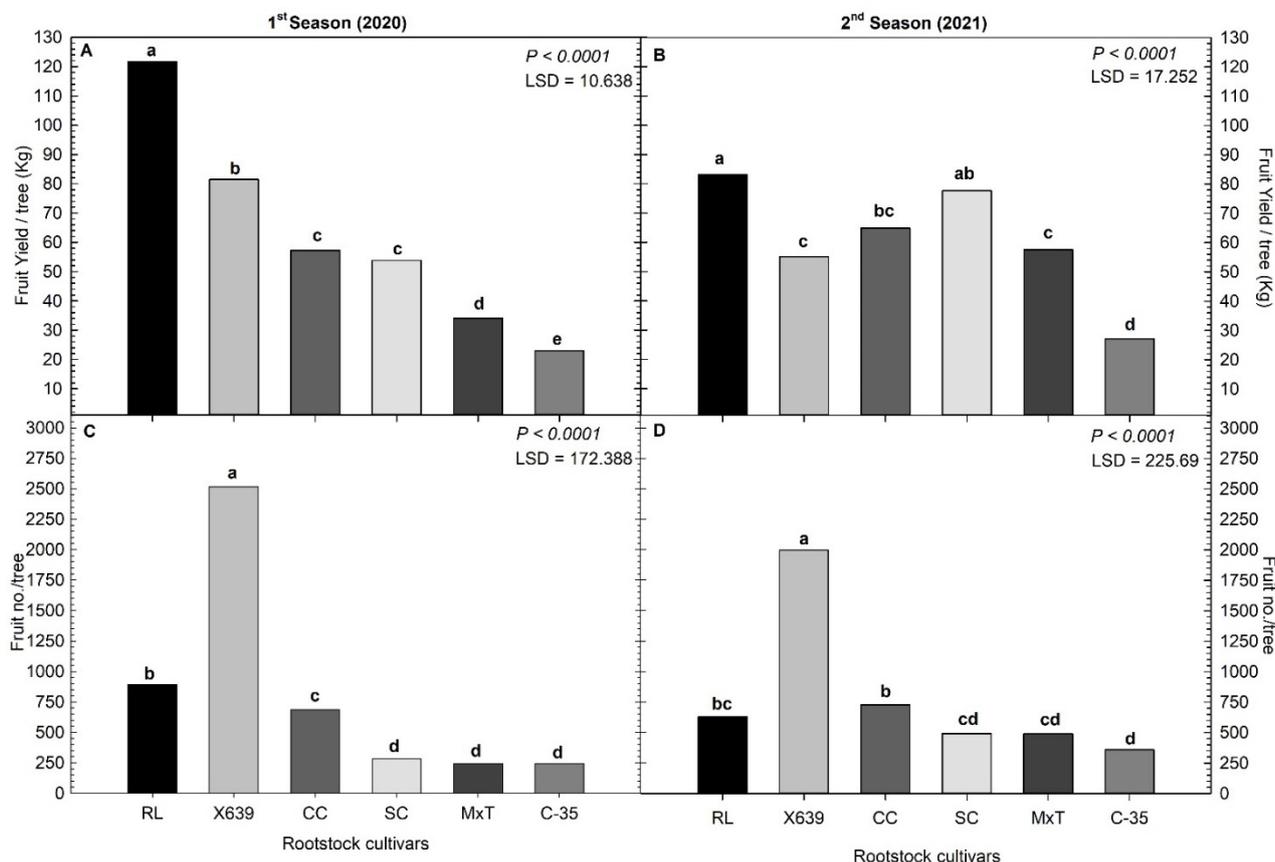
**Average fruit yield and fruit number per tree.** During the first season (2020) RL trees produced the highest fruit yield (121.61 kg) followed by X639 (81.48 kg), CC (57.30 kg), SC (53.81 kg), MxT (34.09 kg) and C-35 (22.97 kg) (Fig. 5.3.2.16A). The yield between cultivars differed significantly, except for CC and SC. For the 2021 season, fewer cultivars differed significantly from each other. RL had the highest yield (83 kg), but it was not significantly different from SC (78 kg). C-35 again had the lowest significant average yield (27 kg) (Fig. 5.3.2.16B). Further did CC, X639 and MxT not differ significantly from each other, and no difference was evident for CC and SC (Fig. 5.3.2.16B).

Overall, when looking at the graph for the two season, some cultivars had a decrease in yield, whilst some showed an increase during the second season. This variation in yield between seasons, is also an important contributor in seed supply, and therefore a, third seasons data for the yield especially will be recorded, to determine if there is an alternate bearing pattern evident for some cultivars.

The highest fruit number per tree was recorded for X639, which produced around 2516 fruit per tree (Fig. 5.3.2.16C) (first season). Since X639 had the lowest average seed per fruit, one can argue that the tree needs

to produce a high number of fruit to ensure that the seed demand is met compared to the other cultivars. The second largest fruit number/tree was recorded for RL (891) and the third largest for CC (687). The lowest fruit numbers per tree were recorded for SC (285), MxT (245), and C-35 (244) respectively.

For the 2021 season, X639 had 1997 fruit/tree which was significantly higher than all other cultivars (Fig. 5.3.2.16D) and CC had the second highest fruit number, but not significantly higher than RL. Although C-35 had the lowest fruit number, was is not significantly lower than SC and MxT.



**Figure 5.3.2.49.** Average fruit yield per tree (A&B) and fruit number per tree (C&D) of the respective rootstocks during the 2019/2020 and 2020/2021 season. The means are a replicate of 10 trees per cultivar (n=10) and 9 trees for MxT (n=9). Different letter on each graph denotes significant differences between rootstock cultivars at 5% significant level.

**Total viable seed production and seed no./fruit.** The seeds were quantified following the entire seed extraction (mechanical) process as is standard practice for the CFB. The seeds were also sorted and graded according to CFB standards and therefore represent the commercial quantity available for nurseries. It should therefore be noted that this data, where the seed no./tree is given, will differ from the previous data where seeds were quantified directly after hand seed extraction, because some seeds get lost in the process of mechanical seed extraction and seed treatment, as well as during the sorting/grading process, where dirty seeds (brown lesions) gets discarded.

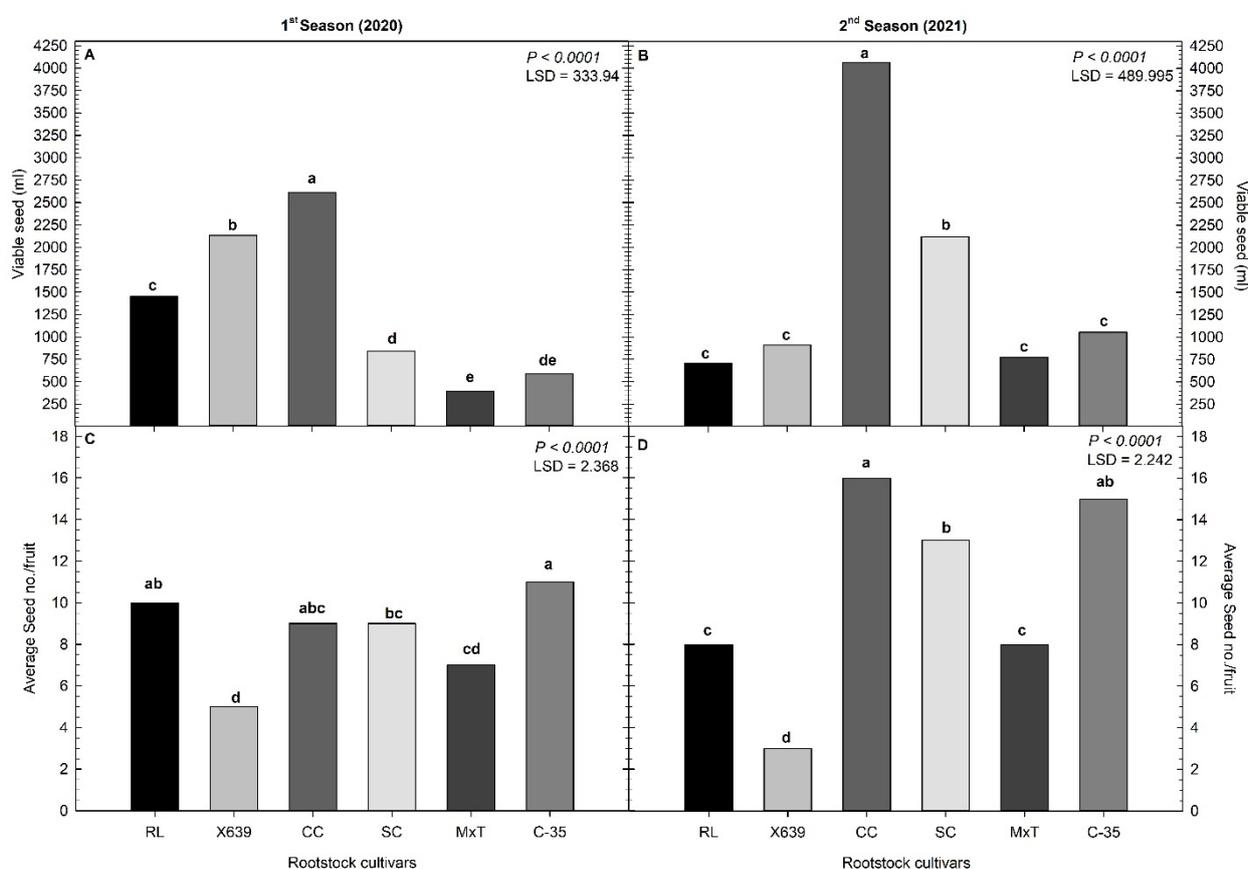
Carrizo citrange produced the highest viable seed (expressed in millimetres (ml) as in commercial seed trade), 2613 ml, while X639 produced 2133 ml, followed by 1450 ml for RL. The lowest viable seeds were found for SC (840 ml), C-35 (590 ml) and MxT (392 ml) (Fig. 5.3.2.17A) for the first season (2020). Results from the 2021 season showed that CC again had the highest seed quantity (4063 ml), followed by SC (2121 ml). No significant differences were seen between RL, X639, MxT and C-35, with all four having the lowest significant millimeter seed.

In the book, *Rootstocks for Florida citrus* (Castle *et al.*, 1993), there is a table indicating the number of seeds for selecting rootstocks/quart. A quart equals a value of 1.13L (1130ml). According to Castle *et al.* (1993) there

are approximately 2600 seeds/1130 ml for CC, 6000 seeds/1130 ml for RL and 3500 seeds/1130 ml for SC. This difference in seed per volume is an indication of the size of the respective seeds. In the current project it was observed that CC had the largest seed, and RL the smallest, with the rest of the cultivars falling in between this range, which concur with Castle *et al.* (1993).

The average number of seeds per fruit from the volume (ml) of viable seed and seeds used in the seed extraction were also determined for both seasons. The average seed no./fruit ranged from 11 (C-35) to 5 seeds per fruit (X639) in the first season (Fig. 5.3.2.17C). There was however, no significant difference between C-35, RL and CC. For the 2021 season, CC (16/fruit) had the highest seed no./fruit, but not significantly higher than C-35 (15/fruit), which was again not significantly higher than SC (13). A similar pattern was seen in the 2020 season, with X639 having on average 3 seed/fruit, the lowest value, following the dry and grading process (Fig. 5.3.2.17 D).

This important commercial data can be used as forecast planning by the CFB in predicting the seed yield for the season, and also to aid in future orchard plantings. One could argue that for cultivars that produce a lower number of seeds, more hectares of that cultivar should be cultivated to meet the seed demand by the industry.



**Figure 5.3.2.50.** The average viable seed in millilitre (A-B) and the average seed count per fruit (C-D) for the respective cultivars during the first (2020) and second (2021) season respectively. The data presented are the means of ten trees ( $n=10$ ) per cultivar except for MxT ( $n=9$ ) and RL ( $n=8$ ). These are seeds that were extracted, treated and air dried. The average seed number per fruit were calculated by the total weight of viable seed (g) divided by the average mass of viable seed (g), and also the (ml seed/15 ml)\*# seed in 15 ml. The average value from the two equations were then divided by the number of fruit used in seed extraction. Different letters on each graph depict significant differences between cultivars at 5% significant level.

**Seed germination percentage at green and colour-break.** The seed germination percentage shown in Figure 5.3.2.18A, represents the germination in an incubator for 20 days at  $\pm 28^{\circ}\text{C}$  from fruit sampled at the green and colour-break stage, respectively. The data from green and colour-break can be compared within a cultivar at the two respective stages, and also between cultivars, since there was an interaction between colour stage and cultivar.

The germination percentage was higher when fruit were sampled at colour-break compared to green fruit for all cultivars except for RL, which showed a high germination percentage at both stages (78 & 73%, respectively). In addition, US-812 also showed good germination at the green stage (64%) compared to the other cultivars harvested at the green stage. MxT showed the lowest germination percentage at the two stages (green and colour-break), although not significantly different from SC at the green stage (Fig. 5.3.2.18A).

For the fruit harvested when a full rind colour developed, RL had a significantly higher germination percentage (95%) than all other cultivars shown in Figure 5.3.2.18B except for US-812 (86%). In general, MxT seeds do not have a very high germination percentage (<59%) with the rest having a good germination percentage above > 65%.

For the second season, there was a significant interaction evident between cultivar and colour stage, which means that the combination of the two factors influences the germination percentage. A trend similar to 2020 for most of the rootstock cultivars was seen where the germination percentage increased from colourbreak to full colour. However, for C-35, the germination percentage decreased significantly from green to colourbreak and full colour. This resulted in C-35 having the lowest germination percentage at harvest for the 2021 season.

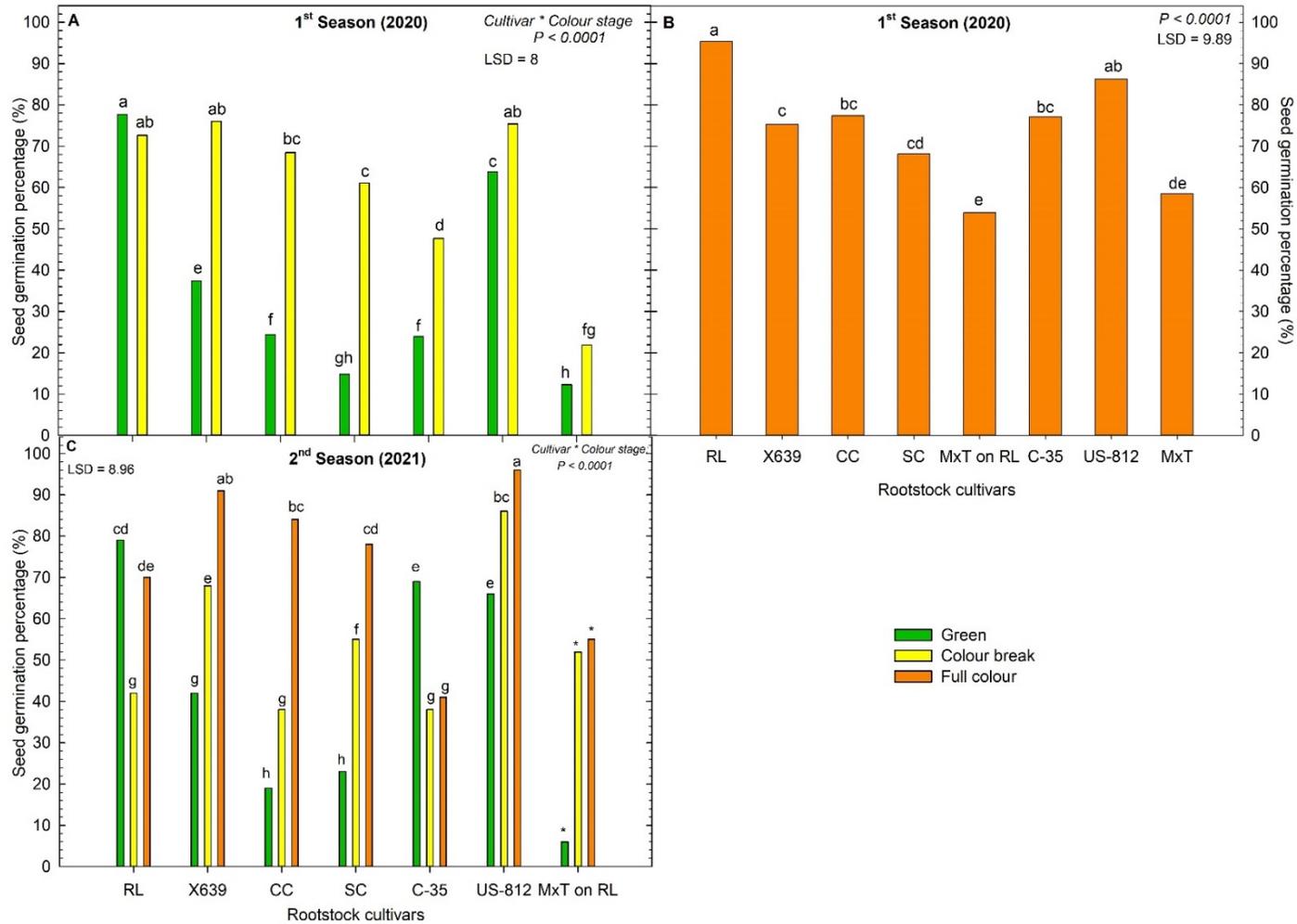
Overall, US-812 seeds from full coloured fruit had the best germination (96%), but not significantly different from X639 (91%). Seeds from green RL fruit had the best germination (79%) compared to the other cultivars at the green stage, which was followed by C-35 (69%) and US-812 (66%). Contradicting to this, were CC and SC, which only had a germination percentage of 19 and 23% respectively (Fig. 5.3.2.18C). The MxT on RL germination was not analyzed statistically due to sampling that was not done from the same trees, and is therefore only indicated on the graph to show a trend.

Temperature are one of the factors that influence germination. In a study done by Rouse and Sherrod (1996), they found that the optimum soil temperature for most citrus rootstock type seeds to germinate ranged between 28.8 – 30.8 °C for CC, RL, SC, C-35 and X639 which was some of the cultivars they included in their study. The germination range was between 5-28 days. It may be required that for MxT which showed a low germination percentage at 20 days, either a higher temperature is required, or a longer germination period.

Chilembwe *et al.* (1992), showed that seed weight or size, did not affect the mean days to seedling emergence and emergence of the first seed for CC and SC investigated in the study. This means that although some seed sizes were not identical, the germination percentage differences, especially with C-35 during the second season, where there was a decrease from green to full colour, are related to the fruit from which the seed were extracted, not the rind colour. De Carvalho *et al.* (2021), proved in their study, that rind colour change in the rind is not the best indicator of seed maturity and that only when the fruit has an increase in the sensitivity for abscission, then the seed are ready to germinate, i.e. physiological ready. Therefore, the fruit sampled may contain seeds that were not physiologically ready.

Viable seeds are already present at an early fruit growth stage; however, it would not be advised to harvest at such an early stage, not only due to the low germination percentage. It is possible that some percentage of seeds need to complete the maturation proses. In terms of a practical observation harvesting at maturity help seed recovery as the juice sacs of most cultivars were not being completely loose at this early green stage. Fruit for the green stage were sampled during the second week in March 2020. As a result, the seed extraction by hand for the later maturing cultivars, as can be seen in the fruit growth figure (Fig. 5.3.2.14), was difficult. In addition, Table 5.3.2.1 indicates the days before harvest for each cultivar, which indicates how mature the fruit was as each stage. This will also not be ideal for machine extraction. It would be best to harvest fruit when the juice sacs are loose from colour-break onwards.

If there are green fruit present on a tree at harvest, due to a second set or delay in coloration, but the amount of green fruit does not justify a second harvest, one can argue that the fruit can be harvested green, as there will be adequate germination occurring in the seeds from green fruit. Removal of all out of season fruit as soon as possible will aid flower development for the subsequent season.



**Figure 5.3.2.51.** The average germination percentage of seeds placed in an incubator for 20 days at 28°C at two different fruit maturity stages (A) and for fruit harvested at full colour (B) during the first season. In figure C, the germination % of the three maturity stages during the 2021 season is shown. Different letters indicate significant differences between cultivars at the different maturity stages on each graph. Significant differences were determined at  $P < 0.05$ . \* Note that MxT on RL for the 2021 season has no statistical analysis and is only shown to indicate the trend.

**Seed germination percentage in the greenhouse.** The germination percentage were determined in the greenhouse 88 days after sowing for all rootstock cultivars (Fig. 5.3.2.19A). MxT again had the lowest germination (72.8%), whereas all other cultivars had a germination percentage above 83%. Bisi *et al.* (2020) investigated seedling characteristics of different citrus hybrid rootstocks, with SC and US-812 included. They found that US-812 and SC had 96 and 98% germination, respectively. However, different optimum soil temperatures for various rootstock cultivars will affect germination, and the days of seedling germination also vary between different rootstock cultivars (Rouse and Sherrod, 1996). It is important to determine the optimum soil temperature for each rootstock in order to ensure the highest germination percentage.

The emerged seedlings were also classified as single or multiple seedlings (Fig. 5.3.2.19 B&C), which was determined by dividing the number of trays with single seedlings, by the number of total trays that had seedlings which emerged. X639 (79%) and C-35 (78%) had a significantly higher percentage of single seedlings, compared to the other cultivars where the percentage was 65% and lower (Fig. 5.3.2.19B). This in turn, led to these two cultivars having the lowest multiple seedling percentage (Fig. 5.3.2.19C). Overall, the multiple seedling percentage was not higher than 55%, with US-812 having the highest percentage (55%), but not significantly higher than RL (47%) and MxT (47%). These results for US-812 and SC were in line with a study done by Bisi *et al.* (2020), who found that US-812 produced 58% of multiple seedlings and SC, 24%. However, the rate of polyembryony may differ between seasons and seeds on the same tree. De Carvalho and Silva (2013), proposed that polyembryony is related to influences on the environment within the seed.

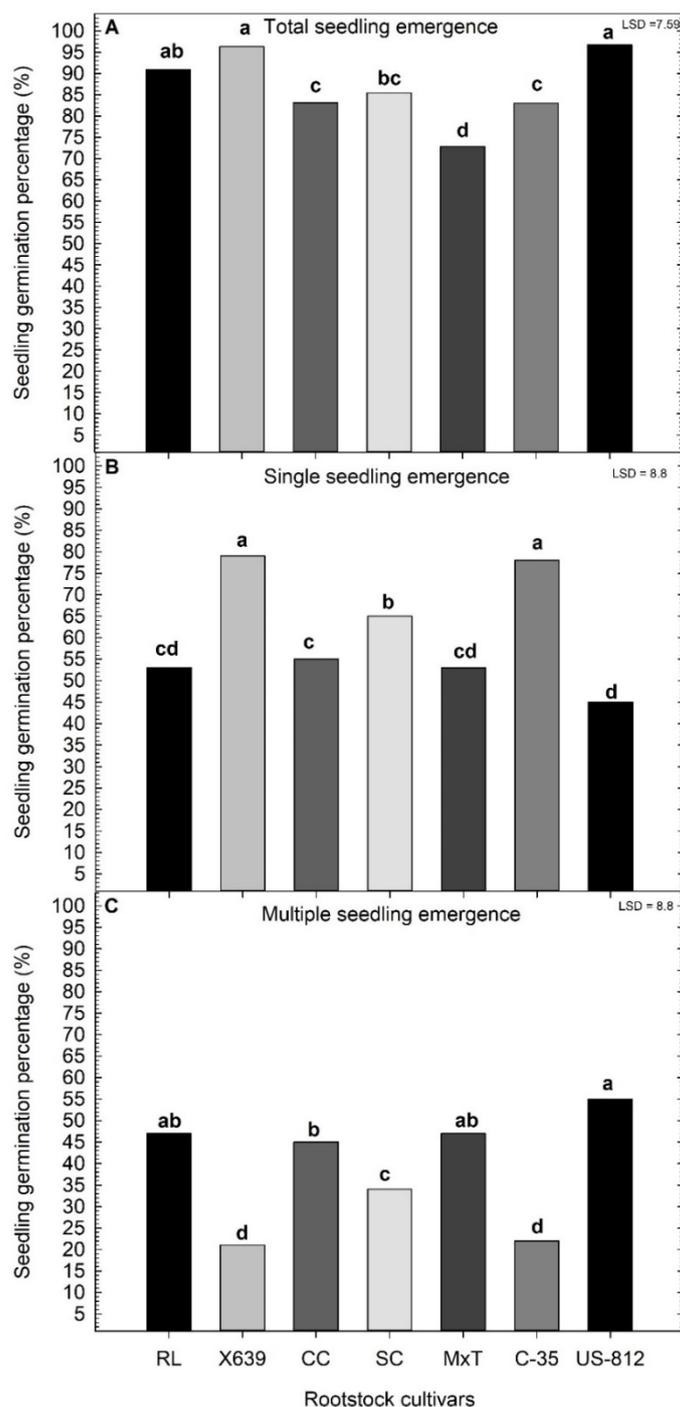
We also determined the percentage multiple and single seedlings for each cultivar, which is a break-up of the germination percentage from Fig. 5.3.2.19A. These values differ from the results in figure 19B-C, because it is worked out per cultivar and the sum of the two values adds up to the germination percentage for the respective cultivar. The data only compares differences in seedling type within each cultivar. Three cultivars, X639, SC, and C-35 had a significantly higher number of single seedlings than multiple seedlings, while the remaining cultivars, RL, CC, MxT, and US-812 did not have a huge difference in seedling type, and it was almost a ratio of 1:1 (Table 5.3.2.5).

In a study done by Anderson *et al.* (1991) they found that when two seedlings was produced from an SC seed, the smaller one of the two was more frequently zygotic (off-type). The zygotic seedlings therefore need to be discarded. The emergence of multiple seedlings per tray, should be monitored carefully to remove smaller seedlings, and to ensure optimal root growth of the true-to-type seedling in the seedling tray. Removal of the extra seedlings per tray should be done at an early stage, before the roots tangle up in the seedling tray, which will make separation difficult at a later stage and possibly damage roots. This can therefore be more labour intensive in the nursery for rootstocks, producing a high amount of multiple seedlings (i.e. RL, CC, MxT and US-812 (Table 5.3.2.5) to rogue out possible zygotic seedlings.

**Table 5.3.2.5.** The percentage of multiple and single seedlings per cultivar based on germination percentage at 88d evaluation in the greenhouse during the 2020 season. The seedling type is a breakup of the germination percentage. Values are the means of 35 seeds per replicate (n=10), except for US-812, where only 18 seeds/rep were sowed.

Seedling type	Rootstock Cultivar						
	RL	X639	CC	SC	MxT	C-35	US-812
Single seedling %	48 a <sup>z</sup>	76 a	45 a	56 a	38 a	65 a	54 a
Multiple seedling %	43 a	21 b	38 a	29 b	35 a	18 b	43 a
<i>P-value</i>	0.442	<0.0001	0.267	0.001	0.487	< 0.0001	0.198
LSD-value	14.5%	7.1%	13.1%	11.6%	10.3%	9%	17.1%

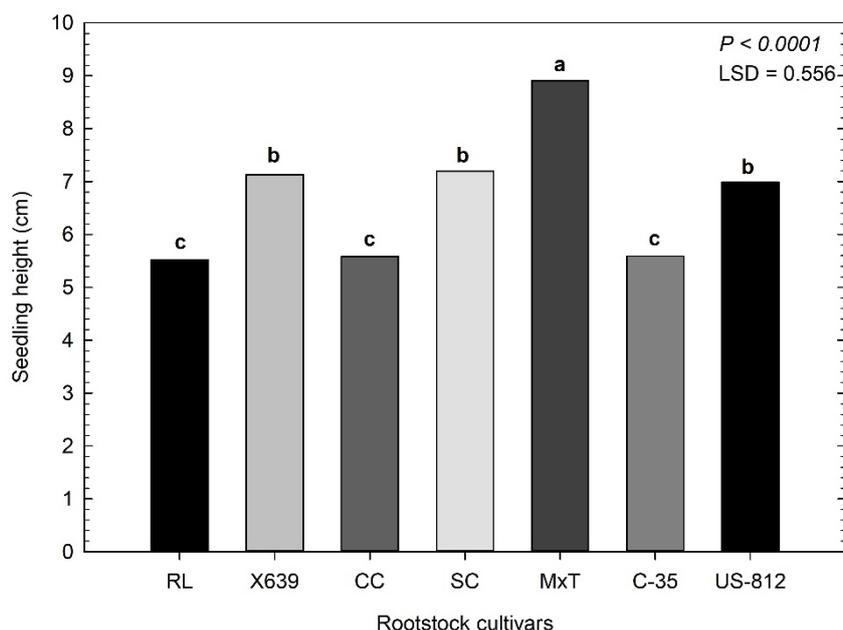
<sup>z</sup> Different letters within a column, indicates significant differences between treatment means at  $P < 0.05$ .



**Figure 5.3.2.19.** The total average germination percentage of seedlings that emerged following 88d after sowing in a temperature-controlled greenhouse (A). Values are the means of 35 seeds per replicate (n=10), except for US-812, where only 18 seeds/rep were sowed. The growth medium is a 50/50 mix of sand and peat. B and C are breakups of the % single and multiple seedlings that emerged at 88d visual assessment in the greenhouse. These percentages were determined by the amount of single seedlings divided by only the # of total seedlings that germinated. Different letters in each graph indicate a significant difference between cultivars at  $P < 0.0001$ .

**Seedling height (mm) at 88 days assessment in the greenhouse.** The seedling height was determined by measuring the stem from the soil level to the growing tip. On average, the MxT seedlings reached a height of 8.91 cm after 88 days in the soil, which was significantly higher than all the other cultivars (Fig. 5.3.2.20). SC (7.20 cm), X639 (7.13 cm) and US-812 (6.99 cm) were the second tallest seedlings, and did not differ significantly from each other. On the other hand, the smallest seedling height was RL (5.52 cm), CC (5.58 cm) and C-35 (5.59 cm). This indicates the vigour of the different rootstocks, with MxT being a more vigorous grower compared to RL, CC, and C-35. In addition, this is an important commercial aspect as the transplant

date, i.e. when a seedling reaches 20 cm and gets transplanted into a larger pot, will be at an earlier stage for MxT compared to the other cultivars and thereby save propagation time.



**Figure 5.3.2.20.** The average seedling height of each cultivar at 88 days visual evaluation in the temperature-controlled greenhouse. Data reported are the means of ten replicates (35 seeds/rep) for all cultivars, except for US-812, which had 18 seeds/rep sowed. Different letters denote a significant difference between cultivars at  $P < 0.05$ .

**Seedling evaluation at 225 days in the greenhouse (Table 5.3.2.6).** The data are presented as percentages based on the number of germinated seedlings, as determined at 88 days for each cultivar. The parameter in the first column, seedling height  $>20$  cm, indicates the percentage of seedlings that can be transplanted to larger planting pots, for grafting. MxT had the highest percentage of seedlings that can be transplanted (93.2%) and was significantly higher than SC, C-35, CC and RL (59.7 %), with the latter being significantly lower than the rest (Table 5.3.2.6). This reflects the seedling height data measured at 88 days, where MxT on average had the highest, and RL the shortest seedling height. This possibly represents the growth vigour of the various rootstock cultivars.

This qualitative parameter, seedling height  $<20$  cm, indicates that a longer period will be required for RL seedlings (24 %  $< 20$  cm), before transplanting as opposed to the other cultivars, except for C-35 (17.2%  $< 20$ cm), which did not differ significantly from RL.

Furthermore, with regards to the other three parameters (dead, off-type or irregular/stunted growth) used to characterize the seedlings at 225 days, no significant differences were found between cultivars for dead seedlings recorded and irregular/stunted growth. The percentage of 'off-type' seedlings was overall very low, ranging from 5.6% (CC) -0% (MxT) (Table 5.3.2.6). The percentage of 'off-type' US-812 seedlings was in accordance with Bisi *et al.* (2020). These low percentages of off-type and irregular/stunted growth of seedlings is a very good trait (Nucellar polyembryony) that ensures the commercial success of rootstocks, being able to continue propagation by means of seeds (Bisi *et al.*, 2020). However, the 'off-type' seedlings were only rogued out based on morphological traits (different leaves and visual appearance) and no Isozymic identification was done were multiple seedlings arise from a single seed. In a previous study by Anderson *et al.* (1991), they found that zygotic seedlings are more frequently found where 2 or more seedlings arise from one seed. Therefore, the percentage of 'off-type' seedlings may be more for each cultivar if not only rogued out on visual appearance.

**Table 5.3.2.6.** Visual assessment of seedling characteristics after 225 days in the greenhouse. The data represents the percentage of seedlings that can be transferred for grafting (height >20 cm) based on the germination percentage/number of seedlings at the 88 day assessment. Data expressed are the means of ten replicates per cultivar (n=10) with 35 seeds/rep for all except for US-812 (18 seeds/rep). Mean separation was done using Fishers LSD test at  $P < 0.05$ .

Cultivar	Seedling height > 20 cm (%)	Seedling height < 20 cm (%)	Dead seedling (%)	Off-type seedling (%)	Irregular growth/stunted (%)
RL	59.7 d <sup>z</sup>	24.3 a	15.1 <sup>NS</sup>	0.6 bc	1.1 <sup>NS</sup>
X639	82.3 ab	1.5 c	14.9	0.3 c	1.4
CC	65.2 cd	11.1 b	18.2	5.6 a	0
SC	75.2 bc	13 b	10.2	1.6 abc	0
MxT	93.2 a	2.3 c	4.5	0.0 c	0
C-35	69.2 bcd	17.2 ab	7.7	4.8 ab	1.9
US-812	81.2 ab	2.8 c	12.6	3.5 abc	1.8
<i>P-value</i>	< 0.0001	< 0.0001	0.237	< 0.0001	0.116

<sup>NS</sup> Non significant differences between treatments at 5% significant level.

<sup>z</sup> Different letters within a column indicates significant differences between means.

#### **Flower number and fruit set percentage for the 2021/2022 season**

The highest average flower numbers were recorded for RL (18.7 flowers/shoot) and CC (16.5), followed by X639, C-35, SC and MxT (7.6) (Table 5.3.2.7) during the 2021 flowering season. The cultivar with the highest fruitset percentage was C-35, with 17.1 %, however, it did not differ significantly from RL (13.6%) (Table 5.3.2.7). Based on these data, some rootstocks produce a higher number of flowers, but also set a higher percentage of fruitlets, i.e. RL, CC and C-35. These cultivars sets on average between 1-2 fruit/shoot, whereas the others ranged below 1/shoot, from the average of 10 shoots/tree.

What is however important to remember is, that there are various factors on orchard level that influences flowering i.e. temperature, light, and soil water (Krajewski and Rabe, 1995). Climate does vary between seasons, therefore flower number for each cultivar is seasonally, and more seasons should be included to determine an overall average per cultivar. This was only done to indicate how the flower number varies between the rootstock cultivars.

**Table 5.3.2.7.** The average flower number per shoot and fruit set percentage which was determined during the 2021 flowering season for the different rootstock cultivars. Values reported are the means of 10 shoots per tree (n=10).

Cultivar	Flower nr./shoot	Fruitset (%)
RL	18.7 a	13.6 ab
X639	11.6 b	7.5 c
CC	16.5 a	10.9 bc
SC	9.4 bc	5.5 c
MxT	7.6 c	6.2 c
C-35	10.9 b	17.1 a
<i>P-value</i>	<i>&lt;0.0001</i>	<i>0.001</i>
<i>LSD</i>	<i>2.51</i>	<i>5.69</i>

**Objective 4:**

Individual Leaf analysis from each cultivar were done for 2020 and 2021 seasons of the 7 respective rootstocks, SC, RL, MxT, CC, C-35, US-812 and X639 (Table 8). No nutritional norms exist for rootstock cultivars probably due to the low number of rootstock orchards requiring nutritional advice. However, the general nutritional norms used in citriculture (Raath, 2021) are supplied in the table to allow comparison and identify noticeable differences between the rootstocks. Different colour shades in the table indicate whether the values are below or above the standard optimum for each element for citrus. Overall, tree health of all cultivars was good, with no various symptoms of yellowing associated with deficiencies.

Nitrogen (N) levels were below optimum levels over both seasons for most of the rootstock cultivars, except for CC and C-35, in the 2020 season. Phosphorus (P), calcium (Ca) and magnesium (Mg) show no extreme deviations from the norms over the two seasons for all cultivars. Potassium (K) levels were lower than optimum in 2020 for most cultivars except for CC and US-812. In 2021, K levels remained below optimum for RL, X639 and MxT.

**Table 5.3.2.8.** Leaf analysis of the 7 different rootstocks from the 2020 and 2021 season. Samples were taken from 25 trees according to industry guidelines on the Eastern and Western side of the tree. Leaves were analysed by Labserve.

Season	Cultivar	<b>N</b> %	<b>P</b> %	<b>K</b> %	<b>Ca</b> %	<b>Mg</b> %	<b>Na</b> mg/kg	<b>Mn</b> mg/kg	<b>Fe</b> mg/kg	<b>Cu</b> mg/kg	<b>Zn</b> mg/kg	<b>B</b> mg/kg	<b>S</b> %	<b>Mo</b> µg/kg
2020	RL	2.09	0.12	0.46	6.04	0.49	<100	400	204	358	82	163	0.34	2515
	X639	2.36	0.11	0.62	4.56	0.33	<100	328	217	334	29	130	0.31	5043
	CC	2.65	0.13	0.75	6.12	0.49	129	401	271	577	56	135	0.38	6408
	SC	2.09	0.09	0.49	5.81	0.42	105	346	279	448	73	190	0.32	6761
	MxT	2.33	0.13	0.60	5.62	0.48	<100	310	272	406	42	108	0.30	6475
	C-35	2.80	0.12	0.68	4.31	0.52	<100	458	221	374	70	120	0.28	1129
	US-812	2.08	0.11	0.82	5.02	0.31	147	137	160	177	33	145	0.25	167
2021	RL	1.50	0.12	0.58	4.23	0.39	137	167	162	149	55	85	0.30	<100
	X639	1.93	0.12	0.66	4.27	0.34	243	234	186	183	49	103	0.29	780
	CC	2.26	0.14	0.76	4.68	0.39	113	144	150	169	53	92	0.38	107
	SC	2.24	0.11	0.71	4.06	0.37	206	116	169	195	29	94	0.27	<100
	MxT	2.14	0.15	0.57	5.25	0.45	111	175	290	143	39	85	0.40	233
	C-35	2.18	0.14	0.74	4.14	0.47	163	209	148	141	82	87	0.27	<100
	US-812	1.93	0.13	0.76	4.76	0.41	172	178	152	195	37	121	0.28	<100
	Norms	2.4-2.60	0.11-0.14	0.70-1.10	3.5-6.0	0.35-0.50	<1600	40-150	-	5-20	25-100	75-200	0.20-0.30	100-1000

## Objective 5

To test the efficacy of GA<sub>3</sub> application on fruit set, ProGibb 40% were applied on RL, X639, CC, SC and C-35; however, these trials were not performed in-depth for MxT due to limited available trees, and so the data for MxT, which will be discussed in the text was not statistically analyzed. Flowers were counted following the first GA<sub>3</sub> (50% petal fall) except for X639, where it was counted before the foliar spray. Very small flowering buds were excluded, since GA<sub>3</sub> would not have an effect on it, because the flower must be open for GA<sub>3</sub> to have an effect.

Overall, no significant differences in fruit-set percentage were observed between the untreated control and the three respective GA<sub>3</sub> concentrations, 10 mg·L<sup>-1</sup>·GA<sub>3</sub>, 15 mg·L<sup>-1</sup>·GA<sub>3</sub>, 20 mg·L<sup>-1</sup>·GA<sub>3</sub> (10, 15 and 20 ppm) applied. However, for X639, the 20 ppm applied resulted in almost double the fruit set % compared to the untreated control and the 10 and 15 ppm. It should be noted that X639 and RL have various flowering periods and fruit sets, making it difficult to apply GA<sub>3</sub> at the right time. For C-35, there was approximately 75% petal fall when flowers were counted. Some fruitlets were present on the tagged branches where the stylar-end was already off, possibly indicating that the fruit was already after the stage for GA<sub>3</sub> to have an effect on the fruitlet, and that the fruitlet set naturally.

The untreated control trees for RL, SC, C-35, and CC showed a high fruit set. It can therefore be argued that the external application of GA<sub>3</sub> is not required, and that the endogenous GA<sub>3</sub>, present in the seeds (Bermejo, 2015; Ben-Cheikh, 1997), is sufficient to ensure fruit set. In addition, fruitlet abscission is dependent on the variety, climate and flower intensity of the tree. Varieties containing seeds also have a higher fruit set ability (Agusti and Primo-Millo, 2020). For MxT, the fruit set percentage ranged from 11.5 (Control), to 8.5% and 7% for 10 and 20 ppm respectively (data not indicated on table).

No significant difference in the fruit yield was recorded between treatments for any of the rootstock cultivars (Table 9). Due to these differences not being significant, the trend for differences in yield between treatments can be ascribed to variation between trees rather than a treatment effect. Further no treatment differences were evident with regards to the fruit number per tree, except for SC, where the 10 ppm had higher fruit number than 20 ppm. Further was the yield recorded for MxT (data not indicated on table), 61.5, 51.39, and 58.17 Kg for control, 10 and 20 ppm respectively. The fruit number for the control was 483, in comparison to 388 for 10 ppm and 434 for 20 ppm. Overall the trend indicated for the control to have higher values, therefor showing no effect of exogenous GA<sub>3</sub> application. However, no GA<sub>3</sub> applied treatments differed from the control indicating that differences is possibly related to variation between the trees.

The average fruit number per m<sup>3</sup> (tree volume) was also determined for the different treatments. No significant differences were evident, again indicating no treatment effect of exogenous GA<sub>3</sub> application on the five different rootstock trees (Table 5.3.2.10).

Overall, based on the results from the 2021 season, the application of GA<sub>3</sub> did not have a significant effect compared to the control, and the fruit set is not a limiting factor, but rather the effect of alternate bearing that might cause some trees to vary in yield from season to season.

In Table 5.3.2.11, the seed volume of viable and cracked seeds is shown for four respective GA<sub>3</sub> applied treatments on the different rootstocks. The data is shown to determine if exogenous applied GA<sub>3</sub> might have had an effect on the seed development. The data is not statistically analyzed because the fruit from each replicate were pooled for each treatment and was not extracted separately. The only large differences were seen for CC where 20 ppm had almost double the amount of seeds compared to the other treatments. For SC, the 10 ppm however had very little seed, only 480 ml, compared to the other treatments who had higher than 2000ml seeds on average per tree. This is not necessarily related to a treatment effect, since for all other rootstocks the seed quantity was relative constant, and variation between fruit in seed number might be the main factor. Further, the CC and SC also had a higher volume of cracked seeds, which is especially very common for SC (Table 5.3.2.11).

**Table 5.3.2.9.** Average fruit set percentage, yield and fruit number/tree of the rootstock cultivars following GA<sub>3</sub> application at 50% and 100% petal fall during the 2020/2021 season. Ten shoots (6-12 month old) per tree were tagged to quantify the flower number. Fruitlets were counted in December 2020, following physiological fruit drop, where after the fruit set percentage was determined. The yield and fruit nr/tree were determined during the 2021 harvest season.

Treatment	RL <sup>z</sup>			X639 <sup>z</sup>			CC <sup>y</sup>			SC <sup>y</sup>			C-35 <sup>z</sup>		
	Fruit set %	Yield (Kg)	Fruit #/tree	Fruit set %	Yield (Kg)	Fruit #/tree	Fruit set %	Yield (Kg)	Fruit #/tree	Fruit set %	Yield (Kg)	Fruit #/tree	Fruit set %	Yield (Kg)	Fruit #/tree
Untreated control	40.8 <sup>NS</sup>	85.31 <sup>NS</sup>	651 <sup>NS</sup>	8.33 <sup>b<sup>v</sup></sup>	29.87 <sup>NS</sup>	834 <sup>NS</sup>	43.20 <sup>NS</sup>	39.01 <sup>NS</sup>	462 <sup>NS</sup>	16.40 <sup>NS</sup>	105.49 <sup>NS</sup>	668 <sup>ab</sup>	42.50 <sup>NS</sup>	42.82 <sup>NS</sup>	410 <sup>NS</sup>
10 mg·L <sup>-1</sup> .GA <sup>3</sup>	42.33	72.96	550	6.00 <sup>b</sup>	28	734	30.80	39.33	443	17.60	125.84	802 <sup>a</sup>	47.17	47.14	462
15 mg·L <sup>-1</sup> .GA <sup>3</sup>	54.33	77.22	654	8.50 <sup>b</sup>	30.54	798	22.80	40.26	458	17.00	104.76	680 <sup>ab</sup>	41.67	43.11	440
20 mg·L <sup>-1</sup> .GA <sup>3</sup>	55.33	77.98	628	16.83 <sup>a</sup>	29.58	854	37.60	45.03	504	17.00	90.26	536 <sup>b</sup>	43.50	44.18	434
<i>P-value</i>	<i>0.173</i>	<i>0.690</i>	<i>0.544</i>	<i>0.004</i>	<i>0.966</i>	<i>0.778</i>	<i>0.144</i>	<i>0.840</i>	<i>0.928</i>	<i>0.993</i>	<i>0.099</i>	<i>0.015</i>	<i>0.946</i>	<i>0.975</i>	<i>0.971</i>
<i>LSD</i>	<i>20.59</i>	<i>21.96</i>	<i>170.69</i>	<i>5.54</i>	<i>10.95</i>	<i>262.97</i>	<i>18.40</i>	<i>16.34</i>	<i>208.50</i>	<i>8.86</i>	<i>27.837</i>	<i>146.27</i>	<i>20.92</i>	<i>22.32</i>	<i>231.98</i>

<sup>NS</sup> Denotes non-significant difference at 5% significant level.

<sup>z</sup> Values reported per treatment are the means of 10 shoots per tree of six single tree replicates (n=6).

<sup>y</sup> Values reported per treatment are the means of 10 shoots per tree five single tree replicates (n=5).

<sup>x</sup> No statistical analysis were performed due to a limited tree nr. Data reported are the mean of two single tree replicates per treatment.

<sup>w</sup> Data not recorded

<sup>v</sup> Different letters within in column denotes significant differences between treatments at 5% significant level. Mean separation was done by means of Fishers LSD.

**Table 5.3.2.10.** The average fruit number per m<sup>3</sup> of the tree volume for the different GA<sub>3</sub> applied treatments during the 2020/2021 flowering season on the 5 different rootstock cultivars. Values reported are the means of 6 single tree replicates for all cultivars, except for SC, which had 5 single trees per replicate.

Treatment	Rootstock cultivars				
	RL	X639	CC	SC	C-35
Untreated control	119 <sup>NS</sup>	94 <sup>NS</sup>	146 <sup>NS</sup>	79 <sup>NS</sup>	69 <sup>NS</sup>
10 mg·L <sup>-1</sup> .GA <sup>3</sup>	122	96	143	93	66
15 mg·L <sup>-1</sup> .GA <sup>3</sup>	141	91	141	87	67
20 mg·L <sup>-1</sup> .GA <sup>3</sup>	132	107	148	74	77

**Table 5.3.2.11.** The average seed quantity in millilitre recorded for the different GA<sub>3</sub> concentration treatments that was applied during the 2020/2021 flowering season. Fruit were harvested at commercial maturity and the seeds were extracted from the fruit to be quantified. No statistical analysis was performed since fruit were pooled for each treatment and seeds were not extracted separately per rep.

Cultivar	Type of seed	Treatment				
		Untreated control	10mg·L <sup>-1</sup> .GA <sup>3</sup>	15 mg·L <sup>-1</sup> .GA <sup>3</sup>	20 mg·L <sup>-1</sup> .GA <sup>3</sup>	
RL	Viable (ml)	692	633	467	642	
	Cracked (ml)	33	37	38	28	
X639	Viable (ml)	592	450	633	625	
	Cracked (ml)	37	50	50	78	
CC	Viable (ml)	3152	3170	2760	6280	
	Cracked (ml)	213	192	211	501	
SC	Viable (ml)	3340	480	2076	2451	
	Cracked (ml)	792	1080	651	757	
MxT <sup>z</sup>	Viable (ml)	1125	775	-	725	
	Cracked (ml)	-	-	-	-	
C-35	Viable (ml)	1592	1783	1792	1458	
	Cracked (ml)	0	0	0	0	
<i>P-value</i>		0.667	0.736	0.998	0.271	0.922
LSD-value		41.563	31.54	83.846	21.638	36.734

<sup>z</sup> There was limited trees available, and therefore no 15 mg·L<sup>-1</sup> GA<sup>3</sup> treatment was applied.

<sup>y</sup> Cracked seed quantity was negligible.

<sup>NS</sup> indicates nonsignificant difference between treatments within a column at 5% significant level.

## Objective 6

This objective was not carried out experimentally due to the limited number of available trees to perform all the trials on. In addition, the 10 data trees of the various cultivars used to determine the yield over various seasons have been pruned to have a more uniform tree canopy, where water shoots were removed, as well as shoots where no branching on the lower side of the branch was present. This was not done for MxT, since it was harvested at a very late stage in the season, and the flowering initiation already started. The main goal for this objective was to prune the trees in such a manner to prevent it from influencing the yield of the following season to a great extent. It was also important to prune the trees from an early stage to prevent the trees from reaching heights in the future that will not only affect the harvesting procedure, but also the general orchard management, i.e. spraying the trees.

## Conclusion

Although this study only shows data from two seasons, it serves as a sound basis to build on to develop different management strategies, ensuring consistent fruit set, yield and seed count per fruit. It is also evident from the data that the seeds are viable, and the germination percentage is higher than 50% for all cultivars. However, since it is known that there are various aspects responsible for the production of a high number of viable seeds (climate), more seasons should be evaluated to build on this data to gain more insight.

The average seed number per fruit, yield per tree, and average fruit count per tree is useful data that can aid in the forecast of the average seed production per cultivar and also to plan the size (hectare) of future plantings for each cultivar to meet the increasing demand of rootstock supply for commercial plantings. For cultivars that produce less millilitres of viable seeds, i.e. RL, SC, C-35, X639 and MxT, more hectares should be planted or at a higher density to ensure an adequate seed supply.

The fruit diameter at harvest and average seed number per fruit remained relatively constant over the two seasons for each cultivar. Further, some cultivars showed a reduction in yield for the second season (RL, X639), whereas, for others, there was a slight increase (CC, SC, MxT). There was evident variation in yield between the trees for each cultivar in the second season. It may be possible that alternate bearing is evident for some cultivars, therefore, a third season's data on yield will be conducted for 2022. This third season's data, will be valuable in order to have a 3-year average of yield/tree for each rootstock cultivar to aid in forecast planning.

The successful and high percentage of seed germination is an important process that determines the success of rootstock propagation. The germination percentage varied between seasons for each cultivar. Therefore, germination is likely dependent on the seed itself and its potential to germinate as seeds of the same size some did germinate while other did not. What would be of interest is to determine the germination percentage of the seed, extracted per fruit, and not a pooled sample.

Generally, it is advised that fruit should be harvested at least when colour-break is evident, but the full colour is better to ensure the best germination percentage possible of all seeds. MxT seeds showed an overall low germination percentage compared to the other cultivars. This can be altered by sowing more seeds to meet the supply amount for MxT rootstocks. As previously mentioned, seeds from fruit of rootstock trees are polyembryonic, however, not all embryos germinate and produce multiple seedlings (Primo-Millo and Agusti, 2020). The advantage of multiple seedlings/seed when true-to-type is that nurseries can plant out seedlings into separate trays, thereby having a higher number of available seedlings to use i.e. US-812 showed 53 % of multiple seedlings that germinated. However, it is not always very accurate to separate seedlings based on morphology, as zygotic seedlings ('off-type') may be present and missed. Therefore, extra caution should be taken when multiple seedlings that emerged are separated to ensure they are true-to-type and that the roots do not interfere with the other seedlings. This may be more time-consuming.

All rootstocks investigated during the trial (RL, X639, CC, SC, C-35, US-812, and MxT) are sufficient to propagate by means of seeds. To summarise, these results provide valuable cultivar-specific information to assist in future planning for rootstock farms to ensure the seed supply is met to assist in HLB management

when the turnaround time for trees are more rapid than it is currently. Therefore, yearly seed supply needs to be consistent.

This remains a very important research field and is of high importance in ensuring the consistency of seed production in citrus rootstocks, especially in South Africa. But, as the saying goes, "only time will tell".

### **Future research**

Germination of seeds per fruit would be of interest to see how much seed per fruit has the potential to germinate. The yield per tree for the different rootstock cultivars should also be recorded to build a data basis over various seasons, as climate differs between seasons, and the alternate bearing factor may come into play.

### **Technology transfer**

Talk or presentation at the future planned CRI Research symposium.

A possible paper/ article on the horticultural differences between rootstock cultivars used in South Africa.

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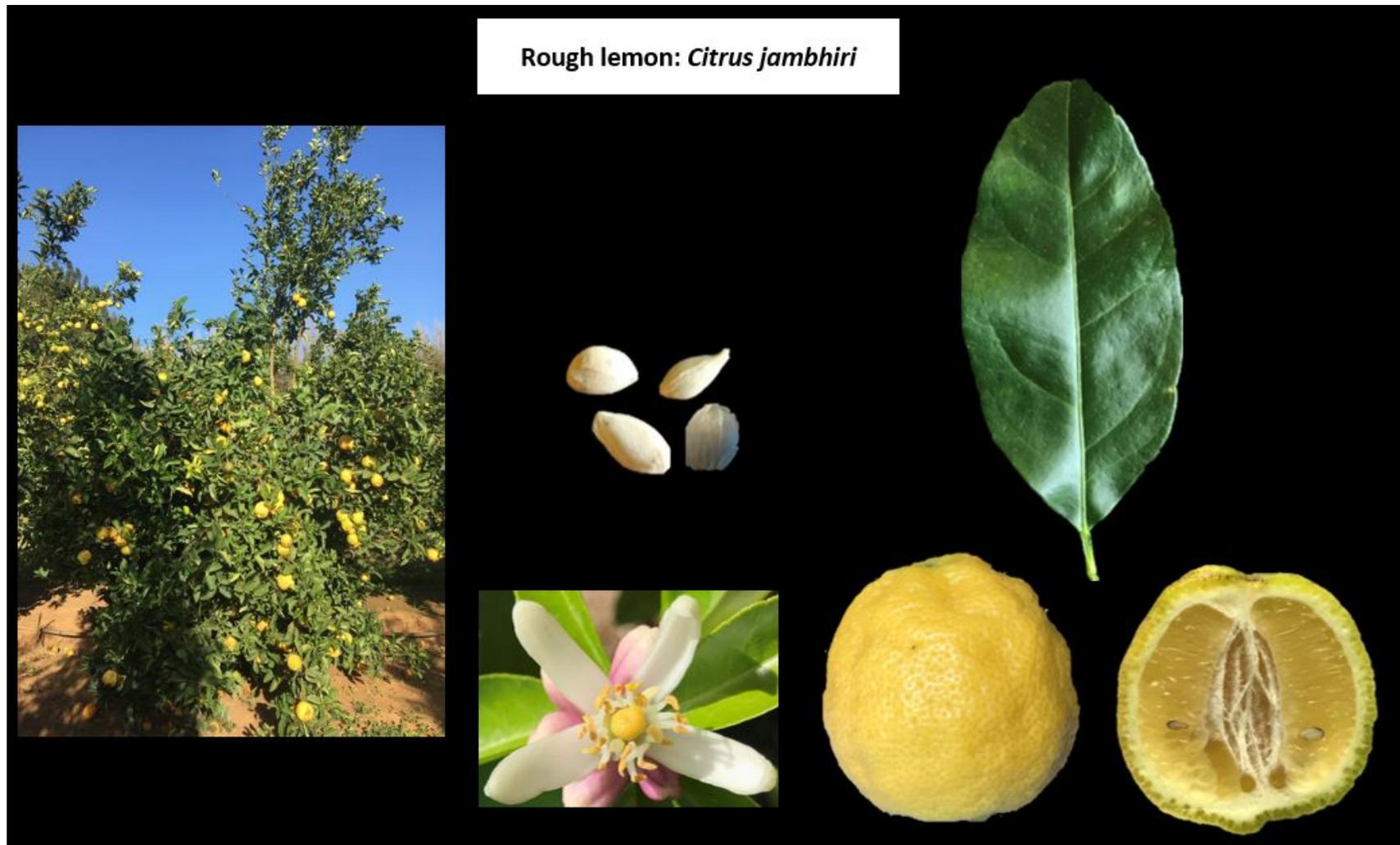
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Addendum A



**Figure 1.** An illustration of tree growth, flower at full bloom, seeds, fruit appearance at harvest, and mono-foliate leaf (no wings at the petiole) of a Rough lemon rootstock.

*\*Note: The photos are not 100% according to scale and merely serve as an illustration.*

X639 citrandarin: Cleopatra mandarin (*Citrus reticulata*) x *Poncirus trifoliata*



**Figure 2.** An illustration of the tree growth, flower at full bloom, seeds, fruit appearance at harvest, and the tri-foliolate leaf of a X639 citrandarin.

*\*Note: The photos are not 100% according to scale and merely serve as an illustration.*

Carrizo citrange: *Citrus sinensis* x *Poncirus trifoliata*



**Figure 3.** An illustration of the tree growth, flower at full bloom, seeds, fruit appearance at harvest, and tri-foliolate leaf of a Carrizo citrange rootstock.  
*\*Note: The photos are not 100% according to scale and merely serve as an illustration.*

Swingle citrumelo: *Citrus paradisi* x *Poncirus trifoliata*



**Figure 4.** An illustration of the tree growth, flower at full bloom, seeds, fruit appearance at harvest, and tri-foliolate leaf of a Swingle citrumelo rootstock.  
*\*Note: The photos are not 100% according to scale and merely serve as an illustration.*

MxT : Minneola (*Citrus paradisi* x *Citrus reticulata*) x *Poncirus trifoliata*



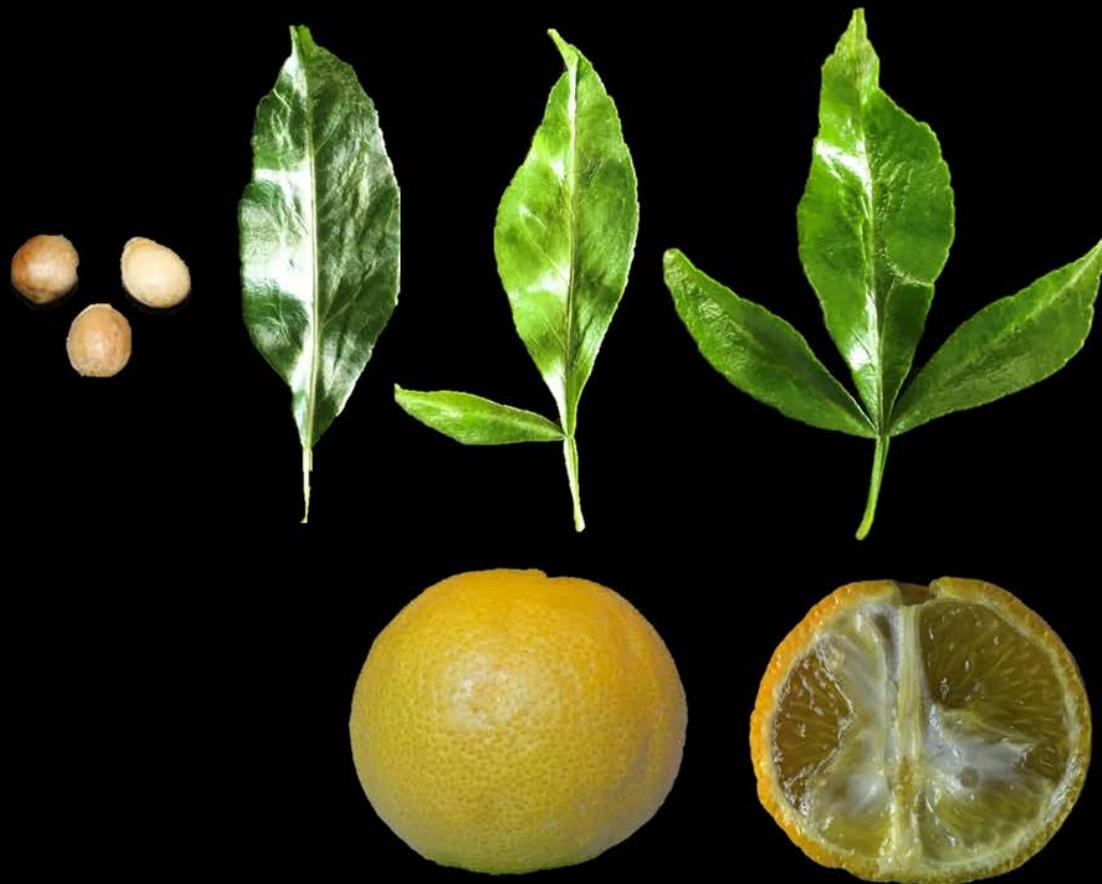
**Figure 5.** An illustration of the tree growth, flower at full bloom, seeds, fruit appearance at harvest, and tri-foliolate leaf of a MxT rootstock.  
*\*Note: The photos are not 100% according to scale and merely serve as an illustration.*

**C-35 citrange: *Citrus sinensis* x *Poncirus trifoliata***



**Figure 6.** An illustration of the tree growth, flower at full bloom, seeds, fruit appearance at harvest, and tri-foliolate leaf of a C-35 rootstock.  
*\*Note: The photos are not 100% according to scale and merely serve as an illustration.*

US-812: Sunki Mandarin (*Citrus reticulata*) x Benecke trifoliolate orange (*Poncirus trifoliata*)



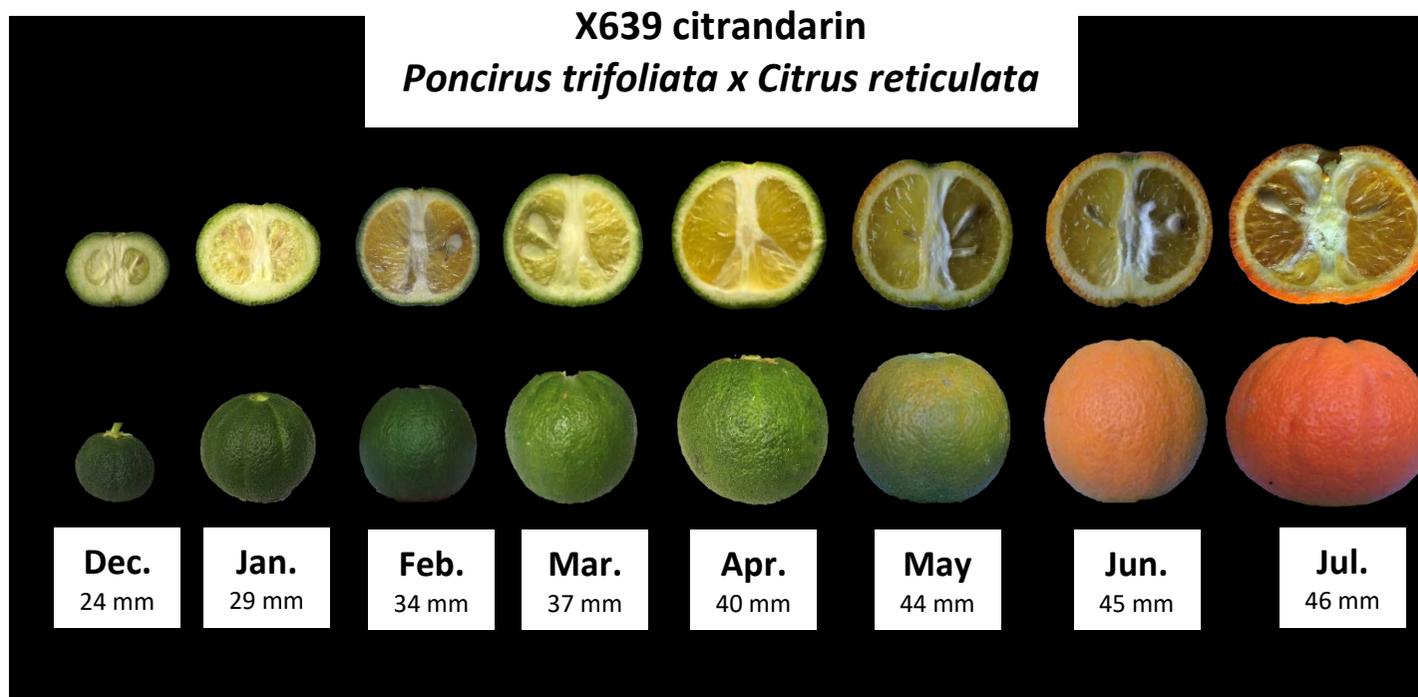
**Figure 7.** An illustration of the tree growth, seeds, fruit appearance at harvest, and the tri-foliolate leaf of US-812.  
*\*Note: The photos are not 100% according to scale and merely serve as an illustration.*

Addendum B



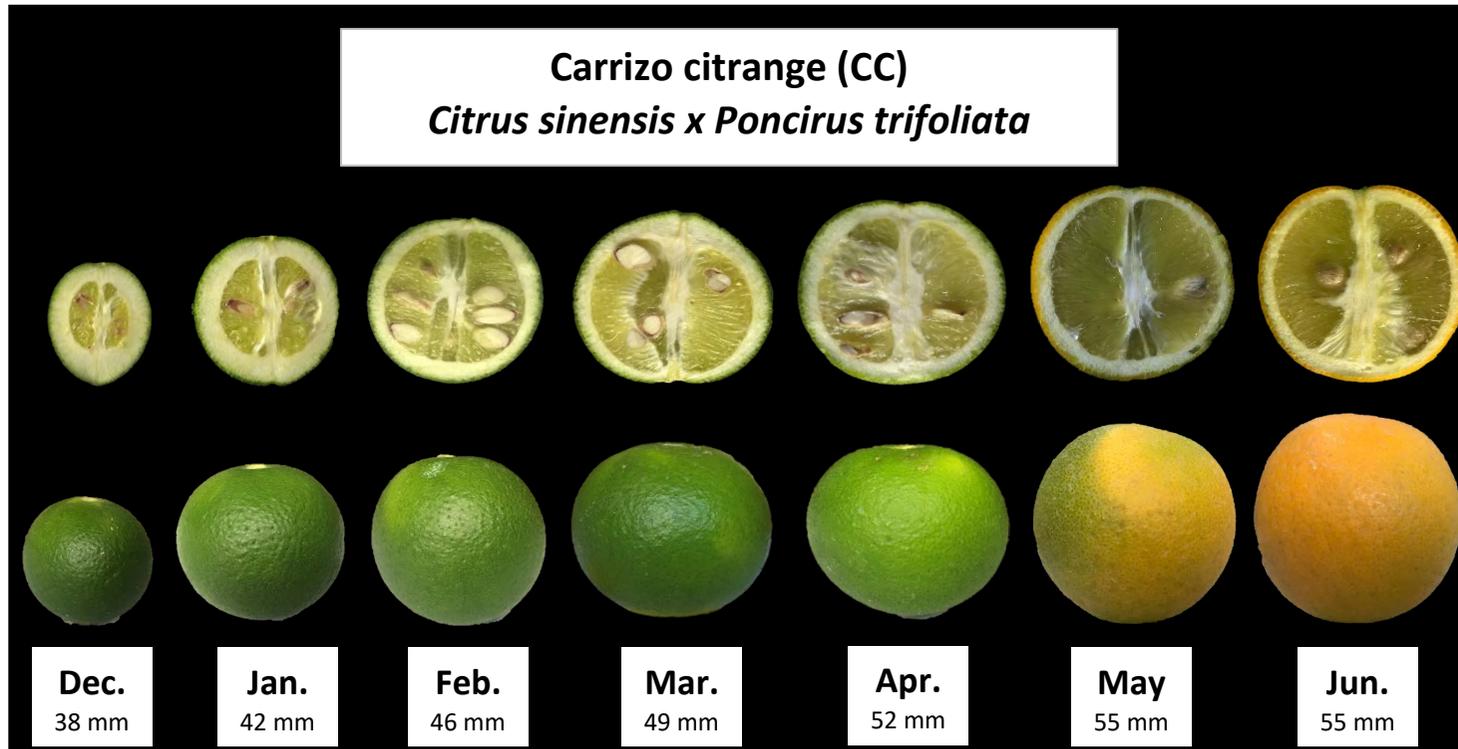
**Figure 1.** Fruit development from Dec. to Harvest for C-35.

*\*Note: the photos of the fruit is a percentage of the fruit diameter shown in the photo.*



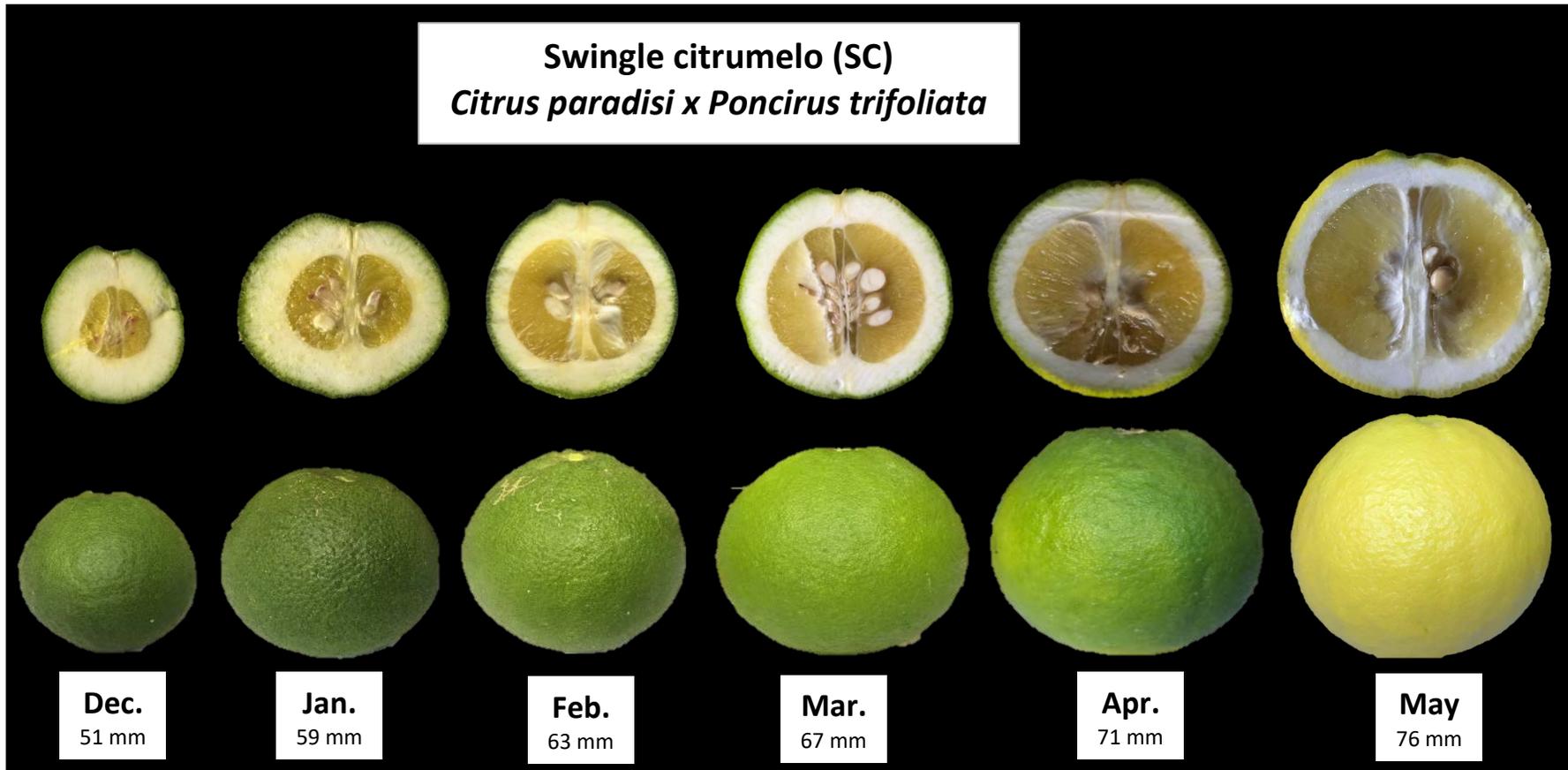
**Figure 2.** Fruit development from Dec. to Harvest for X639.

*\*Note: the photos of the fruit is a percentage of the fruit diameter shown in the photo.*



**Figure 3.** Fruit development from Dec. to Harvest for CC.

*\*Note: the photos of the fruit is a percentage of the fruit diameter shown in the photo.*



**Figure 4.** Fruit development from Dec. to Harvest for SC.

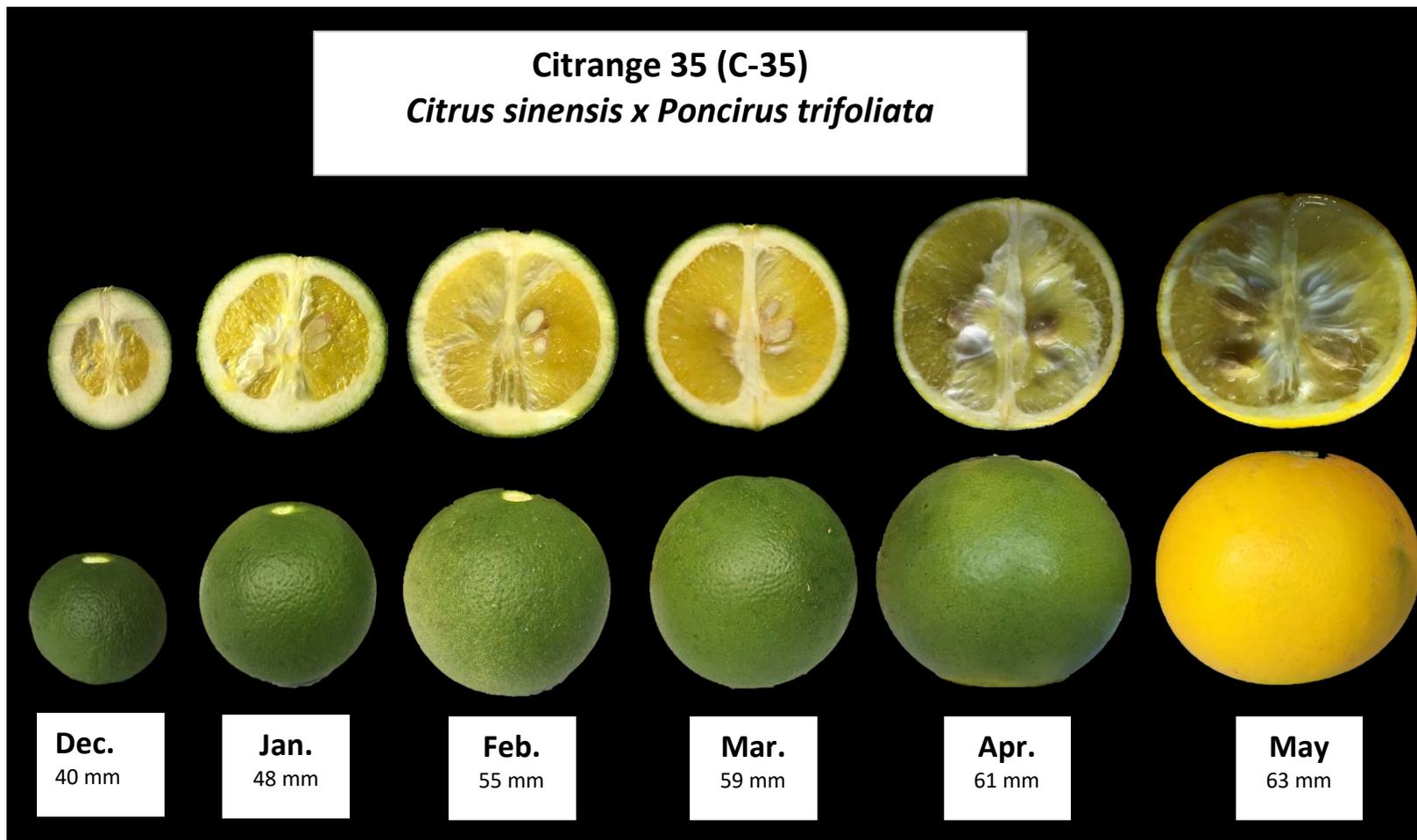
*\*Note the photos of the fruit is a percentage of the fruit diameter shown in the photo.*

**Mineola x trifoliata hybrid (MxT)**  
*(Citrus paradisi x C. reticulata) x Poncirus trifoliata*



**Figure 5.** Fruit development from Dec. to Harvest for MxT.

*\*Note: the photos of the fruit is a percentage of the fruit diameter shown in the photo.*



**Figure 6.** Fruit development from Dec. to Harvest for C-35.

*\*Note: the photos of the fruit is a percentage of the fruit diameter shown in the photo.*

**US-812 (Sunki x Benecke)**  
*Citrus reticulata x Poncirus trifoliata*



**Figure 7.** Fruit development from Dec. to Harvest for US-812.

*\*Note the photos of the fruit is a percentage of the fruit diameter shown in the photo.*

### Addendum C

**Table 1.** A summary of important horticultural properties of 7 important rootstocks used commercially in South Africa. The trees used to describe the horticultural properties are located at the Citrus Foundation Block in Uitenhage, Eastern Cape. All trees were planted in 2015 except for US-812, which was planted in 2010. The data presented here are an average of trials conducted during the 2019/2020 and 2020/2021 season.

	Rough Lemon <i>C. jambhiri</i>	Mineola x trifoliolate hybrid (MxT) ( <i>C. paradisi</i> x <i>C. reticulata</i> ) x <i>P. trifoliata</i>	Carrizo citrange <i>C. sinensis</i> x <i>P. trifoliata</i>	Swingle citrumelo <i>C. paradisi</i> x <i>P. trifoliata</i>	Citrange 35 (C-35) <i>C. sinensis</i> x <i>P. trifoliata</i>	X639 citrandarin <i>P. trifoliata</i> x <i>C. reticulata</i>	US-812 (US x Benecke) <i>C. reticulata</i> x <i>P. trifoliata</i>
Tree vigor	Vigorous	Vigorous	Intermediate	Intermediate	Intermediate	Intermediate	Vigorous
Type of leaves	Unifoliolate with no petiole wings.	High presence of unifoliolate (winged petioles) compared to trifoliolate leaves.	High presence of trifoliolate leaves, and low % of unifoliolate (winged petiole)	High presence of trifoliolate leaves, and low % of unifoliolate (winged petiole)	High presence of trifoliolate leaves, and low % of unifoliolate (winged petiole)	Trifoliolate and unifoliolate (winged petiole) leaves	High presence of unifoliolate (winged petiole) and low % of trifoliolate leaves.
Fruit size	68 mm	67mm	56 mm	76 mm	61 mm	46 mm	39 mm
Average seed no./fruit quantified at colour-break stage before seed treatment process	16	16	17	19	19	10	3
Average Yield/tree (kg/tree)	102.5 Kg/tree	46 Kg/tree	61 Kg/tree	66 Kg/tree	25 Kg/tree	68 Kg/tree	n/a
Average fruit number/ tree.	761	367	707	388	301	2256.6	n/a.
	Rough Lemon <i>C. jambhiri</i>	Mineola x trifoliolate hybrid (MxT)	Carrizo citrange <i>C. sinensis</i> x <i>P. trifoliata</i>	Swingle citrumelo <i>C. paradisi</i> x <i>P. trifoliata</i>	Citrange 35 (C-35) <i>C. sinensis</i> x <i>P. trifoliata</i>	X639 citrandarin <i>P. trifoliata</i> x <i>C. reticulata</i>	US-812 (US x Benecke) <i>C. reticulata</i> x <i>P. trifoliata</i>

		<i>(C. paradisi x C. reticulata) x P. trifoliata</i>					
Viable seed in ml following seed extraction and grading.	1080 ml	584 ml	3338 ml	1480 ml	823 ml	3045 ml	n/a
Average Seediness/fruit after seed extraction and grading	8	7	13	10	13	4	n/a
Seed germination in oven (26-28 °C)	83%	59%	81%	73%	59%	83%	91%
Seed germination in greenhouse (30-40 °C) at 88d after sowed	91%	73%	83%	85%	83%	96%	97%
Multiple seedling emergence at 88d after sowing in greenhouse	47%	47%	45%	34%	22%	21%	55%
Average seedling height at 88d in greenhouse visual assessment.	5.5 cm	8.9 cm	5.6 cm	7.2 cm	5.6 cm	7.1 cm	7.0 cm
Average % 'off-type' seedlings from total seeds germinated at 88d	0.6%	0%	5.6%	1.6%	4.8%	0.3%	3.5%
Maturation period (2019/2020)	Mid (Mid-June)	Late (Early September)	Early (End of May)	Early (Mid-May)	Early (Mid-April)	Mid (Early- July)	Mid (Mid-June)

### 5.3.3 FINAL REPORT: Adaptive Nutrition Management Strategies for Improved Fruit Quality Project 1231(April 2019 – March 2022) by PJ Raath (CRI)

#### Summary

In this trial the response of Midnight Valencias in Nelspruit and Orri mandarins in De Wet to excessive fertilisation with N, P, K and Mg was investigated. A specific focus was the changes in the trees' nutritional status as expressed in leaf and fruit analysis, as well as vegetative growth responses, fruit set and effects on fruit quality. The goal was to determine whether fertilisation requirements could be established from early season leaf and/or fruit mineral composition data so that for in-season adjustments of the fertilisation programme and/or manipulation of mandarin and Valencia quality could be achieved. Ultimately, development of a strategy for improved fertilisation management and fertiliser usage, possibly through better monitoring of tree nutrient (especially N, P and K) status, was the intention. A notable lack of responsiveness of both Valencia and mandarin trees to both excessive and low rates of mineral nutrition was observed – with the exception of nitrogen (N). Consequently, it was concluded that in-season regular short-term changes in fertilisation rates in orchards that are amply fertilised have little value, and therefore early season leaf analysis also. Long-term tendencies should rather be used to establish the effect of over-fertilisation and adjust fertilisation programmes. The limited usefulness of foliar analysis in conditions of ample to over-supply of nutrients was emphasised – the maximum norms was particularly found to be irrelevant. The sufficiency ranges should be regarded as secondary to observation of long-term trends in the foliar analysis, and foliar analysis should not be the only indication whether the fertilisation programme followed are appropriate, especially in conditions of ample to high rates of fertilisation. Either leaves from non-fruit bearing or fruit-bearing shoots can be used to evaluate tree nutritional status, but the proposed adjustments from the norms for fruit-bearing shoots should be made if leaves are sampled from non-fruit bearing shoots. Finally, the lack of response in fruit quality in these situations of excessive fertilisation highlighted the senselessness of over-fertilisation - the ability to manipulate tree performance or fruit quality is completely lost.

#### Opsomming

In hierdie projek is die reaksie van Midnight Valencias in Nelspruit en Orri mandaryne in De Wet op oormatige bemesting met N, P, K en Mg ondersoek. Daar is gefokus op veranderinge in die bome se voedingstatus, soos uitgedruk in blaar- en vrugontledings, asook vegetatiewe groei, vrugset en die effek op vrugkwaliteit. Die doel was om vas te stel of bemestingsbehoefte bepaal kan word uit vroeë, in-seisoen, blaar- en/of vrugontledings, sodat aanpassings in die seisoen reeds gemaak kan word om vrugkwaliteit te manipuleer. Die uiteindelijke doelwit is om 'n strategie vir verbeterde bemestingsbestuur en verhoogde kunsmis gebruikseffektiwiteit met behulp van meer akkurate monitering daar te stel. Met uitsondering van stikstof (N), was daar 'n afwesigheid van waargenome reaksie op oormatige bemesting by beide die Valencia en mandaryn bome. Die gevolgtrekking was dat dit nie moontlik is om in-seisoen, korttermyn veranderinge in bemestingstoedienings te maak as die bome voldoende tot oorvoorsien aan voedingstowwe is nie. Langtermyn neigings moet eerder gebruik word om die effek van oorbemesting te monitor en gebruik word om aanpassings in bemestingsprogramme te maak. Blaarontledings in die herfs ( $\pm 180$  dae na volblom) is bevestig as geskik vir die evaluasie van bome se algemene voedingstatus en hul reaksie op die seisoen se bemestingsprogram – daar is 'n baie beperkte waarde in die neem van vroeëre monsters. Die beperkte waarde van blaarontledings in toestande van volop of oormatige bemesting is egter aangetoon, met die maksimum norme wat spesifiek as waardeloos bevind is. Die optimale grenswaardes moet as sekondêr tot die waarneming van langtermyn neigings beskou word, en dus nie as die enigste aanduiding van die geskiktheid van 'n bemestingsprogram beskou word nie – veral nie in toestande van volop of oorbemesting nie. Hetsy blare van nie-vrugdraende lote of vrugdraende lote kan ontleed word om bome se voedingstatus mee te evalueer, maar die voorgestelde aanpassings in die norme van vrugdraende lote se blare moet gedoen word. Laastens, die tekort aan reaksie op oorbemesting sover dit vrugkwaliteit aangaan, het die tekort aan logika van oorbemesting beklemtoon – die vermoë om vrugkwaliteit te manipuleer gaan verlore.

#### Introduction

Fertilisation of citrus trees is regarded as an important factor that influences photosynthesis, flowering, fruit set, fruit growth and fruit quality. According to Ladaniya (2008) balanced nutrient management is the key for producing good quality citrus fruit with desired storage ability. Progress in citrus tree nutrition has led to the incorporation of various practices to better manipulate tree physiology and ensure improved set, higher production and superior fruit quality (Coetzee, 2007). Lately producers are confronted by concepts such as geoinformatics (to address spatial variation), site-specific nutrient management strategies, open field hydroponics in a low-flow context, or the use of both organic matter and soil microbiology, to improve fertilisation efficiency and improve the conventional methods of nutrient management (Srivastava, 2012). Despite all these concepts, citrus producers need the ability to practice effective decision making with regards to tree nutrition, *viz.*, to do “nutrient management through soil and foliar applications, as the need arises”. Alternatively phrased, they need verified “adaptive management strategies” to use so that a specific objective (e.g., improved set or fruit size, thicker skins, larger fruit, etc.) can be achieved.

This research project elucidated the possibility of improved decision-making regarding N, P and K fertilisation through possible development and the use of norms for early leaf sampling, fruit growth patterns and changes in mineral composition of fruit. The goals of achieving improved set, and enhanced management of fruit size, skin- and internal quality, was also addressed.

### **Stated objectives**

- To establish whether a fertilisation requirement model can be developed from early season leaf and/or fruit mineral composition data for *in-season* (i) adjustments of the fertilisation programme and (ii) manipulation of mandarin and Valencia quality.
- To establish to what extent *higher rates* of N, P, K and Mg fertilisation rates affect fruit set, fruit development, and fruit quality.
- Improve fertilisation management and optimise fertiliser usage (so called “best management practice”) by better monitoring tree nutrient (especially N, P and K) status.

### **Materials and methods**

#### Trial plots

The experiment was conducted in two locations, on two varieties, respectively:

Plot 1: ‘Orri’ mandarin trees budded onto ‘Carrizo’ citrange rootstock in a commercial four-year-old orchard in the De Wet area, Western Cape;

Plot 2: A mature, 10-year-old commercial ‘Midnight’ Valencia/C35 citrange orchard at Croc valley, Nelspruit.

All trees received the standard farm practice fertiliser applications, with the rate of application based on OHS principles (“Orri” Mandarin block); and annual leaf mineral nutrient analysis together with an average target fruit yield (“Midnight” Valencia block).

#### Treatments and experimental design

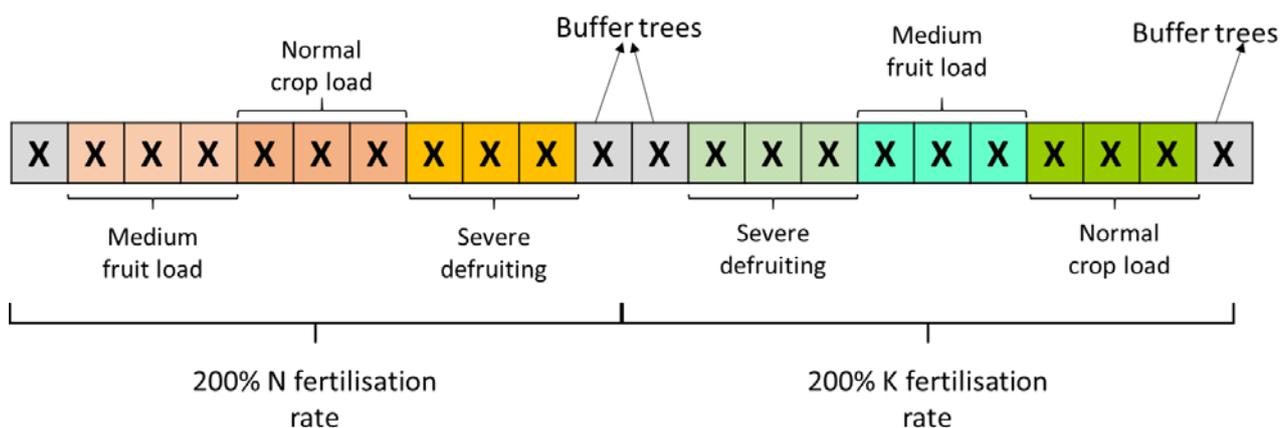
The experiment consists of two phases:

Phase 1 (July 2018 to harvest 2019): In two locations, i.e., Stellenbosch and Nelspruit, two mandarin (Orri and Nadorcott) and Valencia (Midnight and Delta) cultivars were respectively selected to establish a baseline trend of changes in leaf and fruit mineral nutrient content throughout the season. In both locations, the respective cultivars were planted in blocks that were similar in age and on the same soil types. In each area, for both varieties, twenty trial trees that are uniform in size, fruit load and health were selected before the start of commercial harvest in 2018. They were divided into ten randomly located two-tree plots (n=10) from which mature leaves, flowers, fruitlets and fruit samples were collected at monthly intervals, starting in July (2018), and finishing in March (2019).

Phase 2 (July 2019 to harvest 2021): An experiment was set up in both locations in a randomised complete block design using plots of 11 trees, replicated four times. Five main treatments of fertiliser application were

applied (Table 5.3.3.1). In each experimental plot the middle nine trees were used for data collection (Figure 5.3.3.1). They were split in three groups of three trees, on which three de-fruiting treatments were respectively applied at random (Table 5.3.3.1) early December 2019. For the moderate de-fruiting treatment, all fruit smaller than 10 mm were removed by hand. For the severe de-fruiting treatment, all fruit smaller than 15 mm were removed.

The fertilisation applied as main treatments are described in Tables 5.3.3.2 and 5.3.3.3, while the actual applied amounts of each nutrient are presented in Tables 5.3.3.4 and 5.3.3.5.



**Figure 5.3.3.1.** Illustration of two neighbouring experimental plots indicating the randomised crop load treatments within each set of nine trees upon which the main fertilisation treatment is applied.

**Table 5.3.3.1.** Exposition of the various treatments that were applied.

Treatment number	Treatment name	Description of treatments
<b>Fertilisation trial</b>		
1.1	Control	Farm's prescribed standard fertilisation programme followed, with moderate defruiting in November/December to obtain medium fruit load.
1.2	N	Application of 200% <b>N</b> of the standard recommended N fertilisation
1.3	P	Application of 200% <b>P</b> of the standard recommended P fertilisation
1.4	K	Application of 200% <b>K</b> of the standard recommended K fertilisation
1.5	Mg	Application of 200% <b>Mg</b> of the standard recommended Mg fertilisation
<b>Crop load trial</b>		
2.1	Control	Untreated trees, heavy fruit load, commercial practice without fruit thinning.
2.2 (also 1.1)	Medium fruit load	Moderate defruiting in November/December to obtain medium fruit load
2.3	Severe defruiting	Severe defruiting in November/December to obtain low fruit load

**Table 5.3.3.2.** Fertilisation programme followed throughout the 2019/2020 and 2020/2021 seasons respectively, applied as standard farm practice on the De Wet/Orrri Mandarin trial plot – the indicated amounts were doubled (applied at 200% rate) for the respective treatments above.

Month 2019/20	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	TOTAL
N kg/ha	12	17	35	52	69	75	75	75	69	35	35	29	577

P		0	0	1	1	1	1	1	1	1	1	1	1	10
K		4	6	13	19	26	28	28	28	26	13	13	11	215
Mg		2	3	5	8	11	12	12	12	11	5	5	5	90
Percentage of total annual N application		2%	3%	6%	9%	12%	13%	13%	13%	12%	6%	6%	5%	100%

**Table 5.3.3.3.** Fertilisation programme followed throughout the 2019/2020 and 2020/2021 seasons respectively, applied as standard farm practice on the Nelspruit/*Midnight Valencia* trial plot – the indicated amounts were doubled (applied at 200% rate) for the respective treatments above.

Month 2019/20		Jul	Aug	Sep	Oct	Nov	TOTAL
N	kg/ha	50	2.5	27	9	2	90
P		27	0	5	2	0.5	35
K		0	0	82	27	5	114
Mg*		0	0 (12)*	0 (12)*	0 (12)*	0	0 (36)*
Percentage of total annual N application		55%	3%	30%	10%	2%	100%

\*The total annual Mg that was applied in Treatment 1.5 was 36 kg/ha, split up in three instalments from August to October.

#### Data generation

*Quantify flowering, fruit set and vegetative shoot development:* The number of flowers and total number of new vegetative shoots after cessation of periods of vegetative shoot flushes in spring (November), summer (February) and autumn (April) were determined per tree. The phenological pattern in individual shoots were followed by randomly selecting ten shoots from each tree.

*Leaf, flower and fruit sampling for mineral nutrient analysis:* On a monthly basis, mature leaves were sampled from spring flushes (collected from vegetative shoots that developed during the previous season's vegetative shoot flushes), through summer (samples collected from vegetative shoots that developed during the current season's spring vegetative shoot flush), and autumn (samples collected from vegetative shoots that developed during the summer vegetative shoot flush). Selecting leaves on fully hardened, non-fruiting and purely vegetative shoots at a height of 1.5 m of the ground from the outer layer of the tree canopy, 20 leaves at the third to fifth position from the latest applicable flush, on at least four shoots per tree were sampled. An additional sample from fruit bearing shoots were also collected in autumn.

At anthesis, 80 full flowers were sampled at random around the tree from terminal positions at about 1.5 m from the ground.

Fifty fruitlets were sampled on a monthly basis from anthesis until autumn. They were taken at random around the tree from single-fruiting shoots on the terminal position, 1.5 m from the ground. In addition, the fruit were also sampled at harvest, at random, i.e., different canopy positions.

*Fruit growth:* When the fruitlets reached a diameter of  $\pm 20$  mm, or after November fruit fall, ten were tagged per tree at 1.5 m from the ground per treatment/replicate. Fruit size (diameter) was then measured on a monthly basis until autumn (March/April), and then again at harvest, but then at random from different canopy positions. Fruit diameter were measured with an electronic fruit size measurer (EFM) and data logger [EFM & Data Logger; Güss Manufacturing (Pty) Ltd, Strand, South Africa].

*Fruit yield:* Total fruit yield was determined when harvest commenced (*viz.*, when the fruit quality indices complied to specifications established by fruit export markets) by stripping the trees, counting, and weighing each tree's fruit separately.

**Table 5.3.3.4.** The actual applied amounts of each nutrient to the *Orri Mandarin* orchard at DeWet during the two seasons.

<b>First season (2019/2020)</b>														
Supply	Nutrient (kg/ha)	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	Apr	May	Jun	TOTAL
Commercial nutrient supply*	N	11.5	17.3	34.6	51.9	69.2	75.0	75.0	75.0	69.2	34.6	34.6	28.9	<b>577</b>
	P	0.2	0.3	0.6	0.9	1.2	1.3	1.3	1.3	1.2	0.6	0.6	0.5	<b>10</b>
	K	4.3	6.5	12.9	19.4	25.8	28.0	28.0	28.0	25.8	12.9	12.9	10.8	<b>215</b>
	Mg	1.8	2.7	5.4	8.1	10.8	11.7	11.7	11.7	10.8	5.4	5.4	4.5	<b>90</b>
Additional treatment supply**	<b>N</b>	11.5	17.3	34.6	51.9	69.2	75.0	75.0	75.0	69.2	34.6	34.6	28.9	<b>577</b>
	<b>P</b>	0.1	0.15	0.3	0.45	0.6	0.65	0.65	0.65	0.6	0.3	0.3	0.25	<b>5</b>
	<b>K</b>	4.3	6.5	12.9	19.4	25.8	28.0	28.0	28.0	25.8	12.9	12.9	10.8	<b>215</b>
	<b>Mg</b>	1.8	2.7	5.4	8.1	10.8	11.7	11.7	11.7	10.8	5.4	5.4	4.5	<b>90</b>
Total Nutrient supply**	<b>N</b>	23.1	34.6	69.2	103.9	138.5	150.0	150.0	150.0	138.5	69.2	69.2	57.7	<b>1154</b>
	<b>P</b>	0.3	0.45	0.9	1.35	1.8	1.95	1.95	1.95	1.8	0.9	0.9	0.75	<b>15</b>
	<b>K</b>	8.6	12.9	25.8	38.7	51.6	55.9	55.9	55.9	51.6	25.8	25.8	21.5	<b>430</b>
	<b>Mg</b>	3.6	5.4	10.8	16.2	21.6	23.4	23.4	23.4	21.6	10.8	10.8	9.0	<b>180</b>
<b>Second season (2020/2021)</b>														
Supply	Nutrient (kg/ha)	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	Apr	May	Jun	TOTAL
Commercial nutrient supply*	N	11.5	17.3	34.6	51.9	69.2	75.0	75.0	75.0	69.2	34.6	34.6	28.9	<b>577</b>
	P	0.2	0.3	0.6	0.9	1.2	1.3	1.3	1.3	1.2	0.6	0.6	0.5	<b>10</b>
	K	4.3	6.5	12.9	19.4	25.8	28.0	28.0	28.0	25.8	12.9	12.9	10.8	<b>215</b>
	Mg	1.8	2.7	5.4	8.1	10.8	11.7	11.7	11.7	10.8	5.4	5.4	4.5	<b>90</b>
Additional treatment supply**	<b>N</b>	11.5	17.3	34.6	51.9	69.2	75.0	75.0	75.0	69.2	34.6	34.6	28.9	<b>577</b>
	<b>P</b>	0.2	0.3	0.6	0.9	1.2	1.3	1.3	1.3	1.2	0.6	0.6	0.5	<b>10</b>
	<b>K</b>	4.3	6.5	12.9	19.4	25.8	28.0	28.0	28.0	25.8	12.9	12.9	10.8	<b>215</b>
	<b>Mg</b>	1.8	2.7	5.4	8.1	10.8	11.7	11.7	11.7	10.8	5.4	5.4	4.5	<b>90</b>
Total Nutrient supply**	<b>N</b>	23.1	34.6	69.2	103.9	138.5	150.0	150.0	150.0	138.5	69.2	69.2	57.7	<b>1154</b>
	<b>P</b>	0.4	0.6	1.2	1.8	2.4	2.6	2.6	2.6	2.4	1.2	1.2	1.0	<b>20</b>
	<b>K</b>	8.6	12.9	25.8	38.7	51.6	55.9	55.9	55.9	51.6	25.8	25.8	21.5	<b>430</b>
	<b>Mg</b>	3.6	5.4	10.8	16.2	21.6	23.4	23.4	23.4	21.6	10.8	10.8	9.0	<b>180</b>

\*The control treatment received only the commercial nutrient supply, i.e., the producer's typical fertilisation rates.

\*\*N, P, K, Mg in this row represents the treatments.

**Table 5.3.3.5.** The actual applied amounts of each nutrient to the *Midknight Valencia* orchard at Nelspruit during the two seasons.

<b>First season (2019/2020)</b>														
Supply	Nutrient (kg/ha)	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	Apr	May	Jun	TOTAL
Commercial nutrient supply*	N	60.8	2.5	27.1	9.0	1.8	-	-	-	-	-	-	-	101
	P	24.3	-	5.4	1.8	0.4	-	-	-	-	-	-	-	32
	K	-	-	81.6	27.2	5.4	-	-	-	-	-	-	-	114
	Mg	-	-	-	-	-	-	-	-	-	-	-	-	0
Additional treatment supply**	N	-	64.7	28.8	9.0	-	-	-	-	-	-	-	-	103
	P	-	24.3	5.8	-	-	-	-	-	-	-	-	-	30
	K	-	-	80.9	27.2	-	-	-	-	-	-	-	-	108
	Mg	-	72.7	-	-	-	-	-	-	-	-	-	-	73
Total nutrient supply*	N	60.8	67.2	55.9	18.0	1.8	-	-	-	-	-	-	-	204
	P	24.3	24.3	11.2	1.8	0.4	-	-	-	-	-	-	-	62
	K	-	-	162.5	54.4	5.4	-	-	-	-	-	-	-	222
	Mg	-	72.7	-	-	-	-	-	-	-	-	-	-	73
<b>Second season (2020/2021)</b>														
Supply	Nutrient (kg/ha)	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	Apr	May	Jun	TOTAL
Commercial nutrient supply*	N	50.0	35.8	27.1	9.0	-	-	-	-	-	-	-	-	122
	P	-	24.3	5.4	1.8	-	-	-	-	-	-	-	-	32
	K	-	-	81.6	27.2	-	-	-	-	-	-	-	-	109
	Mg	-	-	-	-	-	-	-	-	-	-	-	-	0
Additional treatment supply**	N	-	85.9	27	9.7	-	-	-	-	-	-	-	-	123
	P	-	24.3	-	-	-	-	-	-	-	-	-	-	24
	K	-	-	82.8	27	-	-	-	-	-	-	-	-	110
	Mg	-	72.7	-	-	-	-	-	-	-	-	-	-	73
Total nutrient supply**	N	50.0	121.7	54.1	10.7	-	-	-	-	-	-	-	-	237
	P	-	44.6	5.4	1.8	-	-	-	-	-	-	-	-	52
	K	-	-	164.4	54.2	-	-	-	-	-	-	-	-	219
	Mg	-	72.7	-	-	-	-	-	-	-	-	-	-	73

\*The control treatment received only the commercial nutrient supply, i.e., the producer's typical fertilisation rates.

\*\*N, P, K, Mg in this row represents the treatments.

**Fruit quality:** One week before the orchards were commercially harvested, a sample of 12 randomly selected fruit per data tree were collected to determine the effects of treatments on fruit quality attributes. This was done in the laboratory and the quality parameters evaluated were fruit size (diameter and weight), colour, rind thickness, total soluble solids (TSS), titratable acidity (TA) and juice percentage (%). Fruit size was measured using the Güss EFM and data logger, and then cut in half to measure rind thickness at opposite sides using an electronic caliper (CD-6" C; Mitutoyo Corp, Tokyo, Japan) and to calculate the average rind thickness. Fruit colour was evaluated using the standard colour charts. A fruit juicer (USst®, Chicago, IL) was then used to extract the juice from the fruit to calculate the juice percentage, measure the TSS with a refractometer (PR-32 Palette, Atago Co., Tokyo, Japan) and determine the TA by titration with NaOH as base and phenolphthalein as indicator. The TSS:TA was calculated by dividing the TSS by the TA.

**Phase 3 (August 2021 to March 2022):** In response to results obtained from the above trials, it was decided to investigate to what extent tree nutritional status, expressed in foliar N and K analysis, is affected in conditions of no or low fertilisation rates. A one-year trial was therefore set up in a Nardocott Mandarin block on the Welgevallen experimental farm, Stellenbosch. The experimental design was a randomised block design using four tree plots replicated four times. Nitrogen and K was applied in combination at increasing annual levels of 0 kg/ha, 50 kg/ha, 100 kg/ha and 150 kg/ha, respectively in the form of LAN and K<sub>2</sub>SO<sub>4</sub>, i.e., 16 treatments. Leaf analysis was conducted on leaves sampled in March 2022 from mature spring flush shoots with terminal fruit.

### **Statistical analysis**

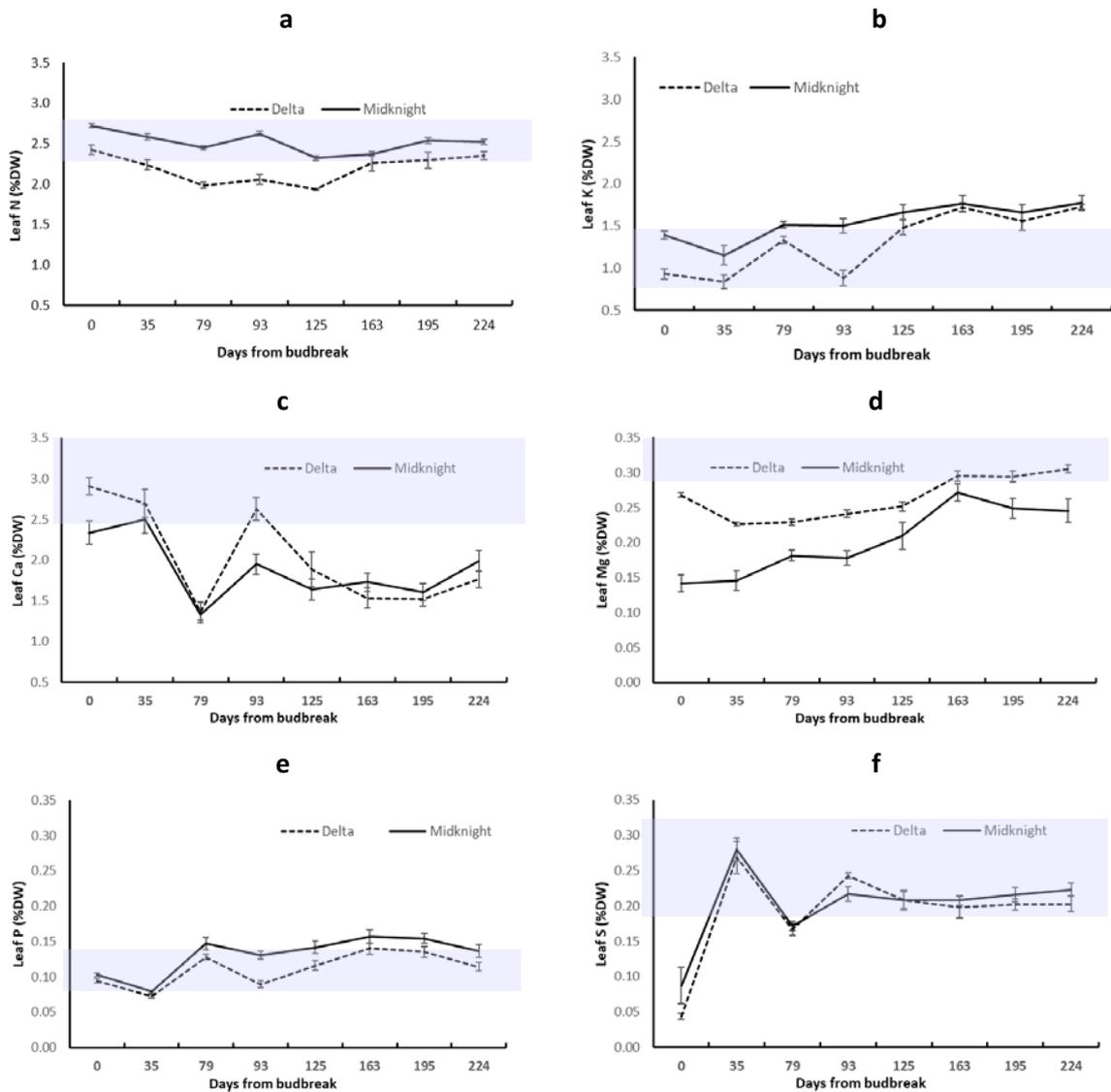
SAS data analysis software (Version 9.4; SAS Institute Inc, Cary, USA) was used to analyse the data. Analysis of variance (ANOVA) or repeated-measures ANOVA was performed when responses were repeated on the same respondent. Mean separations were carried out using Fisher's least significant difference test where it was applicable, at a  $P \leq 0.05$ .

## **Results and discussion**

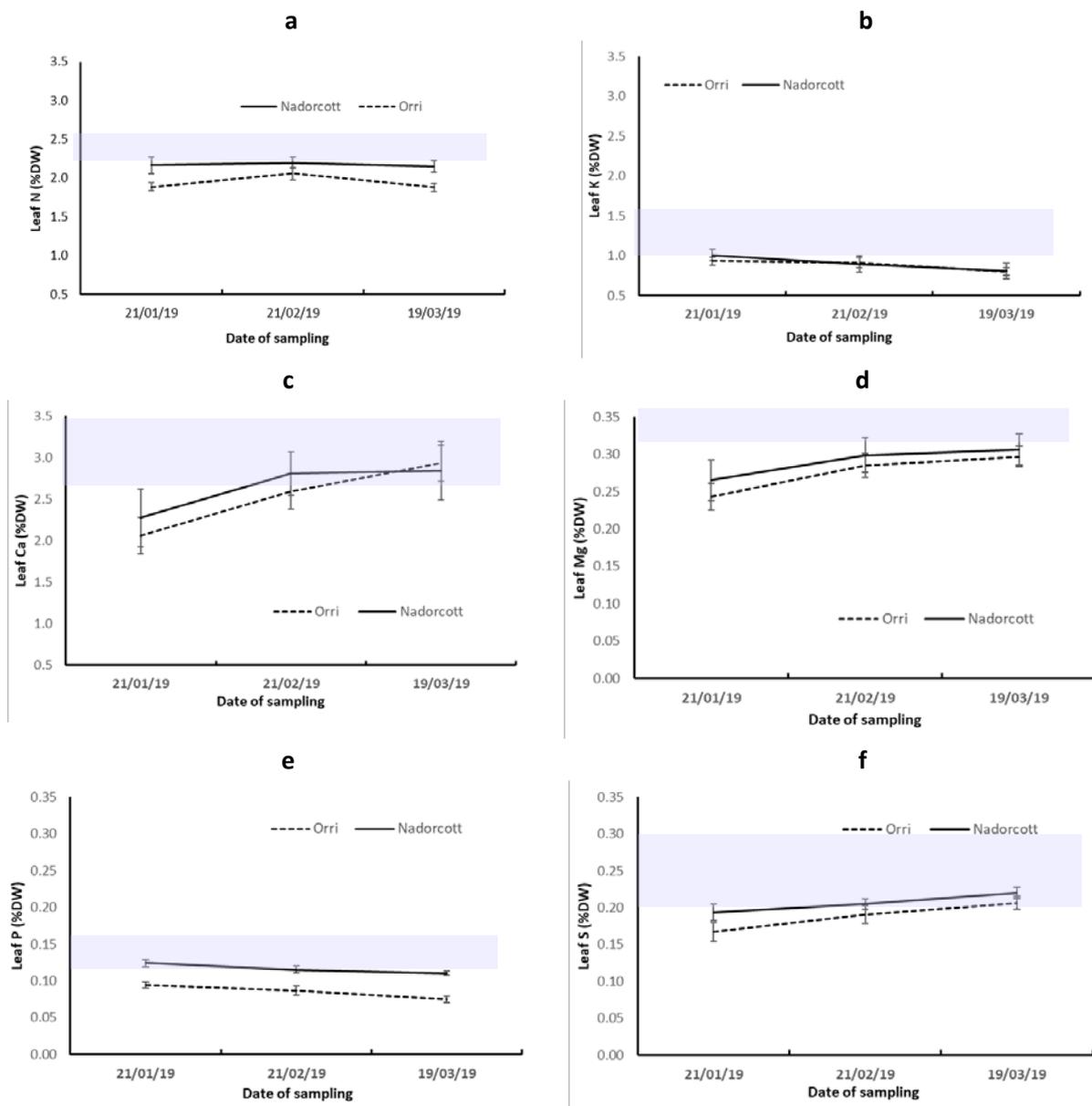
### **Phase 1**

Although significant differences were obtained between the Delta and Midnight Valencia leaf N, K, Ca, Mg, P and S concentrations, the seasonal trends in leaf nutrient concentrations were very similar (Figure 5.3.3.2). Likewise, for the Orri and Nardocott mandarins (Figure 5.3.3.3). The differences in absolute concentrations between the respective cultivars can be due to various factors, e.g., differences in soil nutrient supply, levels of fertilisation, different rootstocks. Sheng *et al.* (2009) also found similar trends in foliar Ca and K nutrient concentrations of the two navel cultivars they studied, i.e., 'Newhall' and 'Skagg's Bonanza'. These patterns corresponded to those obtained in this trial and can be described as relatively constant throughout the whole season. The exception is Mg, where leaf Mg concentration in their studied cultivars showed a steady declining trend throughout the season. Except for a slight but steady decline in foliar K concentration of "Star Ruby" grapefruit, Singh *et al.* (2016) also found that the nutrient concentrations of both fruiting and non-fruiting terminals remained stable throughout the year.

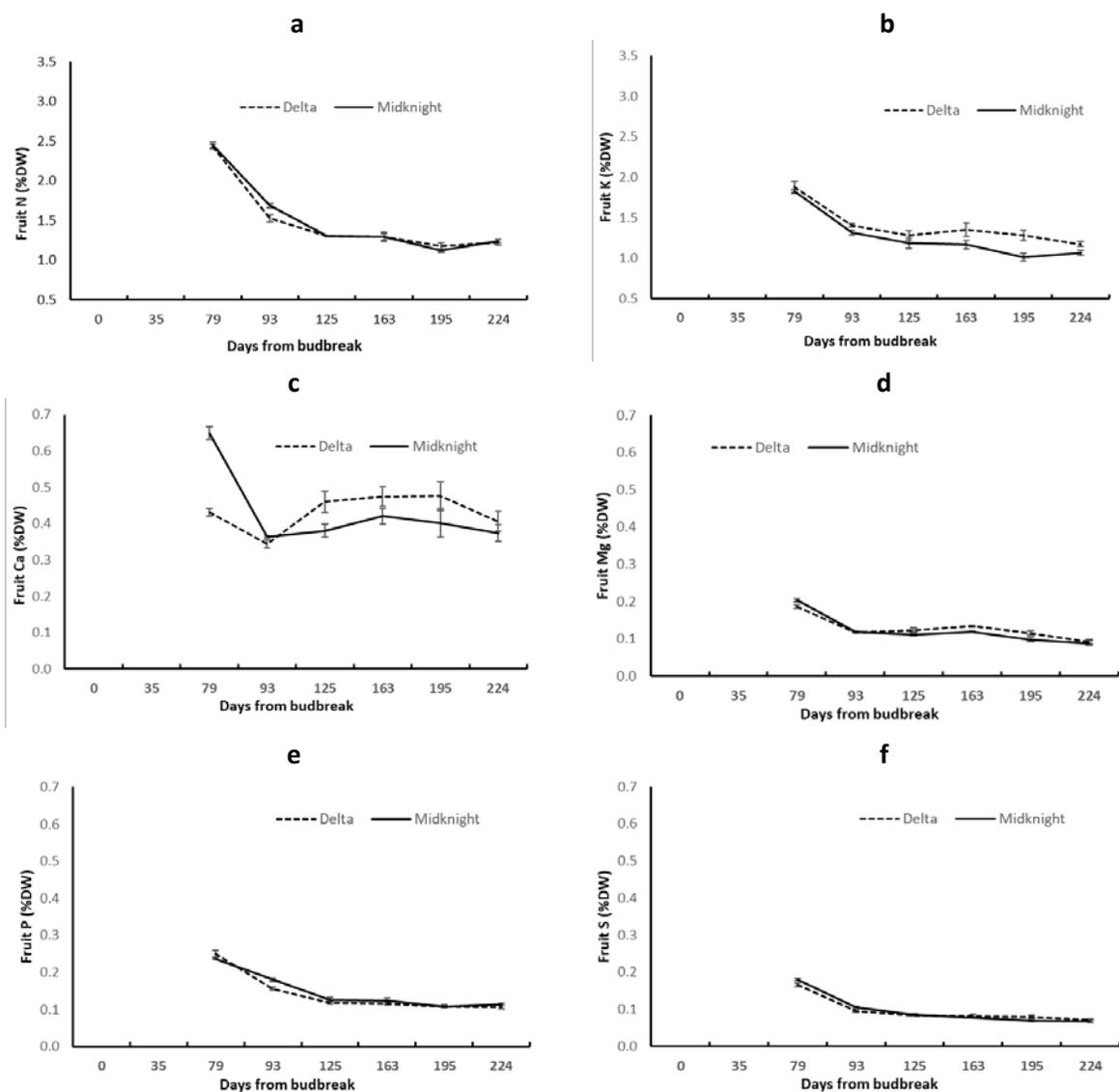
Fruit nutrient concentration differences between the respective pairs of cultivars were less pronounced, with slightly higher concentrations of only K and Ca in the Delta fruit compared to Midnight Valencias (Figure 5.3.3.4) and K in the Nardocott compared to Orri Mandarins (Figure 5.3.3.5). The general trend of nutrient concentration during fruit development was to decrease rapidly during the first 14 days after fruit set (Figure 5.3.3.4). This corresponded to the trends obtained by Storey & Treeby (2000) for navel oranges, with the exception that they found an increase in the concentration of all elements during Stage I of fruit growth. Although different trends to that of Storey & Treeby (2000) were obtained regarding the concentration of nutrients in the fruit, these results confirmed their conclusion that Stage I is the period of fruit development in which there are the most rapid and extensive changes in nutrient concentration.



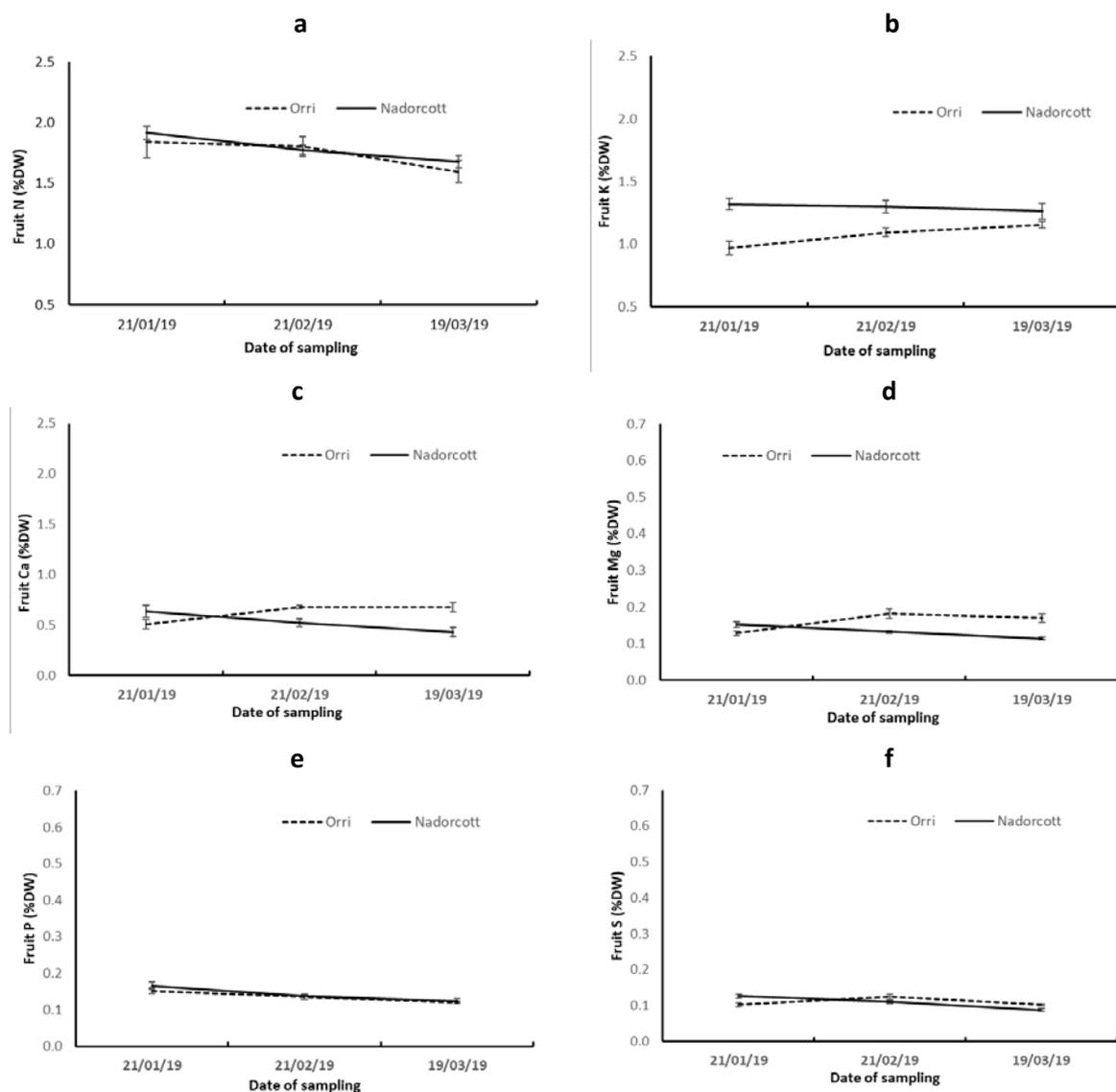
**Figure 5.3.3.2.** Concentration of nitrogen (a), potassium (b), calcium (c) magnesium (d), phosphorous (e) and sulphur (f) in leaves of mature *Delta* and *Midnight* Valencia trees during the 2018/2019 growing season in Nelspruit – mature leaves on the latest flush were respectively sampled. Shaded areas indicate the optimal range for fruiting terminals sampled in March-April.



**Figure 5.3.3.3.** Concentration of nitrogen (a), potassium (b), calcium (c) magnesium (d), phosphorous (e) and sulphur (f) in leaves of mature *Orri* and *Nadorcott* mandarin trees during the 2018/2019 growing season in Stellenbosch – mature leaves on the latest flush were respectively sampled. Shaded areas indicate the optimal range for fruiting terminals sampled in March-April.



**Figure 5.3.3.4.** Changes in the nitrogen (a), potassium (b), calcium (c) magnesium (d), phosphorous (e) and sulphur (f) concentrations of fruit from mature *Delta* and *Midnight* Valencia trees during the 2018/2019 growing season in Nelspruit.

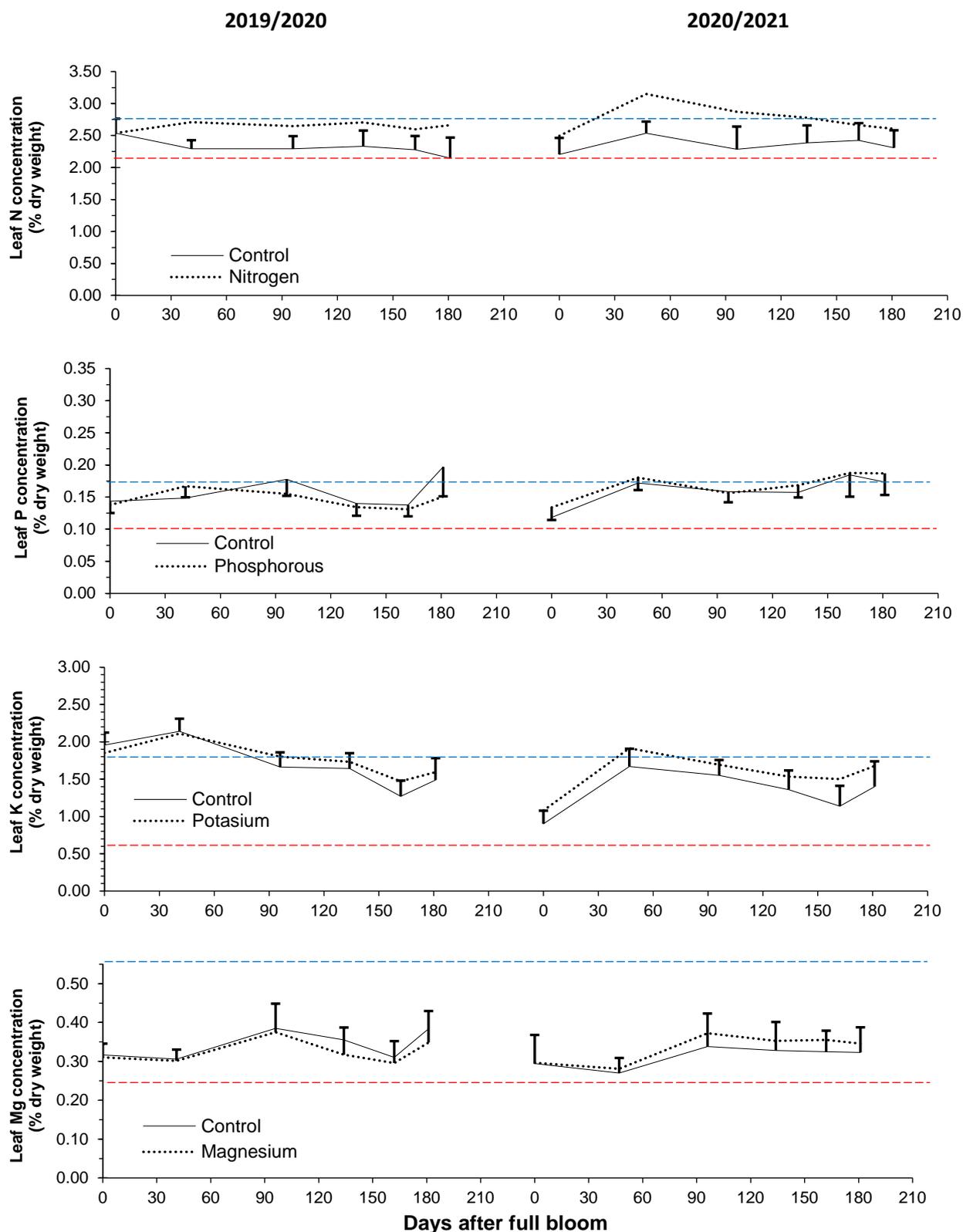


**Figure 5.3.3.5.** Changes in the nitrogen (a), potassium (b), calcium (c) magnesium (d), phosphorous (e) and sulphur (f) concentrations of fruit from mature *Orri* and *Nadorcott* mandarin trees during the 2018/2019 growing season in Stellenbosch.

## Phase 2

### Seasonal changes in leaf nutrient concentration and the effect of the fertilisation treatments on leaf nutrient concentration

*Midknight Valencia trees (Nelspruit):* A response in *only the leaf N* concentration of the trees that were fertilised at a 200% rate of the standard practices could be observed from 30 days after full bloom in the first season (Figure 5.3.3.6). This indicates to an ability of citrus trees to respond quickly to additional N fertilisation – Scholberg *et al.* (2002) even found that a residence time of 8 hours of increased N is sufficient to obtain a response. The same rapid response to the excessive fertilisation, however, did not occur for P, K or Mg (Figure 5.3.3.6). The trees were especially non-responsive to the additionally applied P, with no shift in the leaf P concentrations even in the second season of the additionally applied P. This corresponds to Coetzee (2007) who maintains that well supplied citrus trees do not respond to additionally applied P – this was the case for this experimental orchard (Table 5.3.3.6). A delayed response to the excessive K applications were observed that only became significant in the second season, i.e., 2020/2021 (Figure 5.3.3.6).



**Figure 5.3.3.6.** Seasonal changes in nutrient concentration of *Midknight Valencia* leaves as affected by fertilisation rates at 200% of the normally applied rate (control). The legend indicates the mineral that was applied – vertical bars indicate the LSD at  $p < 0.05$ . The blue horizontal line indicates the concentration that is regarded as an excessive level, while the red line indicates the minimum acceptable nutritional status.

**Table 5.3.3.6.** Chemical and nutrient analysis of the soil (0-40 cm depth) of the two experimental orchards prior to application of the treatments (n=24).

Experimental orchard	pH (KCl)	P	Ambic 1 (mg/kg)		
			K	Ca	Mg
Midnight Valencia (Nelspruit)	5.9 ± 0.4	38 ± 16	49 ± 17	506 ± 76	84 ± 36
Orri mandarin (De Wet)	6.1 ± 0.8	118 ± 81	233 ± 146	1 694 ± 622	121 ± 50

*Orri mandarins (De Wet)*: No response in the leaf nutrient concentration of the trees that were fertilised at a 200% rate compared to the standard practices were obtained in the first season (Figure 5.3.3.7). During the second season, it is *only for K* that an increase in the leaf concentration was achieved - this became significant only from the middle of the 2020/21 season. Despite excessive levels of fertilisation (Table 5.3.3.4), the foliar nutrient concentration did not exceed any of the levels regarded as excessive for any of the nutrients (Figure 5.3.3.7). And, similar to the Midnight Valencia block, the trees also were especially non-responsive to the additionally applied P and Mg.

The above results from the two sites showed that N was the only nutrient being raised through over-fertilisation to levels above the generally accepted limit associated with an excessive concentration – this means that the maximum limits are too high and not helpful on its own to evaluate the accuracy of fertilisation rates, especially of P and K. Furthermore, additional fertilisation in a scenario of luxurious to excessive fertilisation will not result in a quick response with regards to leaf nutrient N and K concentration, and not at all for P and Mg.

#### **Effect of excessive fertilisation on the mineral composition of leaves from both non-fruit bearing terminals and from fruit bearing terminals, sampled in autumn**

The effect of the excessive fertilisation on the mineral composition of leaves from both non-fruit bearing terminals and from fruit bearing terminals, sampled in autumn is demonstrated in Tables 5.3.3.7 and 5.3.3.8 for the Midnight Valencia and Orri mandarin trial sites respectively. A consistent, significant increase in the nutrient concentration in response to the fertilisation treatments was not obtained for any of the nutrients. Only N and K concentrations did show some response, but a definite distinction between the types of leaves cannot be observed. The suppression of N uptake in conditions of excessive K supply, and *vice-a-versa*, was noticeable in both leaf types and for both trial sites, albeit not consistently significant. As for the other nutrients (i.e., P & Mg), additional fertilisation rates did not affect the leaf concentration of the respective leaf types, and also not the Ca-supply to the leaves.

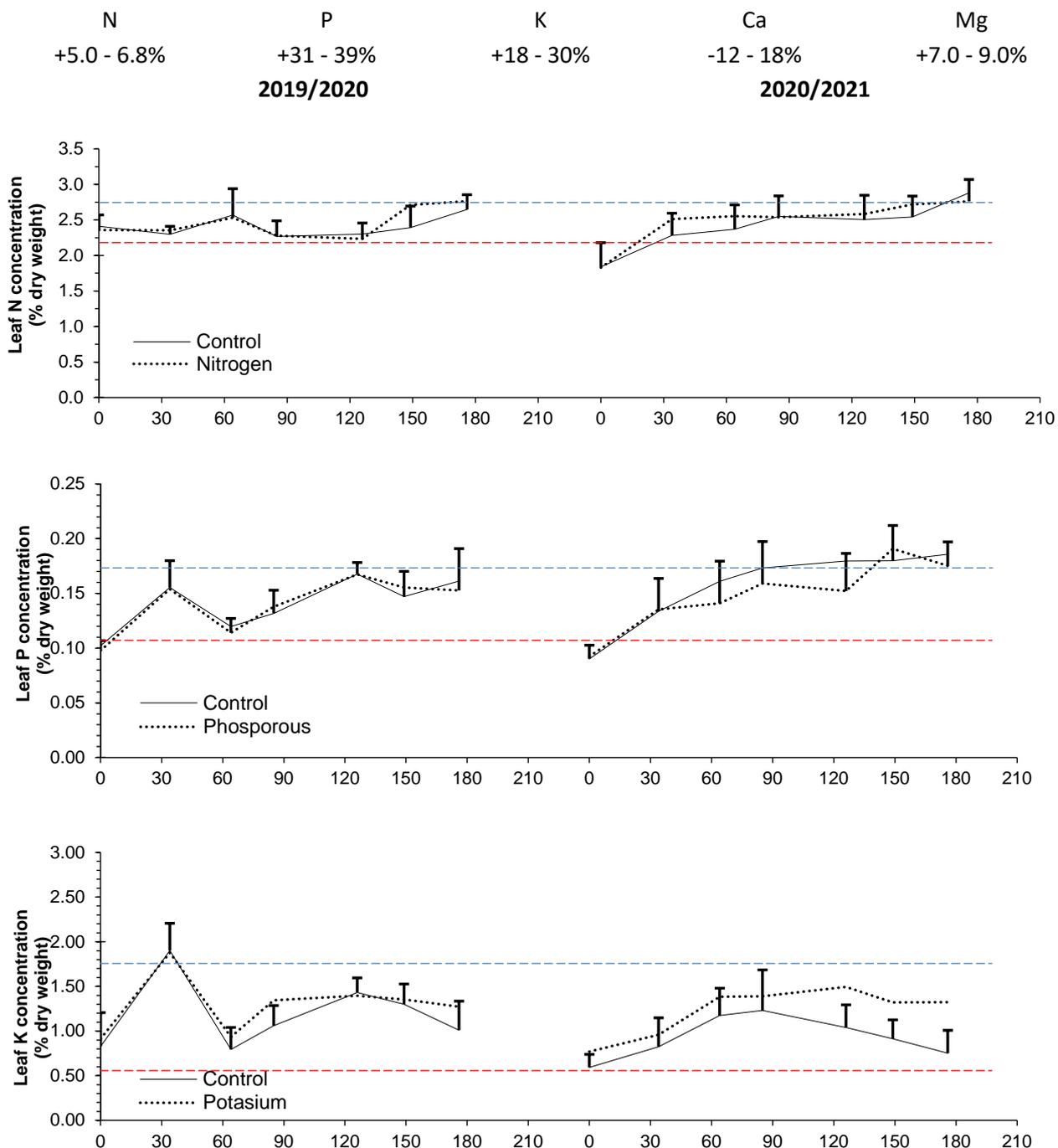
#### **Comparison between the mineral nutrient concentration of leaves from non-fruit bearing terminals and leaves from fruit bearing terminals sampled in autumn**

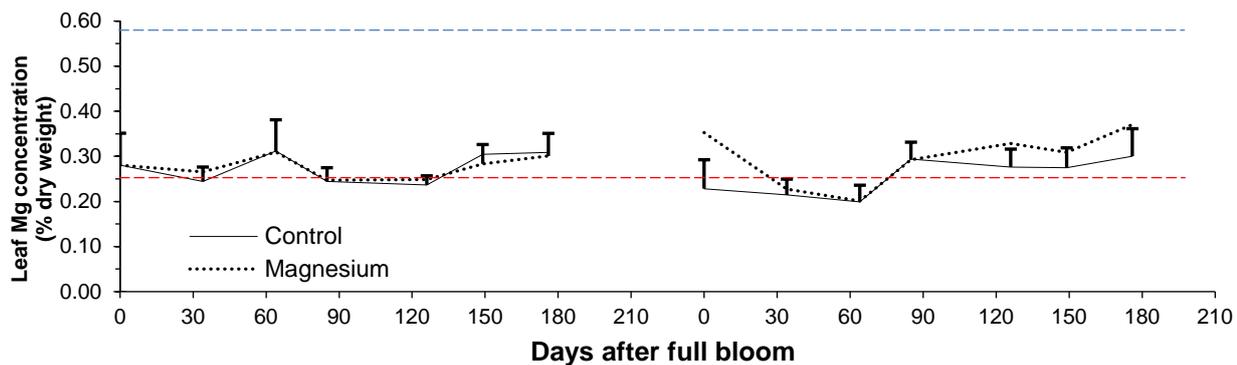
To elucidate whether the mineral nutrient concentration of leaves from non-fruit bearing terminals differ from those on fruit bearing terminals, samples from both positions were taken at the normal autumn sampling time ( $\pm$  180 days after full bloom), and the averages over all the treatments compared. The result of this is presented in Figure 5.3.3.8 for both varieties/sites. With the exception of S and Mg for the Midnight Valentias in 2019/20, the differences between leaves from the different positions are consistent for both varieties. The following observations were made: (i) the N, P and K concentration of leaves from non-fruit bearing terminals were higher than that of fruit bearing terminals – this was also found by Carranca *et al.* (1993) for Valencia late; (ii) despite not showing a consistently significant trend, Mg concentration also was lower in fruit bearing terminals; (iii) the Ca concentration of leaves from fruit bearing terminals is higher than that of non-fruit bearing terminals.

The higher concentration of N, P, K and Mg in the leaves from non-fruit bearing terminals is ascribed to the mobility of these nutrients, i.e., the crop is a strong sink for these nutrients (Mirsoleimani, 2014). On the other

hand, the higher Ca concentration in the fruit-bearing terminals is ascribed to the immobility of Ca, i.e., progressive accumulation, without transfer, of Ca has taken place in these leaves.

Analysis of leaves from non-fruit bearing terminals can also be used to assess tree nutritional status. Adjustments, as provided below is, however, required for leaves from non-fruit bearing terminals compared to the reference norms for leaves from fruit bearing terminals (as provided in Coetzee (2007)). These adjustments are assumed to apply only to Valencias and mandarins.





**Figure 5.3.3.7.** Seasonal changes in nutrient concentration of *Orrri mandarin* leaves as affected by fertilisation rates at 200% of the normally applied rate (control). The legend indicates the mineral that was applied – vertical bars indicate the LSD at  $p < 0.05$ . The blue horizontal line indicates the concentration that is regarded as an excessive level, while the red line indicates the minimum acceptable nutritional status.

**Table 5.3.3.7.** Mineral nutrient concentration of the *Midnight Valencias* leaves sampled in autumn, as affected by fertilisation rates at 200% the normally applied rate (control) – numbers followed by different letters differ significantly at  $p < 0.05$ .

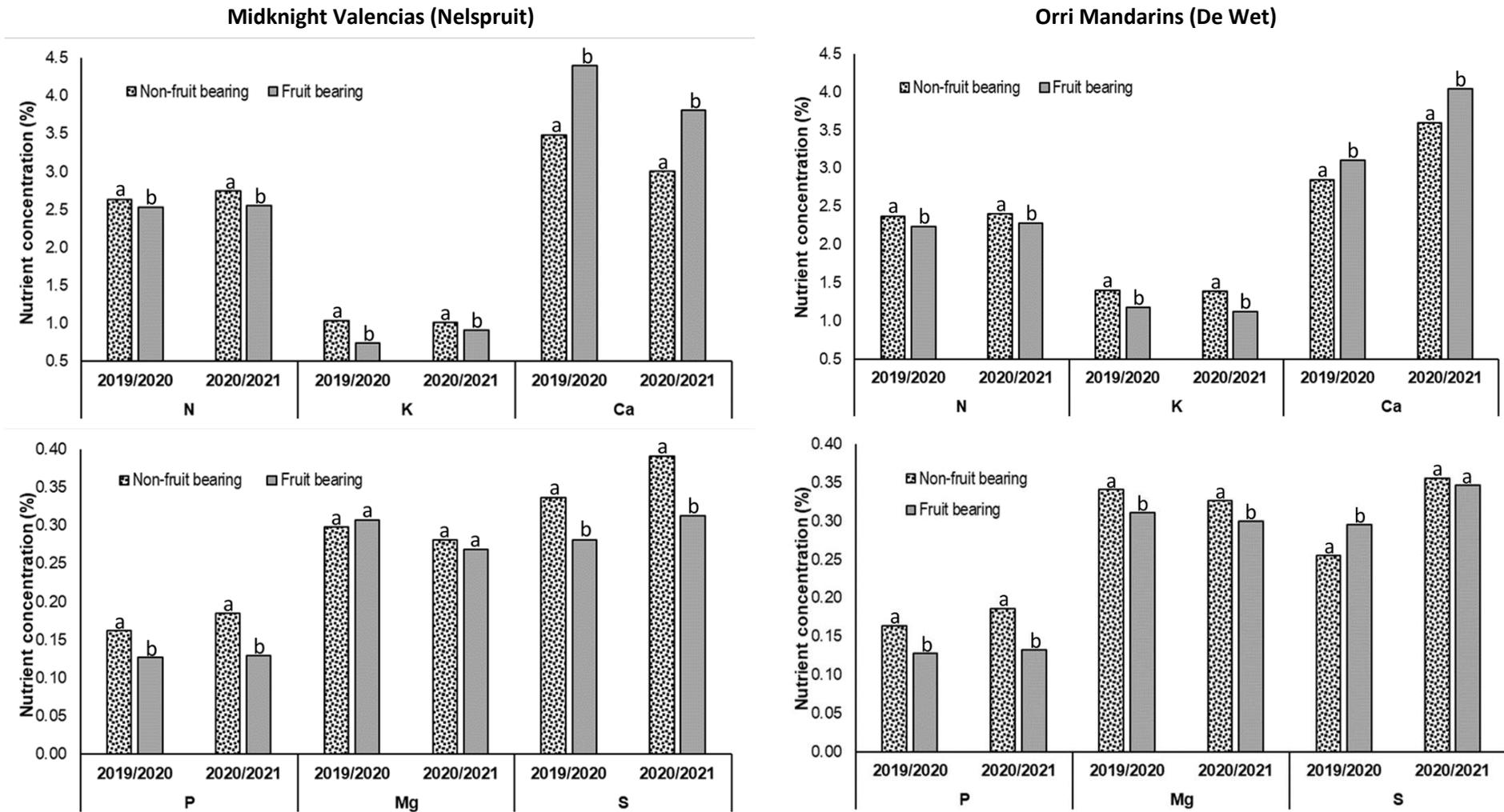
Type of leaf sample	Leaves on non-fruit bearing terminals										Leaves on fruit bearing terminals											
	Treatment	2019/2020					2020/2021					Treatment	2019/2020					2020/2021				
		N	P	K	Ca	Mg	N	P	K	Ca	Mg		N	P	K	Ca	Mg	N	P	K	Ca	Mg
Control	2.15b	0.20a	1.49ab	2.84	0.38a	2.31b	0.17b	1.40ab	3.61ab	0.32	2.41a	0.12b	1.15a	3.01b	0.27b	2.23b	0.14	1.25a	4.14ab	0.35a		
Nitrogen	<b>2.66a</b>	0.16ab	1.29b	2.80	0.33ab	<b>2.61a</b>	0.18ab	<u>1.17b</u>	3.09b	0.32	<b>2.31a</b>	0.14a	<u>0.92b</u>	3.05b	0.32a	<b>2.50a</b>	0.12	<u>0.82b</u>	3.79ab	0.29b		
Phosphorus	2.32b	0.15b	1.34ab	3.11	0.35ab	2.28b	0.19ab	1.27b	3.66ab	0.31	2.02b	0.13a	1.20a	3.13ab	0.33a	2.25b	0.14	1.06ab	3.99ab	0.27b		
Potassium	2.32b	0.17ab	<b>1.59a</b>	2.53	0.30b	2.34ab	0.21a	<b>1.68a</b>	3.69ab	0.33	2.05b	0.13b	1.18a	3.29a	0.32a	<u>2.17b</u>	0.13	1.22a	3.77b	<u>0.24b</u>		
Magnesium	2.36ab	0.15b	1.29b	2.95	0.35ab	2.46ab	0.17b	1.44ab	3.90a	0.35	2.39a	0.12b	1.11a	3.05b	0.31a	2.27b	0.13	1.25a	4.51a	0.36a		
LSD ( $p < 0.05$ )	0.32	0.046	0.29	NS	0.081	0.27	0.034	0.34	0.75	NS	0.24	0.006	0.11	0.18	0.04	0.17	NS	0.26	0.73	0.05		

NS = not significant

**Table 5.3.3.8.** Mineral nutrient concentration of the *Orri mandarin* leaves sampled in autumn, as affected by fertilisation rates at 200% the normally applied rate (control) – numbers followed by different letters differ significantly.

Type of leaf sample	Leaves on non-fruit bearing terminals										Leaves on fruit bearing terminals											
	Treatment	2019/2020					2020/2021					Treatment	2019/2020					2020/2021				
		N	P	K	Ca	Mg	N	P	K	Ca	Mg		N	P	K	Ca	Mg	N	P	K	Ca	Mg
Control	2.65a	0.16	1.01ab	3.75	0.31	2.54b	0.18ab	0.91b	2.93	0.27ab	2.52	0.13	0.69b	4.97	0.32ab	2.50	0.12	0.70b	4.00	0.28bc		
Nitrogen	<b>2.86a</b>	0.16	0.93b	3.14	0.28	2.72ab	0.18ab	<u>0.91b</u>	3.07	0.28ab	2.63	0.13	<u>0.61b</u>	4.28	0.31ab	2.62	0.13	<u>0.76b</u>	3.72	0.25bc		
Phosphorus	2.40b	0.15	0.93b	3.62	0.32	2.85a	0.19ab	0.86b	3.15	0.29ab	2.38	0.12	0.74b	4.34	0.33ab	2.61	0.13	0.88ab	4.03	0.29ab		
Potassium	2.78a	0.17	<b>1.27a</b>	3.37	0.27	2.77ab	0.20a	<b>1.32a</b>	2.83	0.25b	2.44	0.13	<b>1.01a</b>	4.29	<u>0.26b</u>	2.51	0.13	<b>1.28a</b>	3.36	<u>0.22c</u>		
Magnesium	2.60ab	0.17	1.07ab	3.54	0.30	2.89a	0.17b	1.06b	3.08	0.31a	2.57	0.12	<u>0.66b</u>	4.37	<b>0.35a</b>	2.48	0.15	0.93ab	4.07	<b>0.34a</b>		
LSD ( $p < 0.05$ )	0.21	NS	0.32	NS	NS	0.29	0.03	0.21	NS	0.04	NS	NS	0.21	NS	0.09	NS	NS	0.47	NS	0.06		

NS = not significant



**Figure 5.3.3.8.** Difference in nutrient concentrations of non-fruit bearing and fruit bearing terminals' leaves of Midnight Valentias and Orri mandarins, sampled in autumn (different symbols denote significant difference at  $p < 0.05$ ).

### **Correlation between nutrient concentrations of leaves sampled at different times after full bloom and (i) that of the normal prescribed autumn sampling time; (ii) as well as with fruit mineral composition and quality parameters at harvest**

The correlation between both Midnight Valencia and Orri mandarin leaf nutrient concentrations at various times after full bloom and that of the normal prescribed autumn sampling time is presented in Tables 5.3.3.9 and 5.3.3.10. In 2019/2020, the Midnight Valencia leaf analysis at most of the sampling dates showed a correlation with the autumn sampled non-fruit bearing terminal leaves for N only. But in 2020/21 there was a significant correlation between all the sampling times for all the nutrients except P (Table 5.3.3.9).

Not one of the in-season sampling times' nutrient concentration correlated with nutrient concentration of autumn sampled leaves from the fruit bearing terminals. However, in the 2020/2021 season there was significant correlations between the concentration of N and K of most of the sampling times with that of the fruit bearing terminal's leaves sampled in autumn at 180 days after full bloom (DAFB) (Table 5.3.3.9). Furthermore, N, K and Ca concentrations in 180 DAFB sampled leaves on non-fruit bearing terminals and fruit bearing terminals were significantly correlated (Table 5.3.3.9) – only in the 2020/2021 season.

The data presented in Table 5.3.3.10 shows that for the Orri mandarin trees in both seasons there was a significant correlation between leaf K and Mg concentration at almost all sampling times and that of the normal sampling time (176 DAFB) for **both** the leaf types. Furthermore, there was a significant correlation between all nutrients' concentrations in the non-fruit bearing terminals and the fruit bearing terminals at 176 DAFB (i.e., the normal sampling time, autumn samples) in the 2019/2020 season, and for K, Ca and Mg in the 2020/2021 season. The data in Tables 5.3.3.9 and 5.3.3.10 therefore suggests that autumn sampled leaves on both non-fruit bearing and fruit bearing terminals can be used to assess the tree nutritional status.

There were no meaningful correlations found between early leaf nutrient concentrations and (i) fruit mineral nutrient concentration, (ii) fruit characteristics or (iii) yield for both cultivars (data not shown).

### **Seasonal changes in fruit nutrient concentration and the effect of fertilisation treatments on fruit nutrient concentration**

*Midnight Valencia trees (Nelspruit):* A similar response than for the leaf N concentration was obtained regarding fruit N concentration, i.e., the trees that were fertilised at a 200% rate to the standard practices showed significantly increased N concentrations, from fruit set (30 days after full bloom) onwards. At harvest in the second season, no differences in the fruit N concentration were however obtained (Figure 5.3.3.9). The other nutrients were not increased by the additional fertilisation.

*Orri mandarins (De Wet):* No response in the fruit nutrient concentration of the trees that were fertilised at a 200% rate to the standard practices were obtained – this is similar to the leaf nutrient concentration results (Figure 5.3.3.10).

The decreasing trend in mineral nutrient concentration of the fruit of both cultivars (trial sites) in the earlier part of fruit development is directly related to fruit growth; while the flattening of the drop is related to accumulation of nutrients and sugar in the maturation stage (which explains the continued increase in weight) while the fruit growth rate decreases (Alva *et al.*, 2001), as illustrated in Figures 5.3.3.11 and 5.3.3.12 respectively.

**Table 5.3.3.9.** Correlations ( $r^2$ ) between mineral analysis results of mature *Midknight Valencia* leaves of the latest in-season flush, sampled at different times in both the 2019/2020 and 2020/2021 seasons, and the mineral nutrient content of leaves on both non-fruit bearing terminals and fruit bearing terminals sampled in March (normal practice) – the indicated  $r^2$  values are all significant at  $p < 0.05$ , while values in the blue blocks are significant at  $p < 0.07$ .

Nutrient	2019/2020 season											2020/2021 season									
	Sampling time (*DAFB)	Nutrient in leaves sampled in March (180 DAFB)										Nutrient in leaves sampled in March (180 DAFB)									
		From non-fruit bearing terminals					From fruit bearing terminals					From non-fruit bearing terminals					From fruit bearing terminals				
		N	P	K	Ca	Mg	N	P	K	Ca	Mg	N	P	K	Ca	Mg	N	P	K	Ca	Mg
N	0	NS				NS					NS					NS					
	**41/47	0.361				NS					0.228					0.388					
	96	0.257				NS					0.177					NS					
	134	NS				NS					0.231					0.328					
	162	0.243				NS					NS					NS					
	181	-				NS					-					0.446					
P	0		NS				NS					NS					NS				
	41/47		NS				NS					NS					NS				
	96		NS				NS					NS					NS				
	134		NS				NS					NS					NS				
	162		NS				NS					NS					NS				
	181		-				NS					-					NS				
K	0			0.218				NS					NS					NS			
	41/47			0.138				NS					0.251					0.331			
	96			0.371				NS					0.234					0.211			
	134			NS				NS					0.354					NS			
	162			NS				NS					0.581					NS			
	181			-				NS					-					0.391			
Ca	0				NS				NS					0.218					NS		
	41/47				NS				NS					NS					NS		
	96				NS				NS					0.292					NS		
	134				NS				NS					0.680					NS		
	162				NS				NS					0.309					0.216		
	181				-				NS					-					0.301		
Mg	0				NS					NS				NS						NS	
	41/47				NS					NS				0.261						NS	
	96				0.402					NS				0.249						0.268	
	134				NS					NS				0.148						NS	
	162				NS					NS				0.278						NS	
	181				-					NS				-						NS	

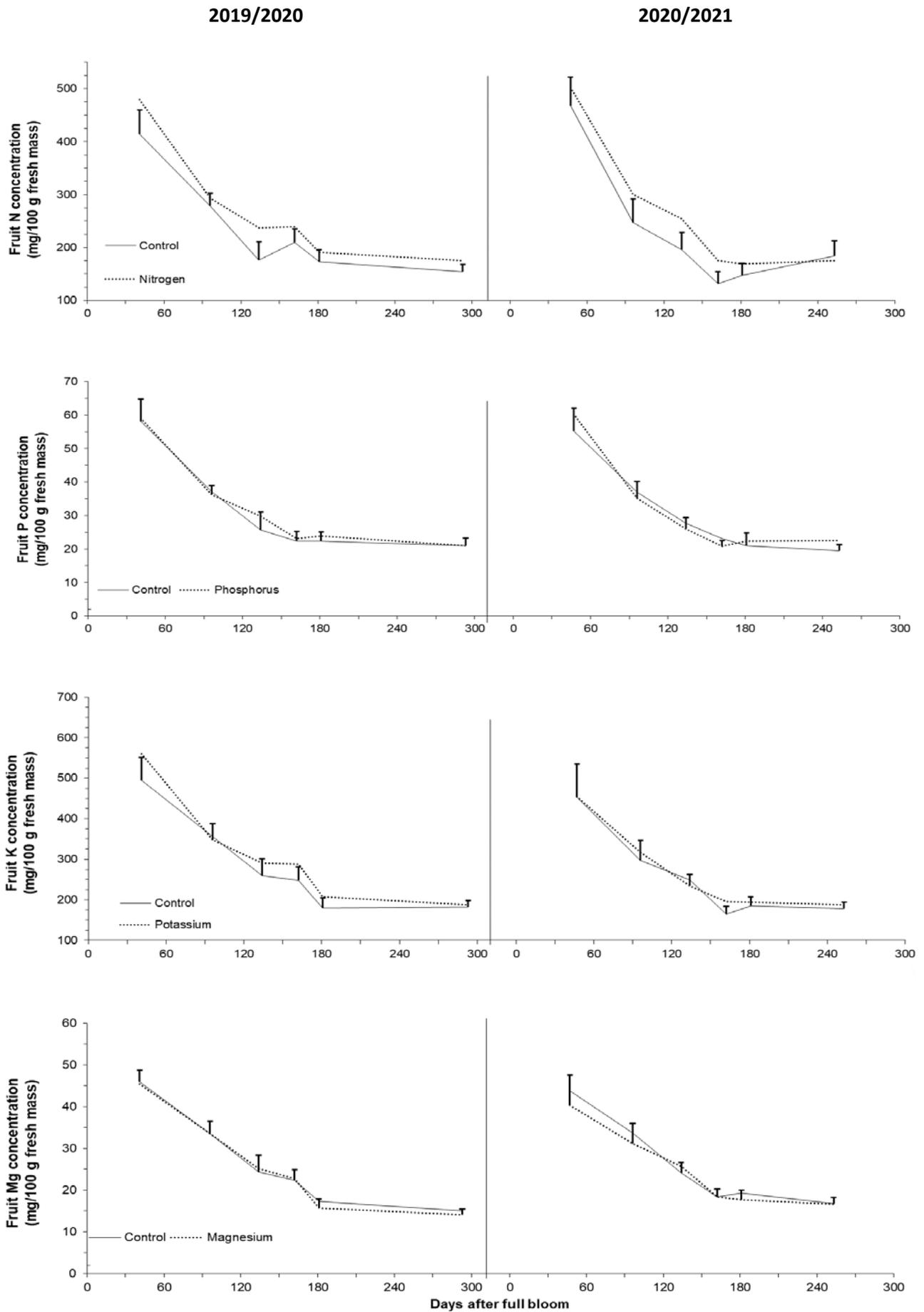
\*DAFB = days after full bloom

\*\* Sampling was at 41 DAFB in 2019/2020 and 47 DAFB in 2020/2021

**Table 5.3.3.10.** Correlations ( $r^2$ ) between mineral analysis results of mature *Orrri mandarin* leaves of the latest in-season flush, sampled at different times in both the 2019/2020 and 2020/2021 seasons, and the mineral nutrient content of leaves on both fruit-bearing terminals and fruit bearing terminals sampled in March (normal practice) – the indicated  $r^2$  values are all significant at  $p < 0.05$ , while values in the blue blocks are significant at  $p < 0.07$ .

Nutrient	Sampling time (*DAFB)	Nutrient in leaves sampled in March (176 DAFB)										Nutrient in leaves sampled in March (176 DAFB)									
		From non-fruit bearing terminals					From fruit bearing terminals					From non-fruit bearing terminals					From fruit bearing terminals				
		N	P	K	Ca	Mg	N	P	K	Ca	Mg	N	P	K	Ca	Mg	N	P	K	Ca	Mg
N	0	NS					NS					NS					NS				
	34	NS					NS					NS					NS				
	64	NS					NS					NS					NS				
	85	NS					0.233					NS					0.347				
	126	NS					NS					NS					NS				
	149	NS					NS					NS					NS				
	176	-					0.504					-					NS				
P	0		NS										NS					NS			
	34		NS										0.267					NS			
	64		NS										NS					NS			
	85		NS										0.378					NS			
	126		NS										0.525					0.316			
	149		NS										NS					NS			
	176		-										-					NS			
K	0			0.356										0.683					0.581		
	34			NS										NS					0.192		
	64			0.524										0.748					0.667		
	85			0.308										0.628					0.546		
	126			0.319										0.807					0.746		
	149			0.496										0.802					0.591		
	176			-										-					0.818		
Ca	0				NS												NS				NS
	34				NS												NS				NS
	64				NS												NS				0.596
	85				NS												0.246				0.495
	126				NS												0.402				0.321
	149				NS												0.582				0.467
	176				-												-				0.615
Mg	0					0.466							0.434					0.752			NS
	34					0.621							0.361					0.524			0.248
	64					0.254							0.397					0.203			0.469
	85					0.579							0.454					0.500			0.656
	126					0.246							0.199					0.538			0.507
	149					0.182							0.336					0.356			0.703
	176					-							0.507					-			0.436

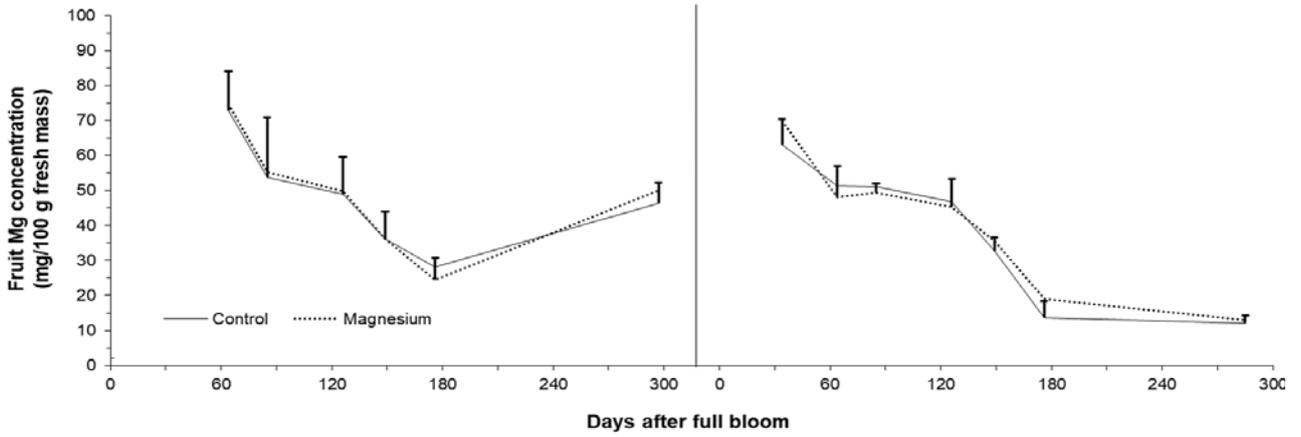
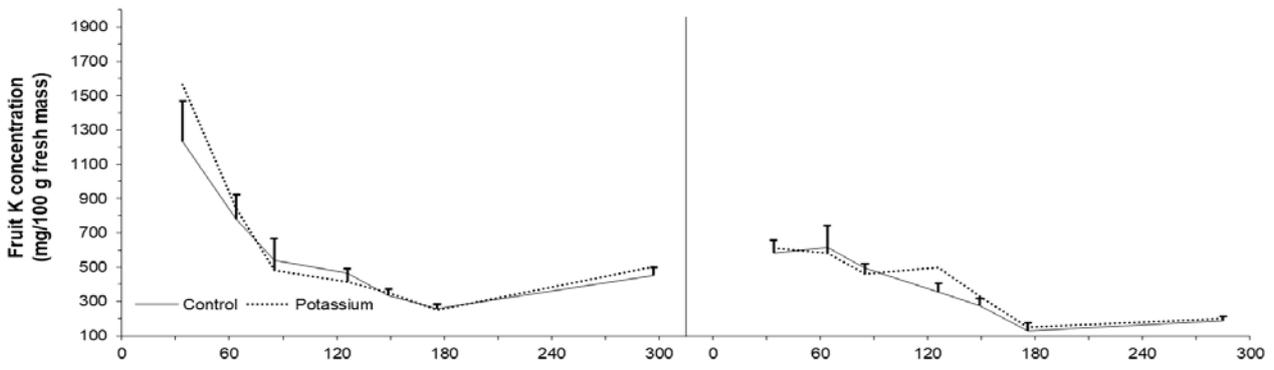
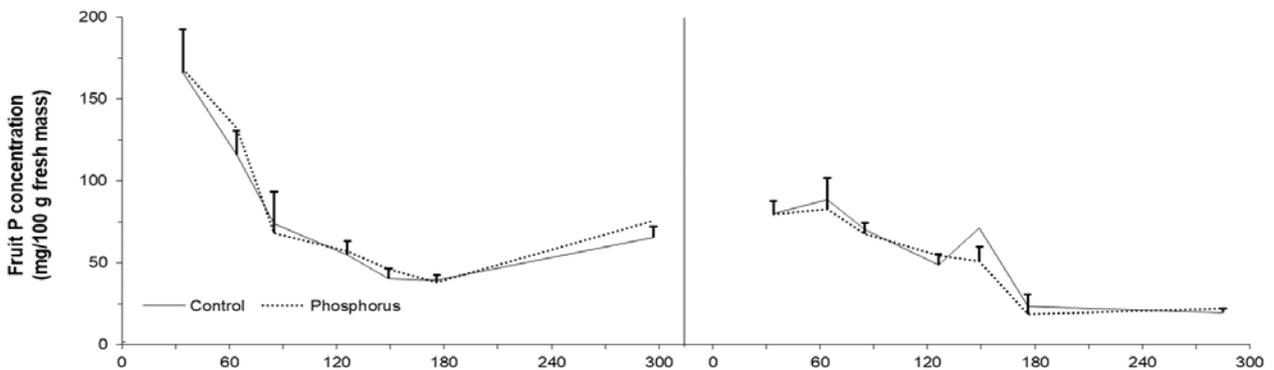
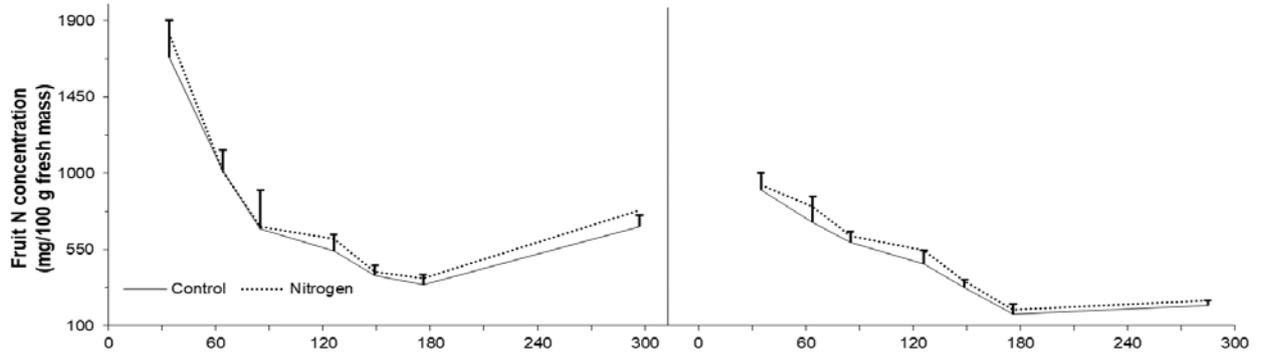
\*DAFB = days after full bloom



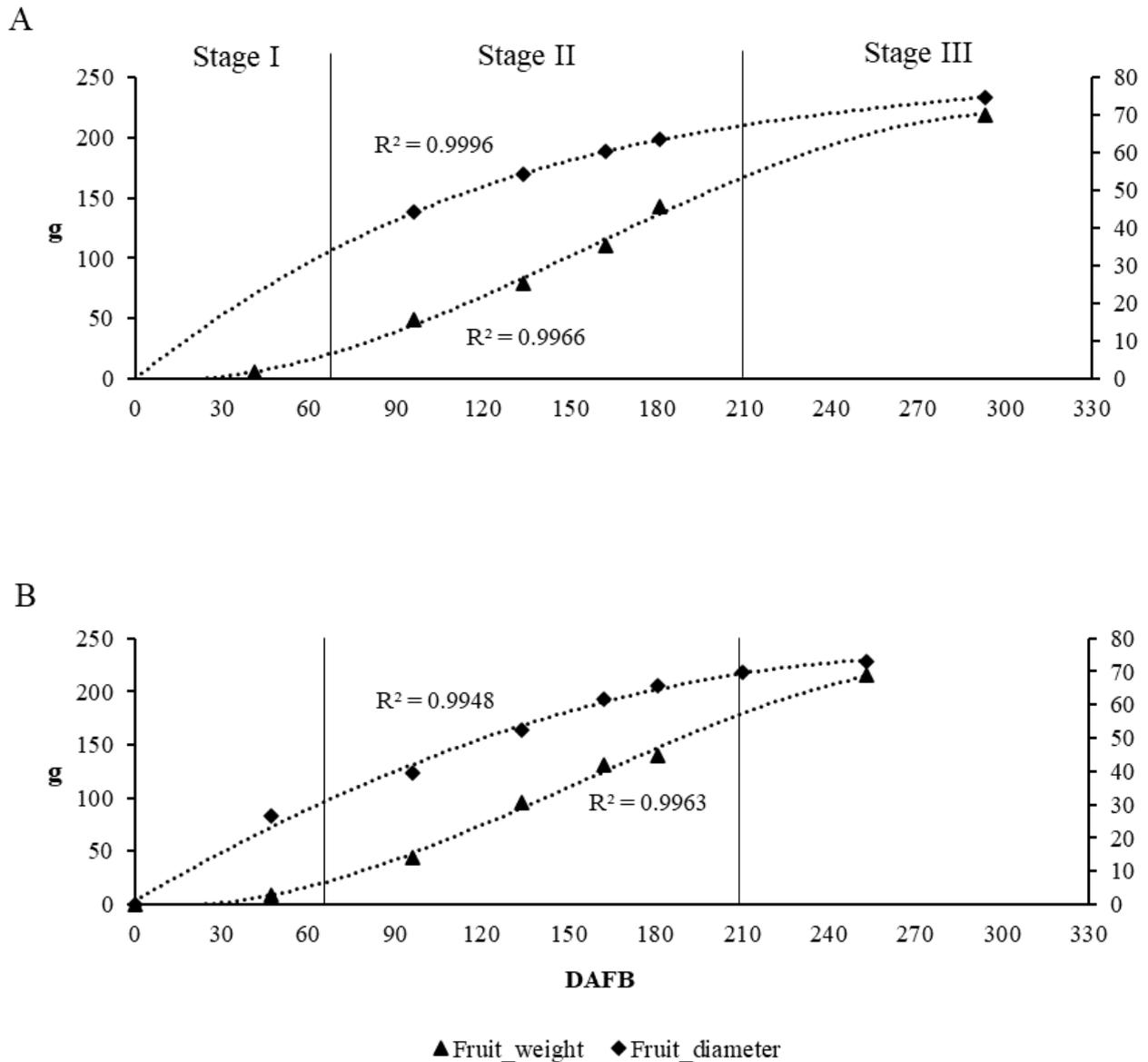
**Figure 5.3.3.9.** Seasonal changes in nutrient concentration of *Midknight Valencia* fruit as affected by fertilisation rates at 200% the normally applied rate (control) where the legend indicates the mineral that was applied – vertical bars indicate the LSD at  $p < 0.05$ .

2019/2020

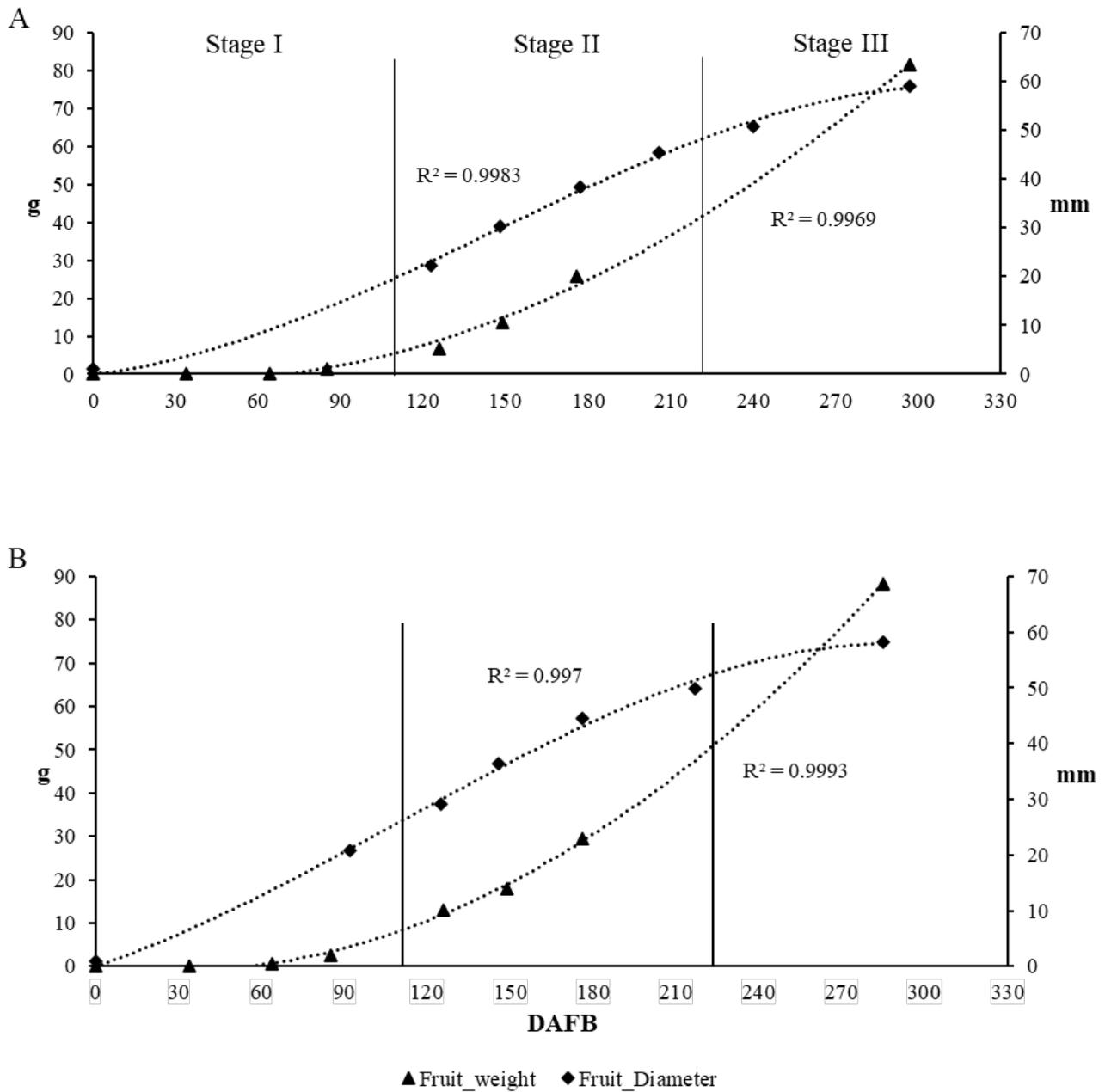
2020/2021



**Figure 5.3.3.10.** Seasonal changes in nutrient concentration of *Orri mandarin* fruit as affected by fertilisation rates at 200% the normally applied rate (control) where the legend indicates the mineral that was applied – vertical bars indicate the LSD at  $p < 0.05$ .



**Figure 5.3.3.11.** Average change in fruit weight (g) and diameter (mm) for the experimental ‘Midnight’ Valencia block in Nelspruit during the 2019/20 (A) and 2020/21 (B) seasons, starting at anthesis (DAFB = 0). Anthesis during the first season was on 04/09/2019 and during the second season it was 18/09/2020. DAFB: Days after full bloom.



**Figure 5.3.3.12.** Average fruit weight (g) and diameter (mm) for ‘Orri’ mandarins during the 2019/20 (A) and 2020/21 (B) seasons, over time, starting at anthesis (DAFB = 0). Anthesis for the first season was on 23/09/2019 and for the second season 22/10/2020. DAFB – Days after full bloom..

### Crop nutrient removal

Fruit analysis from both seasons were used to calculate the nutrient removal per ton of fruit. It is shown in Table 5.3.3.11. For both cultivars this is lower than that reported in Raath (2021), especially the P and Mg removal values. The reported values are similar to that found by Alva *et al.* (2001) for Valencias.

**Table 5.3.3.11.** Nutrient removal per ton of Midnight Valencia and Orri mandarin fruit produced in Nelspruit and De Wet respectively.

Midnight Valentias					Orri mandarins				
N	P	K	Ca	Mg	N	P	K	Ca	Mg
kg/ton fruit					kg/ton fruit				
1.6 ± 0.1	0.22 ± 0.01	1.8 ± 0.1	0.54 ± 0.02	0.17 ± 0.02	2.2 ± 0.5	0.23 ± 0.04	2.0 ± 0.3	0.57 ± 0.2	0.13 ± 0.02
*2.0-2.3	0.4	2.3	0.65	0.45	1.9-2.3	0.3	2.4	0.65	0.45

\*Removal of nutrients by the citrus crop as per Raath (2021)

### Fruit characteristics at harvest

The Nelspruit trial ('Midnight' Valencia) was conservatively fertilised and during the *first season* the N-treatment resulted in a significantly higher mean rind colour score than the other treatments (i.e., poorer colour) and a lower TSS:TA ratio – last mentioned was significantly lower than the Mg-treatment which had the highest TSS:TA ratio (Table 5.3.3.12). The rind colour score is a range from 1 - 8 with one being the best developed orange fruit and 8 being entirely dark green. Application of additional Mg at adequate leaf Mg levels could result in an increased TSS:TA ratio (Koo, 1988; Obreza et al., 2020). Both the rind colour and the TSS:TA ratio responses to the N applications are in accordance with Raath (2021) who stated that increases in N fertilisation levels result in delayed colour break and a decreased TSS:TA ratio. Rind thickness was significantly increased by the additional K application compared to the Mg application ( $p \leq 0.05$ ). It is well known that K nutrition can increase rind thickness and that excessive soil Mg levels can suppress K uptake (Raath, 2021), potentially at the cost of rind thickness. Although juice %, fruit diameter (Table 5.3.3.12) and yield (data not shown) are commonly known to be affected by excessive fertilisation (Koo, 1988; Obreza et al., 2020; Raath, 2021) it was not affected by any of the nutrient treatments. This alludes to the possibility that soil amendments requires more than one season to have an effect.

**Table 5.3.3.12.** The effect of over-fertilisation (200% of the normal supply) of N, P, K and Mg on 'Midnight' Valencia fruit quality during the 2019/2020 season.

Quality parameters	Treatments										LSD
	Control		N		P		K		Mg		
Rind colour (1-8)	2.18	b	2.94	a	2.25	b	2.44	b	2.31	B	0.404
Rind thickness (mm)	4.27	ab	4.62	ab	4.28	ab	4.76	a	4.02	B	0.696
TSS	11.30	a	10.78	a	11.15	a	11.21	a	11.19	A	0.673
TA	0.72	a	0.75	a	0.72	a	0.71	a	0.68	A	0.088
TSS:TA	15.81	ab	14.41	b	15.62	ab	15.94	ab	16.48	A	2.016
Juice %	57.92	a	58.14	a	59.93	a	58.9	a	60.06	A	2.393
Diameter (mm)	75.43	a	74.26	a	73.67	a	75.98	a	74.90	A	3.004

Different letters from left to right indicate a significant difference ( $p \leq 0.05$ ) between two treatments.

TSS-Total soluble solids; TA-Titratable acidity

The *second season* (Table 5.3.3.13) yielded more significant effects for N and K treatments on 'Midnight' Valencia. The K- and Mg-treatments increased rind colour development in comparison to the control and the N-treatment by having a significantly lower colour score (Koo, 1988; Wutscher and Smith, 1993). The TA and the TSS:TA ratio was increased and decreased respectively as would be expected for additional N application when N leaf concentrations are within the established norms (Obreza et al., 2020). Similarly, additional K application increased and decreased the TA and TSS:TA ratio, which is in accordance with other studies (Obreza et al., 2020). Contrary to the expected response (Obreza et al., 2020), additional P application

increased rind thickness compared to the control. Potassium increased rind thickness compared to the control, as is expected (Koo, 1988; Raath, 2021).

The fruit quality factors of most economic interest were primarily affected during the second season (Table 5.3.3.13). The TA increase during season two, due to the N- and K-treatments would have major economic consequences, by moving the fruit from locally marketable (TA < 0.6 (Directorate Food Safety and Quality Assurance, 1999)) to exportable as Valencia's (TA > 0.6 (Directorate Food Safety and Quality Assurance, 1999)), increasing the price per carton.

**Table 5.3.3.13.** The effect of over-fertilisation (200% of the normal supply) of N, P, K and Mg on 'Midnight' Valencia fruit quality during the 2020/2021 season.

Quality parameters	Treatments										
	Control		N		P		K		Mg		LSD
Rind colour (1-8)	1.70	a	1.69	a	1.57	ab	1.44	b	1.48	b	0.188
Rind thickness (mm)	3.98	c	4.30	ab	4.54	a	4.39	ab	4.16	bc	0.253
TSS	12.15	a	11.86	a	12.13	a	11.84	a	12.58	a	0.752
TA	0.59	b	0.69	a	0.59	b	0.69	a	0.60	b	0.082
TSS:TA	20.81	a	17.24	b	20.55	a	17.31	b	21.16	a	2.918
Juice %	61.13	a	58.82	b	59.07	ab	60.06	ab	59.25	ab	2.239
Diameter (mm)	72.73	a	74.36	a	71.98	a	73.24	a	73.09	a	3.330

Differing letters from left to right indicate a significant difference ( $p \leq 0.05$ ) between two treatments.  
TSS-Total soluble solids; TA-Titratable acidity

The 'Orri' mandarin trees, received excessive N and moderate K fertilisation. Despite the high levels of N applied in the normal fertilisation programme (Control), the N-treatment significantly affected fruit rind colour in comparison to the Control in the first season, as well as TA and TSS:TA ratio during the second season (Table 5.3.3.14). During the second season more quality parameters were affected by the N-treatment (Table 5.3.3.13 vs. 5.3.3.14). In the first season the P treatment resulted in a significant increase in the TSS:TA ratio in comparison to the control, but this trend did not persist in the second season. This increase in the TSS:TA ratio is in accordance with the expected response as stated by Koo (1988) .

All of the responses in fruit quality associated with the K-treatment was in accordance with current literature (Koo, 1988; Obreza et al., 2020; Raath, 2021; Wutscher and Smith, 1993), i.e., the TA and fruit diameter (mm) both increased whereas the TSS:TA ratio and the juice percentage decreased. The decreased juice % is more likely an indirect result of the increased fruit size, because larger fruit generally have lower juice %, TSS, and TA, which could also be the reason for the decreased TSS:TA ratio (Koo, 1985). The decrease in the juice percentage was severe enough to change the marketability of the fruit from exportable (juice % > 48%) to locally marketable, which would have economic implications (Directorate Food Safety and Quality Assurance, 1999).

**Table 5.3.3.14.** The effect of over-fertilisation (200% of the normal supply) of N, P, K and Mg on 'Orri' mandarin fruit quality during the 2019/2020 season.

Quality parameters	Treatments										
	Control		N		P		K		Mg		LSD
Rind colour (1-8)	1.88	b	2.38	a	2.06	ab	2.19	ab	2.31	ab	0.473
Rind thickness (mm)	4.39	a	4.33	a	4.55	a	4.62	a	4.43	a	0.350
TSS	13.83	a	13.90	a	13.64	a	13.54	a	13.5	a	0.584
TA	1.17	a	1.14	a	1.08	a	1.13	a	1.14	a	0.097

TSS:TA	11.89	b	12.26	ab	12.66	a	12.01	b	11.89	b	0.643
Juice %	56.71	a	55.90	a	55.41	a	55.37	a	53.75	a	3.049
Diameter (mm)	59.38	a	58.99	a	59.15	a	58.92	a	58.55	a	2.072

Letters that differ from left to right indicate a significant difference ( $p \leq 0.05$ ) between two treatments.  
TSS-Total soluble solids; TA-Titratable acidity

**Table 5.3.3.15.** The effect of over-fertilisation (200% of the normal supply) of N, P, K and Mg on 'Orri' mandarin fruit quality during the 2020/2021 season.

Quality parameters	Treatments										
	Control	N		P		K		Mg		LSD	
Rind colour (1-8)	1.79	a	1.82	a	2.14	a	1.75	a	2.00	a	0.425
Rind thickness (mm)	3.12	a	3.09	a	3.13	a	3.56	a	3.41	a	0.481
TSS	13.78	ab	13.86	a	13.28	ab	12.99	b	13.31	ab	0.790
TA	1.06	b	1.17	a	1.07	b	1.18	a	1.03	b	0.080
TSS:TA	13.05	a	11.81	bc	12.47	ab	11.00	c	12.94	a	0.922
Juice %	59.23	a	47.38	ab	58.71	a	36.79	b	54.76	a	13.575
Diameter (mm)	58.03	b	55.92	b	58.03	b	61.04	a	57.80	b	2.906

Letters that differ from left to right indicate a significant difference ( $p \leq 0.05$ ) between two treatments.  
TSS-Total soluble solids; TA-Titratable acidity

### The effect of fruit thinning in combination with excessively applied nutrients

The level of fruit set was quantified during both seasons. No significant effect on fruit set was obtained response to the fertilisation or fruit thinning treatments (data not shown) – probably due to the success of fruit thinning being time-dependent and the slow, or lack, of response of the trees to the additional fertilisation applied. Furthermore, fruit thinning also did not have a consistent effect on the nutrient concentration of leaves on non-fruit bearing terminals or on fruit bearing terminals (data not shown).

### Phase 3

#### Tree nutrient status as affected by zero, low and increasing rates of N and K fertilisation

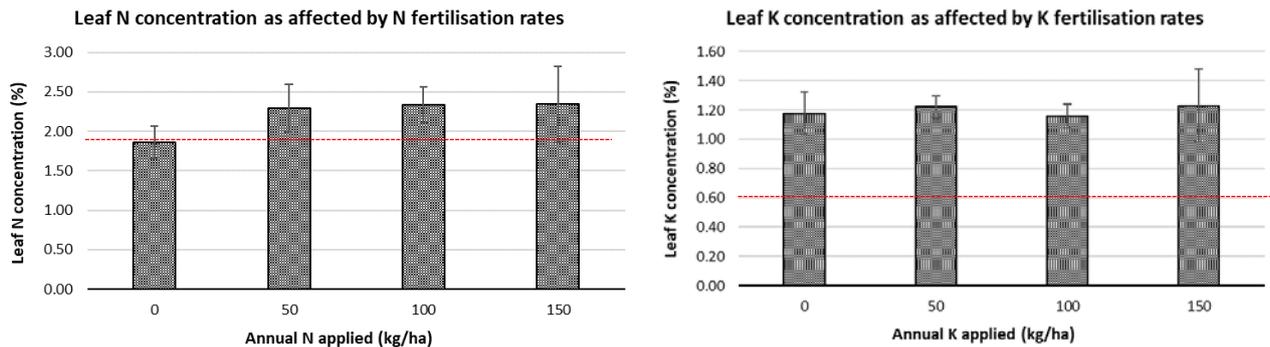
Increased rates of seasonal N and K applications resulted only in an increasing trend in the soil mineral N and exchangeable K concentrations by the end of the growing season, March 2022 (Table 5.3.3.16).

Analysis of leaves sampled in March 2022 from mature spring flush shoots with terminal fruit did not show significantly increased N or K concentrations in response to increased levels of N fertilisation (Figure 5.3.3.13). Despite clear differences in leaf colour (Figure 5.3.3.14), the increasing trend in foliar N concentration that is observed is not significant. This is due to the large variation in foliar N concentration between the individual trees within each treatment. It could also be due to slightly more (albeit not significant) vegetative growth obtained with increased N fertilisation (Figure 5.3.3.15), causing a dilution effect.

The **lack** of differences in foliar K concentration in response to increasing levels of K fertilisation is even more apparent. In the case of K, it is ascribed to the inherently sufficient concentrations of K in the soil (Table 5.3.3.14). The lack of response to N and K fertilisation after a season of zero fertilisation, compared to 150 kg/ha N and K fertilisation, corresponds to the results obtained in Phase 2 of this project. **It can therefore be concluded that a significant response to changed N and K fertilisation rates in citrus tree leaf nutrient concentrations must not be expected within one season, especially not in conditions where the K concentration of the soil is sufficient or if the trees' nutritional status has been maintained above the minimum norms.**

**Table 5.3.3.16.** The effect of increased applications of N and K fertilisation on soil mineral N and exchangeable K concentration, as well as total plant available N and K over a soil depth of 40 cm at the end of the 2021/2022 growing season (NS = not significant at  $p < 0.05$ ).

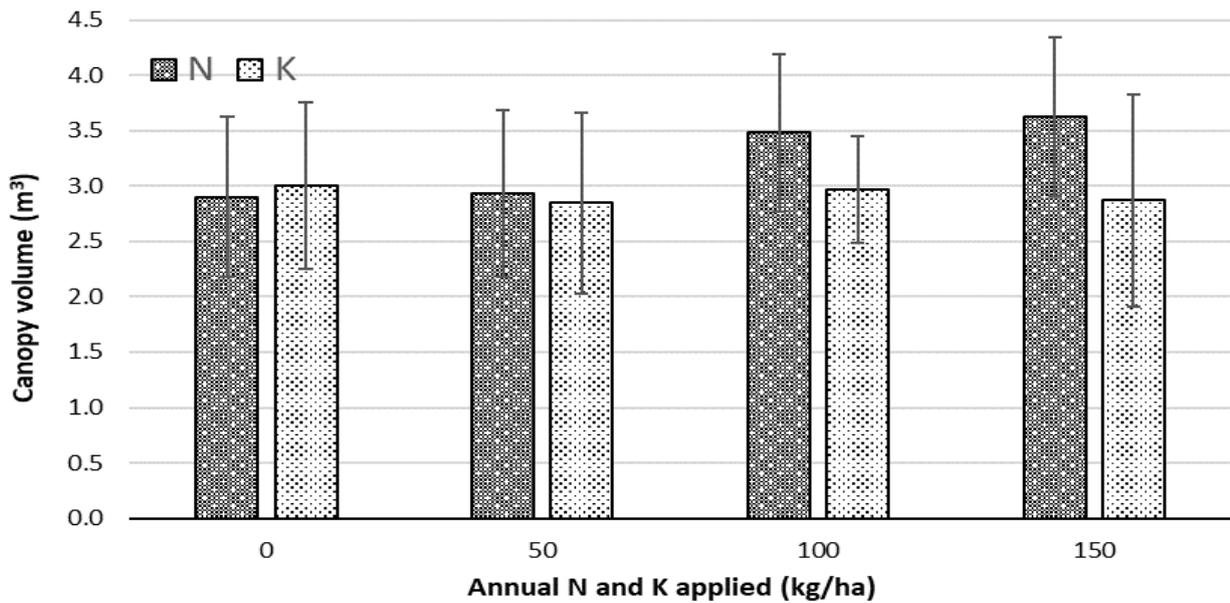
Soil N					LSD ( $p < 0.05$ )
Application rates (kg/ha/year)	0 kg	50 kg	100 kg	150 kg	
Mineral N ( $\text{NH}_4^+$ & $\text{NO}_3^-$ )	11.5 mg/kg	11.3 mg/kg	14.8 mg/kg	18.1 mg/kg	NS
Total plant available N up to 40 cm	61 kg	60 kg	79 kg	97 kg	NS
Soil K					
Exchangeable K	128 mg/kg	148 mg/kg	166 mg/kg	170 mg/kg	NS
Total plant available K up to 40 cm	685 kg	789 kg	884 kg	907 kg	NS



**Figure 5.3.3.13.** Nitrogen and K concentration of leaves sampled in March 2022 on mature (spring flush) shoots bearing terminal fruit – the red line indicates the minimum norms for N and K respectively (Raath 2021).

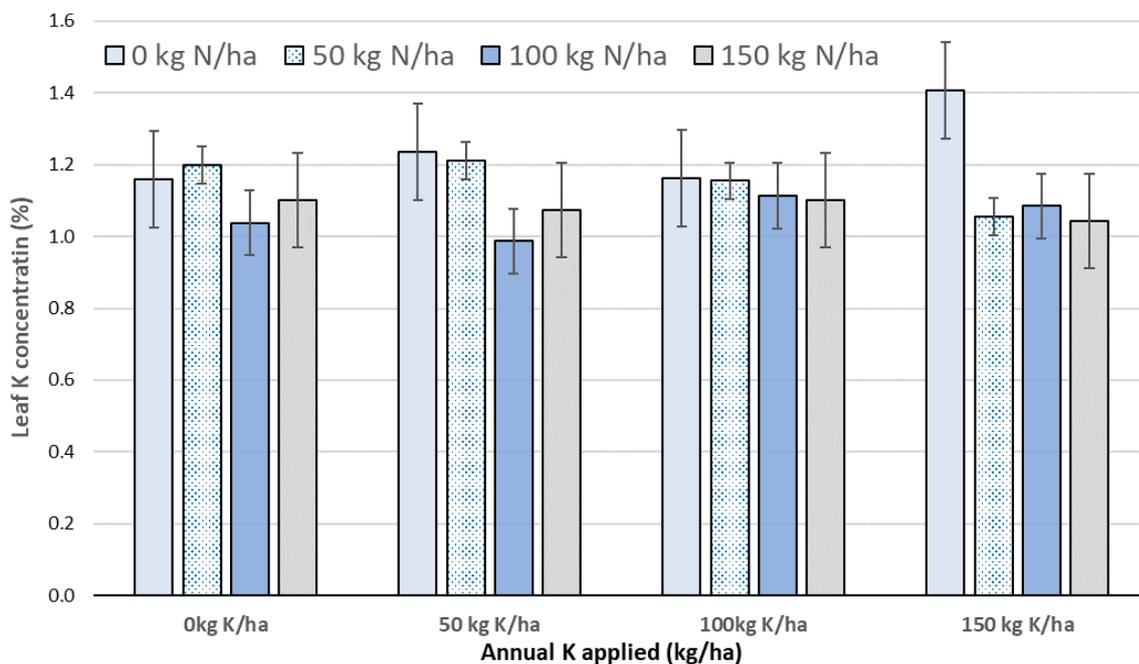


**Figure 5.3.3.14.** Differences observed in tree colour between trees that received zero N (left) during the 2021/22 season and those that received 150 kg N/ha (right).



**Figure 5.3.3.15.** Tree volumes as measured after a season (2021/22) of differentiated fertilisation applications of N and K.

Despite the antagonistic effect of high rates of K fertilisation on N uptake (Raath 2021), increased application rates of K did not affect the leaf N concentration (data not shown). However, in line with the vice-a-versa effect of N fertilisation of K uptake, the N fertilisation resulted in lower leaf K concentrations, most significantly in the treatments that received the highest rate of K fertilisation (Figure 5.3.3.16) – last mentioned was contrary to expectations.



**Figure 5.3.3.16.** Potassium concentration of leaves sampled in March 2022 on mature (spring flush) shoots bearing terminal fruit as determined by the rate of N fertilisation.

## Conclusion

Significant insight was gained regarding the responsiveness of both Valencia and mandarin trees to mineral nutrition. Bearing the objectives of the project in mind, the outcomes that was obtained is helpful. They are discussed below as conclusions to this trial.

A fertilisation requirement model, developed from early season leaf mineral analysis data for in-season adjustments of the N fertilisation programme can be developed, but not for other nutrients. Manipulation of mandarin and Valencia quality from this data will be found redundant due to the minimal, or lack of response to fertilisation in the trees' nutritional status – both in foliar and fruit nutrient concentrations. Except for N, it can be concluded from this study that citrus trees have an ability to regulate its nutritional status in conditions of excessive fertilisation, making its leaf nutrient concentration fairly to non-responsive to over-supplied levels of P, K, and Mg. This can explain the trend found in the Industry to over-fertilise, in an attempt to avoid deficiencies, without experiencing a negative effect on fruit quality. Long-term tendencies should rather be used to establish the effect of over-fertilisation, and be used to compile and manage fertilisation programmes and avoid over-fertilisation, instead of in-season regular short-term changes in fertilisation rates.

With regards to improving fertilisation management and optimise fertiliser usage (so called “best management practice”) it must be pointed out that the maximum limits used to evaluate foliar analysis is of little help to assess the accuracy of fertilisation rates of both Valencias and mandarins, since it was not exceeded despite excessively supplied nutritional conditions. The limited usefulness of foliar analysis in conditions of ample to over-supply of nutrients therefore needs to be emphasised. The autumn sampling time ( $\pm 180$  days after full bloom) for foliar analysis was shown to sufficiently reveal the trees' overall nutritional response to a season's fertilisation and there is very limited value in using other sampling times. The sufficiency ranges also decrease diagnostic precision because they are too wide – they should therefore be regarded as secondary to observation of long-term trends in the foliar analysis, and foliar analysis should not be the only indication whether the fertilisation programme followed are appropriate, especially in conditions of ample to high rates of fertilisation.

When leaves are sampled for analysis, this trial showed that analysis of leaves from non-fruit bearing terminals can also be used successfully to assess tree nutritional status. Higher norms for N, P, K, Mg should however be used, while for Ca it must be slightly lower.

High rates of N fertilisation do not necessarily affect fruit colour - it must be applied early enough on Midnight Valencias in Nelspruit, but can even be applied as late as February on Orri mandarins in the Western Cape. The positive effect of K application on fruit size and rind thickness seems to apply mainly to situations of conservative or low supply of K.

This study has highlighted the futility of over-fertilisation, in that the ability to manipulate tree performance or fruit quality is completely lost. In addition, the anticipated increased cost-price squeeze as well as pressure on Industry to responsibly manage mineral fertilisation, will force producers to reduce their fertiliser inputs – and they will indeed find it to their advantage.

## Future research

Given that continuous excessive fertilisation has no positive, nor detrimental effect on tree nutritional status or fruit quality, the ability to manipulate fruit quality and tree performance in conditions of limited nutrient supply, with the goal of improving nutrient use efficiency, and reduced impact on the environment, needs to be elucidated.

Furthermore, the general lack of response to increased fertilisation rates raises curiosity regarding in the nutritional management of HLB, given that a strong emphasis is presently placed on promotion of optimal to high tree nutritional conditions to avoid tree decline.

## Technology transfer

- Two presentations were given at a technical training event organised by Yara fertilisers (Paarl, June 2021).
- Some of the project's data was used in the Fertilisation Courses presented in October 2021.
- Two MSc thesis was generated from this project – the students graduated in April 2022 and will present posters at the 2022 CRI symposium. Titles and electronic links to these two theses are as follows:
  - Broeksma, C.R., 2022. In-season mineral nutrient management of Citrus by early (spring/summer) season sampling of leaves, fruitlets, and flowers. MScAgric., Dept. Horticultural Science, Stellenbosch University [Online]. Available: <http://scholar.sun.ac.za/handle/10019.1/124995>
  - Sofberg, M., 2002. Mineral nutrition of citrus trees in relation to flowering, fruit set and yield. MScAgric., Dept. Horticultural Science, Stellenbosch University [Online]. Available: <http://scholar.sun.ac.za/handle/10019.1/124953>

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#### 5.3.4 **PROGRESS REPORT: The influence of shade netting and rootstock choice on the oleocellosis incidence of citrus varieties, Navel oranges, and Eureka lemons**

Project 1301(Apr 2021-Mar 2022) by J Niemann and P Cronjé (CRI)

##### **Summary**

Oleocellosis is a common post-harvest rind blemish that occurs on citrus fruit, because of oil that leaks out on the surface, following rough handling. The fruit rind turgidity influences the degree to which oleocellosis symptoms develop, with sensitivity also varying between varieties, cultivars, and rootstocks in addition to ambient temperature and RH during harvest. Commercial reports of oleocellosis on the 'Washington' navel that occurs irrespective of a month-long wilting period under shade netting, have led to the current study. The aim was to investigate the effect of shade netting, irrigation, and rootstock on oleocellosis incidence and development. The study was conducted in the Sundays River Valley (SRV) in the Eastern Cape on orchards of 'Eureka' lemon (*Citrus limon* L. Burm. f.) and 'Washington', 'Cara Cara' and 'Palmer' navel trees (*C. sinensis* Osbeck). For the irrigation trial under shade netting, plastic sheets were placed under trees for 7-days before harvest (dbh) (lemons), 8 dbh ('Washingtons'), and 11 dbh ('Cara Cara') to affect a wilting treatment. As a contrasting treatment, the irrigation was continued as per normal and  $\pm 45$  L/tree was applied 1 dbh. To compare the shade vs non-shade net and the different rootstocks, 10 single trees/treatment was selected randomly for fruit sampling. Irrigation continued as per normal and  $\pm 45$  L/tree was applied 1 dbh to wet the soil profiles before harvesting as an attempt to induce oleo. From each selected tree, 10 fruit were sampled and 5 fruit were allocated to the drop test, and 5 to the penetrometer oleo inducing test. For the drop test, the fruit/rep were dropped simultaneously from a 1 m height. For the penetrometer test, an 8 mm tip was used on one side of the surface of the fruit in two positions. A 4 kg (lemons) and 3.5 kg (oranges) force were applied. The fruit were evaluated after 7d by measuring the length of the lesion from one side to the other, and the sum of the length of the two lesions was calculated. For the drop test, the fruit was scored on a scale that ranged from 0= no incidence to 5 = highly severe. Shade net significantly increased the susceptibility of 'Eureka' lemon for oleocellosis. The wilting period of 7d before harvest did not affect the lemon fruit sensitivity under nets. For 'Cara Cara' navel, shade net fruit had a significantly higher oleocellosis incidence, however, there was temperature variations during harvest evident between the two treatments, 12.9 °C (shade net) and 15.2 °C (Control). There was no effect of wilting the 'Cara Cara' trees 11 dbh on oleocellosis incidence. Cara Cara trees grafted on RL had a higher index and lesion size than CC. Wilting before harvest (8 d) did not reduce oleocellosis on 'Washington' and no shade net treatment effect was evident at  $P \leq 0.05$  except for trees grafted on CC, which had a larger lesion for shade net fruit compared to control. No rootstock differences were seen, however, both treatments were harvested at air temperatures  $< 13^{\circ}\text{C}$  when fruit turgidity is very high. It is suggested that later during the day, when the transpiration of the trees increases and fruit turgor decrease, rootstock differences might be evident. Shade net had no effect on 'Palmer' navel, oleocellosis sensitivity for CC grafted trees, but control trees (no net) on RL were more sensitive and for SC trees the shade netted fruit had a higher sensitivity. However, there was variation in air temperature between treatments (shade net and control) for each rootstock type, indicating that shade netted fruit might have to be harvested later during the day to reduce its oleo sensitivity in comparison to control orchards. 'Palmer' grafted on RL had a significantly higher sensitivity to oleo compared to CC and SC. However, there were differences in air temperature at sampling, which might increase the sensitivity. In the following season specific attention will be given to sampling at the same ambient conditions i.e. air, fruit temperature and RH to allow a final conclusion. The

findings, therefore, suggest that air temperature during harvest plays a critical role in the fruit sensitivity to oleocellosis and that the sensitivity of the fruit is not solely related to one factor and that shade netting and rootstocks are important contributable factors and further studies should be done to develop clear guidelines.

## Opsomming

Oleosellose is 'n algemene na-oes skil-afwyking wat op sitrusvrugte voorkom as gevolg van olie wat ná rowwe hantering op die oppervlak uitlek. Die turgiditeit van die vrugskil is die hoof faktor wat die mate waarin oleosellose-simptome ontwikkel, beïnvloed, met sensitiwiteit wat ook wissel tussen variëteite, kultivars en onderstamme, benewens omgewingstemperatuur en RH tydens oes. Kommersiële verslae van oleosellose op 'Washington' nawels wat voorkom, ongeag 'n maand-lange verwelkperiode onder skadunet, het tot die huidige studie gelei. Die doel is om die effek van skadunet, besproeiing en onderstam op oleosellose voorkoms en ontwikkeling te ondersoek. Die studie is in die Sondagsrivier vallei (SRV) in die Oos-Kaap op boorde van 'Eureka' suurlemoen (*Citrus limon* L. Burm. f.) en 'Washington', 'Cara Cara' en 'Palmer' nawel bome (*C. sinensis* Osbeck) uitgevoer. Vir die besproeiingsproef onder skadunet is plastiekseile onder bome geplaas vir 7 dae voor oes (dvo) (suurlemoene), 8 dvo ('Washington'), en 11 dvo ('Cara Cara') om verwelking te bewerkstellig. Vir die ander behandeling is besproeiing soos normaal voortgesit en ± 45 L/boom is 1 dvo toegedien. Om die skadu vs. nie-skadunet behandelings sowel as die verskillende onderstamme te vergelyk, is 10 enkel bome/behandeling vir vrugmonsters geselekteer. Besproeiing het voortgegaan soos normaal en ± 45 L/boom is 1 dvo toegedien om die grondprofiel voor oes te benat as 'n poging om oleosellose te indueer. Van elke geselekteerde boom is 10 vrugte gemonster en 5 vrugte is toegeken aan die valtoets, en 5 aan die penetrometer oleosellose induserende toets. Vir die valtoets is die vrugte/rep gelyktydig vanaf 'n 1m hoogte laat val. Vir die penetrometer toets, is 'n 8mm punt aan die een kant van die vrugoppervlak op twee posisies gebruik. 'n 4 kg (suurlemoene) en 3.5 kg (lemoene) krag is toegepas. Die vrugte is na 7 d geëvalueer deur die lengte van die letsel van die een kant na die ander te meet, en die som van die lengte van die twee letsels is bereken. Vir die valtoets is die vrugte beoordeel op 'n skaal van 0 = geen voorkoms tot 5 = hoogs ernstig. Skadunet het die vatbaarheid van 'Eureka' suurlemoen vir oleosellose verhoog. Die verwelkperiode van 7d voor oes beïnvloed nie suurlemoene se sensitiwiteit onder nette nie. Vir 'Cara Cara' het skadunet vrugte 'n aansienlik hoër voorkoms van oleosellose gehad, maar daar was temperatuurvariasies tydens oes duidelik tussen die twee behandelings, 12.9 °C (skadunet) en 15.2 °C (kontrole). Daar was geen effek van verwelking van die 'Cara Cara' bome 11 dvo op oleosellose voorkoms nie. 'Cara Cara' bome geënt op GS het 'n hoër indeks en letselgrootte as CC gehad. Verwelking voor oes (8d) het nie oleosellose op 'Washington' verminder nie en geen skadunet behandelingseffek was duidelik ( $P > 0.05$ ) behalwe vir bome geënt op CC, wat 'n groter letsel vir skadunet vrugte gehad het in vergelyking met kontrole nie. Geen onderstam verskilte is gesien nie, maar beide behandelings is by lugtemperatuur  $< 13^{\circ}\text{C}$  geoes wanneer vrug turgordruk baie hoog is. Daar word voorgestel dat later deur die dag, wanneer die transpirasie van die bome toeneem en turgordruk afneem, onderstam verskilte sigbaar kan wees. Vir 'Palmer' onder skadunet, was daar geen effek op oleosellose sensitiwiteit vir CC geënte bome, maar kontrole bome (geen net) op GS was meer sensitief, en vir SC bome het die skadunet vrugte 'n hoër sensitiwiteit gehad. Daar was egter variasie in lugtemperatuur tussen behandelings (skadunet en kontrole) vir elke onderstam tipe, wat aandui dat skadunet vrugte dalk later deur die dag geoes moet word om die oleo-sensitiwiteit daarvan te verminder in vergelyking met geen skadu boorde. 'Palmer' geënt op GS het 'n hoër sensitiwiteit vir oleosellose in vergelyking met CC en SC. Daar was egter verskilte in lugtemperatuur tydens monsterneming, wat die sensitiwiteit kan verhoog. In die volgende seisoen sal spesifieke aandag gegee word aan monsterneming by dieselfde omgewingstoestand d.w.s. lug, vrugtemperatuur en RH om 'n finale gevolgtrekking moontlik te maak. Die bevindinge dui dus daarop dat lugtemperatuur tydens oes 'n kritieke rol speel in die vrugsensitiwiteit vir oleosellose en dat die sensitiwiteit van die vrugte nie net met een faktor verband hou nie en dat skadunet en onderstamme belangrike bydraende faktore is en verdere studies moet gedoen word om duidelike riglyne te ontwikkel.

### 5.3.5 PROGRESS REPORT: The use of root growth restricting soil management practices to improve Valencia tree vigour, yield and fruit quality

Project 1340 (2021/22 – 2022/23) by V G White and P J Raath (CRI)

## Summary

This project aims to investigate the effect of a root restricting trench on above ground vegetative growth, thereby further elucidating the relationship between root and shoot growth, as well as the effect on yield and fruit quality. A Further aim is the compare soil preparation practices i.e., ridging and not-ridging. The trial consists of three treatments (i.e., trench, ridge and non-ridge) replicated seven times, laid out in in randomised block design. Each treatment replicate was sub-divided into two sub-treatments i.e., conventional irrigation and reduced irrigation. The trial was laid out in September 2021, the soil was ameliorated with lime, phosphorous and potassium chloride, successful amelioration has been confirmed with soil analyses in February 2022. Trenches was created with dimensions 60cm (depth)×120cm(width) and drainage has been installed. Compaction has been confirmed with a penetrometer, soil strength ranged from 320-1000kpa and is expected to restrict root growth. 'Turkey' valencia trees grafted on Carizzo citrange (*Poncirus trifoliata* x *Citrus sinensis*) rootstock was planted in December 2021 and base line tree heights and trunk circumferences has been measured in February 2022. All experimental equipment i.e., loggers and soil moisture sensors, has been purchased and installed. Root growth will be evaluated in December 2022 and irrigations sub-treatments will be employed if roots have sufficiently filled the trench volume.

## Opsomming

Hierdie projek beoog om die effek van 'n wortelbeperkende sloot ("trench") op bogrondse vegetatiewe groei te ondersoek, om sodoende die verband tussen wortel- en lootgroei verder toe te lig, asook die effek op opbrengs en vrugkwaliteit. 'n Verdere doelwit is om grondvoorbereidingspraktyke te vergelyk, d.w.s. op-erd walle en geen op-erd walle. Die proef bestaan uit drie behandelings wat sewe keer herhaal word, uiteengesit in ewekansige blokontwerp. Elke behandelingsherhaling is in twee sub-behandelings onderverdeel, naamlik konvensionele besproeiing en verminderde besproeiing. Die proef is in September 2021 uitgelê, die grond is met kalk, fosfaat en kaliumchloried reggestel. Suksesvolle regstelling is met grond-ontledings in Februarie 2022 bevestig. Slote met afmetings 60cm (diepte) x 120cm (breedte) is geskep en dreineringspype is geïnstalleer. Grondverdigting is met 'n penetrometer bevestig. Grondsterkte het van 320-1000kpa gewissel en sal na verwagting wortelgroei beperk. 'Turkey' Valencia bome, geënt op Carizzo citrange (*Poncirus trifoliata* x *Citrus sinensis*) onderstam is in Desember 2021 geplant en basislyn boomhoogtes en stam-omtrek is in Februarie 2022 gemeet. Alle eksperimentele toerusting, d.w.s. grondvogssensors, is aangekoop en geïnstalleer. Wortelgroei sal in Desember 2022 geëvalueer word en besproeiing sub-behandelings sal aangewend word indien wortels die slootvolume voldoende gevul het.

### 5.3.6 PROGRESS REPORT: Evaluation of strategies to improve water use efficiencies in citrus production

Project 1341 (2021/22 – 2024/25) by Nicky Taylor, John Annandale and Michael van der Laan (University of Pretoria), Sebinasi Dzikiti (Stellenbosch University) and Stephanie Midgley (Department of Agriculture Western Cape)

## Summary

Pressure is mounting on growers to use water more wisely or to improve their water use efficiency, which often implies using less water to produce the same yield. One solution is to use a more efficient irrigation system, which has led to the adoption of low flow drip systems, where delivery rates are less than 1 L h<sup>-1</sup>. While the benefits of these system are widely publicised, there have been few attempts to systematically quantify the water balance of an orchard under low flow drip in order to determine where and how water savings are realised. It is for this reason that a replicated trial has been established in a 'Nadorcott' mandarin orchard in Nelspruit. The trial consists of five treatments each replicated four times, with 10 trees in each replicate. Three treatments receive the same amount of water, with delivery rates of 2.3 L h<sup>-1</sup>, 1.6 L h<sup>-1</sup> and 0.7 L h<sup>-1</sup>. The remaining two treatments receive 20% and 40% less water at a delivery rate of 0.7 L h<sup>-1</sup>. Irrigation volumes are determined weekly based on projected reference evapotranspiration and site-specific crop coefficients. The water balance in each treatment is determined by measuring transpiration, deep drainage, and soil water content. Key periods are targeted for plant physiological measurements, which include stomatal conductance, fluorescence and predawn and midday leaf water potentials. A 2-D grid of soil water sensors is used to determine differences in wetting patterns for the different treatments and to parameterise the Hydrus 2-D model to further explore how wetting patterns differ with different soil textures and how plant water uptake differs

between the different delivery rates. Yield and quality will be determined from three trees in each replicate at the end of each season.

## Opsomming

Daar is toenemend druk op produsente om water met meer oorleg te gebruik, te wete hul watergebruikseffektiwiteit te verbeter. Dit beteken gewoonlik dat minder water gebruik moet word om dieselfde opbrengs te verkry. Een benadering is om 'n meer effektiewe besproeiingsstelsel te gebruik – die gevolg is dat lae vloei dripsisteme, met lewerings van minder as  $1 \text{ L}\cdot\text{h}^{-1}$ , deesdae gebruik word. Hoewel die voordele van hierdie sisteme wyd verkondig word, is daar tot hede min gedoen om die waterbalans van 'n boord met 'n lae-vloei dripsisteme te kwantifiseer en dus vas te stel waar/hoe, indien wel, waterbesparings gerealiseer word. Gevolglik is 'n goed-ontwerpte statistiese proef in 'n 'Nadorcott' mandaryn boord in Nelspruit begin. Die proef bestaan uit vyf behandelings, wat vier keer herhaal word, met 10 bome in elke herhaling. Drie van die behandelings ontvang dieselfde hoeveelheid water, maar teen dripper leweringstempo's van onderskeidelik  $2.3 \text{ L h}^{-1}$ ,  $1.6 \text{ L h}^{-1}$  en  $0.7 \text{ L h}^{-1}$ . Die ander twee behandelings ontvang onderskeidelik 20% en 40% minder water, en dan teen 'n leweringstempo van  $0.7 \text{ L h}^{-1}$ . Besproeiingsbehoefte word weekliks bepaal n.a.v. voorspelde verwysing evapotranspirasies en ligging-spesifieke gewasfaktore. Die waterbalans van elke behandeling word bepaal deur transpirasie, diepdreinerings en grondwaterinhoud te meet. Die belangrikste fenologiese stadiums is ook gekies waartydens plantfisiologiese metings gedoen word, wat o.a. stomatale geleiding en fluorissensie metings insluit, asook blaarwaterpotensiale voor sonopkoms en in die middel van die dag. 'n Tweedimensionele rooster van grondwatersensors word gebruik om verskille in benattingspatrone van die grond in die verskillende behandelings te meet. Verder word hierdie data ook gebruik om grense vir die Hydrus 2-D model vas te stel sodat verdere ondersoek ingestel kan word na die verskille in die benattingspatrone in verskillende grondteksture, asook om vas te stel hoe wateropname tussen die verskillende behandelings verskil. Opbrengs en gehalte van die vrugte word vir alle herhalings aan die einde van elke seisoen bepaal.

### 5.3.7 **PROGRESS REPORT: Water stress at different phenological stages of Nadorcott Mandarin trees in combination with two irrigation systems to mitigate water shortages in citrus growing areas**

Project number 1342 (Apr 2021 – Dec 2024) by EL Lategan and JL van Zyl (Stellenbosch University)

## Summary

Recent droughts in large parts of South Africa gave crop producers a glimpse of how water availability could be affected in the future. This is further aggravated by increased demand for water for agriculture, domestic, industrial usages, and ecological requirements. Crop production industries will have to adapt practices to produce economical viable yields with less water than in the past. Water constraints affects every aspect of citrus yield including flowering, fruit drop, fruit size, fruit yield, fruit quality and canopy size. The specific negative effect of water stress depends mainly on three factors, namely the phenological stage when it occurs, the severity of the stress, and the duration of the water stress. The Department of Soil Science (Stellenbosch University) proposed a project to the CRI to investigate the effect of soil water constraints during three phenological stages through the production year, i.e., from flowering to harvest, on the vegetative, production and quality responses of Mandarins. Eight different irrigation strategies will be applied. A control treatment will be irrigated once *ca.* 20% plant available water (PAW) in the rootzone has been depleted, while six strategies irrigated at either *ca.* 20% or 80% PAW depletion, depending on the phenological stage, and a final treatment irrigated at *ca.* 80% PAW depletion throughout the year. Drip and micro-sprinkler systems will be used to compare their different water usages. A suitable Tango orchard to conduct the experiment in was identified near Addo, Sundays River Valley. The project could not start in September 2021 due to the irrigation company that committed themselves to sponsor the irrigation equipment only being able to supply the equipment in April 2022. Although soil water monitoring equipment and a weather station was installed by September 2021, it was decided to postpone the commencement of the applications to the onset of flowering in September 2022.

## Opsomming

Onlangse droogtes in groot dele van Suid-Afrika het gewasprodusente 'n blik gegee op hoe waterbeskikbaarheid in die toekoms geaffekteer kan word. Dit word verder vererger deur die groter vraag na water vir landbou, huishoudelike, industriële gebruike en ekologiese vereistes. Gewasproduksiebedrywe sal bestuurspraktyke moet aanpas om ekonomies volhoubare produksies met minder water as in die verlede te produseer. Watertekorte beïnvloed elke aspek van sitrusopbrengs, insluitend blom, vrugval, vruggrootte, vrugopbrengs, vrugkwaliteit en blaredakgrootte. Die spesifieke negatiewe effek van waterstres hang hoofsaaklik van drie faktore af, naamlik die fenologiese stadium wanneer dit voorkom, die erns van die stres en die duurte van sodanige waterstres. Die Departement Grondkunde (Stellenbosch Universiteit) het 'n projek vir die CRI geïnisieer om die effek van grondwaterbepelings gedurende drie fenologiese stadiums tussen blom en oes op die vegetatiewe groei, produksie en vruggehalte van Mandaryne te ondersoek. Agt verskillende besproeiingsstrategieë sal toegepas word. 'n Kontrolebehandeling sal besproei word sodra ca. 20% plantbeskikbare water (PBW) in die wortelzone onttrek is, ses strategieë sodra ca. 20% of 80% PBW onttrekking bereik is, afhangende van die fenologiese stadium, en 'n finale behandeling by ca. 80% PBW onttrekking deur die jaar. Drup- en mikrosprinkelstelsels sal gebruik word om hul verskillende waterbehoefes te vergelyk. 'n Geskikte Tango-boord vir die uitvoer van die eksperiment is naby Addo, Sondagsriviervallei, geïdentifiseer. Die projek kon nie soos beplan in September 2021 begin nie, omdat die besproeiingsmaatskappy wat hulle daartoe verbind het om die besproeiingstoerusting te borg eers in April 2022 die toerusting kon verskaf. Alhoewel grondwatermoniteringstoerusting en 'n weerstasie teen September 2021 geïnstalleer is, is daar besluit om die toediening van behandelings uit te stel tot die aanvang van blom gedurende September 2022.

#### 5.4 **PROGRAMME: CULTIVAR EVALUATION**

Programme coordinator: Johan Joubert (CRI)

##### 5.4.1 **Programme summary**

To address the Satsuma and Clementine requirements, there are numerous trials representing the cooler citrus production areas of South-Africa (mainly Western and Eastern Cape), focusing on the early mandarin demand (5.4.15, 5.4.25, 5.4.26, 5.4.27, 5.4.28).

The mandarin trials in the hot citrus production areas (5.4.4, 5.4.7) remain a priority with the reality of good quality fruit with good colour development early in the season and optimum Brix:acid ratios being critical (low acids biggest concern), options are improving with new selections becoming available, but require good management practices. The cool and intermediate production areas remain the best mandarin producing options (5.4.6, 5.4.8, 5.4.10, 5.4.16, 5.4.17, 5.4.18, 5.4.19) due to specific climatic requirements (better early colour and acids). The focus remains to plant earlier or later maturing cultivars outside the Tango, ARCCIT9 (Nadorcott LS), and Nadorcott picking windows since very high numbers of these trees have been planted. The best quality fruit produced in this picking window will be in high demand, but marginal fruit quality areas will be in trouble. The consumer demands fruit with low seed numbers, or seedless fruit, that peels easily, has good colour development and excellent flavour. Numerous new experimental options went into trial sites in the main citrus production areas, on different rootstocks, to determine the commercial value of these cultivars, including in the hotter production regions (early- and late maturing possibilities).

Navel prices remained fairly low due to a combination of shelf-life problems (low acid levels), postharvest performance (more sensitive rinds) and open navel ends, all contributing to the decrease in new navel plantings (5.4.9, 5.4.20, 5.4.21, 5.4.22). There is a demand for red pigmented navel fruit in the market and specifically new Cara Cara plantings increased in the navel production areas. The focus will remain on lemon, mandarin and Valencia options to fill the requirements in the production cycle of the packhouse programme.

We need early maturing Valencia options to replace or supplement Turkey because of the rind disorders and shelf life restrictions (adding experimental options in trials; Ngonini and Rato Early). The focus on late-maturing Valencia selections increased (5.4.2, 5.4.3, 5.4.5, 5.4.12, 5.4.23, 5.4.24) in the suitable citrus production areas (Letsitele) where demand for low seeded or seedless Valencia with good crop production increased. The problem with high chimera numbers on some of the late Valencia selections stimulated the need for alternative options to replace problem orchards or to establish new plantings (Jasi, Kobus du Toit Late, McClean SL etc).

Star Ruby remains the number one grapefruit planted in South Africa due to excellent internal colour development and very good internal quality with high Brix content. The Star Ruby early and late were included at trial sites to allow the possibility of a longer picking window for red grapefruit. There are numerous new red grapefruit selections and a white included in the trial sites with lower naringin (bitter taste) levels to improve flavour and eating experience.

Eureka, 2 PH Seedless Eureka, followed by Lisbon, Limoneira and Genoa (single crop production per season) plantings remain high in all the citrus-producing areas, as lemon establishment is possible in any citrus climatic region. One of the most important fruit characteristics remains oblong (longer) fruit shape, followed by prolonged picking window (best prices early in the season) and low seed numbers. Another important aspect on the tree's side is low thorn numbers or smaller thorns on the bearing branches to facilitate the harvesting process. Eureka remains the number one lemon selection preferred by citrus growers and consumers for several reasons: good quality fruit with high juice content; fairly long fruit shape; ability to bear a good crop on the trees with two to three main crops; and limited thorns on the bearing branches for optimal picking. Lisbon and Limoneira will be the next option for commercial plantings, due to compatibility on citrumelo and citrange rootstocks (Carizzo citrange, Swingle citrumelo and C35) and good production and fruit quality. There are several new lemon selections included in the trial sites to challenge Eureka, with a specific goal towards completely seedless fruit along with optimum yield on the trees (5.4.11).

Rootstock evaluations (including semi-commercial trials) expand the range of new rootstock trials, including the mainstream (commercial), semi-commercial range, as well as several Florida options (with the possibility of HLB tolerance) to evaluate production performance and suitability in different soil types (pH, clay content, salinity etc). There is also a range of new Argentinian rootstocks in the new trials (experimental and semi-commercial) to address the need for lemon scion compatibility and specific conditions and smaller tree volumes (5.4.13, 5.4.14).

### **Program-opsomming**

Die Satsuma en Clementine behoeftes word aangepreek in verskeie proewe wat die koeler sitrus produserende areas van Suid-Afrika insluit (hoofsaaklik Wes en Oos Kaap) en die vroeë mandaryn aanvraag bedien (5.4.15, 5.4.25, 5.4.26, 5.4.27, 5.4.28).

Die mandaryn proewe in die warm sitrus produksie areas (5.4.4, 5.4.7) bly 'n prioriteit van goeie vrugkwaliteit (optimale Brix:suur) met goeie kleurontwikkeling vroeg in die seisoen (lae suurvlakke groot uitdaging). Die nuwe seleksie brei die opsies uit vir hierdie doelwit, maar met goeie bestuurs praktyke. Sitrus wat in die koel en intermedieë produksieareas verbou word, bly die beste mandaryn produserende opsies (5.4.6, 5.4.8, 5.4.10, 5.4.16, 5.4.17, 5.4.18, 5.4.19) as gevolg van spesifieke klimaatvereistes (beter vroeë kleur en sure). Die fokus bly op die aanplant van vroer of later rypwordende kultivars buiten die Tango, ARCCIT9 (Nadorcott LS) en Nadorcott plukvensters, aangesien groot volumes van hierdie kultivars geplant is. Die beste kwaliteit vrugte wat in hierdie plukvenster geproduseer word, sal in groot aanvraag wees, maar die gebiede met marginale vrugkwaliteit kan problematies wees. Die verbruikers voorkeur bly vrugte met 'n lae saadinhoud, of totaal saadlose vrugte, wat maklik skil met 'n goeie kleurontwikkeling en uitstekende smaak. Verskeie nuwe eksperimentele opsies word ingesluit in proefpersele in die belangrikste sitrus produkserende areas, op verskillende onderstam opsies, om die kommersiële waarde van hierdie kultivars te bepaal, insluitend die warmer produksie areas (vroeë en laat rypwordende opsies).

Die nawel pryse het redelik laag gebly as gevolg van die kombinasie van rakleef tyd probleme (lae suurvlakke), na-oes prestasie (meer sensitiewe skille) en oop nawel-ente, wat alles bygedra het tot die afname in nuwe nawel aanplantings (5.4.9, 5.4.20, 5.4.21, 5.4.22). Daar is wel 'n aanvraag vir rooi gepigmenteerde vrugte in die mark en spesifiek het nuwe Cara Cara aanplantings toegeneem in die nawel produksie areas. Die fokus bly egter op suurlemoen, mandaryn en Valencia opsies om die vereistes in die produksie siklus van die pakhuisprogram aan te spreek.

Vroeë Valencia opsies word benodig om die Turkey aan te vul of te vervang, a.g.v. problematiese skildefekte en hou vermoë (nuwe opsies word ingesluit in eksperimente; Ngonini and Rato Early)). Die fokus op laat rypwordende Valencia-seleksies bly hoog (5.4.2, 5.4.3, 5.4.5, 5.4.12, 5.4.23, 5.4.24) in die geskikte sitrusproduksie areas (Letsitele), waar die vraag na Valencia met 'n lae saadinhoud of totaal saadlose vrugte met goeie produksie toegeneem het. Die probleem van 'n hoë chimera voorkoms by sommige van die laat Valencia seleksies, het die behoefte laat ontstaan om alternatiewe opsies vir die vervanging van probleem boorde of nuwe aanplantings te gebruik (Jasi, Kobus du Toit Late, McClean SL ens.).

Star Ruby is die nommer een pomelo wat in Suid-Afrika aangeplant word a.g.v. uitstekende interne kleurontwikkeling en baie goeie interne gehalte met 'n hoë Brix-inhoud. Die eksperimentele vroeë- en laat rypwordende Star Ruby seleksie word in proef persele ingesluit met die moontlikheid om die plukvenster te verleng van die rooi pomelos. Daar is verskeie nuwe rooi pomelo seleksies en een wit opsie wat by die proefpersele ingesluit word met laer naringien vlakke (bitter smaak) om die smaak en eet-ervaring te verbeter.

Eureka, 2 PH Saadlose Eureka, gevolg deur Lisbon, Limoneira en Genoa (enkel oes produksie per seisoen) aanplantings, bly hoog in al die sitrus produserende areas; deurdat suurlemoen vestiging moontlik is in enige sitrus klimaatzone. Een van die belangrikste vrug eienskappe is steeds langwerpige (silindriese) vrug vorm, gevolg deur verlengde plukvenster (beste pryse vroeg in die seisoen) en lae saad getalle. Ander belangrike aspekte is lae en of kleiner dorings op die draetakke om die oes proses te vergemaklik. Eureka bly steeds die nommer een suurlemoenseleksie wat deur Sitrusprodusente en verbruikers verkies word om verskillende redes: vrugte van goeie gehalte met 'n hoë sapinhoud; redelike lang vrugvorm; potensiaal vir 'n goeie drag met twee tot drie vrugsette asook min dorings. Lisbon en Limoneira is die volgende kommersiele opsie vir aanplantings, a.g.v. verenigbaarheid op citrumelo en citrange onderstamme (carizzo citange, swingle citrumelo en citrange 35) asook goeie produksie en vrugkwaliteit. Lemongold (2 PH SL Eureka) aanplantings neem toe as 'n kommersiele saadlose opsie. Daar is 'n aantal nuwe suurlemoen seleksies wat by die proefpersele ingesluit word om met Eureka te vergelyk, met die spesifieke doel om totaal saadlose langwerpige vrugte met optimale opbrengs te produseer (5.4.11).

Onderstam evaluasies word uitgebrei (insluitend semi-kommersiele proewe) met 'n nuwe reeks onderstam proewe, ingesluit die hoofstroom reeks (kommersiele opsies), semi-kommersiele reeks asook verskeie Florida opsies (met moontlike HLB weerstandbiedendheid) om produksie potensiaal te evalueer en geskiktheid vir verskillende grond tipes (pH, klei inhoud, sout vlakke) te bepaal. Daar is 'n reek Argentynse onderstamme in nuwe proewe ingesluit (eksperimenteel en semi-kommersieel) om die suurlemoen-bostam verenigbaarheids kwessie aan te spreek asook kleiner boom volumes te bevorder (5.4.13, 5.4.14).

#### 5.4.2 **PROGRESS REPORT: Evaluation of Valencia selections in hot humid inland areas (Onderberg)** Project 75A by J Joubert (CRI)

##### **Summary**

Selections that performed well in this season, in this hot, humid production area, according to optimal maturity from early to late, were as follows. Val early, one of the new early maturing (internal quality) cultivars that matures before Turkey. There was a better colour development on the fruit by the time of optimum maturity with Val early, but deeper orange colour compared to the more yellow of Turkey when fully coloured. Turkey will follow, but bear in mind that this selection has a sensitive rind. Do not allow the fruit to hang for too long because the optimal picking period is no longer than 4-6 weeks. Delta would follow, with good internal quality, production and fruit size, followed by McClean SL and Gusocora representing the middle of the Valencia season for this area. Alpha will be next in line, maturing later in this area than other areas (Letsitele), but keep the younger tree age in mind. The later selections can broaden the list of choices to extend the season, commencing with Skilderkrans, Kobus du Toit Late and Jasi (optimum fruit size distribution) and followed by Lavalley, with higher acid levels, an ultra-late possibility.

## Opsomming

Seleksies wat hierdie seisoen, volgens optimum rypheid van vroeg tot laat goed presteer het vir hierdie vrotte warm produksiegebied, is soos volg. Valearly is een van die nuwe vroeë Valencia-opsies (vroeg intern ryp) wat voor Turkey inpas. Daar was 'n beter kleurontwikkeling op die vrugte gewees met optimum rypheid by Valearly, maar wel dieper oranje kleur wanneer opgekleur in vergelyking met meer geel by Turkey. Turkey sal dan volg, wees net versigtig om nie die seleksie te lank te hang nie. Baie skilprobleme kan ontwikkel, want die optimum oestydperk strek oor gemiddeld vier weke, ses weke maksimum dan moet die vrugte af wees. Delta kan dan volg wat goeie interne kwaliteit, produksie en vruggrootte lewer, gevolg deur McClean SL en Gusocora wat dan die middel van die Valencia-seisoen vir hierdie area verteenwoordig. Alpha pas dan in, wat later in hierdie area rypword in vergelyking met ander areas (Letsitele), maar hou die jong boomouderdom in gedagte. Die later seleksies wat kan bydra tot die keuse om die seisoen te verleng, kan bestaan uit Skilderkrans, Kobus du Toit Laat en Jasi (optimum vruggrootte verspreiding) gevolg deur Lavallo met hoër suurvlakke as die ultra-laat opsie.

## 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 (control), Gusocora, Jasi, Kobus du Toit Late, Lavallo, McClean SL, Skilderkrans, Turkey (control) and Valearly at Riverside in Malelane, Mpumalanga.

**Table 5.4.2.1.** Internal fruit quality minimum export requirements for Valencia types.

Variety	Juice %	Brix °	Min Acids	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 Riverside (Malelane) during 2021.

Selection	Rootstock	Year Planted	Topworked	No. of trees
Alpha	CC	NA	2015	4
Delta	C35/CC/SC	2012	NA	5/5/5
Gusocora	CC	NA	2015	4
Jasi	C35/CC/SC	2012	NA	5/5/5
Kobus du Toit Late	C35/CC/SC	2012	NA	5/5/5
Lavallo	CC	NA	2015	4
McClean SL	C35/CC/SC	2012	NA	5/5/5
Skilderkrans	C35/CC	2012	NA	5/5
Turkey	C35/CC/SC	2012	NA	5/5/5
Valearly	C35/CC/SC	2012	NA	5/5/5

## Results and discussion

This project is ongoing – all evaluations and tasks have been completed to date. Trees were visually evaluated at Riverside (Malelane) during the 2021 season.

### Delta (control)

Delta produced a good crop this season as seedless control for the evaluations. Fruit size range was slightly smaller this season and peaked from medium to large/extra-large (count 88 to 56/48) with good internal quality values, juice levels above 55, Brix of up to 11.7 and acids above 0.95%. Colour development at peak maturity on all three rootstocks were very similar (between T1 and T3). Delta remained completely seedless and complied with the export requirements. Based on the internal quality results in Table 5.4.2.3, maturity will be from the beginning to the middle of July, and slightly later on swingle due to higher acid levels.

### Jasi

The trees were bearing another good crop this year on all three rootstock combinations. The fruit size was smaller and varied from medium to large, count 88/72 to 56 (average). The rind texture improved this season, becoming smoother with time. Seed count per fruit was even lower (low pollination) this season and varied from 2.2 to 3.8 seeds per fruit. Internal quality improved with tree age and produced better juice levels (above 56% at peak maturity), good Brix (above 11 at maturity) and higher acids (above 1.2). External colour development improved and peaked between T1 and 2/3 with the final evaluations. Maturity seems to be the middle of July (C35 and CC) to the beginning of August (SC) based on the results in Table 5.4.2.3.

### Kobus du Toit Late

Kobus du Toit Late was evaluated at the Riverside trial site on two rootstocks (CC and SC) and produced small/medium to large fruit size (count 105/88 to 56) on the trees due to a better crop, with 2.3 seeds average. The colour development was very similar on both rootstocks at peak maturity. The internal quality was good, juice levels above 56%, Brix up to 12.0, and lower acids earlier in the season (below 1.0 on CC) for the later maturing selection. External colour peaked from T1 to 4. Maturity seems to be end of June to end of July (trees on SC), according to Table 5.4.2.3.

### McClellan SL

The standard McClellan will be included in future trials as a control to compare the SL selection's performance, although McClellan developed high chimera incidences on the fruit (up to 40%) in commercial plantings. McClellan SL produced fairly round fruit with soft fibre strength that peeled easily, containing low rind oil levels. All the fruit evaluated remained 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 (GA<sub>3</sub> sprays are recommended). The fruit size increased and peaked at medium-large to large/extra-large (count 88/72-56/48). The internal quality improved from good (2017) to very good with high juice levels for the trial site up to 61%, Brix up to 11.7 and higher acid levels (above 0.95%). There was an improvement on external colour ranging from T1-2/3 compared to a delay the previous season. Based on the internal quality results in Table 5.4.2.3, maturity will be mid to end of July.

### Skilderkrans

Skilderkrans bore fruit on C35, Carrizo and Swingle at the Riverside trial site. Fruit size was smaller due to a better crop on the trees and varied from medium to large/extra-large (count 88/72-56/48). Internally the Brix content improved (up to 12.5) and the acid level of 1.2 to 1.5% indicated a later maturing Valencia selection. Juice level decreased slightly to an average 56.1%; above the minimum required export figure. There was no delay in external colour (T1-3) on any of the rootstocks evaluated. The fruit developed a smooth rind, fibre strength was fairly soft and the fruit shape was round. Ratios were lower this season (except the final evaluation on C35) due to the higher acid levels, delaying peak maturity to the end of July and mid-August on all three rootstocks (Table 5.4.2.3).

### Turkey (Control)

Fruit size was smaller this season, ranging from count 88 to 56 average, with medium to large fruit size. Fruit characteristics for Turkey were round fruit shape, smooth rind texture, very good flavour, soft rag, fairly thin rind, easy fruit peeling, and lower seed count per fruit ranging from 1.0 to 2.5 seeds per fruit (higher than 2019). The internal colour was light yellow, and externally the fruit remained yellow up to over-matured fruit. It should be borne in mind that this selection is not a true Valencia and has the qualities of a mid-season orange; for instance, the exceptionally soft rag of the fruit, and the soft rind result in rind problems if managed incorrectly.

Turkey should not be harvested over more than four weeks as extending the harvesting season can lead to rind disorders. Based on the internal quality results in Table 5.4.2.3, the estimated maturity will be middle to end of May.

#### Val early

Valearly, bearing a good crop on the trees, developed low seed numbers (0.0 to 0.3 seeds per fruit) this season. The internal quality of the fruit was good early in the season with medium-high juice (above 53%, highest for Swingle – 59.1%), Brix above 10.3 and acid above 0.8 on Swingle and Carrizo. Compared to the other early maturing selections, Valearly seems to be at least two weeks earlier, with good internal quality but slightly delayed external colour ranging from T2-3. Estimated maturity according to Table 5.4.2.3, seems to be the end of April.

#### Additional selection

Alpha, Gusocora and Lavallo bore a good crop for the third time this season and evaluations were possible, all topworked onto Carrizo rootstocks. All three selections were completely seedless. Alpha matured first; middle to end of July with good acid levels and good Brix, ranging from 10.0 to 11.4. The highest acid level between the three cultivars was on Lavallo; also developing very good Brix and juice content. Colour development remained very similar between T1 and T3 up to peak maturity. Fruit size peaked on two of the three selections, except for Lavallo with bigger fruit, between count 88/72 and 56/48; very good for Valencia production and export.

### **Conclusions**

The internal quality for this season for all the selections evaluated, complied with the export standards at peak maturity of the fruit. These acid levels will decrease towards the end of the season, indicating extended shelf-life of the selection for example, Jasi and Lavallo. Jasi also indicated low chimera fruit numbers on the trees, providing another good late maturing Valencia option to be included in future plantings. There was no Brix: acid ratio below 7.5:1 at peak maturity this season, which is often associated with later maturing selections having higher acid levels, but rather the opposite scenario with lower acids in general on most of the fruit samples being evaluated. When the acid levels decrease, the ratio increases. There was a better colour development with most of the selections towards peak maturity time, even in the case of Valearly, where the colour development was slightly delayed even after peak quality. The average seed count for this season remained fairly low, including Kobus du Toit Late and Jasi (average 2.3 and 3.0 seeds per fruit), indicating lower cross pollination in the mixed trial block. McClean SL remained completely seedless. Jasi and Kobus du Toit Late will be future possibilities to include in new Valencia plantings (optimum Valencia fruit size distribution, high juice levels, low seed counts and late maturing). Fruit size improved on the trees as they matured, between count 88/72 and up to count 56/48 on selections with lighter yields.

**Table 5.4.2.3.** Internal fruit quality data for Valencia and late orange selections at Riverside (Malelane) during the 2022 season.

Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
Alpha	CC	11/05/2021	76 - 80	72 - 64	54,2	9,8	1,60	6,1	0,0	T4 - 6
Alpha	CC	01/06/2021	75 - 86	72 - 48	52,9	11,0	1,30	8,5	0,0	T1 - 3
Alpha	CC	28/06/2021	75 - 83	72 - 56	53,1	11,0	1,60	6,9	0,0	T1
Alpha	CC	15/07/2021	72 - 86	88 - 48	57,6	11,4	1,00	11,4	0,0	T1 - 2
Delta	C35	01/06/2021	77 - 80	72 - 64	58,5	10,8	1,20	9,0	0,0	T1 - 3
Delta	C35	28/06/2021	76 - 80	72 - 64	59,2	11,0	1,25	8,8	0,0	T1 - 3
Delta	C35	15/07/2021	69 - 72	72 - 64	59,6	11,7	1,10	10,6	0,0	T1 - 2

Delta	CC	01/06/2021	71 - 81	88 - 64	53,8	10,4	1,20	8,7	0,0	T1 - 3
Delta	CC	28/06/2021	75 - 82	72 - 56	59,4	10,9	1,05	10,4	0,0	T1 - 2
Delta	CC	15/07/2021	65 - 79	105 - 64	63,1	11,2	0,95	11,8	0,0	T1 - 2
Delta	SC	01/06/2021	73 - 80	72 - 64	55,6	10,6	1,25	8,5	0,0	T1 - 2
Delta	SC	28/06/2021	71 - 87	88 - 48	59,3	10,0	1,20	8,3	0,0	T1 - 2
Delta	SC	15/07/2021	61 - 73	125 - 72	62,3	11,2	1,25	9,0	0,0	T1
Gusocora	CC	01/06/2021	72 - 77	88 - 72	52,3	10,7	1,05	10,2	0,0	T1 - 3
Gusocora	CC	28/06/2021	72 - 79	88 - 64	50,4	11,0	1,05	10,5	0,0	T1 - 3
Gusocora	CC	15/07/2021	66 - 80	105 - 64	61,0	11,8	1,15	10,3	0,0	T1 - 2
Gusocora	CC	13/08/2021	76 - 88	72 - 48	57,5	12,0	0,75	16,0	0,0	T1
Jasi	C35	01/06/2021	81 - 82	64 - 56	53,0	10,1	1,80	5,6	3,2	T1 - 2
Jasi	C35	28/06/2021	75 - 83	72 - 56	60,4	11,5	1,35	8,5	3,8	T1 - 2
Jasi	C35	15/07/2021	72 - 85	88 - 56	63,1	12,0	1,20	10,0	2,2	T1 - 2
Jasi	CC	01/06/2021	74 - 77	72	56,7	10,6	1,55	6,8	3,0	T1 - 2
Jasi	CC	28/06/2021	75 - 80	72 - 64	54,7	11,4	1,60	7,1	3,2	T1 - 3
Jasi	CC	15/07/2021	77 - 84	72 - 56	58,3	11,6	1,15	10,1	3,3	T1 - 2
Jasi	SC	01/06/2021	75 - 81	72 - 64	53,0	11,8	1,80	6,6	3,3	T1 - 2
Jasi	SC	28/06/2021	73 - 80	72 - 64	56,1	11,3	1,50	7,5	2,2	T1
Jasi	SC	15/07/2021	75 - 81	72 - 64	56,7	11,5	1,25	9,2	2,5	T1 - 2
K du Toit Late	CC	01/06/2021	74 - 80	72 - 64	54,8	9,9	1,15	8,6	2,0	T1 - 3
K du Toit Late	CC	28/06/2021	71 - 84	72 - 56	57,3	11,2	1,40	8,0	2,7	T1 - 2
K du Toit Late	CC	15/07/2021	64 - 77	125 - 72	57,5	12,0	0,90	13,3	0,0	T1 - 2
K du Toit Late	CC	13/08/2021	77 - 83	72 - 56	59,9	11,1	0,95	11,7	3,0	T1 - 2
K du Toit Late	SC	01/06/2021	77 - 80	72 - 64	56,1	10,1	1,55	6,5	3,0	T2 - 3
K du Toit Late	SC	28/06/2021	72 - 81	88 - 64	58,7	11,1	1,40	7,9	1,7	T1 - 2
K du Toit Late	SC	15/07/2021	66 - 81	105 - 64	58,7	11,8	1,25	9,4	2,3	T1 - 2
K du Toit Late	SC	13/08/2021	73 - 79	72 - 64	61,9	10,9	1,05	10,4	3,3	T1
Lavalle	CC	01/06/2021	79 - 85	64 - 56	56,7	10,8	1,85	5,8	0,0	T2 - 3
Lavalle	CC	28/06/2021	77 - 87	72 - 48	59,3	11,2	1,65	6,8	0,0	T1 - 3
Lavalle	CC	15/07/2021	73 - 81	72 - 64	60,0	11,1	1,55	7,2	0,0	T1 - 2
Lavalle	CC	13/08/2021	79 - 90	64 - 40	57,1	11,3	1,35	8,4	0,0	T1
McClellan SL	C35	01/06/2021	71 - 83	88 - 56	54,3	10,6	1,60	6,6	0,0	T1 - 3
McClellan SL	C35	28/06/2021	76 - 82	72 - 56	59,6	10,7	1,20	8,9	0,0	T1
McClellan SL	C35	15/07/2021	70 - 85	88 - 56	53,1	11,0	1,40	7,9	0,0	T1

McClellan SL	C35	13/08/2021	77 - 84	72 - 56	58,7	11,5	1,15	10,0	0,0	T1
McClellan SL	CC	01/06/2021	77 - 81	72 - 64	53,1	10,7	1,55	6,9	0,0	T1 - 2
McClellan SL	CC	28/06/2021	76 - 85	72 - 56	56,2	10,7	1,25	8,6	0,0	T1 - 2
McClellan SL	CC	15/07/2021	76 - 84	72 - 56	59,1	11,3	1,20	9,4	0,0	T1
McClellan SL	CC	13/08/2021	77 - 86	72 - 48	57,7	11,7	0,95	12,3	0,0	T1 - 3
McClellan SL	SC	01/06/2021	77 - 87	72 - 48	57,1	11,5	1,40	8,2	0,0	T1 - 2
McClellan SL	SC	28/06/2021	75 - 86	72 - 48	60,9	11,8	1,20	9,8	0,0	T1 - 2
McClellan SL	SC	15/07/2021	78 - 85	64 - 56	59,3	11,7	1,60	7,3	0,0	T1 - 2
McClellan SL	SC	13/08/2021	77 - 85	72 - 56	60,6	10,5	1,00	10,5	0,0	T1
Skilderkrans	C35	01/06/2021	75 - 81	72 - 64	54,3	10,8	1,50	7,2	0,0	T1 - 3
Skilderkrans	C35	28/06/2021	75 - 83	72 - 64	56,0	11,8	1,35	8,7	0,0	T1 - 2
Skilderkrans	C35	15/07/2021	74 - 84	72 - 56	57,3	11,1	1,25	8,9	0,0	T1 - 2
Skilderkrans	C35	13/08/2021	77 - 82	72 - 56	58,2	12,5	0,85	14,7	0,0	T - 1
Skilderkrans	CC	01/06/2021	76 - 83	72 - 56	53,4	11,0	1,50	7,3	0,0	T1 - 3
Skilderkrans	CC	28/06/2021	75 - 87	72 - 48	56,4	11,3	1,40	8,1	0,0	T1 - 2
Skilderkrans	CC	15/07/2021	76 - 83	72 - 56	54,7	12,3	1,25	9,8	0,0	T1 - 3
Skilderkrans	CC	13/08/2021	76 - 85	72 - 56	59,7	11,5	1,20	9,6	0,0	T - 1
Skilderkrans	SC	01/06/2021	72 - 80	88 - 64	52,4	11,4	1,55	7,4	0,0	T1 - 2
Skilderkrans	SC	28/06/2021	75 - 80	72 - 64	54,4	11,0	1,30	8,5	0,0	T1 - 3
Skilderkrans	SC	15/07/2021	74 - 86	72 - 48	57,2	11,2	1,25	9,0	0,0	T1 - 2
Skilderkrans	SC	13/08/2021	77 - 80	72 - 64	58,7	11,2	1,15	9,7	0,0	T - 1
Turkey	C35	11/05/2021	78 - 85	64 - 56	61,2	11,2	1,00	11,2	2,5	T1 - 3
Turkey	C35	01/06/2021	79 - 81	64	47,2	11,3	0,95	11,9	1,7	T - 1
Turkey	CC	11/05/2021	70 - 81	88 - 64	59,3	10,7	1,10	9,7	1,3	T2 - 3
Turkey	CC	01/06/2021	73 - 84	72 - 56	56,5	10,7	0,95	11,3	1,5	T1 - 2
Turkey	SC	11/05/2021	71 - 80	88 - 64	59,3	10,8	0,80	13,5	1,0	T2 - 3
Turkey	SC	01/06/2021	80 - 87	64 - 48	52,2	10,8	0,90	12,0	1,8	T1 - 2
Valearly	C35	11/05/2021	70 - 85	88 - 56	57,5	11,2	0,95	11,8	0,0	T2 - 3
Valearly	C35	01/06/2021	70 - 82	88 - 56	53,1	11,2	0,75	14,9	0,0	T - 1
Valearly	CC	11/05/2021	70 - 83	88 - 56	58,2	10,9	0,95	11,5	0,0	T1 - 3
Valearly	CC	01/06/2021	77 - 91	72 - 40	55,9	10,5	1,05	10,0	0,0	T1 - 2
Valearly	SC	11/05/2021	77 - 82	72 - 56	59,1	10,3	0,95	10,8	0,3	T1 - 3

**5.4.3 PROGRESS REPORT: Evaluation of Valencia selections in the hot, dry inland areas (Letsitele and Hoedspruit)**  
Project 75B by J Joubert (CRI)

**Summary**

The season starts with early selections and proceeds to the late maturing selections suitable for these hot-dry production areas. Recommendations have therefore been made accordingly. Valearly will start the season as an early maturing Valencia. Turkey will follow, producing large fruit size with good internal quality and soft fibre. The optimal picking window will be within the first four weeks of peak maturity. Bennie 1 and 2 can follow after

Turkey with good production and medium to large fruit size, but we recommend harvesting the cultivar after the middle of the Valencia season to prevent rind pitting problems (in problem areas). Delta, as a control fits in before Gusocora. Gusocora and McClean SL follow 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 an excellent crop on the trees and medium to medium-large fruit size (count 72 to 64). Valencia Late also as control, followed by Lavalley are currently the latest maturing Valencia selections that are being planted commercially, developing large to extra-large fruit size, pebbly rind texture, delayed colour development and average yields. A series of experimental/semi-commercial selections have also been included in the hot production areas. The selection range follows from mid-, to late-maturing options. The mid-season starts with Skilderkrans. Jassie with optimum fruit size as well as good internal quality and Kobus Du Toit late mature more towards the end of the Valencia season with medium to large fruit size.

## Opsomming

Die seisoen begin met vroeg rypwordende seleksies en duur voort met die laat rypwordende seleksies in die warm droë produksie areas en aanbevelings is daarvolgens gebaseer. Valeyly kan die seisoen begin as 'n vroeg rypwordende Valencia. Turkey kan nou volg, wat groot vrugte produseer met goeie interne kwaliteit en sagte vesel. Optimum plukvenster is binne die eerste vier weke van piek rypheid. Bennie 1 en 2 kan na Turkey volg met goeie produksie en medium tot groot vuggrootte, maar ons beveel aan om die kultivar na die middel van die Valencia seisoen te oes om gepokte skil te voorkom (in probleem areas). Delta as kontrole pas in voor Gusocora. Gusocora en McClean SL volg dan met totaal saadlose vrugte en goeie Brix: suur verhoudings. Midnight 1 en 2 vul die middel van die Valencia seisoen met goeie interne kwaliteit vrugte, groot 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 72 tot 64). Valencia late as kontrole, gevolg deur Lavalley, wat huidiglik die laatste rypwordende Valencia seleksie wat kommersieel aangeplant word is, met groot tot ekstra-groot vuggrootte, growwe skil tekstuur, vertraagde kleurontwikkeling en gemiddelde oeste. Daar is 'n reeks eksperimentele/semi-kommersiele seleksies wat ook vir die warm produksie areas ingesluit is. Hier volg die seleksies van middel, tot laat rypwordende kultivars. Die mid-seisoen kan begin word met Skilderkrans. Jassie met optimum vuggrootte asook goeie interne kwaliteit en Kobus Du Toit Laat word meer aan die einde van die Valencia seisoen ryp met medium tot groot vuggroote.

## Objective

- To find suitable Valencia selections with superior characteristics for the hot inland citrus production areas.

## Materials and methods

Field evaluations and laboratory analyses were conducted on Alpha, Bennie 1 & 2, Delta, Du Roi, Gusocora, Jasi, Kobus Du Toit Late, Lavalley 1&2, McClean, McClean SL, Midnight 1 & 2, Skilderkrans, Turkey, Valeyly and Val Late at Bosveld Citrus (Letsitele), Groep 91 (Letsitele) and Unifrutti (Hoedspruit).

**Table 5.4.3.1.** List of Valencia selections evaluated at Bosveld Citrus (Letsitele) during the 2021 season.

Selection	Rootstock	Planted
Alpha	SC	2009
Bennie 1	SC	2009
Delta (control)	C35/CC/SC	2011
Du Roi	SC	2009
Gusocora	SC	2009
Jasi	C35/CC/SC	2011
Kobus Du Toit Late	C35/CC/SC	2011
Lavalley 1	SC	2009
McClean SL	C35/CC/SC	2011

Midknight 1	SC	2009
Midknight 2	SC	2009
Skilderkrans	C35/CC/SC	2011
Turkey	C35/CC/SC	2011
Valearly	C35/CC	2011
Val Late	SC	2009

**Table 5.4.3.2.** List of Valencia selections evaluated at Groep 91 (Letsitele) during the 2021 season.

<b>Selection</b>	<b>Rootstock</b>	<b>Planted</b>
Bennie 1	CC/SC	2006
Bennie 2	CC/SC	2006
Jasi	CC/SC	2006
Kobus du Toit Late	CC/SC/US-812/X639	2013
McClean	CC/SC/US-812/X639	2013
McClean SL	CC/SC/US-812/X639	2013
Midknight 1	CC/SC	2006
Ruby	CC/SC	2006
Skilderkrans	CC/SC	2006
Turkey	C35/CC/SC	2006
Valearly	SC/US-812/X639	2013

**Table 5.4.3.3.** List of Valencia selections evaluated at Unifrutti (Hoedspruit) during the 2021 season.

<b>Selection</b>	<b>Rootstock</b>	<b>Topworked</b>
Alpha	CC/SC/US-812	2017
Gusocora	CC/SC/US-812	2017
Jasi	CC/SC/US-812	2017
Ruby	CC/SC/US-812	2017
Lavalle 2	CC/SC/US-812	2017

## Results and discussion

The new trial block at Unifrutti in Hoedspruit was evaluated for the first time this season, bearing in mind the young tree age and fruit quality associated with the scenario.

### Alpha

Fruit production on the Alpha trees remained good this season in the Letsitele area, with good rains in the area compared to the previous season. Alpha was evaluated on Swingle at the Bosveld trial site and on Carizzo, Swingle and US-812 at Unifrutti to compare tree development (vigour) and yield production. The internal quality was still very good compared to 2019, juice levels peaked at 57%, Brix was above 11 and acids were lower (between 1.2 and 1.4%). Fruit size increased slightly and varied from count 72 to 56/48, excellent for Valencia production and export. External colour was delayed on Swingle (typical characteristic on this rootstock) and peaked from T1 to T2/3. Maturity seems to be the middle to end of July (Table 5.4.3.4 & 5.4.3.5).

### Bennie 1 and 2

Bennie was evaluated at two trial sites this season: Bosveld and Groep 91. There was a good crop on the Bennie trees at both sites with improved water available after good seasonal rains. Fruit size peaked between count 64 and 56 (Bosveld) and 88 up to 56 (Groep 91); fruit size was smaller at Groep 91 compared to Bosveld. The internal colour of the fruit was deep yellow, fruit shape round, rind texture fairly smooth, medium rag content and medium rind thickness. Bennie 1 and 2 internally produced similar juice levels (average 55.6%), Brix (average 12), acid (1.3%) and seed counts (average 3.4 seeds per fruit). External colour on both selections

by the time of harvest varied between T1 and T3 (better colour on SC this season). Based on ratios, Bennie 1 and 2 mature end of June to the beginning of July (Tables 5.4.3.4 & 5.4.3.5).

#### Delta (control)

Delta on C35, Carrizo and Swingle rootstock, as control cultivar, produced completely seedless fruit and a good yield. Fruit size peaked between count 88 and 56/48 (lighter crop) with good internal quality, reaching juice levels of 61%, Brix of 12.7 and acid content of 1.4%. The external colour of the fruit was between T1 and T2 by the time of harvest. Maturity is middle to the end of June (Table 5.4.3.4).

#### Du Roi (control)

Du Roi was planted on two rootstocks, C35 and Swingle at the Bosveld trial, and for this season the Swingle combination was evaluated as a control selection due to C35's severe susceptibility to Blight in the Letsitele production area. There was a good yield and fruit size peaked between count 72 and 56/48 (better crop load on the trees). The external colour peaked between T1 and T3 (maturity) and the average seed count was 2.2 seeds per fruit (slightly higher). Swingle developed a juice content of average 59.8%, Brix of 11.2 and acids of 1.4%. Maturity is end of July to the middle August (Table 5.4.3.4).

#### Gusocora

Gusocora was evaluated at Bosveld Citrus this year on Swingle and on Carrizo, Swingle and US-812 rootstock at Unifrutti. The fruit was completely seedless and developed a good internal quality where juice content was lower (52.2%), Brix higher (12) and acid lower (1.1), but complied with export requirements. The external colour varied from T1 to T3, correlating with the internal quality and Brix:acid ratio of 12; slightly delayed. Fruit size was smaller and peaked between counts 88 and 56 (lighter crop), optimal fruit size for export Valencia (medium to large). There was a good crop on the trees, bearing in mind that Swingle induces good yields and internal quality. It is apparent that Gusocora maturity is middle to end of June (Table 5.4.3.4 & 5.4.3.5).

#### Jasi

Fruit size at Bosveld, Groep 91 and Unifrutti was similar to the previous season and peaked between count 88 and 56. Production was good on all the rootstock combinations. Internal quality was good with juice levels of 59%, Brix up to 13 (CC) and average acid levels at Bosveld of 1.2, with higher levels at Groep 91 (1.4%). Seed count increased even more this year and varied from 2.4 to 7.4 (avg. 3.8) seeds per fruit. Fruit shape was round, with a smooth rind texture, internal colour was light yellow, and juice flavour was good. Fibre strength was fairly soft, rind thickness was medium and the fruit peeled easily. Jassie bore high numbers of fruit inside the tree (good quality and colour). Maturity is the middle of July to the beginning of August in this area (Tables 5.4.3.4, 5.4.3.5 & 5.4.3.6).

#### Kobus Du Toit Late

Kobus Du Toit Late was evaluated at the Bosveld trial site on three rootstocks (C35, CC and SC) and Groep 91 on four rootstock (CC, SC, US 812, X639) and produced medium and large fruit size (count 88 to 56) on the trees, with 2.3 to 3.6 seeds average (similar compared to 2020). The colour development was very similar on all the rootstock combinations this season (T1 to T3). The internal quality was good, juice levels above 54%, Brix up to 12.1 (lower levels at Groep 91 – average 11.7) and good acids for the later maturing selection. Maturity seems to be middle to end of July to middle August according to Tables 5.4.3.4. & 5.4.3.5.

#### Lavalle 1

This season's seed production decreased, and Lavalle still produced no seeds per fruit at Bosveld. The internal quality complied with export requirements and acid level was above 1.0% at the final evaluation in the middle of August, juice levels went up to 60% and Brix up to 11.4. Keep in mind that Lavalle is a late Valencia selection with good shelf life and the optimal harvest time will be in August. The navel end on some fruit seems to develop a button and there was split fruit on some of the trees evaluated, but this varies from season to season (seen mainly in 2013), as well as a fairly coarse rind texture. From the ratio on this date, it is apparent that Lavalle 1 maturity is the end of August to middle of September (Table 5.4.3.4).

#### McClellan and McClellan SL

Both selections were planted and evaluated this season on Carrizo, Swingle, X639 and US 812 at Groep 91 to compare the performance of McClean SL with the old clone Mclean selection. McClean SL was planted on C35, Carrizo and Swingle at the Bosveld trial site with good crop production and remained completely seedless (Bosveld & Groep 91); similar to all the other trial sites where the selection was included. Fruit quality improved at the Groep 91 site due to trees bearing fifth crop now; the US 812 combination followed by Swingle were the best for 2021. Fruit size peaked from count 88 to 56 (excellent for Valencia production). External colour varied from T1-2/3 at Bosveld and Groep 91; improved from 2020 where SC had a delayed colour. At both sites juice was 54% and above (as high as 62% on CC), Brix improved to as high as 13.6 and acids were above 1.0% (peak maturity) towards the end of the season, resulting in good Brix:acid ratios (above 12:1). Maturity seems to be the end of June to the middle of July (Tables 5.4.3.4. & 5.4.3.5).

#### Midnight 1 & 2

Midnight 1 and 2 bore an average to good yield on the two rootstocks Carrizo and Swingle. This year's fruit size was very uniform and ranged between count 72/64 and 56/48, juice content was around 52% at Groep 91 and 57.2% at Bosveld, Brix levels better around 11.1 (average) and acids around 1.1%. The two Midnight selections performed very similarly this season. Midnight 1 and 2 developed low seed numbers in the fruit, ranging from seedless up to 1.8 seeds per fruit. The characteristic Midnight die-back was more visible on Midnight 1 compared to Midnight 2. Fruit shape was round, rind texture was fairly smooth, and fruit was raggy with a medium rind thickness which peeled moderately. Maturity seems to be the middle of July to the end of July (Table 5.4.3.4. & 5.4.3.5).

#### Skilderkrans

Skilderkrans at Group 91 is the back-up site (was evaluated) and the trial block at Bosveld bore fruit on C35, Carrizo and Swingle due to good rains this season (irrigation water available). Fruit size was bigger this season and more favourable for good quality fruit; varied from medium to large (count 88-56). Internally the Brix content was good (up to 13.2) and high acid level of 1.2% (CC & SC) indicated a later maturing Valencia selection. Juice level increased to an average 57% later in the season; above the minimum required export figure. There was a good external colour at Bosveld (T1-3). The fruit developed a smooth rind, fibre strength was fairly soft and the fruit shape was round. This season, ratios were lower at Bosveld due to the higher acid levels on both Carrizo and Swingle, delaying peak maturity to the end of July and mid-August. Peak maturity by the end of July (Tables 5.4.3.4).

#### Turkey

Turkey was planted on three rootstocks at both trial sites: 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 (count 88-56), good Brix content with highest levels at Groep 91 (average 11.8), similar acid levels at Bosveld compared to Groep 91 and Brix:acid ratio. The average seed count per fruit decreased to 1.3 and peaked at 3.8 seeds. The external colour (between T1 and T3) in the middle of June was similar for all three rootstocks and both trial sites. Yield production and tree size showed Carrizo to be the best rootstock combination for Turkey. C35 developed the smallest tree size (2.5 m) in combination with Turkey. The Swingle trees were declining and die-back was visible. Based on the ratios, maturity will be end of May to middle June (Tables 5.4.3.4. & 5.4.3.5).

#### Valearly

Valearly, bearing an average to good crop, remained low-seeded this season (1.9 seeds per fruit). The internal quality of the fruit was fair to good with juice levels above 50% at both trial sites early in the season (peak maturity), higher Brix on C35 (up to 12.7) compared to Carrizo (11.8) at Bosveld and acid of 0.9 to 1.0 average. Compared with the other early maturing selections (Turkey), Valearly seems to be at least two weeks earlier, with delayed external colour development. Estimated maturity, according to Table 5.4.3.4 & 5.4.3.5 seems to be second week to the end of May.

#### Valencia Late (control)

The Valencia Late was included as one of the control selections in this trial at Bosveld Citrus. Yield production on the trees improved this season and fruit size peaked at medium to large (count 72/64), optimal Valencia export quality. Acid levels were above 1.1 % (average) when the evaluation was completed, indicating the late

maturity qualities of the selection. The juice content was similar to 2018, 2019 and 2020 this season at 55% and Brix 11.6 with the last evaluation. Seed count went up from 1.5 seeds per fruit to 1.8. Maturity will be late in the season and according to Table 5.4.3.4, peak in the middle to end of August.

## Conclusion

Alpha performed well compared to the 2020 evaluation, developing a good crop on the trees. The internal quality was good (juice levels lower – 57%) and fruit size peaked between counts 72 to 56/48 (picked up one count size on the bigger side).

Bennie 1 and 2 produced similar fruit quality this season, as well as yield production and improved medium to large fruit size (peaked from count 88 to 56 between the two trial sites). Delta was the control cultivar for the trial; fruit size peaked between count 88 and 56 (slightly smaller than 2020 due to a better crop) with good internal quality, reaching juice levels of 61%, Brix of 12.7 and acid content of 1.4%.

Du Roi was evaluated on Swingle this season with a smaller fruit size ranging from count 72 to 64 (down by one count size compared to 2020 season). Kobus du Toit Late performed well with good fruit size and promising juice and Brix levels. Gusocora performed well on Swingle (delayed colour development), meeting the export standards (acid levels improved).

Jasi produced an excellent internal quality (high Brix and acid) on Carrizo and Swingle, with medium to large fruit size (count 88-56) with a good crop load on the trees. Lavalley 1 was ultra-late, peak maturity middle of August on Swingle rootstock; colour peaked from T1 to 2.

McClellan SL remained completely seedless at Bosveld and Groep 91 with good internal quality and optimum fruit size (count 88-56).

Fruit quality on Midnight 1 was better, with higher Brix than Midnight 2 (Bosveld trial site). The external colour was similar on both Midnight selections this season (better compared to 2020); range between T1 and T2.

Turkey performed best with Carrizo when Brix:acid ratio and yield production were considered. Valearly produced a better crop on the trees, average juice levels and improved colour development compared to Turkey this season. Future evaluations will determine the value of this cultivar for the citrus industry.

**Table 5.4.3.4.** Internal fruit quality data for Valencia orange selections at Bosveld Citrus (Letsitele) during the 2021 season.

Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
Alpha	SC	16/6/2021	82 - 92	72 - 56	55,7	11,6	1,40	8,3	0,0	T2 - 4
Alpha	SC	6/7/2021	73 - 87	56 - 48	51,8	10,8	1,30	8,3	1,7	T1 - 3
Alpha	SC	29/7/2021	73 - 90	72 - 56	57,6	11,5	1,20	9,6	0,0	T1 - 2
Bennie 1	SC	29/7/2021	78 - 85	64 - 56	56,4	11,6	1,05	11,0	2,0	T1 - 2
Delta	C35	25/5/2021	79 - 81	64	54,9	10,9	1,40	7,8	0,0	T1 - 2
Delta	C35	16/6/2021	75 - 85	72 - 56	54,7	11,8	1,10	10,7	0,0	T1 - 2
Delta	C35	7/7/2021	74 - 81	72 - 64	58,8	11,6	1,20	9,7	0,0	T1 - 2
Delta	C35	29/7/2021	80 - 88	72 - 48	55,6	12,7	1,10	11,5	0,0	T1
Delta	CC	25/5/2021	75 - 88	72 - 48	58,3	11,3	1,40	8,1	0,0	T2 - 3
Delta	CC	16/6/2021	74 - 82	72 - 56	57,4	11,3	1,10	10,3	0,0	T1

Delta	CC	7/7/2021	77 - 85	72 - 56	57,9	12,0	1,00	12,0	0,0	T1 - 2
Delta	CC	29/7/2021	73 - 80	72 - 64	58,4	12,2	0,95	12,8	0,0	T1
Delta	SC	25/5/2021	71 - 82	88 - 56	56,0	11,3	1,15	9,8	0,0	T3 - 4
Delta	SC	16/6/2021	74 - 82	72 - 56	53,6	11,3	0,70	16,1	0,0	T1 - 2
Delta	SC	7/7/2021	76 - 82	72 - 56	58,0	11,8	1,25	9,4	0,0	T1
Delta	SC	29/7/2021	71 - 76	88 - 48	61,0	12,2	1,15	10,6	0,0	T1
Du Roi	SC	16/6/2021	76 - 86	72 - 64	57,4	10,5	1,45	7,2	2,0	T1 - 3
Du Roi	SC	6/7/2021	73 - 78	72 - 48	56,1	11,2	1,20	9,3	3,0	T1 - 2
Du Roi	SC	29/7/2021	73 - 80	72 - 64	65,9	11,9	1,50	7,9	1,7	T1 - 2
Gusocora	SC	16/6/2021	72 - 82	88 - 56	52,3	11,7	1,20	9,8	0,0	T1
Gusocora	SC	6/7/2021	72 - 83	88 - 56	51,5	11,8	0,90	13,1	0,0	T1 - 2
Gusocora	SC	29/7/2021	77 - 83	88 - 56	52,8	12,6	1,05	12,0	0,0	T1 - 3
Jasi	C35	25/5/2021	73 - 76	72	57,0	11,9	1,45	8,2	3,3	T1 - 2
Jasi	C35	16/6/2021	73 - 84	72 - 56	55,0	12,4	1,10	11,3	4,0	T1 - 2
Jasi	C35	7/7/2021	72 - 80	88 - 64	58,1	12,5	1,10	11,4	4,0	T1 - 2
Jasi	C35	29/7/2021	72 - 86	88 - 48	56,2	12,0	1,05	11,4	3,5	T1 - 2
Jasi	C35	17/8/2021	73 - 89	72 - 48	55,1	11,9	1,05	11,3	2,4	T1 - 2
Jasi	CC	25/5/2021	75 - 78	72 - 64	57,8	11,4	1,40	8,1	3,0	T1 - 2
Jasi	CC	16/6/2021	71 - 83	88 - 56	58,6	11,4	1,05	10,9	5,8	T1 - 2
Jasi	CC	7/7/2021	73 - 83	72 - 56	56,0	12,9	1,00	12,9	3,3	T1 - 2
Jasi	CC	29/7/2021	73 - 83	72 - 56	58,9	9,4	1,25	7,5	4,8	T1 - 2
Jasi	CC	17/8/2021	77 - 81	72 - 64	58,3	13,1	1,00	13,1	7,4	T1 - 2
Jasi	SC	25/5/2021	70 - 79	88 - 64	56,9	11,2	1,45	7,7	2,5	T1 - 2
Jasi	SC	6/6/2021	75 - 83	72 - 56	50,7	11,2	1,50	7,5	4,0	T1
Jasi	SC	7/7/2021	75 - 82	72 - 56	59,6	12,4	1,20	10,3	4,8	T1 - 2
Jasi	SC	29/7/2021	75 - 82	72 - 56	59,1	12,7	1,25	10,2	2,7	T1 - 2
Jasi	SC	17/8/2021	70 - 82	88 - 56	60,7	12,4	1,05	11,8	7,3	T1 - 2
K du Toit Late	C35	25/5/2021	68 - 74	88 - 72	56,7	11,3	1,25	9,0	3,0	T2 - 3
K du Toit Late	C35	16/6/2021	77 - 82	72 - 56	58,2	11,9	1,30	9,2	3,6	T1 - 3
K du Toit Late	C35	16/6/2021	73 - 84	72 - 56	60,1	11,7	1,20	9,8	4,3	T1 - 2
K du Toit Late	C35	29/7/2021	74 - 78	72 - 64	59,8	11,6	0,90	12,9	4,8	T1 - 2
K du Toit Late	CC	25/5/2021	72 - 74	88 - 72	56,3	11,0	1,30	8,5	3,8	T1 - 2
K du Toit Late	CC	16/6/2021	74 - 79	72 - 64	55,6	11,3	1,40	8,1	2,7	T1
K du Toit Late	CC	7/7/2021	74 - 78	72 - 64	57,3	12,1	1,15	10,5	5,3	T1
K du Toit Late	CC	29/7/2021	73 - 84	72 - 56	59,1	11,9	1,00	11,9	3,8	T1 - 2

K du Toit Late	SC	25/5/2021	69 - 73	88 - 72	57,9	11,0	1,45	7,6	3,8	T2 - 3
K du Toit Late	SC	16/6/2021	69 - 79	88 - 64	59,0	11,4	1,45	7,9	3,3	T1
K du Toit Late	SC	7/7/2021	64 - 71	125 - 88	58,0	11,5	1,15	10,0	2,3	T1
K du Toit Late	SC	29/7/2021	74 - 77	72	60,5	11,1	1,15	9,7	2,4	T1 - 2
Lavalle 1	SC	16/6/2021	81 - 85	64 - 56	54,5	10,3	1,30	7,9	0,0	T1 - 2
Lavalle 1	SC	6/7/2021	80 - 85	64 - 56	56,4	11,0	0,90	12,2	0,0	T1 - 3
Lavalle 1	SC	29/7/2021	77 - 85	72 - 56	60,6	11,4	0,95	12,0	0,0	T1 - 3
Lavalle 1	SC	17/8/2021	77 - 90	72 - 40	54,1	11,0	0,95	11,6	0,0	T1 - 3
McClellan SL	C35	25/05/2021	71 - 81	88 - 64	53,7	11,5	1,25	9,2	0,0	T1 - 2
McClellan SL	C35	16/06/2021	72 - 83	88 - 56	57,6	12,0	1,20	10,0	0,0	T1
McClellan SL	C35	07/07/2021	75 - 81	72 - 64	57,1	12,0	1,10	10,9	0,0	T1
McClellan SL	C35	29/7/2021	75 - 81	72 - 64	49,3	11,4	1,10	10,4	0,0	T1 - 3
McClellan SL	C35	17/8/2021	74 - 82	72 - 56	58,4	12,7	0,95	13,4	0,0	T1 - 2
McClellan SL	CC	25/05/2021	72 - 81	88 - 64	56,3	10,9	1,50	7,3	0,0	T1 - 3
McClellan SL	CC	16/06/2021	75 - 80	72 - 64	56,1	11,1	1,10	10,1	0,0	T1
McClellan SL	CC	07/07/2021	74 - 84	72 - 56	62,3	13,0	0,95	13,7	0,0	T1 - 2
McClellan SL	CC	29/7/2021	74 - 93	72 - 40	56,8	13,1	1,10	11,9	0,0	T1 - 2
McClellan SL	CC	17/8/2021	74 - 82	72 - 56	56,8	13,6	0,90	15,1	0,0	T1 - 2
McClellan SL	SC	25/05/2021	74 - 80	72 - 64	56,9	11,1	1,35	8,2	0,0	T1 - 3
McClellan SL	SC	16/06/2021	73 - 82	72 - 56	59,9	11,3	1,10	10,3	0,0	T1 - 2
McClellan SL	SC	07/07/2021	72 - 80	88 - 64	60,5	12,0	1,10	10,9	0,0	T1
McClellan SL	SC	29/7/2021	74 - 83	72 - 56	59,7	11,4	1,10	10,4	0,0	T1
McClellan SL	SC	17/8/2021	76 - 84	72 - 56	57,9	11,3	0,90	12,6	0,0	T1 - 2
Midknight 1	SC	15/6/2021	82 - 88	56 - 48	67,3	10,5	1,00	10,5	0,0	T1 - 3
Midknight 1	SC	6/7/2021	74 - 87	72 - 48	57,1	11,9	1,25	9,5	0,0	T1 - 2
Midknight 1	SC	29/7/2021	82 - 90	56 - 40	56,0	11,8	1,00	11,8	0,0	T1 - 2
Midknight 2	SC	16/6/2021	76 - 86	72 - 48	49,6	10,5	1,10	9,5	0,0	T1 - 2
Midknight 2	SC	6/7/2021	77 - 81	72 - 64	58,2	10,4	1,25	8,3	0,0	T1
Midknight 2	SC	29/7/2021	79 - 84	64 - 56	55,1	11,4	1,00	11,4	0,0	T1 - 2
Skilderkrans	C35	25/5/2021	74 - 80	72 - 56	59,6	11,1	1,35	8,2	0,0	T1 - 2
Skilderkrans	C35	16/6/2021	71 - 80	72 - 56	56,2	11,8	1,35	8,7	0,0	T1
Skilderkrans	C35	7/7/2021	74 - 84	72 - 48	60,1	9,5	1,00	9,5	0,0	T1 - 3
Skilderkrans	C35	29/7/2021	77 - 87	88 - 64	59,6	12,1	1,10	11,0	0,0	T1 - 2
Skilderkrans	C35	17/8/2021	77 - 81	72 - 48	59,0	13,2	0,95	13,9	0,0	T1 - 2
Skilderkrans	CC	25/5/2021	76 - 82	88 - 56	52,2	10,5	1,65	6,4	0,0	T1
Skilderkrans	CC	16/6/2021	74 - 81	72 - 64	52,5	11,2	1,45	7,7	0,0	T1 - 2
Skilderkrans	CC	7/7/2021	75 - 83	72 - 56	57,3	11,8	1,20	9,8	0,0	T1
Skilderkrans	CC	29/7/2021	77 - 86	72 - 56	58,1	11,9	1,15	10,3	0,0	T1 - 2

Skilderkrans	CC	17/8/2021	74 - 84	72 - 56	56,2	12,0	1,20	10,0	0,0	T1 - 2
Skilderkrans	SC	25/5/2021	74 - 80	72 - 64	58,8	10,6	1,85	5,7	0,0	T2 - 3
Skilderkrans	SC	16/6/2021	77 - 83	72 - 56	52,6	11,0	1,45	7,6	0,0	T2 - 4
Skilderkrans	SC	7/7/2021	71 - 83	72 - 64	57,9	11,7	1,60	7,3	0,0	T1 - 3
Skilderkrans	SC	29/7/2021	77 - 85	72 - 64	57,2	12,1	1,35	9,0	1,9	T1 - 2
Skilderkrans	SC	17/8/2021	75 - 81	72 - 64	56,0	11,7	1,20	9,8	0,0	T1
Turkey	C35	4/5/2021	72 - 82	72 - 64	58,5	10,9	0,95	11,5	3,8	T1
Turkey	C35	25/5/2021	82 - 90	72 - 56	56,5	11,2	1,05	10,7	1,0	T1 - 2
Turkey	C35	16/6/2021	76 - 79	56 - 40	52,0	12,0	0,90	13,3	1,7	T - 1
Turkey	CC	4/5/2021	74 - 85	88 - 64	61,2	11,2	1,15	9,7	1,8	T1 - 2
Turkey	CC	25/5/2021	77 - 84	72 - 56	57,1	11,6	1,05	11,0	3,7	T1
Turkey	CC	16/6/2021	77 - 84	88 - 56	54,8	11,5	1,00	11,5	1,3	T1 - 2
Turkey	SC	4/5/2021	72 - 81	72 - 64	58,6	10,9	0,95	11,5	0,0	T2 - 4
Turkey	SC	25/5/2021	77 - 80	72 - 56	55,5	11,4	1,00	11,4	2,2	T1 - 3
Turkey	SC	16/6/2021	76 - 81	72 - 64	59,1	12,0	1,00	12,0	0,0	T1 - 2
Valearly	C35	16/6/2021	74 - 85	56 - 48	45,1	12,7	0,85	14,9	5,2	T1 - 2
Valearly	C35	25/5/2021	83 - 87	72 - 48	50,9	11,9	1,05	11,3	3,0	T1 - 2
Valearly	C35	4/5/2021	76 - 84	56 - 40	52,3	11,2	1,15	9,7	2,7	T1 - 2
Valearly	CC	4/5/2021	74 - 89	72 - 48	57,7	10,0	0,95	10,5	0,0	T1 - 2
Valearly	CC	25/5/2021	84 - 89	72 - 40	50,2	11,8	0,85	13,9	0,0	T1
Valearly	CC	16/6/2021	76 - 83	72 - 56	49,8	11,5	0,85	13,5	0,8	T1 - 2
Val Late	SC	16/6/2021	71 - 79	72 - 64	53,4	11,1	1,40	7,9	1,8	T1 - 2
Val Late	SC	6/7/2021	74 - 80	88 - 64	53,6	11,5	1,10	10,5	0,0	T1 - 2
Val Late	SC	29/7/2021	79 - 87	72 - 64	60,9	11,8	1,05	11,2	0,0	T1 - 2
Val Late	SC	17/8/2021	72 - 83	64 - 48	53,4	12,5	1,05	11,9	0,0	T1 - 2

**Table 5.4.3.5.** Internal fruit quality data for Valencia orange selections at Groep 91 (Letsitele) during the 2021 season.

Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
Bennie 1	CC	25/5/2021	70 - 82	88 - 56	53,8	11,3	1,35	8,4	5,7	T1 - 2
Bennie 1	CC	15/6/2021	75 - 80	72 - 64	53,7	11,6	1,40	8,3	3,6	T1
Bennie 1	CC	6/7/2021	74 - 80	72 - 64	58,4	12,4	1,25	9,9	4,5	T1
Bennie 1	CC	29/7/2021	77 - 85	72 - 56	57,8	12,5	1,05	11,9	4,0	T1 - 2
Bennie 1	CC	17/8/2021	76 - 80	72 - 64	58,7	12,8	0,95	13,5	7,0	T1
Bennie 1	SC	25/5/2021	73 - 80	72 - 64	54,1	10,8	1,55	7,0	2,8	T1 - 2
Bennie 1	SC	15/6/2021	78 - 87	64 - 48	54,4	11,5	1,55	7,4	1,6	T1 - 3
Bennie 1	SC	6/7/2021	72 - 77	88 - 72	57,5	12,5	1,45	8,6	3,7	T - 1
Bennie 1	SC	29/7/2021	74 - 80	72 - 64	50,2	12,2	1,25	9,8	3,5	T1 - 2

Bennie 1	SC	17/8/2021	77 - 83	72 - 56	56,4	13,1	1,10	11,9	4,0	T1 - 3
Bennie 2	CC	25/5/2021	73 - 77	72	53,4	11,0	1,50	7,3	4,3	T2 - 3
Bennie 2	CC	15/6/2021	73 - 79	72 - 64	54,5	11,5	1,25	9,2	4,7	T1
Bennie 2	CC	6/7/2021	73 - 78	72 - 64	55,3	12,4	1,00	12,4	4,0	T1
Bennie 2	CC	29/7/202	71 - 81	88 - 64	60,2	12,6	1,25	10,1	0,0	T1 - 2
Bennie 2	CC	17/8/2021	75 - 85	72 - 56	58,0	12,4	1,10	11,3	3,6	T1 - 2
Bennie 2	SC	25/5/2021	74 - 82	72 - 56	49,3	12,0	1,35	8,9	0,0	T1 - 2
Bennie 2	SC	15/6/2021	73 - 81	73 - 64	53,5	11,4	1,30	8,8	2,3	T1 - 2
Bennie 2	SC	6/7/2021	74 - 80	72 - 64	54,5	12,3	1,15	10,7	2,5	T1 - 2
Bennie 2	SC	29/7/2021	73 - 80	72 - 64	59,5	12,2	1,30	9,4	3,0	T1
Bennie 2	SC	17/8/2021	73 - 79	72 - 64	59,8	12,3	1,00	12,3	3,3	T1 - 3
Jasi	CC	15/6/2021	75 - 83	72 - 56	55,4	11,2	1,35	8,3	3,5	T1 - 2
Jasi	CC	6/7/2021	70 - 76	88 - 72	57,7	12,1	1,45	8,3	3,8	T1 - 2
Jasi	CC	29/7/2021	72 - 84	88 - 56	55,8	11,4	1,30	8,8	2,7	T1 - 2
Jasi	SC	15/6/2021	72 - 79	88 - 64	57,3	11,3	1,50	7,5	3,0	T1
Jasi	SC	6/7/2021	71 - 83	88 - 56	56,8	11,8	1,30	9,1	4,0	T1
Jasi	SC	29/7/2021	74 - 80	72 - 64	57,6	11,8	1,55	7,6	3,6	T1 - 2
K du Toit Late	CC	25/5/2021	69 - 80	88 - 64	55,4	10,6	1,85	5,7	2,3	T2 - 4
K du Toit Late	CC	15/6/2021	72 - 82	88 - 56	54,1	11,2	1,30	8,6	2,7	T1 - 2
K du Toit Late	CC	6/7/2021	69 - 76	88 - 72	55,8	11,1	1,05	10,6	1,8	T1 - 2
K du Toit Late	CC	29/7/2021	73 - 80	72 - 64	57,5	11,7	1,05	11,1	1,8	T1 - 2
K du Toit Late	CC	17/8/2021	72 - 80	88 - 64	57,1	11,0	1,00	11,0	3,7	T1 - 2
K du Toit Late	SC	25/5/2021	69 - 81	88 - 64	55,6	9,9	1,35	7,3	1,0	T3 - 4
K du Toit Late	SC	15/6/2021	76 - 88	72 - 48	50,5	9,5	1,50	6,3	0,8	T1 - 3
K du Toit Late	SC	6/7/2021	75 - 80	72 - 64	58,0	10,6	1,05	10,1	4,0	T1 - 2
K du Toit Late	SC	29/7/2021	79 - 80	64	56,5	10,8	0,95	11,4	0,0	T1
K du Toit Late	SC	17/8/2021	79 - 85	64 - 56	58,3	12,6	0,80	15,8	0,0	T1 - 2
K du Toit Late	X639	25/5/2021	70 - 75	88 - 72	57,4	10,5	1,40	7,5	3,8	T2 - 4
K du Toit Late	X639	15/6/2021	72 - 79	88 - 64	55,6	10,8	1,30	8,3	1,7	T1 - 3
K du Toit Late	X639	6/7/2021	72 - 84	88 - 56	56,4	11,1	1,20	9,3	2,7	T1 - 2
K du Toit Late	X639	29/7/2021	77 - 85	72 - 56	56,9	11,5	1,05	11,0	2,8	T1 - 2
K du Toit Late	X639	17/8/2021	76 - 83	72 - 56	58,2	11,2	0,95	11,8	2,8	T1 - 2

K du Toit Late	US 812	25/5/2021	71 - 76	88 - 72	54,6	10,8	1,80	6,0	1,5	T2 - 4
K du Toit Late	US 812	15/6/2021	72 - 80	88 - 64	56,4	11,0	1,25	8,8	0,8	T1 - 2
K du Toit Late	US 812	6/7/2021	77 - 81	72 - 64	55,2	12,8	1,30	9,8	3,3	T1 - 2
K du Toit Late	US 812	29/7/2021	74 - 79	72 - 64	58,5	12,0	1,10	10,9	1,7	T1 - 2
K du Toit Late	US 812	17/8/2021	75 - 83	72 - 56	58,4	12,3	0,95	12,9	3,5	T1 - 3
McClean	CC	25/5/2021	72 - 79	88 - 56	58,3	10,8	1,80	6,0	0,0	T3 - 4
McClean	CC	15/6/2021	73 - 84	72 - 56	56,4	11,3	1,10	10,3	0,0	T1
McClean	CC	6/7/2021	74 - 85	72 - 56	57,5	11,6	1,25	9,3	0,8	T1 - 2
McClean	CC	29/7/2021	69 - 81	88 - 64	58,3	12,3	1,30	9,5	1,7	T1 - 3
McClean	CC	17/8/2021	73 - 82	72 - 56	58,5	12,1	1,20	10,1	2,4	T1 - 2
McClean	SC	25/5/2021	71 - 77	88 - 72	57,5	11,0	1,65	6,7	0,0	T3 - 4
McClean	SC	15/6/2021	72 - 80	88 - 64	55,7	11,1	1,45	7,7	0,8	T1 - 2
McClean	SC	6/7/2021	72 - 78	88 - 56	55,3	11,8	1,60	7,4	2,3	T1 - 2
McClean	SC	29/7/2021	72 - 84	88 - 56	54,5	12,5	1,45	8,6	2,0	T1
McClean	SC	17/8/2021	74 - 80	72 - 64	58,5	11,8	1,30	9,1	0,0	T1
McClean	X639	25/5/2021	70 - 79	88 - 64	57,0	10,6	1,50	7,1	1,3	T1 - 3
McClean	X639	15/6/2021	77 - 82	72 - 56	54,4	11,0	1,60	6,9	1,7	T1 - 2
McClean	X639	6/7/2021	74 - 81	88 - 64	51,8	10,8	1,45	7,4	2,5	T1 - 2
McClean	X639	29/7/2021	72 - 77	88 - 72	57,5	11,4	1,10	10,4	0,0	T1 - 3
McClean	X639	17/8/2021	75 - 85	72 - 56	55,6	12,0	1,00	12,0	1,5	T1 - 2
McClean	US 812	25/5/2021	74 - 84	72 - 56	56,8	11,1	1,75	6,3	2,0	T2 - 3
McClean	US 812	15/6/2021	71 - 85	88 - 56	57,8	11,8	1,50	7,9	1,4	T1 - 2
McClean	US 812	6/7/2021	73 - 84	72 - 56	57,3	11,5	1,40	8,2	3,0	T1
McClean	US 812	29/7/2021	75 - 84	72 - 56	57,5	11,3	1,35	8,4	1,5	T1 - 2
McClean	US 812	17/8/2021	74 - 84	72 - 56	55,9	12,9	1,10	11,7	2,7	T1 - 2
McClean SL	CC	25/5/2021	74 - 79	72 - 64	48,9	10,6	1,60	6,6	0,0	T1 - 2
McClean SL	CC	15/6/2021	75 - 84	72 - 56	52,5	11,2	0,80	14,0	0,0	T1
McClean SL	CC	06/7/2021	74 - 84	72 - 56	53,8	11,9	1,20	9,9	0,0	T1 - 2
McClean SL	CC	29/7/2021	74 - 82	72 - 56	56,3	12,5	1,10	11,4	0,0	T1 - 3
McClean SL	CC	17/8/2021	78 - 88	64 - 48	53,2	12,4	0,90	13,8	0,0	T1 - 2
McClean SL	SC	25/5/2021	71 - 85	88 - 56	51,5	10,9	1,80	6,1	0,0	T1 - 3
McClean SL	SC	15/6/2021	77 - 84	72 - 56	49,0	11,4	1,20	9,5	0,0	T1
McClean SL	SC	6/7/2021	72 - 77	88 - 72	57,1	11,8	1,65	7,2	0,0	T1
McClean SL	SC	17/8/2021	75 - 86	72 - 48	55,5	12,6	1,05	12,0	0,0	T1 - 3
McClean SL	X639	25/5/2021	74 - 80	72 - 64	64,5	10,8	1,20	9,0	0,0	T1 - 3

McClellan SL	X639	15/6/2021	72 - 86	88 - 48	53,4	11,2	1,20	9,3	0,0	T1 - 2
McClellan SL	X639	6/7/2021	76 - 85	88 - 56	54,4	11,4	1,10	10,4	0,0	T1
McClellan SL	X639	29/7/2021	74 - 82	72 - 56	54,6	12,1	0,95	12,7	0,0	T1 - 2
McClellan SL	X639	17/8/2021	74 - 89	72 - 48	56,6	12,5	0,80	15,6	0,0	T1
McClellan SL	US 812	25/5/2021	76 - 84	72 - 56	56,6	10,1	1,55	6,5	0,0	T3 - 4
McClellan SL	US 812	15/6/2021	75 - 91	72 - 40	52,2	10,6	1,15	9,2	0,0	T1 - 3
McClellan SL	US 812	06/7/2021	74 - 82	72 - 56	54,8	12,0	1,00	12,0	0,0	T1 - 2
McClellan SL	US 812	29/7/2021	76 - 90	72 - 40	53,8	11,8	1,10	10,7	0,0	T1 - 2
McClellan SL	US 812	17/8/2021	75 - 85	72 - 56	41,8	11,9	0,95	12,5	0,0	T1 - 2
Midnight 1	CC	25/5/2021	77 - 86	72 - 48	53,0	11,4	1,20	9,5	0,0	T1 - 2
Midnight 1	CC	15/6/2021	74 - 82	72 - 56	50,7	12,0	1,25	9,6	0,0	T1
Midnight 1	CC	6/7/2021	75 - 81	72 - 64	53,7	12,5	1,00	12,5	0,0	T1
Midnight 1	CC	29/7/2021	77 - 85	72 - 56	55,4	12,9	1,00	12,9	1,0	T1 - 3
Midnight 1	SC	25/5/2021	72 - 84	88 - 56	51,6	10,9	1,10	9,9	0,0	T1 - 2
Midnight 1	SC	15/6/2021	78 - 84	64 - 56	44,4	11,6	1,30	8,9	0,0	T1
Midnight 1	SC	6/7/2021	70 - 82	88 - 56	50,6	12,8	1,05	12,2	0,0	T1
Midnight 1	SC	29/7/2021	78 - 87	64 - 48	53,4	12,8	0,95	13,5	0,0	T1 - 2
Ruby	CC	15/6/2021	72 - 76	88 - 72	59,5	11,5	1,45	7,9	3,0	T1
Ruby	CC	6/7/2021	69 - 84	88 - 56	56,6	11,6	1,60	7,3	3,8	T1 - 2
Ruby	CC	29/7/2021	71 - 79	88 - 64	58,7	12,3	1,25	9,8	5,3	T1 - 3
Ruby	CC	17/8/2021	71 - 88	88 - 48	58,6	11,8	1,10	10,7	0,0	T1 - 2
Ruby	SC	15/6/2021	71 - 78	88 - 64	52,0	11,3	1,50	7,5	3,6	T1
Ruby	SC	6/7/2021	73 - 76	72	57,3	11,7	1,35	8,7	3,3	T1
Ruby	SC	29/7/2021	71 - 75	88 - 72	59,1	11,9	1,05	11,3	3,3	T1
Ruby	SC	17/8/2021	72 - 89	88 - 48	57,8	11,7	1,05	11,1	3,5	T1 - 2
Skilderkrans	CC	15/6/2021	71 - 89	88 - 48	66,3	11,3	1,50	7,5	0,0	T1 - 2
Skilderkrans	CC	6/7/2021	73 - 78	72 - 64	59,1	11,8	1,15	10,3	0,0	T1 - 3
Skilderkrans	CC	29/7/2021	77 - 81	72 - 64	44,3	12,5	1,15	10,9	0,0	T1 - 2
Skilderkrans	CC	17/8/2021	78 - 84	64 - 56	54,5	12,1	1,10	11,0	0,0	T1 - 2
Skilderkrans	SC	15/6/2021	71 - 84	88 - 56	31,8	11,8	1,50	7,9	0,0	T1 - 2
Skilderkrans	SC	6/7/2021	71 - 78	88 - 64	57,5	12,5	1,70	7,4	0,0	T1
Skilderkrans	SC	29/7/2021	73 - 82	72 - 56	56,2	12,3	1,40	8,8	0,0	T1 - 2
Skilderkrans	SC	17/8/2021	77 - 81	72 - 64	57,4	10,8	1,35	8,0	0,0	T1 - 2
Turkey	C35	4/5/2021	71 - 80	88 - 64	58,8	11,2	1,35	8,3	3,0	T1 - 3
Turkey	C35	25/5/2021	72 - 78	88 - 64	57,4	11,6	1,15	10,1	2,1	T1 - 2
Turkey	CC	4/5/2021	70 - 73	88 - 72	59,4	12,3	1,14	10,8	3,2	T1 - 3
Turkey	CC	25/5/2021	73 - 79	72 - 64	57,3	12,4	1,40	8,9	1,5	T1 - 2

Turkey	SC	4/5/2021	76 - 78	72 - 64	55,7	11,2	1,30	8,6	1,3	T1 - 3
Turkey	SC	25/5/2021	70 - 80	88 - 64	49,1	11,8	1,40	8,4	1,7	T1 - 2
Valearly	CC	4/5/2021	72 - 79	88 - 64	53,5	9,7	0,90	10,8	0,0	T2 - 3
Valearly	CC	25/5/2021	73 - 82	72 - 56	50,8	10,2	0,80	12,8	0,0	T1 - 2
Valearly	CC	15/6/2021	74 - 80	72 - 64	49,6	10,3	0,80	12,9	0,0	T1
Valearly	SC	4/5/2021	74 - 83	72 - 56	56,1	10,2	0,90	11,3	0,0	T1 - 2
Valearly	SC	25/5/2021	75 - 82	72 - 56	50,4	10,4	0,95	10,9	0,0	T1 - 2
Valearly	SC	15/6/2021	75 - 83	72 - 56	45,0	11,8	0,95	12,4	0,0	T1 - 2
Valearly	X639	4/5/2021	74 - 91	72 - 40	55,6	9,7	0,75	12,9	0,0	T2 - 4
Valearly	X639	25/5/2021	70 - 99	88 - 36	49,8	10,1	0,75	13,5	0,0	T1 - 2
Valearly	X639	15/6/2021	74 - 82	72 - 56	46,9	10,9	0,90	12,1	0,0	T1
Valearly	US 812	4/5/2021	75 - 86	72 - 56	52,5	9,7	0,85	11,4	0,0	T2 - 3
Valearly	US 812	25/5/2021	71 - 84	88 - 56	53,3	10,7	0,95	11,3	0,0	T1 - 2
Valearly	US 812	15/6/2021	77 - 89	72 - 48	46,5	11,7	0,75	15,6	0,0	T1 - 2

**Table 5.4.3.6.** Internal fruit quality data for Valencia orange selections at Unifrutti (Hoedspruit) during the 2021 season.

Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
Alpha	CC	26/05/2021	73 - 78	72 - 64	51,9	11,1	1,15	9,7	0,0	T1 - 3
Alpha	CC	16/06/2021	73 - 79	72 - 64	54,0	11,1	1,25	8,9	0,0	T1 - 2
Alpha	CC	07/07/2021	75 - 81	72 - 64	52,9	13,0	1,05	12,4	0,0	T1 - 2
Alpha	SC	26/05/2021	73 - 78	88 - 64	55,2	10,9	1,30	8,4	0,0	T2 - 3
Alpha	SC	16/06/2021	74 - 80	72 - 64	54,5	12,3	0,95	12,9	0,0	T1
Alpha	SC	07/07/2021	72 - 81	88 - 64	54,3	12,9	1,00	12,9	0,0	T1
Alpha	US-812	26/05/2021	76 - 80	72 - 64	49,2	10,8	1,05	10,3	0,0	T1 - 3
Alpha	US-812	16/06/2021	75 - 87	72 - 48	53,5	11,4	0,90	12,7	0,0	T1 - 3
Alpha	US-812	07/07/2021	74 - 85	72 - 56	53,5	11,6	0,85	13,6	0,0	T1
Gusocora	CC	26/05/2021	73 - 81	72 - 64	50,8	11,5	0,90	12,8	0,0	T2 - 4
Gusocora	CC	16/06/2021	75 - 84	72 - 56	46,6	11,6	0,90	12,9	0,0	T1
Gusocora	CC	07/07/2021	69 - 81	88 - 64	52,6	11,9	0,85	14,0	0,0	T1
Gusocora	SC	26/05/2021	69 - 75	88 - 72	59,3	10,8	0,90	12,0	0,0	T1 - 5
Gusocora	SC	16/06/2021	72 - 79	88 - 64	59,8	11,4	0,80	14,3	0,0	T1 - 3
Gusocora	SC	07/07/2021	72 - 79	88 - 64	37,0	12,4	0,75	16,5	0,0	T1
Gusocora	US-812	26/05/2021	70 - 78	88 - 64	57,0	10,7	1,00	10,7	0,0	T2 - 4

Gusocora	US-812	16/06/2021	74 - 83	72 - 56	50,6	11,2	0,80	14,0	0,0	T1 - 3
Gusocora	US-812	07/07/2021	72 - 83	88 - 56	54,6	12,0	0,85	14,1	0,0	T1
Jasi	CC	26/05/2021	75 - 78	72 - 64	57,9	11,6	1,55	7,5	4,7	T1 - 3
Jasi	CC	16/06/2021	69 - 84	88 - 56	56,6	12,8	1,25	10,2	2,7	T1 - 2
Jasi	CC	07/07/2021	73 - 80	72 - 64	57,3	12,6	1,15	11,0	4,3	T1 - 2
Jasi	CC	30/07/2021	69 - 83	88 - 56	53,6	13,0	1,00	13,0	2,3	T1 - 3
Jasi	SC	26/05/2021	73 - 79	72 - 64	56,3	11,3	1,35	8,4	3,0	T1 - 3
Jasi	SC	16/06/2021	74 - 78	72 - 64	56,7	10,4	1,25	8,3	3,8	T1
Jasi	SC	07/07/2021	72 - 79	88 - 64	57,6	12,4	1,15	10,8	1,8	T1
Jasi	SC	30/07/2021	75 - 83	72 - 56	55,8	12,7	1,10	11,5	1,7	T1 - 2
Jasi	US-812	26/05/2021	72 - 80	88 - 64	55,0	10,9	1,15	9,5	2,3	T1 - 3
Jasi	US-812	16/06/2021	73 - 81	72 - 64	56,6	11,2	1,00	11,2	4,1	T1 - 2
Jasi	US-812	07/07/2021	72 - 80	88 - 64	58,6	12,2	1,30	9,4	1,3	T1
Jasi	US-812	30/07/2021	73 - 76	72	57,9	12,7	0,85	14,9	2,2	T1 - 2
Lavalle	CC	16/06/2021	82 - 90	56 - 40	56,1	11,4	1,10	10,4	0,0	T1
Lavalle	CC	07/07/2021	74 - 85	72 - 86	59,2	11,8	1,30	9,1	0,0	T1 - 2
Lavalle	CC	11/08/2021	77 - 86	72 - 48	58,6	12,6	0,85	14,8	0,0	T1 - 2
Lavalle	SC	16/06/2021	75 - 84	72 - 56	57,6	10,3	1,25	8,2	0,0	T1 - 2
Lavalle	SC	07/07/2021	76 - 83	72 - 56	57,8	11,2	1,15	9,7	0,0	T1 - 3
Lavalle	SC	11/08/2021	75 - 85	72 - 56	57,1	11,2	1,00	11,2	0,0	T1 - 2
Lavalle	US-812	16/06/2021	75 - 83	72 - 56	61,1	10,7	1,30	8,2	0,0	T1 - 2
Lavalle	US-812	07/07/2021	79 - 87	64 - 48	63,4	10,6	1,05	10,1	0,0	T1 - 3
Lavalle	US-812	11/08/2021	78 - 85	64 - 56	60,9	11,8	1,25	9,4	0,0	T1 - 3
Ruby	CC	16/06/2021	71 - 76	88 - 72	60,0	11,6	1,10	10,5	1,7	T1 - 3
Ruby	CC	07/07/2021	65 - 81	105 - 64	58,5	11,1	1,10	10,1	2,1	T1 - 3
Ruby	CC	30/07/2021	73 - 82	72 - 56	58,3	11,6	1,05	11,0	3,3	T1 - 3
Ruby	SC	16/06/2021	64 - 72	125 - 88	62,1	10,9	1,40	7,8	3,3	T1 - 3
Ruby	SC	07/07/2021	65 - 72	105 - 88	61,6	11,5	1,35	8,5	3,5	T1 - 2
Ruby	SC	30/07/2021	66 - 72	105 - 88	62,5	12,4	1,15	10,8	3,0	T1 - 3
Ruby	US-812	16/06/2021	60 - 79	125 - 64	57,7	11,5	1,20	9,6	3,0	T1 - 3
Ruby	US-812	07/07/2021	73 - 77	72	70,2	11,9	1,25	9,5	3,3	T1 - 2
Ruby	US-812	30/07/2021	67 - 72	105 - 88	61,2	11,8	1,00	11,8	3,7	T1 - 2

#### 5.4.4 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the hot inland areas (Letsitele and Hoedspruit) Project 75C by J Joubert (CRI)

##### Summary

Etna, Sirio and RHM mature first according to the results of the 2021 season for the warm production areas, and RHM developed a delayed external colour with low acid levels. Samba and Leanri with high juice levels, fit in before Furr that followed with the highest seed count per fruit for this trial. Next will be Orah, also developing fairly high seed counts per fruit. The mid-maturing mandarins are represented by Ma'ayana, which developed high juice levels compared to the other selections (up to juice of 64%). Tambor, followed by Tanor Late and then Sugar Belle, were the last selections to mature at these trial sites, ending the Mandarin Hybrid season. Picking periods should not be longer than 3 - 4 weeks to maintain good internal quality and avoid rind disorders.

##### Opsomming

Etna, Sirio en RHM word die vroegste ryp volgens resultate van die 2021 seisoen vir hierdie warm produksie area, met RHM wat 'n vertraagde kleur ontwikkeling toon met lae suur vlakke. Samba en Leanri, met hoë sap vlakke, pas in voor Furr, wat daarna volg met die hoogste saadtelling per vrug vir hierdie proef. Volgende is Orah, met redelike hoë saad tellings per vrug. Die middel van die mandaryne word verteenwoordig deur Ma'ayana met die hoë sap vlakke in vergelyking met die ander seleksies (tot sap van 64%). Tambor, gevolg deur Tanor Late, en dan Sugar Belle, was die laaste seleksie om ryp te word op hierdie proef persele, wat ook die Mandaryn Hibried seisoen afsluit vir hierdie proef. Daar word aanbeveel om nie die oesperiode langer as 3 tot 4 weke te verleng nie om goeie interne kwaliteit te verseker met minimum skil probleme.

##### Objectives

- To select Mandarin Hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new Mandarin Hybrid cultivars and to determine the climatic suitability of these cultivars in hot production regions.

##### Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from Bosveld Citrus (Letsitele), Mahela Citrus (Letsitele) and Moriah Citrus (Hoedspruit) from the Limpopo region.

The following cultivars were evaluated:

**Table 5.4.4.1.** List of Mandarin Hybrid selections evaluated at Bosveld Citrus (Letsitele) during the 2021 season.

Selection	Rootstock	Topwork
Ma'ayana (Dina)	CC	2018
Samba	CC	2015
Sugar Belle	CC	2015

**Table 5.4.4.2.** List of Mandarin Hybrid selections evaluated at Mahela (Letsitele) during the 2021 season.

Selection	Rootstock	Planted
Etna	CC	2014
Furr	CC	2014

Saint André	CC	2014
Samba	CC	2014
Sirio	CC	2014
Tanor Late	CC	2014
Tasty 1	CC	2014

**Table 5.4.4.3.** List of Mandarin Hybrid selections evaluated at Moriah (Hoedspruit) during the 2021 season.

Selection	Rootstock	Topwork
ARCCIT 9 LS	C35	2015
Furr (Clemcott)	MxT	2011
IRM 1 & 2	C35	2015
Leanri	C35	2015
Nova SL ARC	C35	2014
Orah	MxT	2011
Orri	C35	2015
RHM	C35	2015
Saint André	C35	2014
Samba	C35	2014
Tambor 1	MxT	2011
Tanor Late	C35	2015

**Table 5.4.4.3.** List of Mandarin Hybrid selections evaluated at Unifrutti (Hoedspruit) during the 2021 season.

Selection	Rootstock	Topwork
Ma'ayana (Dina)	CC	2017
Leanri	CC	2017
RHM	CC	2017
Samba	CC	2017
Tambor 2	CC	2017

## Results and discussion

For Mandarin Hybrid selections, a ratio of 11:1 is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over-mature. This process from the start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a higher instance of quality and rind issues.

### Etna

Etna bore another good crop with large to extra-large fruit (count 1X to 1XXX) and good internal quality for a hot production area (juice up to 62%), but low acids (below 1.0%). External colour was delayed from the start (varied from T3 to 5). The fruit developed seeds this season (avg 1.9 seeds per fruit), due to cross-pollination in the trial block (seedless in 2020). Maturity seems to be the middle of April for the hot production areas, according to the information in Table 5.4.4.5.

### Furr (Clemcott)

Furr developed large to extra-large fruit size (count 1XX – 1XXX) on the trees at Mahela and Moriah Estate, one of the characteristics of the cultivar, as well as a good to excellent crop on the trees. The external colour development on the fruit was good for the Hoedspruit and Letsitele area (T1-3) this season, where Letsitele was delayed in the past. Internally the fruit quality was very good, developing high juice (up to 63%) and Brix (up to 13/14) levels with good acids. Another quality of the fruit is the high seed count which peaked at 24

seeds per fruit (high self-and cross pollination). Maturity seems to be middle to the end of May to the middle of June for the hot production areas, according to the information in Table 5.4.4.5.

#### ARCCIT 9 LS

The crop was average to good this season to evaluate (management improvement). The fruit shape was very similar to the Nadorcott selection. Rind texture was very smooth with a natural shine (similar to packhouse waxing). The fruit size was bigger this season, ranging from count 1 and peaked at count 1XX even with the medium crop load on the trees. ARCCIT 9 LS produced seedless fruit (2020 up to 1.4 seeds per fruit) this season. Maturity seems to be two weeks earlier on the ARCCIT 9 LS selection, according to Table 5.4.4.5, but the information was limited due to only three evaluations (end of May to beginning of June).

#### Orah

Orah, producing a good crop with medium to large/extra-large (avg. count 2-1XX) fruit size. The average seed count in the fruit went down from 2020 to 8.6 seeds per fruit, one of the selection characteristics (higher seed numbers). Internal quality was good, the Brix levels were above 11 by time of harvest, good juice levels (above 61%) and acceptable acids (1.0%). External colour development was delayed and ranged from T4 to T5 (only two evaluations). Based on the internal quality results in Table 5.4.4.5, estimated maturity will be middle of April (degreening).

#### Samba

Samba on Carrizo rootstock produced a good to very good crop with good internal quality on the large, fast-growing thornless trees at Mahela and Bosveld (Letsitele). The trees at Moriah planted on C35 bore their fourth crop this season, producing large fruit size due to the young tree age (from count 2/1 up to count 1 XX). Colour development was early in the season (deep orange) with a smooth rind texture on the fruit. Fruit were completely seedless this season in the combined trial blocks, except two evaluations at Mahela with avg 0.5 seeds per fruit (future evaluations will confirm low seed numbers) and peaked from medium to large/extra-large fruit size at Bosveld and Mahela (average count 2 to 1XX). Internal quality was good with high juice and Brix levels, and lower acids (average 0.8%). Based on the internal quality results in Table 5.4.4.5, the estimated maturity will be middle to the end of April.

#### Tambor 1&2

Tambor 1 and 2 is an addition to the late maturing mandarin selections for the hot production areas, producing seedless fruit this season, except for one evaluation at Unifrutti (2 seeds per fruit), fairly low compared to the Furr and Orah selections. The external colour was on the yellow side at peak maturity, but with good internal quality, developing juice levels above 64%, Brix above 11 and acids above 1.0 at the last evaluation. Fruit size peaked from count 1XX to count 1XXX, very large for mandarin cultivars. Based on the internal quality results in Table 5.4.4.5, estimated maturity will be the end of June to the middle of July.

#### Additional selections

The internal quality of Tasty (Bruce) remained below average this season with fairly low juice levels (50.4%), higher Brix (above 11) and acids were better (0.80%), questioning the potential of the cultivar in the hot production areas. The fruit size peaked from count 1XX to count 1XXX with high seed numbers (7.0 seeds/fruit).

Sirio produced large to extra-large fruit (count 1/1X to 1XXX) on the trees due to a fair crop (large fruit size in hot areas and younger trees) with average internal quality (low Brix and acid levels) in the hot production areas. The fruit was not seedless with four evaluations completed (avg. 7.4 seeds per fruit).

Ma'yana (Dina) bore fruit on the Unifrutti (Hoedspruit) and Bosveld (Letsitele) trial trees this year. The tree shape is very upright with a dark scion bark colour. Fruit size varied from count 2/1 to 1XX/XXX (large fruit size similar to 2020), and seedless fruit in the crosspollination environment. The internal quality was good on the young trees; juice was above 58 up to 64.5%, Brix levels average 11.4 and better acid levels (remained on the lower but stable side).

Leanri cropped fruit this season at Moriah and Unifrutti, the fruit size varied from large to extra-large (1/1X-1XXX) with good internal quality; high juice levels and good flavoured fruit, good Brix (average 12.6 & 11.8) and better acids (average 1.1%). Seed numbers added up to 1.0 seeds per fruit; not completely seedless.

One of the new ultra-late selections to include at the Bosveld trial site will be Sugar Belle, bearing medium to large fruit (count 3 to 1X) this season with good Brix and high acids (avg. 1.4%).

Nova SL was included as a control for Saint André in the trial, the fruit is fairly difficult to peel and seedless fruit was cropped on the trees this season compared to low seeded fruit with the previous season. External colour was late and the fruit size varied between count 1 and count 1XX (large fruit). Nova SL (ARC) produced a coarse rind texture on the fruit. The acid levels in the fruit were similar compared to Saint André (slightly lower) and the external colour development delayed (T4 to 5).

Tanor Late cropped fruit at Mahela in Letsitele and Moriah in Hoedspruit; the peak maturity was by the middle of June (high Brix and acids this season at Mahela). Fruit size was extra-large (count 1XX to 1XXX) and completely seedless.

ARCCIT 9 LS, IRM 1&2, Orri and RHM bore third-second crop at Moriah Estate this season. IRM 1, ARCCIT 9 LS and Orri cropped large to extra-large fruit on the trees with acceptable internal quality and low seed numbers (Orri and RHM) in the crosspollination trial block. RHM performed well on C35 and developed good internal qualities with lower acid levels, with delayed external colour. The fruit size varied from count 1 to 1XX/XXX.

## Conclusion

There was an improvement in the external colour delay in the hot areas, that was a problem in the past; future evaluations will clarify the situation. Degreening may be an option for RHM, Leanri, Orri, IRM 1, Furr, Tambor and Orah (fruit colour development was yellow with degreening), but ethylene reacted slowly with Tango and Nadorcott LS (W. Murcott selection). In the hot areas, it will be crucial to cover the mandarin orchards with shade net, to minimise sunburn and improve the fruit packout percentage.

Furr and Tanor Late had the larger fruit size, followed by Tambor, Samba and then Orah. The smaller fruit size was produced on ARCCIT 9 (Nadorcott LS). Furr and Orah developed the highest number of seeds, followed by Leanri, Saint Andre, RHM and Orri.

Ma'yana (Dina), Tanor Late and Leanri were evaluated for the fourth time; and Etna, Sirio and Tasty 1 for the fifth time this season; future evaluations will continue to determine the suitability for this production area.

**Table 5.4.4.5.** Internal fruit quality data for Mandarin hybrid selections at Bosveld (Letsitele), Mahela (Letsitele) and Moriah (Hoedspruit) during the 2021 season.

Bosveld										
Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
Ma'ayana (Dina)	CC	24/3/2021	60 - 69	2 - 1X	60,0	10,2	0,70	14,6	0,0	T6 - 7
Ma'ayana (Dina)	CC	8/4/2021	67 - 79	1 - 1XXX	60,0	10,9	0,75	14,5	0,0	T5 - 6
Ma'ayana (Dina)	CC	4/5/2021	64 - 79	1 - 1XXX	58,8	11,0	0,75	14,7	0,0	T2 - 3
Samba	CC	24/3/2021	59 - 72	2 - 1XX	61,3	9,7	0,80	12,1	0,0	T4 - 6
Samba	CC	8/4/2021	55 - 71	3 - 1X	62,6	11,2	0,75	14,9	0,0	T3 - 5
Samba	CC	4/5/2021	59 - 70	2 - 1X	62,0	11,5	0,80	14,4	0,0	T1 - 2

Samba	CC	25/5/2021	59 - 76	2 - 1XX	58,5	10,9	0,85	12,8	0,0	T1 - 2
Sugar Belle	CC	25/5/2021	59 - 71	2 - 1X	59,3	12,3	1,35	9,1	2,0	T1 - 2
Sugar Belle	CC	4/5/2021	57 - 70	3 - 1X	63,4	11,4	1,50	7,6	2,7	T1 - 3

Mahela										
Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed per fruit	Fruit external colour
Etna	CC	24/03/2021	67 - 77	1 - 1XX	62,8	9,6	0,85	11,3	0,0	T5 - 6
Etna	CC	08/04/2021	65 - 74	1 - 1XX	62,7	10,5	0,90	11,7	1,9	T3 - 5
Etna	CC	04/05/2021	65 - 80	1 - 1XXX	60,3	12,3	1,45	8,5	5,7	T1 - 3
Etna	CC	15/06/2021	67 - 80	1 - 1XXX	53,1	11,8	0,85	13,9	0,0	T1
Furr	CC	24/03/2021	74 - 82	1XX - 1XXX	55,5	10,5	1,20	8,8	24,2	T5 - 6
Furr	CC	08/04/2021	75 - 83	1XX - 1XXX	56,8	11,0	0,85	12,9	17,2	T5 - 6
Furr	CC	04/05/2021	75 - 84	1XX - 1XXX	63,8	12,3	1,00	12,3	16,0	T1 - 2
Furr	CC	25/05/2021	75 - 85	1XX - 1XXX	62,9	13,9	1,15	12,1	13,2	T1 - 2
Furr	CC	15/06/2021	73 - 84	1XX - 1XXX	60,0	14,5	1,00	14,5	15,8	T1
Saint André	CC	24/03/2021	69 - 73	1X - 1XX	54,9	10,9	0,80	13,6	7,7	T5 - 6
Saint André	CC	08/04/2021	69 - 81	1X - 1XXX	62,0	10,9	0,75	14,5	1,5	T4 - 5
Saint André	CC	04/05/2021	68 - 78	1X - 1XXX	60,9	12,1	0,80	15,1	2,1	T1 - 2
Samba	CC	24/03/2021	64 - 70	1 - 1X	61,1	9,6	0,90	10,7	0,0	T5 - 6
Samba	CC	08/04/2021	57 - 69	3 - 1X	70,1	10,5	0,85	12,4	0,0	T3 - 5
Samba	CC	04/05/2021	67 - 73	1 - 1XX	58,9	12,2	0,80	15,3	0,0	T1 - 3
Samba	CC	25/05/2021	63 - 75	2 - 1XX	63,5	12,9	0,90	14,3	0,8	T1 - 2
Samba	CC	15/06/2021	63 - 74	2 - 1XX	58,5	14,8	0,85	17,4	1,7	T1
Sirio	CC	24/03/2021	68 - 76	1X - 1XX	54,1	9,6	0,90	10,7	10,3	T6 - 7
Sirio	CC	08/04/2021	64 - 81	1 - 1XXX	56,2	10,6	0,85	12,5	2,3	T2 - 4
Sirio	CC	04/05/2021	70 - 84	1X - 1XXX	51,6	10,9	0,80	13,6	8,5	T1 - 2
Sirio	CC	25/05/2021	71 - 82	1X - 1XXX	52,9	11,3	0,80	14,1	8,5	T1 - 3
Tanor Late 1	CC	25/05/2021	77 - 90	1XX - 1XXX	54,3	12,1	1,45	8,3	0,0	T1 - 3
Tanor Late 1	CC	15/06/2021	76 - 90	1XX - 1XXX	53,4	14,1	1,40	10,1	0,0	T1 - 2
Tanor Late 1	CC	06/07/2021	78 - 98	1XXX	54,8	13,7	1,35	10,1	0,0	T1 - 2

Tanor Late 1	CC	30/07/2021	84 - 91	1XXX	57,2	14,2	1,20	11,8	0,0	T1
Tasty 1	CC	04/05/2021	76 - 85	1XX - 1XXX	50,4	11,9	0,80	14,9	7,0	T1 - 2

Moriah										
Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed per fruit	Fruit external colour
ARCCIT 9 LS	C35	25/03/2021	55 - 83	1 - 1XX	69,6	9,5	1,10	8,6	0,0	T6 - 7
ARCCIT 9 LS	C35	09/04/2021	68 - 76	1X - 1XX	60,6	10,2	1,15	8,9	0,0	T4 - 5
ARCCIT 9 LS	C35	26/05/2021	69 - 74	1X - 1XX	60,5	12,3	1,05	11,7	0,0	T1 - 2
Furr	MxT	25/03/2021	68 - 83	1X - 1XXX	56,2	11,6	1,45	8,0	18,7	T5 - 6
Furr	MxT	09/04/2021	74 - 87	1XX - 1XXX	57,1	11,2	1,10	10,2	12,7	T4 - 5
Furr	MxT	05/05/2021	74 - 79	1XX - 1XXX	59,0	12,8	1,15	11,1	15,3	T1 - 3
Furr	MxT	25/05/2021	69 - 84	1X - 1XXX	62,9	13,8	1,05	13,1	5,3	T1
IRM1	C35	05/05/2021	68 - 83	1X - 1XXX	61,2	11,7	1,20	9,8	0,0	T1 - 3
IRM1	C35	26/05/2021	74 - 84	1X - 1XXX	61,1	12,7	1,10	11,5	0,0	T1 - 2
IRM1	C35	16/06/2021	65 - 71	1 - 1X	63,1	13,8	1,05	13,1	0,0	T1
IRM2	C35	05/05/2021	66 - 75	1 - 1XX	60,4	12,1	0,95	12,7	0,0	T1 - 2
IRM2	C35	26/05/2021	68 - 78	1X - 1XXX	57,2	12,7	1,10	11,5	0,0	T1 - 2
IRM2	C35	16/06/2021	65 - 78	1 - 1XXX	62,9	13,5	1,00	13,5	0,0	T1 - 2
Leanri	C35	25/03/2021	64 - 74	1 - 1XX	61,3	11,4	1,10	10,4	0,8	T4 - 6
Leanri	C35	09/04/2021	65 - 78	1 - 1XXX	62,0	10,1	1,05	9,6	0,7	T3 - 5
Leanri	C35	16/06/2021	71 - 79	1 - 1XXX	60,8	14,9	1,20	12,4	0,3	T1 - 2
Leanri	C35	26/05/2021	66 - 75	1 - 1XX	62,1	13,8	1,10	12,5	0,0	T1 - 2
Nova SL	C35	25/03/2021	67 - 74	1 - 1XX	62,8	11,0	0,75	14,7	0,0	T5 - 6
Nova SL	C35	09/04/2021	63 - 68	1 - 1XX	64,0	11,3	0,90	12,6	0,0	T4 - 5
Orah	C35	25/03/2021	57 - 79	3 - 1XXX	58,0	10,4	1,70	6,1	10,3	T5 - 6
Orah	C35	09/04/2021	62 - 69	2 - 1X	61,3	11,0	1,00	11,0	5,3	T4 - 5
Orri	C35	25/03/2021	64 - 70	1 - 1X	54,8	10,7	1,70	6,3	0,0	T5 - 7
Orri	C35	09/04/2021	60 - 77	2 - 1XX	58,0	11,5	1,40	8,2	0,0	T4 - 6
Orri	C35	05/05/2021	63 - 77	2 - 1XX	59,9	12,4	1,40	8,9	0,0	T1 - 3
Orri	C35	26/05/2021	71 - 79	2 - 1XX	61,1	13,0	1,15	11,3	0,0	T1 - 2
Orri	C35	07/07/2021	71 - 80	1X - 1XXX	60,2	14,0	0,90	15,6	0,0	T1
Orri	C35	30/07/2021	63 - 67	1X - 1XXX	60,3	14,9	0,90	16,6	0,8	T1 - 2
RHM	C35	25/03/2021	65 - 74	2 - 1X	60,9	9,8	1,05	9,3	2,8	T5 - 7
RHM	C35	09/04/2021	64 - 77	1 - 1XX	55,0	10,9	0,80	13,6	0,0	T4 - 5
RHM	C35	05/05/2021	72 - 77	1 - 1XX	51,1	11,6	0,65	17,8	0,0	T1 - 3
RHM	C35	26/05/2021	62 - 67	1XX	55,9	12,5	0,85	14,7	0,0	T1 - 2
Saint André	C35	25/03/2021	67 - 78	1X - 1XX	55,2	10,4	0,90	11,6	0,0	T5 - 6
Saint André	C35	09/04/2021	69 - 74	1 - 1XXX	54,6	11,7	0,75	15,6	0,0	T3 - 5

Saint André	C35	05/05/2021	60 - 68	1X - 1XX	63,4	11,5	1,25	9,2	0,0	T1 - 2
Samba	C35	25/03/2021	66 - 74	2 - 1	61,3	10,5	0,85	12,4	0,0	T4 - 6
Samba	C35	09/04/2021	68 - 75	1 - 1XX	70,2	11,1	0,70	15,9	0,0	T2 - 4
Samba	C35	05/05/2021	69 - 73	1X - 1XX	63,1	11,1	0,85	13,1	0,0	T1
Tambor 1	MxT	16/06/2021	77 - 94	1XX - 1XXX	59,5	11,9	1,40	8,5	0,0	T1
Tambor 1	MxT	07/07/2021	76 - 86	1XX - 1XXX	59,7	12,3	1,15	10,7	0,0	T1
Tambor 1	MxT	30/07/2021	70 - 88	1XX - 1XXX	62,8	13,3	1,15	11,6	0,0	T1 - 2
Tambor 1	MxT	19/08/2021	64 - 71	1X - 1XXX	64,6	13,1	1,00	13,1	0,0	T1
Tanor Late	C35	26/05/2021	77 - 93	1XXX	47,9	11,0	1,25	8,8	0,0	T2 - 4
Tanor Late	C35	16/06/2021	76 - 92	1XX - 1XXX	53,4	11,5	0,90	12,8	0,0	T1 - 2
Tanor Late	C35	07/07/2021	81 - 103	1XX - 1XXX	53,8	12,9	0,90	14,3	0,0	T1
Tanor Late	C35	30/07/2021	92 - 106	1XXX	52,0	12,5	0,75	16,7	0,0	T1

Unifrutti										
Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
Ma'ayana (Dina)	CC	24/03/2021	57 - 69	2 - 1X	64,5	11,6	1,00	11,6	0,0	T5 - 7
Ma'ayana (Dina)	CC	09/04/2021	60 - 65	2 - 1	63,0	12,6	0,90	14,0	0,0	T4 - 5
Leanri	CC	25/03/2021	73 - 80	1XX - 1XXX	58,0	10,5	1,20	8,8	1,0	T5 - 6
Leanri	CC	09/04/2021	65 - 81	1 - 1XXX	61,0	12,0	1,10	10,9	1,0	T4 - 5
Leanri	CC	05/05/2021	69 - 76	1X - 1XX	60,1	12,9	0,95	13,6	0,9	T1 - 2
RHM	CC	25/03/2021	63 - 73	2 - 1XX	58,5	10,7	0,85	12,6	5,3	T6 - 7
RHM	CC	09/04/2021	60 - 74	2 - 1XX	58,3	11,3	0,75	15,1	1,8	T4 - 6
RHM	CC	05/05/2021	63 - 73	2 - 1XX	61,7	12,5	0,70	17,9	0,0	T1 - 3
Samba	CC	25/03/2021	56 - 64	3 - 1	59,4	11,4	0,85	13,4	0,0	T4 - 6
Samba	CC	09/04/2021	56 - 65	3 - 1	63,8	12,2	0,80	15,3	0,0	T3 - 5
Tambor 2	SC	26/05/2021	75 - 89	1XX - 1XXX	59,4	12,3	1,15	10,7	0,0	T1 - 3
Tambor 2	CC	26/05/2021	85 - 81	1XXX	62,5	12,3	1,10	11,2	2,0	T1 - 3

#### 5.4.5 PROGRESS REPORT: Evaluation of Valencia selections in the hot, dry production areas (Tshipise)

Project 899A by J Joubert (CRI)

#### Summary

This was the sixth season for the Alicedale site due to fruit numbers on the trees, and meaningful data was collected. Turkey will start the season as the earliest maturing Valencia. Delta will be next in line, followed by Alpha, Skilderkrans and McClean SL with improved colour and completely seedless fruit. Kobus du Toit Late follows as part of the mid-maturing Valencia section. Gusocora with seedless fruit and Henrietta and Bennie will be next, followed by Louisa and Jassie, towards the late Valencia section, with excellent internal quality and optimal colour development. Finally, Lavalley will end of the Valencia season in the warm, dry production areas.

## Opsomming

Hierdie was die vyfde seisoen vir Alicedale as gevolg van voldoende vrugte aan die bome, betekenisvolle data kon versamel word. Turkey begin die seisoen as die vroegste Valencia. Delta sal volgende in lyn wees, gevolg deur Alpha, Skilderkrans en McClean SL met later vrugkleur en totaal saadlose vrugte. Kobus du Toit Laat volg as deel van die mid-rypwordende Valencia gedeelte. Gusocora met saadlose vrugte, asook Henrietta en Bennie is volgende, gevolg deur Louisa en Jassie, nader aan die laat Valencia periode met uitstekende interne kwaliteit en optimum kleur ontwikkeling. Lavalle sal die Valencia seisoen afsluit in die warm droë produksie areas.

## Objective

- To find suitable Valencia selections with superior characteristics for the hot dry inland citrus production areas.

## Materials and methods

Field evaluations and laboratory analyses were conducted on the list below at Alicedale (Tshipise).

**Table 5.4.5.1.** List of Valencia selections evaluated at Alicedale (Tshipise) during 2021.

Selection	Rootstock	Planted
Alpha	C35/US-812 (SxB)/RL/X639	2013
Bennie 2	C35/US-812 (SxB)/RL/X639	2013
Delta	C35/US-812 (SxB)/RL/X639	2013
Gusocora	C35/US-812 (SxB)/RL/X639	2013
Henrietta	C35/US-812 (SxB)/RL/X639	2013
Jassie	C35/US-812 (SxB)/RL/X639	2013
Kobus du Toit Late	C35/US-812 (SxB)/RL/X639	2013
Lavalle	C35/US-812 (SxB)/RL/X639	2013
Louisa	C35/US-812 (SxB)/RL/X639	2013
McClean SL	C35/US-812 (SxB)/RL/X639	2013
Skilderkrans	C35/US-812 (SxB)/RL/X639	2013
Turkey	RL/X639	2013

## Results and discussion

The Alicedale trial site at Tshipise bore fruit on all the cultivars on different rootstocks and evaluations were done accordingly. There was a good fruit set on the trees for 2021 (determine yield production) and all cultivar combinations will be evaluated in the next season.

### Alpha

The fruit was completely seedless on all four rootstock combinations with medium to large/extra-large fruit size (count 72 to 48/40). C35 and X639 matured first, Brix:acid ratio above 10.0 and average juice content (above 50%). It is apparent that Alpha's maturity is middle to end of July (Table 5.4.5.3).

### Bennie 2

There was a good crop on Bennie and the fruit size peaked between count 72 and 48/40 (very good for Valencia production, but on the larger side). The internal colour of the fruit was deep yellow, fruit shape round, rind texture fairly smooth, medium rag content and medium rind thickness. Bennie produced good juice levels (average 53%), Brix (average 11.1) and acid (1.2%) and low seed counts (average 0.4 seeds per fruit). External colour by the time of harvest varied between T1 and T2. Based on ratios, maturity end of June to the middle of July (Table 5.4.5.3).

### Delta

The Delta (control) trees were planted at Alicedale on four rootstocks. Fruit size distribution was uniform and ranged from count 88/72 to 56/48, medium to large fruit and optimum Valencia requirements. Internal quality was good with high juice (avg 51%), higher Brix (11.1) and good acid levels through the season (average 1.1%). Based on the internal quality results in Table 5.4.5.3, the estimated maturity will be mid-June to mid-July.

### Gusocora

Gusocora was evaluated at Alicedale this year on C35, RL, US 812 and X639 rootstocks. The fruit was completely seedless and developed a good internal quality where juice (up to 55%), Brix (avg 11.7) and acid above 0.90 complied with export requirements. The external colour (improved) varied from T1 to T3, correlating with the internal quality and Brix:acid ratio (10:1 for maturity). Fruit size was slightly smaller and peaked between counts 88/72 and 56/48, optimal fruit size for export Valencia (medium to large). There was an average crop on the trees. It is apparent that Gusocora maturity is end of June to middle of July this year (Table 5.4.5.3).

### Henrietta

Henrietta was evaluated on all four rootstock combinations at Alicedale, Tshipise this season. Juice levels peaked above 55% average with higher Brix (up to 12.1) and acids 1.20. The external colour development improved and peaked between T1 and T3 for the season. Average seeds per fruit increased to 0.1 seeds per fruit (0.9 seeds for 2020). Based on the internal quality results in Table 5.4.5.3, estimated maturity will be mid-July to mid-August.

### Jasi

Jasi seems to be one of the most promising new Valencia selections being tested and evaluated in the different citrus production and climatic areas. Fruit size distribution was bigger with the good yield on the trees; the counts were from 72 to 56/48. Fruit quality was good with high juice (average 54.1%), Brix of up to 12.7 (C35) and fairly high acid levels (above 0.95%) at the final evaluation, indicating the late characteristics of the cultivar. The seed counts varied from seedless up to 1.8 seeds per fruit (average 0.5 seeds per fruit – very low). Based on the internal quality results in Table 5.4.5.3, the estimated maturity will be mid-July to mid-August.

### Kobus du Toit Late

There was an external colour delay that improved even more on the fruit during the season, ranging from T1 to T3/4 up until the last evaluation. Fruit average size varied from medium to large/extra-large, count 72 to 48/40. Internal quality was good depending on the age of the trees and the rootstock combinations. Kobus du Toit Late performed the best on C35 and US 812 at Alicedale, Tshipise. Seed production was very low this season for a seeded selection (average 0.1 seeds per fruit). Acid levels were above 0.80% the entire season. Maturity, based on the internal quality results in Table 5.4.5.3, is estimated to be the end of June to the middle of July for these hot production areas.

### Lavalle

Lavalle was evaluated on all four rootstocks this season and produced completely seedless fruit for the rest of the evaluations. The internal quality complied with export requirements and acid level was above 1.2% at the final evaluation (Alicedale harvested fruit to determine final crop). Keep in mind that Lavalle is a late Valencia selection with good shelf life and the optimal harvest time will be in August. The navel end on some fruit seems to develop a button and there were split fruit on some of the trees evaluated, but this varies from season to season. From the ratio on this date it is apparent that Lavelle's maturity is end of August to middle of September (Table 5.4.5.3).

### Louisa

There was a lighter crop on all four rootstock combinations at Alicedale. The fruit was completely seedless and internal quality was average this season with lower juice (50.6% vs 63% in 2020) and good Brix levels (up to 12.7). The fruit colour was fairly yellow by the time of peak maturity between T1 and T3. Fruit size peaked from medium to large/ extra-large, count 88/72 to count 48/40. Based on the internal quality results in Table 5.4.5.3, estimated maturity will be middle to the end of July.

### McCleane SL

Compared to all the other Valencia trial sites, McCleane SL remained completely seedless. This year all the combinations with McCleane SL were bearing fruit at Alicedale to evaluate, indicating the potential for the future (crop improvement due to Gibb spray). The fruit size peaked between count 72 and 48/40 (medium to large/very large) with average internal quality (juice up to 53%, Brix 12.3, acid 0.9). The acid levels remained fairly low during the duration of the production season. Maturity (Table 5.4.5.3) is estimated to be the middle/end of June to middle of July.

### Skilderkrans

Skilderkrans was evaluated at Alicedale in the hot production areas. Fruit size varied from medium to large/extra-large (count 88-48), smaller compared to the 2020 season (better crop). Internally the Brix content was good (up to 12.7) and the acid level of 1.1% (peak maturity) indicated a later maturing Valencia selection. Juice level decreased to an average 51.4% later in the season; above the minimum required export figure. There was an improvement in external colour at Alicedale on all four of the rootstocks evaluated, including on RL (T1-2/3). The fruit developed a smooth rind, fibre strength was fairly soft and the fruit shape was round. Ratios were better this season due to the more acceptable acid levels, improving peak maturity from middle to the end of July (Table 5.4.5.3).

### **Conclusions**

Bennie matures well on the trees and reduces rind pitting problems. The recommendation will be to harvest the fruit from middle July onwards (stronger rind). Gusocora seems to have an improved colour development at peak maturity, peaked between T1 and T2/3 with good Brix levels.

Skilderkrans still developed high numbers of Chimeras on the fruit this season, questioning the selections' stability.

All the selections evaluated developed low seed numbers in their fruit, except for Alpha, Delta, Gusocora, Louisa, Lavalle 2, McCleane seedless and Skilderkrans being completely seedless. All the selections comply with the minimum export standards. The ideal fruit size distribution for Valencia exports was achieved and peaked from count 88 to count 56 (excellent).

**Table 5.4.5.3.** Internal fruit quality data for Valencia orange selections at Alicedale (Tshipise) during the 2021 season.

Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
Alpha	C35	24/05/2021	80 - 90	64 - 40	48,3	10,6	1,25	8,5	0,0	T1 - 3
Alpha	C35	15/06/2021	77 - 87	72 - 48	52,0	10,8	1,30	8,3	0,0	T1
Alpha	C35	06/07/2021	77 - 85	72 - 56	50,3	11,9	1,15	0,0	0,0	T1 - 3
Alpha	C35	29/07/2021	77 - 95	72 - 40	50,9	11,9	1,20	9,9	0,0	T1 - 2
Alpha	X639	24/05/2021	78 - 90	64 - 40	49,1	10,5	1,65	6,4	0,0	T2 - 3
Alpha	X639	15/06/2021	76 - 86	72 - 48	41,3	10,8	1,40	7,7	0,0	T1
Alpha	X639	06/07/2021	72 - 82	64 - 56	47,4	11,3	1,10	10,3	0,0	T1 - 3
Alpha	X639	29/07/2021	81 - 85	64 - 56	50,6	11,6	1,10	10,5	0,0	T1 - 2
Alpha	US-812	24/05/2021	79 - 94	64 - 40	53,0	10,7	1,75	6,1	0,0	T1 - 3
Alpha	US-812	15/06/2021	80 - 90	64 - 40	50,3	11,1	1,30	8,5	0,0	T1

Alpha	US-812	06/07/2021	84 - 95	64 - 40	44,1	11,7	1,30	9,0	0,0	T1 - 2
Alpha	US-812	29/07/2021	81 - 94	64 - 40	53,6	12,0	1,20	10,0	0,0	T1
Alpha	RL	24/05/2021	81 - 85	64 - 56	46,3	10,2	1,70	6,0	0,0	T2 - 4
Alpha	RL	15/06/2021	75 - 86	72 - 48	45,3	11,0	1,50	7,3	0,0	T1 - 2
Alpha	RL	06/07/2021	78 - 85	64 - 56	53,1	10,8	1,20	9,0	0,0	T1 - 2
Alpha	RL	29/07/2021	75 - 86	72 - 48	53,0	11,8	1,20	9,8	0,0	T1 - 2
Bennie 2	C35	24/05/2021	80 - 86	64 - 48	49,1	11,0	1,45	7,6	0,0	T1 - 2
Bennie 2	C35	15/06/2021	79 - 88	64 - 48	54,7	11,1	1,10	10,1	0,0	T1
Bennie 2	C35	06/07/2021	82 - 89	56 - 48	52,5	11,5	1,05	11,0	0,0	T1 - 2
Bennie 2	C35	29/07/2021	75 - 84	72 - 56	53,8	10,7	0,95	11,3	1,1	T1
Bennie 2	C35	17/08/2021	83 - 89	56 - 48	56,8	11,9	0,95	12,5	0,0	T1 - 2
Bennie 2	X639	24/05/2021	76 - 90	72 - 40	54,2	10,4	1,30	8,0	0,0	T1 - 3
Bennie 2	X639	15/06/2021	77 - 80	72 - 64	50,7	11,1	1,15	9,7	1,0	T1
Bennie 2	X639	06/07/2021	77 - 89	72 - 48	54,5	11,1	1,20	9,3	0,0	T1 - 2
Bennie 2	X639	29/07/2021	80 - 89	64 - 48	54,1	11,5	0,95	12,1	0,0	T1 - 2
Bennie 2	X639	17/08/2021	80 - 90	64 - 40	53,5	12,0	1,00	12,0	0,0	T1 - 2
Bennie 2	US-812	24/05/2021	78 - 92	64 - 40	52,0	11,2	1,65	6,8	0,0	T1 - 3
Bennie 2	US-812	15/06/2021	77 - 99	72 - 36	55,4	9,9	1,45	6,8	2,0	T1
Bennie 2	US-812	06/07/2021	80 - 87	64 - 48	52,1	11,8	1,15	10,3	0,0	T1 - 2
Bennie 2	US-812	29/07/2021	82 - 89	56 - 48	55,0	11,0	1,15	9,6	0,0	T1 - 2
Bennie 2	US-812	17/08/2021	85 - 94	56 - 40	43,5	12,0	1,05	11,4	0,0	T1 - 2
Bennie 2	RL	24/05/2021	80 - 88	64 - 48	52,2	9,8	1,60	6,1	0,0	T2 - 3
Bennie 2	RL	15/06/2021	74 - 85	72 - 56	54,1	10,9	1,45	7,5	1,8	T1
Bennie 2	RL	06/07/2021	79 - 91	64 - 40	54,4	10,9	1,49	7,3	1,3	T1
Bennie 2	RL	29/07/2021	82 - 92	56 - 40	55,8	11,4	1,10	10,4	0,0	T1
Bennie 2	RL	17/08//2021	80 - 87	64 - 48	50,3	11,3	0,85	13,3	0,0	T1 - 2
Delta	C35	24/05/2021	73 - 85	72 - 56	52,5	10,9	1,20	9,1	0,0	T3 - 4
Delta	C35	15/06/2021	71 - 85	88 - 56	45,2	11,4	1,15	9,9	0,0	T1 - 2
Delta	C35	06/07/2021	76 - 86	72 - 86	53,2	12,6	1,00	12,6	0,0	T1 - 3
Delta	C35	29/07/2021	80 - 86	64 - 48	52,1	11,7	1,50	7,8	0,0	T1 - 2
Delta	X639	24/05/2021	72 - 84	88 - 56	52,0	10,5	1,25	8,4	0,0	T4 - 5
Delta	X639	15/06/2021	79 - 82	64 - 56	49,0	10,9	1,40	7,8	0,0	T1 - 3
Delta	X639	06/07/2021	79 - 90	64 - 40	50,0	11,1	0,90	12,3	0,0	T1 - 3
Delta	X639	29/07/2021	78 - 84	64 - 56	51,0	11,1	0,95	11,7	0,0	T1 - 3
Delta	US-812	24/05/2021	75 - 90	72 - 40	48,8	11,0	1,35	8,1	0,0	T3 - 5
Delta	US-812	15/06/2021	77 - 84	72 - 56	50,4	12,9	0,95	13,6	0,0	T1 - 3
Delta	US-812	06/07/2021	82 - 90	56 - 40	52,5	11,3	1,10	10,3	0,0	T1 - 3
Delta	US-812	29/07/2021	75 - 84	72 - 56	53,6	11,9	1,15	10,3	0,0	T1 - 2
Delta	RL	24/05/2021	74 - 80	72 - 64	47,7	10,0	1,20	8,3	0,0	T3 - 6
Delta	RL	15/06/2021	73 - 88	72 - 48	47,0	10,4	1,20	8,7	0,0	T1 - 2

Delta	RL	06/07/2021	74 - 83	72 - 56	52,3	9,8	0,95	10,3	0,0	T1 - 2
Delta	RL	29/07/2021	73 - 89	72 - 48	72,4	11,7	1,00	11,7	0,0	T1 - 3
Delta	RL	06/07/2021	74 - 83	72 - 56	52,3	9,8	0,95	10,3	0,0	T1 - 2
Gusocora	C35	24/05/2021	74 - 84	72 - 56	50,4	11,3	1,05	10,8	0,0	T3 - 5
Gusocora	C35	15/06/2021	70 - 85	88 - 56	51,9	12,5	1,10	11,4	0,0	T1 - 2
Gusocora	C35	06/07/2021	70 - 83	88 - 56	53,7	13,6	0,95	14,3	0,0	T1
Gusocora	C35	29/07/2021	77 - 85	72 - 56	54,3	12,6	0,95	13,3	0,0	T1 - 2
Gusocora	X639	24/05/2021	75 - 84	72 - 56	50,7	10,8	1,15	9,4	0,0	T3 - 5
Gusocora	X639	15/06/2021	77 - 84	72 - 56	51,4	11,1	1,15	9,7	0,0	T1 - 2
Gusocora	X639	06/07/2021	75 - 83	72 - 56	50,0	11,7	1,00	11,7	0,0	T1 - 3
Gusocora	X639	29/07/2021	76 - 88	72 - 48	50,8	11,6	0,90	12,9	0,0	T1 - 2
Gusocora	US-812	24/05/2021	76 - 82	72 - 56	54,1	12,1	1,20	10,1	0,0	T3 - 4
Gusocora	US-812	15/06/2021	73 - 86	72 - 48	50,0	11,7	1,05	11,1	0,0	T1 - 3
Gusocora	US-812	06/07/2021	77 - 81	72 - 64	52,4	12,4	1,05	11,8	0,0	T1 - 2
Gusocora	US-812	29/07/2021	80 - 89	64 - 48	52,0	11,9	1,10	10,8	0,0	T1 - 2
Gusocora	RL	24/05/2021	74 - 78	72 - 64	49,6	10,5	1,40	7,5	0,0	T1 - 3
Gusocora	RL	15/06/2021	70 - 85	88 - 56	49,6	11,0	1,00	11,0	0,0	T1 - 3
Gusocora	RL	06/07/2021	75 - 83	72 - 56	55,1	11,4	0,95	12,0	0,0	T1 - 2
Gusocora	RL	29/07/2021	74 - 77	72	55,3	11,4	1,05	10,9	0,0	T1 - 3
Henrietta	C35	24/05/2021	82 - 90	56 - 40	51,1	10,2	1,25	8,2	0,0	T2 - 3
Henrietta	C35	15/06/2021	82 - 90	56 - 40	55,6	10,2	1,15	8,9	0,0	T1
Henrietta	C35	06/07/2021	77 - 90	72 - 40	54,9	11,2	1,20	9,3	0,0	T1 - 2
Henrietta	C35	29/07/2021	82 - 93	56 - 40	52,8	11,7	0,85	13,8	0,0	T1 - 3
Henrietta	X639	24/05/2021	76 - 86	72 - 48	54,5	10,7	1,05	10,2	0,0	T2 - 3
Henrietta	X639	15/06/2021	78 - 89	64 - 48	52,6	10,7	1,05	10,2	0,0	T1 - 3
Henrietta	X639	06/07/2021	80 - 86	64 - 48	54,9	11,3	1,25	9,0	0,0	T1 - 2
Henrietta	X639	29/07/2021	77 - 84	72 - 56	57,1	12,1	1,05	11,5	0,0	T1 - 2
Henrietta	X639	17/08/2021	72 - 89	88 - 48	50,6	10,8	1,00	10,8	0,0	T1 - 3
Henrietta	US-812	24/05/2021	77 - 89	72 - 48	58,7	10,6	1,05	10,1	0,0	T3 - 4
Henrietta	US-812	15/06/2021	74 - 90	72 - 40	56,8	9,9	1,20	8,3	0,0	T1 - 3
Henrietta	US-812	06/07/2021	83 - 89	56 - 48	54,9	11,7	1,20	9,8	0,0	T1 - 2
Henrietta	US-812	29/07/2021	73 - 84	72 - 56	56,7	11,2	1,00	11,2	0,0	T1 - 3
Henrietta	US-812	17/08/2021	80 - 88	64 - 48	56,0	10,6	1,05	10,1	0,0	T1 - 2
Henrietta	RL	24/05/2021	76 - 91	72 - 40	49,4	10,2	1,30	7,8	0,0	T1 - 3
Henrietta	RL	15/06/2021	77 - 87	72 - 48	55,7	10,2	1,20	8,5	0,0	T1 - 2
Henrietta	RL	06/07/2021	74 - 89	72 - 48	56,7	10,8	1,20	9,0	1,3	T1 - 3
Henrietta	RL	29/07/2021	72 - 84	88 - 56	56,9	10,9	0,95	11,5	0,0	T1 - 2
Henrietta	RL	17/08/2021	80 - 90	64 - 40	54,3	11,7	0,95	12,3	0,0	T1 - 3
Jasi	C35	24/05/2021	82 - 97	56 - 36	54,5	10,0	1,25	8,0	0,0	T2 - 4
Jasi	C35	15/06/2021	83 - 89	56 - 48	51,7	10,7	1,30	8,2	0,8	T1 - 2

Jasi	C35	06/07/2021	78 - 89	64 - 48	54,3	10,9	1,00	10,9	0,0	T1 - 2
Jasi	C35	29/07/2021	80 - 88	64 - 48	52,8	12,2	1,10	11,1	0,0	T1 - 2
Jasi	C35	17/08/2021	84 - 92	56 - 40	53,8	12,7	0,95	13,4	1,8	T1 - 3
Jasi	X639	24/05/2021	76 - 88	72 - 48	54,5	10,4	1,35	7,7	0,0	T1 - 3
Jasi	X639	15/06/2021	75 - 84	72 - 56	54,2	11,3	1,15	9,8	0,0	T1 - 3
Jasi	X639	06/07/2021	75 - 91	72 - 40	52,7	11,1	1,05	10,6	0,0	T1
Jasi	X639	29/07/2021	82 - 89	56 - 48	55,9	11,3	1,00	11,3	0,9	T1 - 2
Jasi	X639	17/08/2021	80 - 90	64 - 40	55,2	12,1	0,95	12,7	0,0	T1 - 2
Jasi	US-812	24/05/2021	77 - 86	72 - 48	56,4	11,4	1,50	7,6	1,0	T2 - 3
Jasi	US-812	15/06/2021	78 - 90	64 - 40	51,1	11,0	1,50	7,3	1,7	T1 - 2
Jasi	US-812	06/07/2021	73 - 82	72 - 56	55,8	10,6	1,06	10,0	0,0	T1
Jasi	US-812	29/07/2021	77 - 89	72 - 48	57,4	11,9	1,30	9,2	0,0	T1 - 2
Jasi	US-812	17/08/2021	81 - 89	64 - 48	53,9	11,7	1,15	10,2	0,0	T1 - 3
Jasi	RL	24/05/2021	75 - 86	72 - 48	52,3	9,6	1,20	8,0	0,0	T1 - 3
Jasi	RL	15/06/2021	71 - 90	88 - 40	53,3	10,1	1,15	8,8	1,5	T1 - 2
Jasi	RL	06/07/2021	77 - 84	72 - 56	58,0	10,9	1,15	9,5	0,0	T1 - 2
Jasi	RL	29/07/2021	75 - 86	72 - 48	52,7	10,7	1,05	10,2	1,7	T1 - 2
Jasi	RL	17/08/2021	81 - 90	64 - 40	50,6	10,8	1,00	10,8	0,0	T1 - 2
K du Toit Late	C35	24/05/2021	77 - 85	72 - 56	54,3	10,5	1,25	8,4	0,0	T2 - 4
K du Toit Late	C35	15/06/2021	77 - 87	72 - 48	54,4	11,4	1,10	10,4	0,0	T1 - 2
K du Toit Late	C35	06/07/2021	76 - 89	72 - 48	52,3	11,2	1,10	10,2	0,0	T1 - 3
K du Toit Late	C35	29/07/2021	76 - 83	72 - 56	55,6	12,3	1,00	12,3	0,0	T1 - 3
K du Toit Late	C35	17/08/2021	80 - 89	64 - 48	51,3	12,3	0,90	13,7	0,0	T1 - 3
K du Toit Late	X639	24/05/2021	73 - 78	72 - 64	56,3	10,3	1,35	7,6	1,7	T4 - 6
K du Toit Late	X639	15/06/2021	76 - 89	72 - 48	52,8	11,3	0,95	11,9	0,0	T1 - 3
K du Toit Late	X639	06/07/2021	76 - 88	72 - 48	51,0	11,1	0,90	12,3	0,0	T1 - 3
K du Toit Late	X639	29/07/2021	65 - 83	105 - 56	53,8	11,2	0,90	12,4	0,0	T1 - 2
K du Toit Late	X639	17/08/2021	84 - 91	56 - 40	53,7	11,7	0,85	13,8	0,0	T1 - 2
K du Toit Late	US-812	24/05/2021	73 - 88	72 - 48	57,7	11,1	1,45	7,7	0,0	T4 - 5
K du Toit Late	US-812	15/06/2021	78 - 87	64 - 48	54,2	11,2	1,30	8,6	0,0	T1 - 3
K du Toit Late	US-812	06/07/2021	79 - 92	64 - 40	54,5	11,6	1,20	9,7	0,0	T1 - 2
K du Toit Late	US-812	29/07/2021	77 - 89	72 - 48	49,7	11,4	1,05	10,9	0,0	T1 - 3

K du Toit Late	US-812	17/08/2021	81 - 90	64 - 40	56,5	12,3	1,05	11,7	0,0	T1 - 2
K du Toit Late	RL	24/05/2021	77 - 86	72 - 48	54,1	10,7	1,20	8,9	0,0	T3 - 5
K du Toit Late	RL	15/06/2021	77 - 85	72 - 56	50,8	10,0	1,05	9,5	0,0	T1 - 3
K du Toit Late	RL	06/07/2021	77 - 87	72 - 48	52,4	10,1	0,90	11,2	0,0	T1 - 3
K du Toit Late	RL	29/07/2021	74 - 81	72 - 64	50,7	10,8	0,85	12,7	0,0	T1 - 3
K du Toit Late	RL	17/08/2021	78 - 87	64 - 48	55,0	11,6	0,80	14,5	0,0	T1 - 2
Lavalle 2	C35	15/06/2021	89 - 94	48 - 40	60,9	10,3	1,55	6,6	0,0	T3 - 4
Lavalle 2	C35	06/07/2021	86 - 92	48 - 40	53,6	11,6	1,45	8,0	0,0	T1 - 2
Lavalle 2	C35	17/07/2021	84 - 96	56 - 40	54,6	12,7	1,35	9,4	0,0	T1 - 3
Lavalle 2	C35	17/08/2021	90 - 101	40 - 36	52,1	13,1	1,30	10,1	0,0	T1 - 3
Lavalle 2	X639	15/06/2021	86 - 94	48 - 40	48,2	11,3	1,40	8,1	0,0	T1 - 3
Lavalle 2	X639	06/07/2021	87 - 95	48 - 36	52,6	11,1	1,30	8,5	0,0	T2 - 3
Lavalle 2	X639	29/07/2021	77 - 86	72 - 48	56,3	13,1	1,35	9,7	0,0	T1 - 3
Lavalle 2	X639	17/08/2021	87 - 99	48 - 36	53,6	12,4	1,30	9,5	0,0	T1 - 3
Lavalle 2	US-812	15/06/2021	82 - 102	56 - 36	51,5	11,7	1,85	6,3	0,0	T2 - 3
Lavalle 2	US-812	06/07/2021	72 - 95	88 - 36	54,2	12,7	1,80	7,1	0,0	T1 - 2
Lavalle 2	US-812	29/07/2021	80 - 92	64 - 40	54,7	12,4	1,60	7,8	0,0	T1 - 3
Lavalle 2	US-812	17/08/2021	80 - 100	64 - 36	53,4	12,5	1,35	9,3	0,0	T1 - 3
Lavalle 2	RL	15/06/2021	81 - 92	64 - 40	50,6	9,4	1,50	6,3	0,0	T2 - 3
Lavalle 2	RL	06/07/2021	81 - 85	64 - 40	55,0	10,8	1,40	7,7	0,0	T1 - 3
Lavalle 2	RL	29/07/2021	86 - 92	48 - 40	55,1	11,0	1,35	8,1	0,0	T1 - 3
Lavalle 2	RL	17/08/2021	81 - 101	64 - 36	53,7	12,0	1,15	10,4	0,0	T1 - 3
Louisa	C35	24/05/2021	81 - 84	64 - 56	49,0	10,3	1,25	8,2	0,0	T2 - 3
Louisa	C35	15/06/2021	81 - 90	64 - 40	52,5	10,6	1,10	9,6	0,0	T1 - 2
Louisa	C35	06/07/2021	70 - 90	88 - 40	49,9	11,3	1,00	11,3	0,0	T1 - 2
Louisa	C35	29/07/2021	77 - 86	72 - 48	49,4	12,7	0,85	14,9	0,0	T1 - 2
Louisa	C35	17/08/2021	84 - 93	56 - 40	48,4	11,2	0,90	12,4	0,0	T1 - 3
Louisa	X639	24/05/2021	78 - 83	64 - 56	48,9	10,5	1,25	8,4	0,0	T2 - 3
Louisa	X639	15/06/2021	74 - 82	72 - 56	51,1	10,9	0,90	12,1	0,0	T1
Louisa	X639	06/07/2021	72 - 85	88 - 56	49,5	11,1	0,95	11,7	0,0	T1 - 2
Louisa	X639	29/07/2021	73 - 82	72 - 56	51,8	10,8	0,95	11,4	0,0	T1 - 3
Louisa	X639	17/08/2021	77 - 88	72 - 48	53,5	11,5	0,90	12,8	0,0	T1 - 3
Louisa	US-812	24/05/2021	81 - 87	64 - 48	50,3	11,7	1,25	9,4	0,0	T1 - 3
Louisa	US-812	15/06/2021	79 - 86	64 - 48	50,6	10,0	1,10	9,1	0,0	T1
Louisa	US-812	06/07/2021	77 - 89	72 - 48	51,3	12,0	1,10	10,9	0,0	T1 - 3
Louisa	US-812	29/07/2021	78 - 88	65 - 48	53,8	11,3	1,10	10,3	0,0	T1 - 2
Louisa	US-812	17/08/2021	83 - 91	56 - 40	51,9	11,9	0,95	12,5	0,0	T1 - 3

Louisa	RL	24/05/2021	78 - 83	64 - 56	51,4	9,1	1,30	7,0	0,0	T3 - 5
Louisa	RL	15/06/2021	75 - 84	72 - 56	48,3	11,1	1,05	10,6	0,0	T1 - 3
Louisa	RL	29/07/2021	79 - 84	64 - 56	49,5	10,1	1,10	9,2	0,0	T1 - 3
Louisa	RL	17/08/2021	78 - 86	64 - 48	49,7	10,8	1,00	10,8	0,0	T1 - 3
McClellan SL	C35	24/05/2021	79 - 87	64 - 48	51,8	10,6	1,10	9,6	0,0	T1 - 3
McClellan SL	C35	14/06/2021	84 - 95	56 - 40	44,4	10,7	1,00	10,7	0,0	T1 - 2
McClellan SL	C35	06/07/2021	79 - 88	64 - 48	52,9	11,6	0,90	12,9	0,0	T1 - 2
McClellan SL	C35	29/07/2021	83 - 88	56 - 48	53,8	11,9	0,85	14,0	0,0	T1 - 3
McClellan SL	C35	17/08/2021	82 - 92	56 - 40	53,3	11,7	0,85	13,8	0,0	T1 - 3
McClellan SL	X639	24/05/2021	72 - 86	88 - 48	51,4	9,8	0,95	10,3	0,0	T2 - 3
McClellan SL	X639	14/06/2021	77 - 88	72 - 48	52,3	10,3	1,25	8,2	0,0	T1
McClellan SL	X639	06/07/2021	77 - 86	72 - 48	55,0	11,0	0,90	12,2	0,0	T1 - 3
McClellan SL	X639	29/07/2021	78 - 87	64 - 48	50,7	11,6	0,75	15,5	0,0	T1 - 2
McClellan SL	X639	17/08/2021	80 - 88	64 - 48	51,4	11,6	0,35	33,1	0,0	T1 - 2
McClellan SL	US-812	24/05/2021	72 - 83	88 - 56	51,0	10,7	1,25	8,6	0,0	T3 - 5
McClellan SL	US-812	14/06/2021	79 - 88	64 - 48	53,6	10,7	1,15	9,3	0,0	T1 - 3
McClellan SL	US-812	06/07/2021	78 - 85	64 - 56	53,0	12,3	1,10	11,2	0,0	T1 - 2
McClellan SL	US-812	29/07/2021	82 - 87	56 - 48	53,5	11,0	0,95	11,6	0,0	T1 - 2
McClellan SL	US-812	17/08/2021	82 - 90	56 - 40	52,4	11,3	1,00	11,3	0,0	T1 - 3
McClellan SL	RL	24/05/2021	77 - 83	72 - 56	45,2	9,7	1,00	9,7	0,0	T2 - 3
McClellan SL	RL	15/06/2021	82 - 85	-56	50,9	10,0	1,05	9,5	0,0	T1 - 2
McClellan SL	RL	06/07/2021	76 - 83	72 - 56	53,0	11,7	0,95	12,3	0,0	T1
McClellan SL	RL	29/07/2021	79 - 84	64 - 56	50,7	9,6	0,95	10,1	0,0	T1 - 2
McClellan SL	RL	17/08/2021	73 - 87	72 - 48	50,6	11,3	0,85	14,5	0,0	T1 - 2
Skilderkrans	C35	24/05/2021	77 - 89	72 - 48	53,5	10,3	1,20	8,6	0,0	T4 - 5
Skilderkrans	C35	14/06/2021	75 - 87	72 - 48	49,4	10,2	1,10	9,3	0,0	T1 - 3
Skilderkrans	C35	06/07/2021	82 - 90	56 - 40	53,4	10,8	1,10	9,8	0,0	T1 - 3
Skilderkrans	C35	29/07/2021	81 - 86	64 - 48	49,7	11,6	1,05	11,0	0,0	T1 - 3
Skilderkrans	X639	24/05/2021	75 - 87	72 - 48	50,0	11,0	1,20	9,2	0,0	T1 - 3
Skilderkrans	X639	14/06/2021	79 - 87	64 - 48	50,6	10,1	1,10	9,2	0,0	T2 - 3
Skilderkrans	X639	06/07/2021	78 - 86	64 - 48	52,9	11,3	1,00	11,3	0,0	T1 - 3
Skilderkrans	X639	29/07/2021	77 - 89	72 - 48	48,8	10,5	0,90	11,7	0,0	T1 - 2
Skilderkrans	US-812	24/05/2021	79 - 88	56 - 48	52,3	10,7	1,35	7,9	0,0	T4 - 5
Skilderkrans	US-812	14/06/2021	83 - 88	56 - 48	49,7	10,9	1,00	10,9	0,0	T1 - 3
Skilderkrans	US-812	06/07/2021	84 - 90	56 - 40	52,5	11,2	1,05	10,7	0,0	T1
Skilderkrans	US-812	29/07/2021	74 - 89	72 - 48	53,5	12,7	1,00	12,7	0,0	T1 - 3
Skilderkrans	RL	24/05/2021	76 - 87	72 - 48	49,3	9,6	1,25	7,7	0,0	T2 - 3
Skilderkrans	RL	14/06/2021	76 - 85	72 - 56	52,0	9,1	1,25	7,3	0,0	T1 - 3
Skilderkrans	RL	06/07/2021	77 - 89	72 - 48	51,0	9,6	1,05	9,1	0,0	T1 - 2
Skilderkrans	RL	29/07/2021	81 - 86	64 - 48	54,5	9,6	0,85	11,3	0,0	T1 - 2

#### 5.4.6 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the intermediate production areas (Marble Hall)

Project 941C by J Joubert (CRI)

##### Summary

The quality of the Mandarin Hybrid fruit improved in this climatic region (intermediate area). The results indicated that RHM with higher seed counts and low acid levels, followed by Leanri mature first. Leanri had a light crop on the trees with large to very large fruit size. There were low seed numbers in all the varieties this season; Ma'ayana was virtually seedless. Ma'ayana seems to fit in with the mid-maturing selections with deep orange rind colour, followed by IRM2 and Taylor Lee. IRM 1 and Orri will end the season as the latest maturing mandarins are evaluated. Picking periods should not be longer than 3 - 4 weeks to maintain good internal quality and to avoid rind disorders.

##### Opsomming

Die kwaliteit van die Mandaryn Hibried vrugte het verbeter in die klimaatsonne (intermediere areas). Die resultate het aangedui dat RHM met hoër saadtellings en lae suurvlaakte, gevolg deur Leanri die vroegste ryp geword het. Leanri het 'n ligte oes op die bome geset, met groot tot baie groot vruggrootte. Tango is volgende, met 'n toename in vruggrootte en gemiddelde tot goeie interne kwaliteit. Daar was lae saad getalle in al die varieteite hierdie seisoen; Ma'ayana was basies saadloos. Ma'ayana pas hier in saam met die mid seleksies met diep oranje skilkleur, gevolg deur IRM 2 en Taylor Lee. IRM 1 en Orri sluit dan die seisoen af as die laaste rypwordende mandaryne vir hierdie evaluasies. 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.

##### 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 Moosrivier Estate (Marble Hall).

**Table 5.4.6.1.** List of Mandarin Hybrid selections evaluated at Moosrivier Estate (Marble Hall) during the 2021 season.

Selection	Rootstock	Planted
IRM 1&2	US-812 (SxB)	2013
Leanri	US 812	2013
Ma'ayana (Dina)	US-812 (SxB)	2013
Orri	C35	2015
RHM	C35/CC/ US-812 (SxB)	2015/2013
Taylor Lee	C35	2015

##### Results and discussion

The trial site at Moosrivier was relocated to a new site and trees were established for future evaluations due to cold damage and soil quality at the old site. All the trees at Moosrivier bore a crop for this season with improved fruit numbers and more mature tree internal quality and fruit size characteristics.

For Mandarin Hybrid selections, a ratio of 11:1 is considered to be the build-up towards peak maturity, with a ratio of 12:1. After reaching the peak, the ratio increases to 13:1, after which the fruit is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater quality and rind issues.

The internal quality of Ma'ayana (Dina) was very good this season with high juice levels (average 57.7%) and no granulation problems in the fruit compared to Nova. Brix (average 13.7) and similar acids (1.1%), indicating the early-mid maturing characteristics of the selection in the intermediate production areas, with low seeded fruit this season (avg. 0.6 seeds per fruit). The fruit size peaked from count 1 to count 1XX.

Leanri developed a fairly large fruit size between count 1X and 1XXX, slightly smaller than the hot production areas. 2021 was the fifth crop on the trees and internal quality was very good on US-812 (average juice 55.9%, Brix 12.2, acid 1.1%). Seed numbers were fairly low; from 0.8 up to 3.8 seeds per fruit.

The tree shape of the IRM selections was very upright (V-shaped) with no thorns and aggressive growth. IRM 1 and 2 produced an alternative crop on the trees, with an on-year this season, and the fruit size peaked on IRM 2 from medium to large/very large (count 1 to 1XX/1XXX). The seed numbers on IRM 1 remained the same this season and averaged from low seeded to 1.8 seeds per fruit, peels easily with some ribbing on the fruit (typical Murcott characteristic). Juice levels on IRM 1&2 decreased compared to 2020 and averaged 54.8%, Brix was very good (up to 15 late in the season) and acids were above 1.1%. The external colour was deep orange and peaked between T1 and 2. Based on the internal quality results in Table 5.4.6.2, estimated maturity will be the middle to end of June

RHM cropped fruit this season with average seed numbers during the evaluation (2.3 seeds per fruit) and are prone to cross-pollination. There was a delayed colour development (T3 to 5) from the second evaluation with low acids (average 0.70 to 0.80), indicating peak maturity Brix: acid ratio over 12. Future evaluations will determine the optimum quality of the fruit evaluated.

Taylor Lee performed well in terms of internal quality (juice 06%, Brix 13, acids 1.1), colour development peaked from T1 to 2 and low seed numbers (avg. 1.9 seeds per fruit), but large to extra-large fruit size (similar to Leanri).

## **Conclusion**

The delay in external colour development improved this season due to the age of the trees (more mature); future evaluation will confirm this.

This was the fifth evaluation of IRM 1&2, RHM on US-812, Leanri and Ma'ayana (Dina) at Moosrivier; so, information is becoming available and future evaluations will improve recommendations on these cultivars (management improvement). The highest seed numbers were on RHM and IRM 1&2 this season, followed by Taylor Lee and Leanri. All the other selections developed very low seed numbers in the fruit. Taylor Lee performed well with deep orange colour development and Ma'ayana's (Dina) internal quality improved substantially (Brix up to 14). RHM continued with the delayed external colour development on the fruit and low acids, similar to the other trial sites.

**Table 5.4.6.2.** Internal fruit quality data for Mandarin hybrid selections at Moosrivier (Marble Hall) during the 2021 season.

Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
IRM 1	US-812	18/5/2021	68 - 80	1X - 1XXX	55,1	11,1	1,75	6,3	3,3	T1 - 2
IRM 1	US-812	10/6/2021	73 - 86	1XX - 1XXX	57,3	13,5	1,45	9,3	1,5	T1 - 2
IRM 1	US-812	30/6/2021	68 - 81	1X - 1XXX	55,8	14,4	1,20	12,0	1,3	T1 - 2
IRM 1	US-812	21/7/2021	70 - 78	1X - 1XXX	57,8	13,4	1,30	10,3	1,1	T1
IRM 2	US-812	18/5/2021	67 - 78	1 - 1XXX	48,1	11,2	1,25	9,0	3,6	T1 - 3
IRM 2	US-812	10/6/2021	65 - 79	1 - 1XXX	55,6	12,9	1,40	9,2	3,2	T2 - 4
IRM 2	US-812	13/7/2021	69 - 79	1X - 1XXX	54,5	14,2	1,10	12,9	1,8	T1
IRM 2	US-812	21/7/2021	67 - 81	1 - 1XXX	54,2	15,0	1,20	12,5	0,0	T1
Leanri	US-812	18/3/2021	70 - 77	1X - 1XX	60,3	11,0	1,25	8,8	1,7	T6 - 7
Leanri	US-812	21/4/2021	71 - 81	1X - 1XXX	54,1	12,2	1,10	11,1	0,0	T3 - 5
Leanri	US-812	18/5/2021	70 - 82	1X - 1XXX	56,3	13,4	0,95	14,1	1,5	T1 - 2
Ma'ayana (Dina)	US-812	21/4/2021	66 - 74	1 - 1XX	59,1	13,0	1,10	11,8	0,0	T4 - 5
Ma'ayana (Dina)	US-812	18/5/2021	65 - 76	1 - 1XX	55,4	14,9	1,30	11,5	0,0	T1 - 3
Ma'ayana (Dina)	US-812	10/6/2021	69 - 77	1X - 1XX	58,5	13,1	0,90	14,6	1,7	T1 - 2
Orri	C35	18/5/2021	63 - 70	2 - 1X	56,7	12,9	1,05	12,3	0,0	T1 - 3
Orri	C35	30/6/2021	63 - 74	2 - 1XX	55,1	15,3	1,10	13,9	0,5	T1 - 2
Orri	C35	21/7/2021	66 - 74	1 - 1XX	55,7	15,7	1,10	14,3	0,0	T1
RHM	C35	18/3/2021	64 - 77	1 - 1XX	57,1	9,6	0,85	11,3	3,5	T6 - 7
RHM	C35	21/4/2020	65 - 75	1 - 1XX	56,3	11,0	0,80	13,8	2,1	T3 - 5
RHM	C35	18/5/2021	65 - 77	1 - 1XX	60,7	11,8	0,75	15,7	0,8	T1 - 2
RHM	C35	10/6/2021	70 - 81	1X - 1XXX	59,4	12,9	0,85	15,2	0,0	T1 - 2
RHM	CC	21/4/2021	65 - 83	1 - 1XXX	61,0	11,3	0,75	15,1	0,0	T5 - 6
RHM	CC	18/5/2021	66 - 76	1 - 1XX	53,0	12,3	0,60	20,5	0,0	T1 - 2
RHM	US-812	21/4/2021	71 - 85	1X - 1XXX	61,0	10,7	0,80	13,4	9,3	T4 - 6
RHM	US-812	18/5/2021	63 - 73	2 - 1XX	60,6	12,7	0,75	16,9	2,7	T1 - 2
Taylor Lee	C35	21/4/2021	73 - 80	1XX - 1XXX	59,7	11,4	1,15	9,9	2,5	T5 - 7
Taylor Lee	C35	18/5/2021	72 - 84	1XX - 1XXX	61,9	12,9	1,05	12,3	2,1	T1 - 3
Taylor Lee	C35	10/6/2021	72 - 85	1XX - 1XXX	59,1	13,5	1,25	10,8	1,9	T1 - 2
Taylor Lee	C35	30/6/2021	74 - 84	1XX - 1XXX	62,6	13,8	1,00	13,8	0,8	T1 - 2
Taylor Lee	C35	21/7/2021	70 - 83	1X - 1XXX	59,1	14,7	1,00	14,7	2,0	T1

#### 5.4.7 **PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the hot, dry inland areas (Tshipise)**

Project 899B by J Joubert (CRI)

##### **Summary**

The quality of the Mandarin Hybrid fruit between the different production areas was very different, indicating how important it is to decide what cultivar to plant and the suitable rootstock for that area. However, the results of the 2021 season still indicated that for the warm production areas Tango matures first with smaller fruit size and good internal quality (acid levels drop early in the season).

Etna was first to mature from the new additional selections, followed by Sirio with low-seeded fruit this season. Saint André, Nova and Nova SL followed with good external colour development and seedless fruit. Next to mature was Samba, followed by Furr with high seed numbers in the fruit. Tambor 1 and 2 mature last, ending off the mandarin season for the hot areas.

Evaluations on the latest additions indicated that Goldup would be very early maturing in the hot areas, followed by RHM with lower acids. Ma'ayana and Leanri will be next in line, cropping large fruit on the trees with good internal quality. Taylor Lee follows; developing low seed number and good internal quality as well as colour on the fruit. The Nadorcott selections will be next, cropping a good crop on the trees; ARCCIT 9 LS had no seeds in the fruit.

Picking periods should not be longer than 3 - 4 weeks to maintain good internal quality and avoid rind disorders.

##### **Opsomming**

Die kwaliteit van die Mandaryn Hibried vrugte het aansienlik verskil tussen die verskillende produksie areas, wat 'n baie belangrike punt uitlig wanneer dit by die keuse van kultivars vir aanplantings kom, sowel as die onderstam wat gebruik word. Die resultate van die 2021 seisoen vir hierdie warm produksie areas het steeds aangedui dat Tango die vroegste ryp geword het met kleiner vruggrootte en goeie interne kwaliteit (suurvlakke daal vinnig in begin van seisoen).

Etna het eerste ryp geword van die nuwe addisionele seleksies, gevolg deur Sirio met lae-saad vrugte vir die seisoen. Saint André, Nova en Nova SL het gevolg met goeie kleur ontwikkeling en saadlose vrugte. Volgende om ryp te word sal Samba wees, gevolg deur Furr met hoë saadtellings in die vrugte. Mor 26 volg nou, met 'n ligte oes op die bome (tussenstam opsie) en goeie interne kwaliteit. Tambor 1 en 2 word laaste ryp en eindig die mandaryn seisoen vir die warm produksie area.

Evaluasies op die nuutste toevoegings het aangedui dat Goldup die vroegste ryp word in die warm area, gevolg deur RHM met lae suur vlakke. Ma'ayana en Leanri is volgende in lyn, wat groot vrugte op die bome dra en goeie interne kwaliteit. Taylor Lee pas nou in; lae saad tellings, goeie interne kwaliteit en eksterne kleur ontwikkeling. Die Nadorcott seleksies pas nou in met 'n goeie oes op die bome; ARCCIT 9 se vrugte was totaal saadloos gewees.

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.

##### **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) in the Limpopo region.

**Table 5.4.7.1.** List of Mandarin Hybrid selections evaluated at Alicedale (Tshipise) during the 2021 season.

Selection	Rootstock	Topworked
ARCCIT 9 (Nadorcott LS)		
Etna	X639	2013/2014
Furr (Clemcott)	X639	2013/2014
Goldup	X639	2015
IRM 2	X639	2015
Leanri	X639	2015
Ma'ayana (Dina)	X639	2015
Nadorcott	X639	2015
Nova	X639	2013/2014
Nova SL (ARC)	X639	2013/2014
Orri	X639	2013/2014
Page	X639	2013/2014
RHM	X639	2015
Samba	X639	2013/2014
Saint Andre	X639	2013/2014
Sirio	X639	2013/2014
Tambor 1&2	X639	2010
Taylor Lee	X639	2015
Winola	X639	2013/2014

## Results and discussion

Due to older trees with better crops, more information was available at Alicedale (new and existing trial site). Tanor Late fruit was picked earlier in the season before late maturing evaluations started and there was no fruit to sample. Evaluations were completed on trees bearing fruit.

For Mandarin Hybrids, a ratio of 11:1 is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which the fruit is considered 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 quality and rind issues.

Etna bore a good crop with medium to very large fruit (count 2/1 to 1XX) and good internal quality (high juice and Brix, and average acids of 0.90). The external colour was delayed at peak maturity (T3 to T5) and all the fruit evaluated were seedless. Furr (Clemcott) was included as a control for the hot production areas and to compare with Leanri. Fruit size was large to very large (count 1X/XX to 1XXX), good internal quality with acceptable acids (average 0.80%) early in the season and lower number of seeds in the fruit (3.5).

Nova was included as a control for Nova SL and Saint André in the trial, the fruit is fairly difficult to peel and low numbers of seed developed in the fruit. The external colour was late and the fruit size peaked from count 1 and 1XX (medium to large). Nova SL (ARC) produced a coarse rind texture on the fruit with similar fruit size, medium to large (count 2 to count 1XX). The acid levels in the fruit were similar compared to Nova (control) and the external colour development was delayed this season at peak maturity. Saint André performed similar to the Nova control with low seed numbers and large to very large fruit size (count 1/1X to 1XXX) and lower acids, as well as delayed external colour development.

Orri trees were very aggressively growing, producing a very light crop on the trees this season and one evaluation was possible.

Samba produced an average fourth (X639 rootstock) crop on the large fast-growing thorn-less trees. Colour development was early in the season (deep orange) with a smooth rind texture on the fruit. Seed counts were very low, completely seedless this season in the mixed block and the fruit size peaked from count 2 to count 1XX. Sirio produced large coarse fruit this season and peaked at count 1XXX (very large fruit) with better internal quality; juice of 56%, Brix 11 and acids below 1.0% and better colour development on the trees (T1 to T3).

The Tambor 1 & 2 fruit matured late this season and all the evaluations was completed. Fruit size was very big; ranged from count 1XX to 1XXX. Juice and Brix levels was good this season, but acids remain above 1.25% with the final evaluation.

Nadorcott (control) and Nadorcott ARCCIT 9 cropped the best yield on the young trees (completely seedless fruit), followed by Leanri with large fruit size and good colour development (with the second evaluation). The yield on Ma'ayana (Dina) improved and two evaluations were possible with good juice and Brix levels; acids improve on the higher side, but with the early colour development Ma'ayana can be harvested as one of the earlier mandarin selections. Ma'ayana developed trees that were very upright in shape and pruning will be crucial to develop proper bearing branches. IRM 1 & 2 cropped minimal fruit this season and evaluations will continue next year.

Taylor Lee bore a better crop this season to evaluate with good juice (up to 56%), Brix (10) and acceptable acids. Seed numbers were on the low side, completely seedless fruit compared to the Furr control (3.5 seeds per fruit).

## **Conclusion**

The external colour delay (internal quality improved with more mature trees) in the hotter areas remained a problem; future evaluations will confirm this. Degreening may be an option for the early selections included in the trial, but ethylene reacted slowly or not at all for Tango and Nadorcott (W. Murcott selections).

This was the sixth evaluation of Etna, Furr (control), Nova (Control), Nova SL, Page, Saint André, Samba, Sirio, Tambor 1 (control) and 2, information is progressing with trees maturing and better fruit quality; so future evaluations will improve recommendations on these varieties. The promising selections at this early stage were Page (good colour development and low seeded fruit), Saint André (bigger fruit size and later maturing) and Samba with good internal quality fruit (early maturing), good colour development and crop on the trees in combination with X639. Seed numbers on these selections were very low to completely seedless in the combination trial block with cross pollinating cultivars included. Furr (control for Leanri) developed the highest seed numbers per fruit, a typical characteristic of the selection with good colour development and internal quality.

This was the fourth evaluation (trees five years old – topworked end of 2015) of Nadorcott, Nadorcott ARCCIT 9, Ma'ayana (Dina), IRM 1&2, Leanri and RHM. The Nadorcott selections performed well with large fruit and good internal quality. Ma'ayana (Dina) and Leanri matures early in the season with improved low acid levels. IRM 1&2 cropped a very light yield possibly due to alternate bearing patterns, future evaluations will confirm and fairly ribbed fruit. RHM had the typical low acids early in the season with delayed colour and very good juice, as well as Brix levels.

Goldup and Taylor Lee were evaluated for the third time this year. The Goldup fruit seems fairly soft and not this suitable for this hot area. Taylor Lee performed well in terms of internal quality, colour development and low seed numbers, but large to extra-large fruit size (similar to Leanri).

**Table 5.4.7.2.** Internal fruit quality data for Mandarin hybrid selections at Alicedale (Tshipise) during the 2021 season.

Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
ARCCIT 9	X639	7/4/2021	67 - 82	1 - 1XXX	57,1	8,5	0,75	11,3	0,0	T5 - 6
ARCCIT 9	X639	3/5/2021	64 - 78	1 - 1XXX	62,2	9,6	0,75	12,8	0,0	T1 - 3
Etna	X639	7/4/2021	63 - 74	2 - 1XX	60,0	12,7	0,90	14,1	0,0	T3 - 5
Etna	X639	3/5/2021	67 - 82	1 - 1XXX	56,3	11,0	0,80	13,8	0,0	T1 - 2
Furr	X639	7/4/2021	71 - 82	1X - 1XXX	52,6	11,4	0,80	14,3	2,2	T5 - 7
Furr	X639	3/5/2021	74 - 88	1XX - 1XXX	64,9	11,5	0,90	12,8	4,8	T1 - 3
Goldup	X639	7/4/2021	55 - 64	3 - 1	57,0	10,2	0,65	15,7	0,0	T3 - 5
Goldup	X639	12/5/2021	53 - 65	4 - 1	55,8	10,7	0,50	21,4	0,0	T1 - 2
IRM2	X639	7/4/2021	66 - 77	1 - 1XX	51,2	9,4	0,75	12,5	0,0	T5 - 6
IRM2	X639	3/5/2021	64 - 79	1 - 1XXX	60,1	10,2	0,80	12,8	0,0	T2 - 3
Leanri	X639	7/4/2021	71 - 85	1X - 1XXX	57,5	10,8	0,95	11,4	0,0	T4 - 6
Leanri	X639	3/5/2021	67 - 87	1 - 1XXX	62,1	10,4	1,06	9,8	0,0	T1 - 3
Ma'ayana (Dina)	X639	7/4/2021	58 - 71	3 - 1X	62,0	12,6	0,80	15,8	0,0	T4 - 5
Ma'ayana (Dina)	X639	3/5/2021	59 - 71	2 - 1X	61,3	12,9	1,00	12,9	0,0	T1 - 2
Nadorcott	X639	7/4/2021	63 - 76	2 - 1XX	57,1	8,8	0,75	11,7	0,0	T5 - 6
Nadorcott	X639	3/5/2021	64 - 80	1 - 1XXX	60,6	9,7	0,80	12,1	0,0	T1 - 2
Nova (control)	X639	7/4/2021	58 - 68	3 - 1X	63,6	12,9	0,70	18,4	0,0	T2 - 5
Nova (control)	X639	3/5/2021	64 - 75	1 - 1XX	59,7	12,2	0,90	13,6	0,0	T1 - 2
Nova SL (ARC)	X639	7/4/2021	63 - 75	2 - 1XX	58,0	12,9	0,95	13,6	0,0	T3 - 5
Nova SL (ARC)	X639	3/5/2021	68 - 76	1X - 1XX	57,8	12,8	1,00	12,8	0,0	T1 - 2
Orri	X639	3/5/2021	64 - 74	1 - 1XX	59,0	10,8	1,00	10,8	0,0	T2 - 3
Page	X639	7/4/2021	60 - 74	2 - 1XX	61,9	11,1	0,75	14,8	0,0	T3 - 5
Page	X639	3/5/2021	66 - 75	1 - 1XX	57,9	10,8	0,70	15,4	0,0	T1 - 2
RHM	X639	7/4/2021	64 - 73	1 - 1XX	61,8	9,2	0,70	13,1	0,0	T4 - 6
RHM	X639	3/5/2021	65 - 74	1 - 1XX	63,5	11,5	0,55	20,9	0,0	T1 - 2
Samba	X639	7/4/2021	60 - 68	2 - 1X	61,8	11,2	0,90	12,4	0,0	T3 - 5
Samba	X639	3/5/2021	59 - 72	2 - 1XX	60,1	11,6	0,90	12,9	0,0	T1 - 2
Saint Andre	X639	7/4/2021	65 - 72	1 - 1XX	58,7	12,5	0,75	16,7	0,0	T4 - 5
Saint Andre	X639	3/5/2021	70 - 85	1X - 1XXX	51,0	11,1	0,80	13,9	1,3	T1 - 3
Sirio	X639	7/4/2021	74 - 87	1XX - 1XXX	57,6	11,2	0,95	11,8	0,0	T5 - 6
Sirio	X639	3/5/2021	71 - 84	1X - 1XXX	54,9	11,0	0,95	11,6	0,0	T1 - 3
Tambor 1	X639	24/5/2021	73 - 85	1XX - 1XXX	60,0	10,5	1,60	6,6	0,0	T1 - 3
Tambor 1	X639	14/6/2021	72 - 85	1XX - 1XXX	60,1	10,9	1,45	7,5	0,7	T1 - 2
Tambor 1	X639	6/7/2021	75 - 91	1XX - 1XXX	48,8	11,4	1,35	8,4	1,1	T1 - 2
Tambor 1	X639	29/7/2021	81 - 87	1XXX	60,4	11,0	1,35	8,1	0,0	T1 - 2
Tambor 1	X639	17/8/2021	80 - 90	1XXX	56,0	11,4	1,25	9,1	0,0	T1 - 2

Tambor 2	X639	24/5/2021	74 - 82	1XX - 1XXX	61,7	10,9	1,55	7,0	0,0	T1 - 3
Tambor 2	X639	14/6/2021	77 - 87	1XX - 1XXX	59,2	11,1	1,55	7,2	0,5	T1 - 3
Tambor 2	X639	6/7/2021	75 - 88	1XX - 1XXX	57,9	11,1	1,25	8,9	0,0	T1 - 2
Tambor 2	X639	29/7/2021	73 - 81	1XX - 1XXX	60,7	12,0	1,20	10,0	2,0	T1
Tambor 2	X639	17/8/2021	77 - 90	1XX - 1XXX	56,0	12,0	1,35	8,9	0,0	T1 - 2
Taylor lee	X639	3/5/2021	73 - 80	1XX - 1XXX	56,4	9,7	0,85	11,4	0,0	T2 - 3
Winola	X639	7/4/2021	64 - 74	1 - 1XX	65,5	10,8	2,10	5,1	0,0	T5 - 6
Winola	X639	5/5/2021	64 - 82	1 - 1XXX	61,6	10,2	1,80	5,7	0,0	T1 - 3

#### 5.4.8 **PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the intermediate production areas (Karino)**

Project 963C by J Joubert (CRI)

##### **Summary**

The quality of the Mandarin Hybrid fruit was good in the Nelspruit production area, due to the similar climatic region (intermediate areas). The results indicated that in the Nelspruit production area, Etna and Sirio matures first with very large fruit size, followed by RHM developing delayed colour. Nova (control), Nova SL and Saint Andre follows, as the early maturing cultivars, with Leanri next in line (large fruit size and seedless fruit). Furr and Taylor Lee follows, representing the early to mid-maturing range. Nadorcott ARCCIT9 LS matures first (two weeks before ARC selection), followed by Nadorcott (control) with medium to large fruit size for this season and excellent colour development. Ma'ayana also indicated to be fairly early maturing selections, followed by Samba and Page with good colour development. IRM 2 mature next towards the middle of the mandarin season with less ribbing on the fruit compared to IRM 1 at the other trial sites. All the fruit evaluated this season had very low seed numbers despite the cross pollination impact from the seeded cultivars close by, except for Furr. Tambor 1&2 and Tanor Late will end of the season, maturing ultra-late.

##### **Opsomming**

Die kwaliteit van die Mandaryn Hibried vrugte was goed in die Nelspruit produksie area, a.g.v. die soortgelyke klimaatsone (intermediêre areas). Die resultate vir die Nelspruit produksie area het aangedui dat Etna en Sirio eerste ryp was met groot vrugte, gevolg deur RHM met vertraagde kleur. Nova (kontrole), Nova SL en Saint Andre volg as deel van die vroeg rypwordende kultivars, met Leanri volgende in lyn (groot vrugte en saadloos). Furr en Taylor Lee was volgende wat die vroeg tot mid-rypwordende reeks vul. Nadorcott ARCCIT9 LS eerste gereed was vir die oesproses (twee weke voor die ARC seleksie), gevolg deur Nadorcott (kontrole) met medium tot groot vrugte vir hierdie seisoen en baie goeie kleur ontwikkeling. Ma'ayana het ook onder die vroeë seleksies ingepas gevolg deur Samba en Page met goeie kleur ontwikkeling. IRM 2 was volgende gereed vir oes gewees meer na die middel van die mandaryn seisoen; met minder ribbing op die vrugte in vergelyking met IRM 1 by die ander proef persele. Al die vrugte geëvalueer het baie lae saadinhoud aangedui ten spite van die kruisbestuivings impak van aangrensende varieteite met saad, behalwe vir Furr. Tambor 1&2 en Tanor Late sal die seisoen afsluit, wat ultra-laat ryp word.

##### **Objectives**

- To select Mandarin Hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new Mandarin Hybrid cultivars and to determine the climatic suitability of these cultivars in intermediate, inland production regions.

##### **Materials and methods**

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from Karino-koop (Nelspruit) in the Mpumalanga region.

**Table 5.4.8.1.** List of Mandarin Hybrid selections evaluated at Karino-koop (Nelspruit) during the 2021 season.

<b>Selection</b>	<b>Rootstock</b>	<b>Planted</b>
ARCCIT9 LS	CC	2017
Etna	CC	2017
Furr	CC	2017
IRM 1	CC	2014
IRM 2	CC	2017
Leanri	CC	2017
Ma'ayana (Dina)	CC	2017
Nadorcott	CC	2017
Nova	CC	2017
Nova SL	CC	2017
Orr 1&4	CC	2017
Page	CC	2017
RHM	CC	2017
Saint Andre	CC	2017
Samba	CC	2017
Sirio	CC	2017
Sugar Belle	CC	2016
Tambor 1&2	CC	2017
Tanor Late	CC	2017
Tasty 2	CC	2017
Taylor Lee	CC	2017
Winola	CC	2014

## **Results and discussion**

The mature trees at Karino-koop's new trial site were evaluated this season; this is having an impact on the quality and quantity of the fruit. More information was available and future evaluations will be conducted.

For Mandarin Hybrids, a ratio of 11:1 is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which the fruit is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

### IRM 1 & IRM 2

The tree shape of the IRM selections was very upright (V-shaped) with no thorns and aggressive growth. IRM 1 and 2 produced an alternative crop on the trees, with an on-year this season, and the fruit size peaked on IRM 2 from medium to large/very large (count 1 to 1XX). The seed numbers on IRM 1 was similar to last season and averaged from seedless to 1.5 seeds per fruit, peels easily with some ribbing on the fruit (typical Murcott characteristic). Juice levels on IRM 1&2 decreased compared to 2020 and averaged above 58.6%, Brix was very good (up to 15.) and acids were above 1.0%. The external colour was deep orange and peaked between T1 and 2.

### Nadorcott & ARCCIT9 LS

The fruit shape was similar to the Nadorcott selection. Rind texture was very smooth with a natural shine (similar to packhouse waxing). Both selections developed good internal quality with high juice levels (up to 63.6%), Brix averaging 11.4 and higher acids (avg. 1.0%). Nadorcott ARCCIT9 LS produced a similar crop on the trees compared to the Nadorcott selection and the fruit size remained the same this season in spite of a good crop load; varied from count 1-1XX/1XXX. ARCCIT9 LS were completely seedless at the Karino trial site.

Maturity seems to be two weeks earlier on the ARCCIT9 LS selection, but the information was limited due to the fourth year of evaluation (end of May to the middle of June), according to Table 5.4.8.2.

#### Additional selections

The internal quality of Ma'ayana (Dina) was good with high juice levels above 60%, no granulation problems in the fruit compared to Nova. Brix (above 12 – all three evaluations) and acceptable acids (0.95%) indicate the selection's early to mid-maturing characteristics in the intermediate production areas, with seedless fruit. The fruit size peaked from count 2/1 to count 1XXX.

Winola were included in the Karino trial site in 2014 and bore their fourth crop on the trees this season. Fruit size peaked from medium to large/extra-large fruit (count 2/1 to 1XX/XX) with seedless fruit and high acid levels with good external colour development. Winola developed juice levels of 64% and Brix up to 12.4.

Sugar Belle was planted at the Karino trial site in 2016 and bore its third crop this season. The fruit size peaked from count 2 to count 1XX/1XXX and colour development was favourable between T1 and T2 early in the season. Internal quality was very good with high juice and Brix levels (up to 15), but exceptionally high acids, 1.0% with the middle August evaluation.

Nova was included as a control for Nova SL and Saint André in the trial, the fruit is fairly difficult to peel and low numbers of seed developed in the fruit. The external colour was late and the fruit size peaked from count 1 and 1XX/XXX (medium to large/very large). Nova SL (ARC) produced a coarse rind texture on the fruit with similar fruit size, medium to large/very large fruit (count 1X to count 1XX/XXX). The acid levels in the fruit were similar compared to Nova (control) and the external colour development was delayed this season at peak maturity. Saint André performed similar to the Nova control with seedless fruit and large to very large fruit size (count 1/1X to 1XXX) and lower acids, as well as delayed external colour development (same for all three).

Orr 1 and Orr (Orr 4) trees were very aggressively growing, producing a very light crop on the trees this season and two evaluation was possible.

Samba produced an average to good crop on the large fast-growing thorn-less trees (CC). Colour development was early in the season (deep orange) with a smooth rind texture on the fruit (similar for Page). Seed counts were very low, completely seedless this season in the mixed block and the fruit size peaked from count 3/2 to count 1XX/XXX. Sirio produced large coarse fruit and peaked at count 1XXX (very large fruit) with better internal quality; juice of 58%, Brix 11 and acids below 1.0% and delayed colour development on the trees (T4 to T5).

The Tambor 1 & 2 fruit matured late this season and all the evaluations was completed. Fruit size was very big; ranged from count 1XX to 1XXX. Juice and Brix levels was good this season, but acids remain above 1.0% with the final evaluation.

Furr (Clemcott) was included as a control for the hot production areas and to compare with Leanri. Fruit size was large to very large (count 1X/XX to 1XXX), good internal quality with good acids (average 1.0%) early in the season and high number of seeds in the fruit (10.3). Leanri developed a fairly large fruit size between count 1X and 1XXX, slightly smaller than the hot production areas. The internal quality was very good on Carizzo (average juice 59.7%, Brix 11.2, acid 1.0%). Seed numbers were very low; seedless fruit with all three evaluations completed. Taylor Lee, cropped fruit for the first time with lower seed numbers compared to Furr (avg. 2.4 seeds per fruit) similar internal quality.

RHM cropped completely seedless fruit this season during the evaluation (compared to the previous) and are prone to cross-pollination. There was a delayed colour development (T2 to 4) from the second evaluation with low acids (average 0.70), indicating peak maturity Brix: acid ratio over 12. Future evaluations will determine the optimum quality of the fruit evaluated.

Tanor Late cropped very large fruit (1XXX) on the trees; average juice (51%), better Brix (11.3) and higher acids later in the season (1.1%).

## Conclusion

Ma'ayana cropped medium to large fruit size on the trees with good colour development (precocious bearing pattern). IRM 1&2, Nadorcott (control) and Nadorcott ARCCIT9 LS was evaluated in the new trial block; where the two Nadorcott selections performed similarly, with seedless fruit on both selections.

The highest seed numbers were on Furr, followed by IRM 1&2, and then Sugar Belle, as well as Taylor Lee and Tambor 1&2. All the other selections developed very low seed numbers or completely seedless fruit. Taylor Lee performed well with deep orange colour development and Ma'ayana's (Dina) internal quality improved substantially (Brix up to 12.5). RHM continued with the delayed external colour development on the fruit and low acids, similar to the other trial sites.

Winola at the Karino site were evaluated for the fourth time on the older trees, so information becomes available and future evaluations will improve recommendations on these varieties. The juice, Brix and acid levels were high during the season.

Sugar Belle bore fruit to evaluate; the cultivar had a good colour development early in the season, but Sugar Bell will be an ultra-late maturing option due to very high acids (elongated fruit shape/long neck).

**Table 5.4.8.2.** Internal fruit quality data for Mandarin hybrid selections at Karino- koöp (Nelspruit) during the 2021 season.

Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
ARCCIT9 LS	CC	30/04/2021	63 - 75	2 - 1XX	58,6	10,7	1,50	7,1	0,0	T1 - 2
ARCCIT9 LS	CC	13/05/2021	68 - 80	1X - 1XXX	58,1	11,1	1,15	9,7	0,0	T1 - 3
ARCCIT9 LS	CC	03/06/2021	75 - 83	1XX - 1XXX	52,2	11,9	1,15	10,3	0,0	T1 - 3
ARCCIT9 LS	CC	24/06/2021	72 - 83	1XX - 1XXX	55,1	11,4	0,95	12,0	0,0	T1
ARCCIT9 LS	CC	19/07/2021	73 - 84	1XX - 1XXX	50,5	12,3	0,90	13,7	0,0	T1 - 2
Etna	CC	29/03/2021	75 - 84	1XX - 1XXX	59,8	9,2	0,70	13,1	0,0	T5 - 6
Etna	CC	12/04/2021	76 - 82	1XX - 1XXX	57,9	10,1	0,70	14,4	0,0	T3 - 5
Etna	CC	30/04/2021	73 - 103	1XX - 1XXX	49,1	9,6	0,55	17,5	0,0	T1 - 4
Furr	CC	30/04/2021	71 - 81	1X - 1XXX	61,8	11,4	1,05	10,9	12,3	T2 - 4
Furr	CC	13/05/2021	75 - 92	1XX - 1XXX	51,6	12,1	1,00	12,1	15,3	T1 - 3
Furr	CC	03/06/2021	78 - 86	1XXX	59,9	13,0	1,05	12,4	6,5	T1
Furr	CC	24/06/2021	69 - 79	1X - 1XXX	58,6	13,9	1,05	13,2	7,0	T1
IRM 1	CC	03/06/2021	69 - 85	1X - 1XXX	59,7	13,0	1,40	9,3	0,0	T1 - 2
IRM 1	CC	19/07/2021	72 - 84	1XX - 1XXX	61,6	14,8	1,15	12,9	1,5	T1
IRM 1	CC	13/08/2021	70 - 82	1X - 1XXX	61,5	15,4	1,15	13,4	0,0	T1
IRM 2	CC	30/04/2021	72 - 86	1XX - 1XXX	55,4	10,3	1,40	7,4	1,2	T2 - 4
IRM 2	CC	03/06/2021	67 - 74	1 - 1XX	55,4	12,7	1,55	8,2	0,0	T1 - 2
IRM 2	CC	24/06/2021	69 - 82	1X - 1XXX	61,1	12,9	1,30	9,9	0,8	T1 - 2
IRM 2	CC	19/07/2021	69 - 84	1X - 1XXX	55,3	13,2	1,00	13,2	1,0	T1 - 2
Leanri	CC	12/04/2021	69 - 79	1X - 1XXX	59,2	10,7	1,10	9,7	0,0	T4 - 5
Leanri	CC	30/04/2021	64 - 75	1 - 1XX	63,1	11,2	1,00	11,2	0,0	T1 - 2

Leanri	CC	13/05/2021	70 - 83	1X - 1XXX	56,9	11,7	1,00	11,7	0,0	T1 - 3
Ma'ayana (Dina)	CC	12/04/2021	63 - 79	2 - 1XXX	61,9	12,0	0,95	12,6	0,0	T4 - 5
Ma'ayana (Dina)	CC	30/04/2021	65 - 78	1 - 1XXX	60,8	12,5	0,95	13,2	0,0	T2 - 3
Ma'ayana (Dina)	CC	13/05/2022	63 - 75	2 - 1XX	61,2	12,3	0,90	13,7	0,0	T1 - 4
Nadorcott	CC	30/04/2021	59 - 74	2 - 1XX	62,6	11,0	1,15	9,6	0,0	T1 - 4
Nadorcott	CC	13/05/2021	66 - 77	1 - 1XX	63,6	11,1	1,15	9,7	0,0	T2 - 3
Nadorcott	CC	03/06/2021	75 - 90	1XX - 1XXX	53,9	10,9	0,95	11,5	0,0	T1 - 3
Nadorcott	CC	24/06/2021	70 - 84	1X - 1XXX	55,3	11,6	0,80	14,5	0,0	T1 - 2
Nova	CC	29/03/2021	65 - 69	1 - 1X	60,7	11,5	0,95	12,1	0,0	T5 - 7
Nova	CC	12/04/2021	69 - 78	1X - 1XXX	61,9	11,3	0,90	12,6	0,0	T4 - 5
Nova	CC	30/04/2021	71 - 81	1X - 1XXX	60,3	11,6	0,85	13,6	0,0	T2 - 3
Nova	CC	13/05/2021	69 - 84	1X - 1XXX	57,7	11,3	0,80	14,1	0,0	T1 - 3
Nova ARC	CC	29/03/2021	65 - 72	1 - 1XX	58,6	9,9	1,10	9,0	0,0	T4 - 7
Nova ARC	CC	12/04/2021	69 - 77	1X - 1XX	57,1	11,2	1,00	11,2	0,0	T4 - 5
Nova ARC	CC	30/04/2021	70 - 85	1X - 1XXX	53,9	11,0	0,90	12,2	0,0	T2 - 3
Nova ARC	CC	13/05/2021	70 - 83	1X - 1XXX	53,7	11,6	0,85	13,6	0,0	T1 - 2
Nova ARC	CC	03/06/2021	71 - 78	1X - 1XXX	55,2	12,6	1,00	12,6	0,0	T1 - 2
Orr 1	CC	19/07/2021	66 - 75	1 - 1XX	59,1	14,0	0,95	14,7	1,3	T1
Orr 1	CC	13/08/2021	64 - 77	1 - 1XX	58,8	15,0	1,00	15,0	0,0	T1
Orr (Orr 4)	CC	19/07/2021	65 - 77	1 - 1XX	59,3	14,8	0,80	18,5	0,0	T1 - 2
Orr (Orr 4)	CC	13/08/2021	65 - 84	1 - 1XXX	58,2	14,1	0,80	17,6	0,0	T1
Page	CC	29/03/2021	71 - 79	1X - 1XXX	57,5	10,1	0,80	12,6	4,7	T4 - 6
Page	CC	12/04/2021	59 - 66	2 - 1	60,8	10,4	0,75	13,9	0,0	T3 - 5
Page	CC	30/04/2021	68 - 74	1X - 1XX	58,4	10,9	0,70	15,6	0,0	T1 - 4
Page	CC	13/05/2021	64 - 75	1 - 1XX	56,9	11,4	0,80	14,3	0,0	T1 - 2
RHM	CC	12/04/2021	67 - 73	1 - 1XX	63,0	10,0	0,90	11,1	0,0	T5 - 7
RHM	CC	30/04/2021	71 - 77	1X - 1XX	61,9	10,7	0,70	15,3	0,0	T2 - 4
RHM	CC	13/05/2021	69 - 78	1X - 1XXX	58,5	11,0	0,65	16,9	0,0	T1 - 3
Samba	CC	29/03/2021	58 - 64	3 - 1	60,9	10,1	0,90	11,2	0,0	T4 - 6
Samba	CC	12/04/2021	60 - 70	2 - 1X	59,2	10,0	0,85	11,8	0,0	T3 - 5
Samba	CC	30/04/2021	64 - 73	1 - 1XX	59,3	11,2	0,80	14,0	0,0	T1 - 3
Samba	CC	13/05/2021	64 - 78	1 - 1XXX	58,8	11,0	0,85	12,9	0,0	T1
Saint Andre	CC	24/03/2021	65 - 73	1 - 1XX	60,2	11,8	0,90	13,1	0,0	T4 - 6
Saint Andre	CC	12/04/2021	67 - 77	1 - 1XX	57,9	12,0	0,90	13,3	0,0	T4 - 5
Saint Andre	CC	30/04/2021	69 - 79	1X - 1XXX	57,7	12,6	0,90	14,0	0,0	T2 - 3
Saint Andre	CC	13/05/2021	67 - 82	1 - 1XXX	57,3	12,7	0,90	14,1	0,0	T1 - 2
Sirio	CC	29/03/2021	72 - 91	1XX - 1XXX	53,9	9,6	0,95	10,1	0,0	T4 - 6

Sirio	CC	12/04/2021	76 - 81	1XX - 1XXX	58,7	10,3	0,95	10,8	0,0	T4 - 5
Sirio	CC	30/04/2021	77 - 87	1XX - 1XXX	56,0	10,9	0,85	12,8	0,0	T1 - 4
Sirio	CC	13/05/2021	72 - 88	1XX - 1XXX	53,7	11,0	0,80	13,8	0,7	T1 - 2
Sugar Belle	CC	13/05/2021	64 - 86	1 - 1XXX	58,9	12,5	1,70	7,4	9,7	T1 - 2
Sugar Belle	CC	19/07/2021	60 - 77	2 - 1XX	61,9	15,3	1,75	8,7	1,3	T1
Sugar Belle	CC	13/08/2021	67 - 78	1 - 1XXX	59,0	14,2	1,00	14,2	0,0	T1
Tambor 1	CC	24/06/2021	71 - 85	1X - 1XXX	59,2	11,0	1,05	10,5	0,8	T1 - 3
Tambor 1	CC	19/07/2021	76 - 86	1XX - 1XXX	58,4	11,5	1,00	11,5	0,0	T1
Tambor 1	CC	13/08/2021	77 - 90	1XX - 1XXX	40,6	11,8	1,05	11,2	1,7	T1 - 2
Tambor 2	CC	24/06/2021	76 - 89	1XX - 1XXX	58,6	11,1	1,10	10,1	1,8	T1 - 2
Tambor 2	CC	19/07/2021	73 - 85	1XX - 1XXX	57,6	11,1	1,00	11,1	1,1	T1 - 2
Tambor 2	CC	13/08/2021	77 - 80	1XX - 1XXX	64,0	12,5	1,00	12,5	0,0	T1
Tanor Late	CC	03/06/2021	75 - 102	1XX - 1XXX	50,7	10,5	1,35	7,8	0,0	T1 - 3
Tanor Late	CC	24/06/2021	89 - 97	1XXX	52,4	11,8	1,00	11,8	0,0	T1 - 2
Tanor Late	CC	19/07/2021	72 - 105	1XX - 1XXX	51,3	11,7	1,00	11,7	0,0	T1 - 2
Tasty 2	CC	30/04/2021	74 - 88	1XX - 1XXX	59,8	10,0	1,05	9,5	0,0	T2 - 3
Tasty 2	CC	13/05/2021	70 - 90	1X - 1XXX	58,0	9,8	0,95	10,3	0,0	T2 - 4
Tasty 2	CC	24/06/2021	76 - 92	1XX - 1XXX	50,0	11,3	0,90	12,6	0,0	T1
Taylor Lee	CC	30/04/2021	73 - 86	1XX - 1XXX	61,1	10,8	0,90	12,0	4,3	T2 - 4
Taylor Lee	CC	13/05/2021	74 - 84	1XX - 1XXX	61,1	11,7	0,95	12,3	2,7	T1 - 3
Taylor Lee	CC	03/06/2021	77 - 87	1XX - 1XXX	59,1	11,7	1,00	11,7	0,0	T1
Taylor Lee	CC	24/06/2021	74 - 84	1XX - 1XXX	58,2	13,1	0,90	14,6	1,5	T1 - 3
Winola	CC	12/04/2021	65 - 73	1 - 1XX	61,6	11,6	1,90	6,1	0,0	T4 - 5
Winola	CC	30/04/2021	61 - 70	2 - 1X	63,8	11,6	1,95	5,9	0,0	T2 - 4
Winola	CC	13/05/2021	61 - 72	2 - 1XX	64,3	11,6	1,70	6,8	0,0	T1 - 2
Winola	CC	03/06/2021	68 - 79	1X - 1XXX	61,0	12,2	1,60	7,6	0,0	T1
Winola	CC	24/06/2021	66 - 80	1 - 1XXX	61,8	12,4	1,35	9,2	0,0	T1

#### 5.4.9 PROGRESS REPORT: Evaluation of Navel selections in the intermediate production areas (Karino)

Project 963B by J Joubert (CRI)

##### Summary

In the Nelspruit production area, M7 matures first with good internal quality (juice and high Brix) for the trial, followed by Fukumoto 1 and 2 with medium/large to very large fruit size for this season and low juice, Brix and acid levels. Bahianinha matures next with good juice levels and earlier external colour on the fruit. Clarke, Fischer and Dream matured next, towards the middle of the navel orange range, with medium to large fruit size and good Brix levels (up to 12.1). De Wet 1 and Hutton grouped well with Dream and Clarke this season, with good Brix and juice content. Kirkwood Red, the only red pigmented navel included in the trial, fits in well with the mid-maturing options. The late selections will include Glenora Late and Gloudi with good acids,

followed by Lane Late and Suitangi. The ultra-late bracket will be filled with Carninka ending of the navel season.

## Opsomming

In die Nelspruit produksie area word M7 eerste ryp met goeie interne kwaliteit (sap en hoë Brix) vir hierdie proef, gevolg deur Fukumoto 1 and 2 met medium/groot tot baie groot vruggrootte vir hierdie seisoen en lae sap, Brix en suur vlakke. Bahianinha word volgende ryp met goeie sap vlakke en vroeër eksterne kleur op die vrugte. Clarke, Fischer en Dream volg, om die middel van die nawel soetleemoen reeks te vul, met medium tot groot vruggrootte en goeie Brix vlakke (tot 12.1). De Wet 1 en Hutton se rypwordings inligting plaas die kultivar saam met Dream en Clarke met goeie Brix en sap inhoud. Kirkwood Red, die enigste rooi gepigmenteerde nawel wat in die proef ingesluit is, pas goed in by die mid-rypwordende opsies. Die laat seleksies sluit in Glenora Late en Gloudi met goeie sure, gevolg deur Lane Late en Suitangi. Die ultra-laat gaping word gevul deur Carninka wat die nawel seisoen afsluit.

## Objectives

- To select navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (juice, Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new navel cultivars and to determine the climatic suitability of these cultivars in intermediate production regions.

## Materials and methods

Field evaluations and laboratory analyses were conducted on navel selections from Karino-koöp (Nelspruit) in the Mpumalanga region. When the ratio between sugar and acid is 10:1, the navel 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 the fruit are considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

**Table 5.4.9.1.** List of navel selections evaluated at Karino-koöp (Nelspruit) during the 2021 season.

Selection	Rootstock	Planted
Bahianinha	CC	2017
Carninka	CC	2017
Clarke	CC	2017
De Wet 1	CC	2017
Dream	CC	2017
Fischer	CC	2017
Fukumoto 1&2	C35/CC/SC	2017
Glenora Late	CC	2017
Gloudi	CC	2017
Hutton	CC	2017
Kirkwood Red	CC	2017
Lane Late	CC	2017
Lazy Boy 1	CC	2017
M7	CC	2017
Suitangi	CC	2017
Witkrans	CC	2017

## Results and discussion

The new trial block trees at Karino-koöp were evaluated for the second time this season; this having an impact on the quality and quantity of the fruit. More information was available and future evaluations will be conducted.

The internal quality varied from fair to good on the second crop and due to high rainfall figures later in the summer season, acids on all the selections (early and late) was better, but still on the lower side (peaked from 0.50 up to 0.90%). The exception on internal quality was Lane late and Glenora Late, two of the late navels included in the trial with acids above 1.0%. Dream as a mid-maturing navel, improved considerable with good internals and higher acids (up to 1.10%) at colour break (T2-3). Juice (average 52.5%) and Brix levels (up to 12.1 and 12.2 – Clarke, Lazyboy 1 and M7, Suitangi) were good to very good this season; external colour peaked from T 1 to T2/3 at peak maturity early in the season. Fruit size distribution peaked from count 88/72 to 48/40 and will be in demand for export purposes.

## Conclusion

This was the second evaluation of all the navel selections at this new trial site in Karino, so information becomes available and future evaluations will improve these cultivars recommendations. The juice levels on most of the combinations improved from average/good to good/very good; above the minimum export requirement of 48% with the exception of Carninka, Lazy Boy 1 and Fukumoto 1&2. Best performers with high juice content was Dream, Kirkwood Red and Witkrans. Acids remained low (below 1.0%) from the beginning of the season up to peak maturity with the exception of Dream, Glenora Late and Lane Late. The external colour development improved on all the season selections, creating a more ideal situation with better internal quality and higher Brix levels (up to 12.2).

Future evaluations will be crucial to determine the performance of these early to mid/late-navel selections for the Karino area.

**Table 5.4.9.2.** Internal fruit quality data for Navel selections at Karino- koöp (Nelspruit) during the 2021 season.

Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
Bahianinha	CC	30/04/2021	76 - 81	72 - 64	54,0	9,5	0,85	11,2	0,0	T2 - 4
Bahianinha	CC	13/05/2021	75 - 84	72 - 56	52,0	10,2	0,85	12,0	0,0	T2 - 5
Bahianinha	CC	03/06/2021	66 - 88	105 - 48	47,8	11,5	0,70	16,4	0,0	T1 - 2
Carninka	CC	03/06/2021	72 - 91	88 - 40	49,0	11,1	0,75	14,8	0,0	T1 - 2
Carninka	CC	24/06/2022	72 - 86	88 - 48	44,8	11,7	0,65	18,0	0,0	T1 - 2
Carninka	CC	19/07/2021	76 - 83	72 - 56	51,7	11,7	0,60	19,5	0,0	T1
Carninka	CC	13/08/2021	79 - 90	64 - 40	48,6	11,6	0,60	19,3	0,0	T1 - 2
Clarke	CC	13/05/2021	78 - 90	64 - 40	52,0	10,3	0,85	12,1	0,0	T2 - 4
Clarke	CC	24/06/2021	78 - 87	64 - 48	53,7	11,6	0,75	15,5	0,0	T1
Clarke	CC	19/07/2021	78 - 89	64 - 48	55,1	12,1	0,70	17,3	0,0	T1 - 2
De Wet 1	CC	30/04/2021	74 - 82	72 - 56	55,8	9,4	0,65	14,5	0,0	T2 - 4
De Wet 1	CC	13/05/2021	73 - 78	72 - 64	56,5	10,1	0,75	13,5	0,0	T2 - 3
De Wet 1	CC	03/06/2021	72 - 80	88 - 64	52,4	10,0	0,65	15,4	0,0	T1 - 2
De Wet 1	CC	24/06/2021	72 - 85	88 - 56	60,3	10,0	0,65	15,4	0,0	T1 - 2
De Wet 2	CC	13/05/2021	85 - 91	56 - 40	51,5	9,8	0,80	12,3	0,0	T2 - 3
De Wet 2	CC	30/05/2021	79 - 89	64 - 48	52,6	11,0	0,80	13,8	0,0	T2 - 4
De Wet 2	CC	03/06/2021	78 - 90	64 - 40	49,9	10,0	0,75	13,3	0,0	T1 - 2
De Wet 2	CC	24/06/2021	78 - 90	64 - 40	49,0	11,1	0,65	17,1	0,0	T1 - 2
Dream	CC	12/04/2021	79 - 89	64 - 48	54,4	9,9	1,05	9,4	0,0	T5 - 6
Dream	CC	30/04/2021	83 - 88	56 - 48	54,8	10,5	1,00	10,5	0,0	T2 - 3

Dream	CC	13/05/2021	76 - 90	72 - 40	60,0	10,6	0,90	11,8	0,0	T1 - 3
Dream	CC	03/06/2021	74 - 84	72 - 56	54,2	11,7	0,85	13,8	0,0	T1 - 2
Dream	CC	24/06/2021	77 - 87	72 - 48	50,3	11,4	0,80	14,3	0,0	T1 - 2
Fischer	CC	13/05/2021	79 - 89	64 - 48	51,7	12,0	0,75	16,0	0,0	T2 - 4
Fischer	CC	30/05/2021	73 - 84	72 - 56	54,0	10,7	0,70	15,3	0,0	T2 - 4
Fukumoto 1	C35	29/03/2021	82 - 89	56 - 48	46,9	8,8	0,70	12,6	0,0	T5 - 6
Fukumoto 1	C35	12/04/2021	85 - 92	56 - 40	48,1	9,5	0,65	14,6	0,0	T4 - 5
Fukumoto 1	C35	13/05/2021	85 - 92	56 - 40	46,1	10,3	0,60	17,2	0,0	T1 - 3
Fukumoto 1	CC	29/03/2021	77 - 89	72 - 48	50,6	8,8	0,70	12,6	0,0	T4 - 5
Fukumoto 1	CC	12/04/2021	80 - 91	64 - 40	50,5	9,3	0,60	15,5	0,0	T4 - 5
Fukumoto 1	CC	13/05/2021	77 - 102	72 - 36	50,3	11,7	0,60	19,5	0,0	T1 - 2
Fukumoto 1	CC	30/05/2021	74 - 92	72 - 40	47,9	9,8	0,60	16,3	0,0	T1 - 3
Fukumoto 1	SC	29/03/2021	82 - 90	56 - 40	47,0	9,2	0,75	12,3	0,0	T4 - 6
Fukumoto 1	SC	12/04/2021	78 - 91	64 - 40	50,3	9,8	0,70	14,0	0,0	T3 - 4
Fukumoto 1	SC	13/05/2021	92 - 95	40 - 36	48,1	10,0	0,50	20,0	0,0	T1 - 2
Fukumoto 2	C35	29/03/2021	84 - 89	56 - 48	46,3	8,5	0,65	13,1	0,0	T3 - 6
Fukumoto 2	C35	12/04/2021	81 - 92	64 - 40	50,0	8,6	0,60	14,3	0,0	T2 - 5
Fukumoto 2	C35	30/04/2021	65 - 93	105 - 40	49,5	10,1	0,65	15,5	0,0	T2 - 4
Fukumoto 2	C35	13/05/2021	85 - 95	56 - 40	49,2	10,4	0,60	17,3	0,0	T1 - 2
Fukumoto 2	CC	31/03/2021	74 - 84	72 - 56	51,0	8,4	0,80	10,5	0,0	T3 - 6
Fukumoto 2	CC	12/04/2021	80 - 92	64 - 40	48,6	9,8	0,65	15,1	0,0	T3 - 5
Fukumoto 2	CC	30/04/2021	76 - 89	72 - 48	48,7	10,3	0,65	15,8	0,0	T1 - 3
Fukumoto 2	CC	13/05/2021	83 - 93	56 - 40	44,4	10,2	0,65	15,7	0,0	T1 - 2
Fukumoto 2	SC	29/03/2021	77 - 84	72 - 56	48,0	9,2	0,65	14,2	0,0	T3 - 6
Fukumoto 2	SC	12/04/2021	79 - 86	64 - 48	49,4	10,0	0,60	16,7	0,0	T4 - 5
Fukumoto 2	SC	30/04/2021	70 - 89	88 - 48	51,6	10,8	0,65	16,6	0,0	T1 - 4
Fukumoto 2	SC	13/05/2021	80 - 96	64 - 36	50,8	9,9	0,55	18,0	0,0	T1 - 3
Glenora Late	CC	20/05/2021	79 - 90	64 - 40	55,1	9,8	1,10	8,9	0,0	T2 - 4
Glenora Late	CC	03/06/2021	78 - 88	64 - 48	51,9	11,1	0,85	13,1	0,0	T1 - 2
Glenora Late	CC	24/06/2021	79 - 89	64 - 48	54,8	10,9	0,89	12,2	0,0	T1
Glenora Late	CC	19/07/2021	79 - 89	64 - 48	50,6	11,6	0,75	15,5	0,0	T1
Gloudi	CC	03/06/2021	74 - 90	72 - 40	56,5	10,5	0,80	13,1	0,0	T1 - 3
Gloudi	CC	24/06/2021	75 - 86	72 - 48	50,7	10,5	0,70	15,0	0,0	T1 - 3
Gloudi	CC	19/07/2021	79 - 82	64 - 56	52,4	11,8	0,75	15,7	0,0	T1 - 3
Gloudi	CC	13/08/2021	83 - 91	56 - 40	53,2	11,4	0,60	19,0	0,0	T1
Hutton	CC	13/05/2021	76 - 86	72 - 48	55,5	9,8	0,85	11,5	0,0	T3 - 5
Hutton	CC	03/06/2021	77 - 90	72 - 40	52,8	10,5	0,85	12,4	0,0	T1 - 3
Hutton	CC	24/06/2021	78 - 87	64 - 48	51,0	11,4	0,80	14,3	0,0	T1 - 2
Hutton	CC	19/07/2021	78 - 85	64 - 56	52,5	11,6	0,65	17,8	0,0	T1 - 3
Kirkwood Red	CC	30/04/2021	75 - 88	72 - 48	100,0	9,5	0,85	11,2	0,0	T3 - 5
Kirkwood Red	CC	13/05/2021	77 - 88	72 - 48	56,1	10,6	0,85	12,5	0,0	T1 - 3
Kirkwood Red	CC	03/06/2021	75 - 85	72 - 56	54,2	11,0	0,80	13,8	0,0	T1 - 3
Kirkwood Red	CC	24/06/2021	72 - 82	88 - 56	52,9	10,7	0,80	13,4	0,0	T1
Lane Late	CC	13/05/2021	79 - 90	64 - 40	55,5	9,8	1,10	8,9	0,0	T2 - 4
Lane Late	CC	03/06/2021	76 - 93	72 - 40	62,9	10,9	0,80	13,6	0,0	T1 - 2
Lane Late	CC	24/06/2021	79 - 99	64 - 36	55,6	11,4	0,80	14,3	0,0	T1 - 2
Lane Late	CC	19/07/2021	75 - 84	72 - 56	55,8	11,4	0,75	15,2	0,0	T1
Lane Late	CC	13/08/2021	78 - 88	64 - 48	54,6	11,2	0,60	18,7	0,0	T1 - 2

Lazy Boy 1	CC	03/06/2021	77 - 91	72 - 40	45,5	11,0	0,75	14,7	0,0	T2 - 3
Lazy Boy 1	CC	24/06/2021	74 - 88	72 - 48	45,4	11,4	0,65	17,5	0,0	T1
Lazy Boy 1	CC	19/07/2021	77 - 83	72 - 56	49,0	12,1	0,55	22,0	0,0	T1 - 3
Lazy Boy 1	CC	13/08/2021	81 - 87	64 - 48	43,0	11,5	0,50	23,0	0,0	T1 - 2
M7	CC	29/03/2021	77 - 90	72 - 40	54,3	11,4	1,00	11,4	0,0	T4 - 6
M7	CC	12/04/2021	68 - 82	88 - 56	55,5	11,7	0,85	13,8	0,0	T2 - 4
M7	CC	13/05/2021	80 - 95	64 - 40	49,0	12,2	0,70	17,4	0,0	T1 - 2
M7	CC	30/05/2021	75 - 89	72 - 48	53,5	12,0	0,65	18,5	0,0	T1 - 3
Suitangi	CC	24/04/2021	76 - 84	72 - 56	49,2	11,2	0,85	13,2	0,0	T1 - 2
Suitangi	CC	03/06/2021	77 - 92	72 - 40	51,9	11,0	0,75	14,7	0,0	T1 - 3
Suitangi	CC	19/07/2021	76 - 81	72 - 64	55,6	12,2	0,70	17,4	0,0	T1 - 2
Suitangi	CC	13/08/2021	83 - 89	56 - 48	53,5	11,9	0,65	18,3	0,0	T1 - 2
Witkrans	CC	13/05/2021	77 - 83	72 - 56	55,2	10,5	0,90	11,7	0,0	T2 - 4
Witkrans	CC	03/06/2021	71 - 94	88 - 40	54,2	11,6	0,85	13,6	0,0	T1 - 2
Witkrans	CC	24/06/2021	77 - 83	72 - 56	52,6	11,2	0,75	14,9	0,0	T1 - 3
Witkrans	CC	19/07/2021	76 - 87	72 - 48	63,6	11,9	0,75	15,9	0,0	T1 - 3
Witkrans	CC	13/08/2021	81 - 87	64 - 48	55,1	11,7	0,75	15,6	0,0	T1

**5.4.10 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the cool-inland production areas (Ngodwana, Orighstad and Burgersfort)**  
Project 990A by J Joubert (CRI)

**Summary**

Nova, Nova SL and Saint André mature first according to the results of the 2021 season for the cool inland production areas, and all three selections developed large to extra-large fruit calibre and good internal quality. RHM, Page, Ma`ayana and Samba with high juice levels, fit in before Leanri with large fruit size, which follows. Furr developed the highest seed count per fruit for this trial under net and in the open blocks followed by IRM 2, developing fairly high seed count per fruit. The mid-maturing mandarins are represented by ARCCIT 9 LS Nadorcott and ARC Nadorcott, which developed good Brix levels and higher acids than the other selections (up to acid of 1.2%) this season. IRM 1, followed by Tambor 1&2 and Tanor Late matures toward the end of the mandarin season, with Sugar Belle ending off the season as ultra-late option. Picking periods should not be longer than 3 - 4 weeks to maintain good internal quality and to avoid rind disorders.

**Opsomming**

Nova, Nova SL and Saint André word die vroegste ryp volgens resultate van die 2020 seisoen vir hierdie koel binnelandse produksie area, met al drie seleksies groot tot ekstra-groot vrugt kaliber en goeie interne kwaliteit. RHM, Page, Ma`ayana en Samba, met hoë sap vlakke, pas in voor Leanri, wat daarna volg met die groot vruggrootte. Furr het die hoogste saadtellings per vrug ontwikkel vir hierdie proef onder net en in die oop blokke. Volgende is IRM 2, met relatiewe hoe saad tellings per vrug. Die middel van die mandaryne word verteenwoordig deur ARCCIT 9 LS Nadorcott en ARC Nadorcott, met goeie Brix vlakke en hoër sure in vergelyking met die ander seleksies (tot suur van 1.2%). IRM 1, gevolg deur Tambor 1&2 en Tanor Late word ryp meer aan die einde van die mandarin seisoen, met Sugar Belle wat die seisoen afsluit as ultra-laas opsie. 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.

**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 cool, inland production regions.

## Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from Mountain Haven (Orighstad), Waterval (Burgersfort) and Futsela Iglobhu Investment (Ryton/Ngodwana).

**Table 5.4.10.1.** List of Mandarin Hybrid selections evaluated at Mountain Haven (Orighstad) during the 2021 season.

Selection	Rootstock	Planted
ARCCIT 9 LS	CC	2017
Etna	CC	2017
Goldup	CC	2017
IRM 1&2	CC	2017
Leanri	CC	2017
Ma`ayana (Dina)	CC	2017
Mor 26	CC	2017
Nadorcott (control)	CC	2017
Nova ARC	CC	2017
Nova	CC	2017
Orr 1&4	CC	2017
Page	CC	2017
RHM	CC	2017
Saint André	CC	2017
Samba	CC	2017
Shani SL	CC	2017
Sirio	CC	2017
Tambor 1&2	CC	2017
Tasty 1&2	CC	2017
Taylor Lee	CC	2017

**Table 5.4.10.2.** List of Mandarin Hybrid selections evaluated at Waterval (Burgersfort) during the 2021 season.

Selection	Rootstock	Planted
ARCCIT 9 LS	CC	2017
Etna	CC	2017
Furr	CC	2017
IRM 1&2	CC	2017
Leanri	CC	2017
Ma`ayana (Dina)	CC	2017
Nova	CC	2017
Page	CC	2017
RHM	CC	2017
Saint André	CC	2017
Samba	CC	2017
Sugar Belle	CC	2016
Tambor 1&2	CC	2017
Tanor Late	CC	2017

**Table 5.4.10.3.** List of Mandarin Hybrid selections evaluated at Futsela Iglobhu Investment (Ryton/Ngodwana) during the 2021 season.

Selection	Rootstock	Planted
ARCCIT 9 LS	CC	2017
Etna	CC	2017
Furr	CC	2017
Goldup	CC	2017
IRM 1&2	CC	2017
Leanri	CC	2017
Ma`ayana (Dina)	CC	2017
Mor 26	CC	2017
Nadorcott (Control)	CC	2017
Nova	CC	2017
Nova ARC	CC	2017
Orr 1&4	CC	2017
Page	CC	2017
RHM	CC	2017
Saint André	CC	2017
Samba	CC	2017
Shani SL	CC	2017
Sirio	CC	2017
Tambor 1&2	CC	2017
Tanor Late	CC	2017
Tasty 1	CC	2017
Taylor Lee	CC	2017

## Results and discussion

The trees at Mountain Haven and Waterval bore their third crop for this season with improved fruit numbers and more mature tree internal quality and fruit size characteristics. Mountain Haven was planted under net completely; Waterval consists of two adjacent trial blocks, one side under net and the other side open, duplicating the same selections.

The new additional trial block at Futsela Iglobhu Investment (Ryton) bore its second crop on the trees; additional information to add for future evaluations.

For Mandarin Hybrid selections, a ratio of 11:1 is considered to be the build-up towards peak maturity, with a ratio of 12:1. After reaching the peak, the ratio increases to 13:1, after which the fruit is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

### Ma`ayana (Dina)

The internal quality of Ma`ayana was good this season at Mountain Haven, with high juice levels (average 56%) and no granulation problems in the fruit compared to Nova. Juice level at Waterval under the net was (58.6%) compared to higher juices (average 59.9%) in the open plantings. Brix (average 13.5) and similar acids (between 1.0 and 1.3%), indicating the early-mid maturing characteristics of the selection in the cool inland production areas, with seedless fruit this season. The fruit size peaked from medium/large to extra-large, count 2/1 to count 1XX/1XXX.

### Furr (Clemcott)

Furr developed large to extra-large fruit size (count 1X – 1XXX) on the trees at Waterval trial site (average bigger size counts at Waterval, smallest at Ryton), one of the cultivar's characteristics as well as a good crop on the trees. The external colour development on the fruit was good for the cool areas, as expected (T1-2/3); slight delay at the Ryton site. Internally the fruit quality was very good, developing high juice (up to 62%) and Brix (up to 15.0) levels with good acids. Another fruit quality is the high seed count (high self-and cross pollination, up to 19 seeds per fruit). Maturity seems to be middle to the end of May to the middle of June for the cool production areas, according to the information in Table 5.4.10.5.

### Leanri

Leanri developed a fairly large to extra-large fruit size between count 2/1 and 1XXX, smaller compared to the hot production areas with the smallest fruit produced this season at Ryton (count 3 to 1XX). 2021 was the second (Ryton) and third (Mountain Haven, Waterval) crop on the trees and internal quality was good (Mountain Haven) to very good (Waterval) on the Carrizo rootstocks (average juice 57%, Brix 13, acid 1.3%). Seed numbers were fairly low; completely seedless up to 3.0 seeds per fruit.

### Nadorcott & ARCCIT 9 LS

The crop on both selections was good this season to evaluate. The fruit shape was very similar to the Nadorcott selection. Rind texture was very smooth with a natural shine (similar to packhouse waxing). Both selections produced a fruit size that ranged from count 2/1 and peaked at count 1XX/1XXX (smaller fruit at Mountain Haven and Ryton) even with the better crop load on the trees. Nadorcott ARC and ARCCIT 9 LS produced low seed numbers in the fruit (up to 2.5 seeds per fruit only at Ryton with higher numbers) this season. Maturity seems to be two weeks earlier on the ARCCIT 9 LS selection, according to Table 5.4.10.5, but the information was limited due to the first crop on the trees being evaluated (end of May to middle of June).

### Nova, Nova SL, Saint André

Nova was included at Waterval as an early maturing control for Nova SL and Saint André in the trial at Mountain Haven. The fruit is fairly difficult to peel and low numbers of seed were discovered in the fruit with some of the evaluations. External colour was later on Nova and Saint André, and the fruit size varied between count 2/1 and count 1XX (medium to large fruit); smaller fruit size at Ryton (cooler areas in summer). Nova SL (ARC) produced a coarse rind texture on the fruit compared to the other two selections. The acid levels at Mountain Haven in the fruit was similar compared to higher acids under the net structures at Waterval than the open block, and the external colour development better (T1 to 2).

### RHM

RHM cropped fruit for the fourth time this season with average seed numbers at Waterval under net and open blocks (from 1.5 up to 10.7 seeds per fruit), and very low numbers in the net block at Mountain Haven (avg.0.3 seeds per fruit) with the crosspollination. There was a delayed colour development (T2 to 4/5) from the first evaluation with low acids (avg. 0.70 to 1.0%), indicating peak maturity Brix: acid ratio over 12. Future evaluations will determine optimum quality of the fruit evaluated.

### Samba

Samba on Carrizo rootstock produced a good crop with good internal quality on the larger fast growing thornless trees at both trial sites. Colour development was good early in the season (deep orange) at Mountain Haven with similar acids (1.0%) compared to Waterval, with a smooth rind texture on the fruit. Fruit was completely seedless at Mountain Haven this season, compared to Ryton and Waterval (3.1 and 1.4 seeds per fruit) in the combined trial blocks (future evaluations will confirm low seed numbers) and peaked from medium to large fruit size at Mountain Haven (average count 2 to 1XX, cooler area) and count 3/2 to 1X at Waterval (medium to large fruit size). Ryton peaked at small to medium fruit (count 4 to 1/1X) for the cool area. Internal quality was good with similar higher juice's at Orighstad and Burgersfort (avg. 57 to 61%), high Brix levels (from 10 to 14.6), and good acids (avg. 1.0 to 1.1%). Based on the internal quality results in Table 5.4.10.5, the estimated maturity will be middle April to middle May.

### Additional selections

Page matured before Samba; juice and Brix was higher at Waterval and the opposite at Mahela with delayed colour development, but similar acids on the lower side for the cooler regions, except for Samba developing good acids throughout the season.

There are new selections in the three trial sites bearing their second light crop on the trees and future evaluations will include performance.

## Conclusion

This was the third and fourth evaluation of all the selections at the three trial sites, with Ryton (Ngodwana) and Mountain Haven (Orighstad) cooler than Burgersfort. Trees are still young and fruit quality will improve with future evaluations.

The highest seed numbers were on Furr, Tambor 1 and 2, Sirio, Nadorcott (control), Page, RHM, Saint André, followed then by Ma'ayana, Leanri and ARCCIT 9 LS with lower numbers in the open trial block at Ryton (cross-pollination). All the other selections developed very low seed numbers in the fruit at Mountain Haven and Waterval, except for Furr. Ma'ayana, Leanri, Samba and the Nadorcott selections performed well with deep orange colour development, as well as the Nova selections and Saint André. RHM continued with delayed external colour development on the fruit and lower acids and large fruit size at Waterval. All the selections planted at Ryton peaked at smaller fruit size compared to the other two sites (cold area).

**Table 5.4.10.5.** Internal fruit quality data for Mandarin hybrid selections at Mountain Haven (Orighstad), Waterval (Burgersfort) and Futsela Iglobhu Investment (Ryton) during the 2021 season.

Mountain Haven										
Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
ARCCIT 9 LS	CC	17/05/2021	59 - 70	2 - 1X	56,9	11,9	1,20	9,9	0,0	T1 - 2
ARCCIT 9 LS	CC	09/06/2021	58 - 77	3 - 1XX	54,6	12,5	1,05	11,9	0,0	T1 - 2
ARCCIT 9 LS	CC	29/06/2021	64 - 75	1 - 1XX	30,8	11,5	0,95	12,1	0,0	T1 - 2
ARCCIT 9 LS	CC	20/07/2021	69 - 83	1X - 1XXX	47,7	13,6	0,85	16,0	0,0	T1 - 2
Etna	CC	17/03/2021	66 - 70	1 - 1X	63,3	9,8	0,95	10,3	0,0	T5 - 6
Etna	CC	21/04/2021	71 - 80	1X - 1XXX	60,5	10,1	0,75	13,5	0,0	T2 - 4
Etna	CC	17/05/2021	65 - 83	1 - 1XXX	61,8	10,1	1,00	10,1	0,0	T1 - 3
Etna	CC	09/06/2021	72 - 82	1XX - 1XXX	52,4	10,8	0,80	13,5	0,0	T1 - 2
Goldup	CC	17/03/2021	47 - 56	5 - 3	52,9	9,0	0,85	10,6	0,0	T4 - 6
Goldup	CC	20/04/2021	53 - 65	4 - 1	51,9	9,7	0,80	12,1	0,9	T1 - 3
Goldup	CC	17/05/2021	50 - 69	5 - 1X	37,2	11,2	0,70	16,0	0,0	T1 - 2
IRM 1	CC	17/05/2021	62 - 75	2 - 1XX	57,1	13,1	1,85	7,1	2,2	T1 - 3
IRM 1	CC	09/06/2021	65 - 77	1 - 1XX	61,9	14,4	1,60	9,0	1,8	T1 - 2
IRM 1	CC	29/06/2021	63 - 74	2 - 1XX	60,5	15,2	1,75	8,7	1,5	T1 - 2
IRM 1	CC	20/07/2021	66 - 74	1 - 1XX	60,1	15,9	1,30	12,2	0,8	T1 - 2
IRM 2	CC	17/05/2021	60 - 73	2 - 1XX	56,7	12,1	1,35	9,0	0,0	T1 - 3
IRM 2	CC	09/06/2021	64 - 74	1 - 1XX	61,3	13,2	1,30	10,2	0,7	T1 - 2
IRM 2	CC	29/06/2021	63 - 73	2 - 1XX	58,0	13,7	1,25	11,0	1,2	T1 - 2
IRM 2	CC	20/07/2021	65 - 85	1 - 1XXX	59,9	13,5	1,00	13,5	1,0	T1 - 2

IRM 2	CC	10/08/2021	62 - 76	2 - 1XX	63,5	14,3	1,05	13,6	1,5	T1 - 2
Leanri	CC	17/03/2021	64 - 76	1 - 1XX	53,4	10,5	1,48	7,1	0,0	T6 - 7
Leanri	CC	20/04/2021	69 - 83	1X - 1XXX	55,9	11,0	1,00	11,0	0,0	T4 - 5
Leanri	CC	17/05/2021	66 - 83	1 - 1XXX	54,4	11,4	1,00	11,4	0,0	T1 - 3
Ma'ayana	CC	20/04/2021	64 - 70	1 - 1X	59,0	9,4	1,00	9,4	0,0	T3 - 4
Ma'ayana	CC	17/05/2021	57 - 74	3 - 1XX	57,2	12,2	1,05	11,6	0,0	T1 - 3
Ma'ayana	CC	09/06/2021	69 - 84	1X - 1XXX	52,1	11,4	1,05	10,9	0,0	T1 - 2
Nadorcott	CC	20/04/2021	62 - 75	2 - 1XX	53,1	9,3	0,95	9,8	0,0	T4 - 6
Nadorcott	CC	17/05/2021	63 - 75	2 - 1XX	52,2	11,4	1,00	11,4	0,0	T1 - 3
Nadorcott	CC	09/06/2021	66 - 83	1 - 1XXX	51,9	12,5	1,05	11,9	0,0	T1 - 2
Nova	CC	17/03/2021	60 - 70	2 - 1X	58,2	9,5	1,05	9,0	0,0	T6 - 7
Nova	CC	20/04/2021	68 - 75	1X - 1XX	58,1	10,6	0,85	12,5	0,0	T3 - 5
Nova	CC	17/05/2021	69 - 75	1X - 1XX	60,8	11,6	1,00	11,6	0,0	T1 - 3
Nova	CC	09/06/2021	68 - 79	1X - 1XXX	53,8	11,6	1,05	11,0	0,0	TT - 2
Nova ARC	CC	17/03/2021	60 - 64	2 - 1	63,3	10,5	1,10	9,5	0,0	T5 - 7
Nova ARC	CC	20/04/2021	63 - 78	2 - 1XXX	54,3	11,6	0,90	12,9	0,0	T3 - 5
Nova ARC	CC	17/05/2021	72 - 80	1XX - 1XXX	59,6	11,9	1,10	10,8	0,0	T1 - 3
Orr 1	CC	17/05/2021	61 - 70	2 - 1X	54,8	12,9	1,60	8,1	0,9	T1 - 3
Orr 1	CC	09/06/2021	61 - 70	2 - 1X	57,9	14,7	1,35	10,9	0,0	T1 - 2
Orr 1	CC	29/06/2021	65 - 76	1 - 1XX	37,3	14,9	1,10	13,5	0,5	T1 - 2
Orr 1	CC	20/07/2021	67 - 73	1 - 1XX	59,0	15,4	1,10	14,0	0,0	T1 - 2
Orr 1	CC	10/08/2021	65 - 80	1 - 1XXX	57,6	15,9	1,00	15,9	0,0	T1 - 2
Orri (Orr 4)	CC	17/05/2021	59 - 67	2 - 1	55,2	13,7	1,45	9,4	0,0	T1 - 2
Orri (Orr 4)	CC	09/06/2021	66 - 75	1 - 1XX	57,9	14,3	1,30	11,0	0,0	T1 - 2
Orri (Orr 4)	CC	29/06/2021	60 - 74	2 - 1XX	36,3	14,6	1,10	13,3	0,0	T1 - 2
Orri (Orr 4)	CC	20/07/2021	63 - 75	2 - 1XX	57,9	15,6	1,10	14,2	0,0	T1 - 2
Orri (Orr 4)	CC	10/08/2021	67 - 78	1 - 1XXX	46,9	16,7	0,85	19,6	0,0	T1 - 2
Page	CC	17/03/2021	61 - 71	2 - 1X	53,9	10,3	1,00	10,3	0,0	T5 - 6
Page	CC	20/04/2021	66 - 75	1 - 1XX	55,1	11,3	0,85	13,3	0,0	T2 - 4
Page	CC	17/05/2021	60 - 71	2 - 1X	55,3	12,0	0,90	13,3	0,0	T1 - 2
RHM	CC	20/04/2021	60 - 66	2 - 1	60,9	10,5	0,70	15,0	0,0	T4 - 6
RHM	CC	17/05/2021	59 - 78	2 - 1XXX	56,5	11,3	0,60	18,8	0,0	T1 - 3
RHM	CC	09/06/2021	64 - 73	1 - 1XX	50,0	12,9	0,85	15,2	0,8	TT - 2
Saint Andre	CC	17/03/2021	55 - 62	3 - 2	61,1	14,4	1,25	11,5	0,0	T6 - 7
Saint Andre	CC	20/04/2021	73 - 78	1XX - 1XXX	56,2	10,3	0,80	12,9	0,0	T3 - 5
Saint Andre	CC	17/05/2021	69 - 89	1X - 1XXX	51,6	11,0	0,90	12,2	0,0	T1 - 2
Saint Andre	CC	09/06/2021	75 - 87	1XX - 1XXX	49,1	10,7	0,85	12,6	0,0	T1 - 2
Samba	CC	17/03/2021	63 - 70	2 - 1X	58,1	10,9	1,05	10,4	0,0	T4 - 7

Samba	CC	20/04/2021	60 - 75	2 - 1XX	54,4	11,4	0,95	12,0	0,0	T2 - 4
Samba	CC	17/05/2021	64 - 72	1 - 1XX	58,5	12,0	0,95	12,6	0,0	T1 - 2
Shani SL	CC	17/05/2021	50 - 66	5 - 1	54,5	13,9	1,90	7,3	0,0	T1 - 3
Shani SL	CC	09/06/2021	62 - 68	2 - 1X	54,2	15,1	1,55	9,7	0,0	T1 - 2
Shani SL	CC	29/06/2021	62 - 68	2 - 1X	56,0	15,7	1,60	9,8	0,0	T1 - 2
Shani SL	CC	20/07/2021	66 - 76	1 - 1XX	55,1	15,3	1,45	10,6	0,0	T1 - 2
Sirio	CC	17/03/2021	64 - 75	1 - 1XX	54,1	9,2	1,05	8,8	0,0	T6 - 7
Sirio	CC	20/04/2021	70 - 85	1X - 1XXX	50,2	11,2	1,15	9,7	0,0	T2 - 4
Sirio	CC	17/05/2021	72 - 80	1XX - 1XXX	57,3	11,4	0,85	13,4	0,0	T1 - 2
Sirio	CC	09/06/2021	72 - 83	1XX - 1XXX	49,1	11,2	0,90	12,4	0,0	T1 - 2
Tambor 1	CC	17/05/2021	75 - 83	1XX - 1XXX	56,4	10,6	1,05	10,1	0,0	T2 - 3
Tambor 1	CC	09/06/2021	79 - 88	1XXX	56,0	11,0	1,10	10,0	0,0	T1 - 2
Tambor 1	CC	29/06/2021	77 - 87	1XX - 1XXX	45,9	12,4	1,35	9,2	0,0	T1 - 2
Tambor 1	CC	20/07/2021	81 - 90	1XXX	51,4	11,0	1,05	10,5	0,0	T1 - 2
Tambor 1	CC	10/08/2021	80 - 86	1XXX	56,0	12,0	1,05	11,4	0,0	T1 - 2
Tambor 2	CC	17/05/2021	71 - 81	1X - 1XXX	57,7	11,2	1,30	8,6	0,0	T1 - 3
Tambor 2	CC	07/06/2021	75 - 85	1XX - 1XXX	57,0	11,3	1,25	9,0	0,0	T1 - 2
Tambor 2	CC	29/06/2021	76 - 88	1XX - 1XXX	51,8	12,2	1,25	9,8	0,0	T1 - 2
Tambor 2	CC	20/07/2021	82 - 94	1XXX	52,2	11,9	1,25	9,5	0,0	T1 - 2
Tambor 2	CC	10/08/2021	77 - 90	1XX - 1XXX	54,7	12,0	1,00	12,0	0,0	T1 - 2
Tanor Late	CC	17/05/2021	75 - 89	1XX - 1XXX	52,3	12,2	1,75	7,0	0,0	T1 - 3
Tanor Late	CC	09/06/2021	76 - 88	1XX - 1XXX	52,6	12,0	1,60	7,5	0,0	T1 - 3
Tanor Late	CC	29/06/2021	79 - 90	1XXX	51,3	12,0	1,40	8,6	0,0	T1 - 2
Tanor Late	CC	20/07/2021	82 - 96	1XXX	52,0	12,1	1,15	10,5	0,0	T1 - 2
Tanor Late	CC	10/08/2021	84 - 96	1XXX	50,0	12,7	1,05	12,1	0,0	T1 - 2
Tasty 1	CC	20/04/2021	70 - 80	1X - 1XXX	48,0	10,8	1,10	9,8	0,0	T2 - 4
Tasty 1	CC	17/05/2021	69 - 76	1X - 1XXX	45,8	14,3	1,00	14,3	0,0	T1 - 2
Tasty 1	CC	09/06/2021	68 - 82	1X - 1XXX	42,9	11,2	0,80	14,0	0,0	T1 - 2
Tasty 1	CC	29/06/2021	76 - 89	1XX - 1XXX	40,6	12,6	0,95	13,3	0,0	T1 - 2
Tasty 1	CC	20/07/2021	77 - 92	1XXX	40,0	12,7	0,85	14,9	0,0	T1
Tasty 2	CC	20/04/2021	65 - 81	1 - 1XXX	54,4	11,3	1,30	8,7	0,0	T4 - 6
Tasty 2	CC	17/05/2021	68 - 84	1X - 1XXX	52,4	12,6	1,35	9,3	0,0	T1 - 2
Tasty 2	CC	09/06/2021	69 - 82	1X - 1XXX	44,1	12,9	1,35	9,6	0,0	T1 - 2
Taylor Lee	CC	20/04/2021	67 - 80	1 - 1XXX	62,0	11,2	1,00	11,2	2,3	T4 - 6
Taylor Lee	CC	17/05/2021	72 - 82	1XX - 1XXX	57,9	12,5	1,05	11,9	3,2	T1 - 3
Taylor Lee	CC	09/06/2021	74 - 88	1XX - 1XXX	54,7	12,7	1,10	11,5	1,5	T1 - 2
Taylor Lee	CC	29/06/2021	71 - 84	1X - 1XXX	54,2	13,3	1,05	12,7	2,7	T1 - 2
Taylor Lee	CC	20/07/2021	72 - 83	1XX - 1XXX	58,6	14	1,00	14,0	4,3	T1 - 2
Taylor Lee	CC	10/08/2021	73 - 80	1XX - 1XXX	57,9	14,6	1,05	13,9	2,2	T1 - 2

Waterval											
Cultivar	Root-stock	Net/Out of Net	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
ARCCIT 9 LS	CC	Net	17/03/2021	60 - 74	2 - 1XX	59,9	14,2	1,60	8,9	0,0	T1 - 3
ARCCIT 9 LS	CC	Net	09/06/2021	65 - 75	1 - 1XX	54,3	14,7	1,35	10,9	0,0	T1 - 2
ARCCIT 9 LS	CC	Net	29/06/2021	65 - 77	1 - 1XX	52,6	15,0	1,40	10,7	0,0	T1 - 2
ARCCIT 9 LS	CC	Net	20/07/2021	67 - 81	1 - 1XXX	52,6	15,1	1,20	12,6	0,0	T1 - 2
ARCCIT 9 LS	CC	Out of Net	17/05/2021	59 - 73	2 - 1XX	60,9	14,2	1,40	10,1	1,2	T1 - 4
ARCCIT 9 LS	CC	Out of Net	09/06/2021	65 - 68	1 - 1X	54,7	14,7	1,30	11,3	0,0	T1 - 2
ARCCIT 9 LS	CC	Out of Net	29/06/2021	69 - 80	1X - 1XXX	49,1	14,7	1,30	11,3	0,5	T1 - 2
ARCCIT 9 LS	CC	Out of Net	20/07/2021	66 - 74	1 - 1XX	52,7	15,7	1,20	13,1	0,0	T1 - 2
Etna	CC	Net	17/03/2021	64 - 68	1 - 1X	62,7	10,9	1,15	9,5	1,8	T5 - 6
Etna	CC	Net	20/04/2021	65 - 74	1 - 1XX	62,6	11,6	1,20	9,7	0,0	T2 - 4
Etna	CC	Net	17/05/2021	61 - 70	2 - 1X	60,8	12,7	0,90	14,1	0,0	T1 - 2
Etna	CC	Net	09/06/2021	61 - 74	2 - 1XX	57,4	13,6	1,10	12,4	0,9	T1 - 2
Etna	CC	Out of Net	17/03/2021	67 - 76	1 - 1XX	60,3	11,3	1,25	9,0	5,7	T5 - 6
Etna	CC	Out of Net	20/04/2021	63 - 77	2 - 1XX	65,4	11,6	1,25	9,3	1,8	T1 - 4
Etna	CC	Out of Net	17/05/2021	62 - 72	2 - 1XX	58,7	13,5	1,25	10,8	1,9	T1 - 2
Furr	CC	Net	20/04/2021	78 - 84	1XXX	57,8	13,3	1,50	8,9	18,3	T3 - 5
Furr	CC	Net	17/05/2021	69 - 86	1X - 1XXX	57,2	14,2	1,30	10,9	16,0	T1 - 2
Furr	CC	Net	09/06/2021	75 - 88	1XX - 1XXX	60,3	16,0	1,35	11,9	15,8	T1 - 2
Furr	CC	Net	29/06/2021	75 - 86	1XX - 1XXX	55,3	16,0	1,35	11,9	15,8	T1 - 2
Furr	CC	Out of Net	20/04/2021	74 - 81	1XX - 1XXX	56,5	13,2	1,30	10,2	15,0	T4 - 6
Furr	CC	Out of Net	17/05/2021	75 - 87	1XX - 1XXX	62,6	14,8	1,50	9,9	19,2	T1 - 3
Furr	CC	Out of Net	09/06/2021	74 - 84	1XX - 1XXX	58,0	15,1	1,20	12,6	16,5	T1 - 2
Furr	CC	Out of Net	29/06/2021	75 - 88	1XX - 1XXX	55,4	15,9	1,35	11,8	14,0	T1 - 2
Furr	CC	Out of Net	20/07/2021	79 - 86	1XXX	57,0	15,9	1,20	13,3	16,5	T1 - 2
IRM 1	CC	Net	09/06/2021	68 - 90	1X - 1XXX	62,2	14,3	1,70	8,4	0,8	T1 - 3
IRM 1	CC	Net	17/05/2021	69 - 77	1X - 1XX	51,3	14,3	1,85	7,7	3,0	T1 - 3

IRM 1	CC	Net	29/06/2021	70 - 78	1X - 1XXX	62,4	15,4	1,80	8,6	0,8	T1 - 2
IRM 1	CC	Net	20/07/2021	72 - 81	1XX - 1XXX	60,5	16,3	1,10	14,8	0,8	T1 - 2
IRM 1	CC	Net	10/08/2021	74 - 78	1XX - 1XXX	61,2	17,3	1,50	11,5	1,7	T1 - 2
IRM 1	CC	Out of Net	06/06/2021	65 - 76	1 - 1XX	61,3	15,7	1,95	8,1	4,0	T1 - 3
IRM 1	CC	Out of Net	17/05/2021	65 - 77	1 - 1XX	55,2	14,4	1,75	8,2	2,8	T2 - 4
IRM 1	CC	Out of Net	29/06/2021	67 - 78	1 - 1XXX	59,3	15,2	1,75	8,7	2,7	T1 - 2
IRM 1	CC	Out of Net	20/07/2021	65 - 78	1 - 1XXX	60,1	16,0	1,45	11,0	1,7	T1 - 2
IRM 2	CC	Net	17/05/2021	62 - 72	2 - 1XX	59,4	13,1	1,50	8,7	0,8	T1 - 3
IRM 2	CC	Net	09/06/2021	65 - 73	1 - 1XX	57,3	13,8	1,30	10,6	0,8	T1 - 2
IRM 2	CC	Net	29/06/2021	65 - 72	1 - 1XX	38,8	14,9	1,25	11,9	0,0	T1 - 2
IRM 2	CC	Net	20/07/2021	69 - 78	1X - 1XXX	60,2	15,2	1,15	13,2	1,8	T1 - 2
IRM 2	CC	Net	10/08/2021	68 - 78	1X - 1XXX	62,7	16,4	1,00	16,4	0,0	T1 - 2
IRM 2	CC	Out of Net	17/05/2021	61 - 69	2 - 1X	66,2	13,3	1,25	10,6	4,1	T1 - 2
IRM 2	CC	Out of Net	09/06/2021	66 - 75	1 - 1XX	61,1	14,3	1,30	11,0	2,3	T1 - 3
IRM 2	CC	Out of Net	29/06/2021	67 - 77	1 - 1XX	45,3	14,7	1,20	12,3	2,2	T1 - 2
IRM 2	CC	Out of Net	20/07/2021	62 - 75	2 - 1XX	63,1	15,2	1,10	13,8	1,8	T1 - 2
IRM 2	CC	Out of Net	10/08/2021	66 - 87	1 - 1XXX	60,0	15,6	0,85	18,4	1,3	T1 - 2
Leanri	CC	Net	17/03/2021	63 - 78	2 - 1XXX	57,3	11,2	1,30	8,6	1,2	T5 - 7
Leanri	CC	Net	20/04/2021	62 - 87	2 - 1XXX	54,5	11,9	1,25	9,5	0,0	T1 - 4
Leanri	CC	Net	17/05/2021	62 - 83	2 - 1XXX	56,8	12,2	1,15	10,6	0,0	T1 - 3
Leanri	CC	Net	09/06/2021	71 - 85	1X - 1XXX	47,5	13,7	1,10	12,5	0,0	T1 - 2
Leanri	CC	Out of Net	17/03/2021	65 - 75	1 - 1XX	63,6	12,7	1,45	8,8	1,2	T5 - 7
Leanri	CC	Out of Net	20/04/2021	68 - 80	1X - 1XXX	59,0	14,0	1,25	11,2	0,9	T3 - 5
Leanri	CC	Out of Net	17/05/2021	69 - 77	1X - 1XX	58,7	15,9	1,40	11,4	1,0	T1 - 3
Leanri	CC	Out of Net	09/06/2021	68 - 80	1X - 1XXX	57,3	15,9	1,25	12,7	1,0	T1 - 2
Ma'ayana	CC	Net	20/04/2021	61 - 72	2 - 1XX	61,8	12,0	1,45	8,3	0,0	T4 - 5
Ma'ayana	CC	Net	17/05/2021	62 - 72	2 - 1XX	57,6	14,4	1,35	10,7	0,0	T1 - 3
Ma'ayana	CC	Net	09/06/2021	64 - 73	1 - 1XX	56,3	14,5	1,10	13,2	0,0	T1 - 2
Ma'ayana	CC	Out of Net	20/04/2021	65 - 73	1 - 1XX	62,6	12,3	1,40	8,8	0,0	T3 - 5

Ma'ayana	CC	Out of Net	17/05/2021	65 - 78	1 - 1XXX	63,8	13,3	1,10	12,1	0,0	T1 - 3
Ma'ayana	CC	Out of Net	09/06/2021	64 - 84	1 - 1XXX	53,2	14,2	1,00	14,2	0,0	T1 - 2
Nova	CC	Net	17/03/2021	57 - 64	3 - 1	57,9	10,7	1,20	8,9	0,0	T6 - 7
Nova	CC	Net	20/04/2021	60 - 70	2 - 1X	60,3	13,6	1,10	12,4	0,0	T3 - 5
Nova	CC	Net	17/05/2021	54 - 67	4 - 1	60,7	14,7	1,25	11,8	0,0	T1 - 2
Nova	CC	Net	09/06/2021	60 - 74	2 - 1XX	58,3	14,7	1,25	11,8	0,0	T1 - 2
Nova	CC	Out of Net	20/04/2021	63 - 76	2 - 1XX	60,7	14,2	1,25	11,4	1,3	T3 - 5
Nova	CC	Out of Net	17/05/2021	61 - 72	2 - 1XX	67,5	15,2	1,25	12,2	1,8	T1 - 2
Nova	CC	Out of Net	09/06/2021	64 - 70	1 - 1X	56,3	15,4	1,20	12,8	0,9	T1 - 2
Orri	CC	Net	17/05/2021	59 - 68	2 - 1X	48,7	15,8	2,85	5,5	2,3	T1 - 3
Orri	CC	Net	09/06/2021	57 - 69	3 - 1X	50,8	16,1	2,50	6,4	1,5	T1 - 2
Orri	CC	Net	29/06/2021	68 - 82	1X - 1XXX	51,6	15,5	1,95	7,9	0,8	T1 - 2
Orri	CC	Net	20/07/2021	61 - 69	2 - 1X	51,9	16,3	1,60	10,2	1,0	T1 - 2
Orri	CC	Out of Net	17/05/2021	59 - 78	2 - 1XXX	56,0	15,0	2,85	5,3	2,2	T1 - 3
Orri	CC	Out of Net	09/06/2021	65 - 77	1 - 1XX	57,0	13,7	2,45	5,6	0,0	T1 - 2
Orri	CC	Out of Net	29/06/2021	60 - 75	2 - 1XX	51,0	16,1	1,90	8,5	1,7	T1 - 2
Orri	CC	Out of Net	20/07/2021	71 - 83	1X - 1XXX	54,2	15,1	1,45	10,4	0,0	T1 - 2
Orri	CC	Out of Net	10/08/2021	74 - 80	1XX - 1XXX	57,5	16,7	1,35	12,4	1,8	T1 - 2
Page	CC	Net	17/03/2021	57 - 63	3 - 2	60,7	11,6	1,15	10,1	0,0	T6 - 7
Page	CC	Net	20/04/2021	58 - 67	3 - 1	61,8	12,5	1,00	12,5	0,0	T3 - 4
Page	CC	Net	17/05/2021	61 - 72	2 - 1XX	61,2	12,9	0,95	13,6	0,0	T1 - 2
Page	CC	Net	09/06/2021	64 - 80	1 - 1XXX	62,3	14,3	1,05	13,6	0,0	T1 - 2
Page	CC	Out of Net	17/03/2021	58 - 67	3 - 1	59,7	11,5	1,05	11,0	2,3	T4 - 6
Page	CC	Out of Net	20/04/2021	62 - 70	2 - 1X	61,8	12,3	1,05	11,7	4,7	T2 - 4
Page	CC	Out of Net	17/05/2021	59 - 68	2 - 1X	61,1	14,1	1,10	12,8	0,0	T1 - 2
Page	CC	Out of Net	09/06/2021	63 - 74	2 - 1XX	55,8	13,3	1,10	12,1	1,0	T1 - 3
RHM	CC	Net	20/04/2021	60 - 71	2 - 1X	63,6	13,3	1,00	13,3	2,0	T3 - 5
RHM	CC	Net	17/05/2021	61 - 74	2 - 1XX	68,2	14,4	1,15	12,5	1,5	T1 - 2
RHM	CC	Net	09/06/2021	69 - 76	1X - 1XX	62,4	14,2	1,05	13,5	2,3	T1
RHM	CC	Out of Net	20/04/2021	64 - 72	1 - 1XX	61,8	13,8	1,10	12,5	10,7	T3 - 5
RHM	CC	Out of Net	17/05/2021	62 - 72	2 - 1XX	61,5	14,8	1,00	14,8	5,0	T1 - 2
RHM	CC	Out of Net	09/06/2021	64 - 76	1 - 1XX	59,3	15,3	1,00	15,3	3,2	T1 - 2

Saint André	CC	Net	17/03/2021	63 - 77	2 - 1XX	52,6	10,5	1,10	9,5	0,0	T6 - 7
Saint André	CC	Net	20/04/2021	60 - 75	2 - 1XX	60,8	13,5	0,90	15,0	0,0	T3 - 4
Saint André	CC	Net	17/05/2021	60 - 66	2 - 1	61,9	15,7	1,10	14,3	0,6	T1 - 2
Saint André	CC	Net	09/06/2021	68 - 81	1X - 1XXX	56,7	13,9	1,00	13,9	0,0	T1
Saint André	CC	Out of Net	17/03/2021	69 - 73	1X - 1XX	56,6	10,4	1,15	9,0	4,9	T6 - 7
Saint André	CC	Out of Net	20-04/2021	74 - 79	1XX - 1XXX	56,3	10,7	0,85	12,6	2,7	T3 - 5
Saint André	CC	Out of Net	17/05/2021	67 - 90	1 - 1XXX	55,7	10,9	0,95	11,5	2,5	T1 - 2
Saint André	CC	Out of Net	09/06/2021	70 - 85	1X - 1XXX	51,6	11,6	1,00	11,6	1,5	T1 - 2
Samba	CC	Net	17/03/2021	57 - 62	3 - 2	60,7	11,3	1,05	10,8	5,8	T5 - 7
Samba	CC	Net	20/04/2021	58 - 71	3 - 1X	61,8	13,3	1,00	13,3	1,3	T3 - 5
Samba	CC	Net	17/05/2021	51 - 60	4 - 2	57,0	13,5	1,00	13,5	0,0	T1 - 2
Samba	CC	Net	09/06/2021	60 - 65	2 - 1	70,8	14,2	1,05	13,5	1,8	T1
Samba	CC	Out of Net	20/04/2021	56 - 69	3 - 1X	60,0	13,4	1,15	11,7	0,0	T2 - 4
Samba	CC	Out of Net	17/05/2021	58 - 64	3 - 1	59,0	14,6	1,10	13,3	0,0	T1 - 2
Samba	CC	Out of Net	09/06/2021	62 - 70	2 - 1X	56,4	14,6	1,00	14,6	1,0	T1
Sirio	CC	Net	17/03/2021	63 - 71	2 - 1X	56,0	10,6	1,50	7,1	6,5	T6 - 7
Sirio	CC	Net	20/04/2021	63 - 79	2 - 1XXX	55,2	12,7	1,25	10,2	3,2	T2 - 4
Sirio	CC	Net	17/05/2021	57 - 88	3 - 1XXX	55,1	14,0	1,35	10,4	0,0	T1
Sirio	CC	Out of Net	17/03/2021	67 - 73	1 - 1XX	54,7	11,8	1,30	9,1	4,8	T6 - 7
Sirio	CC	Out of Net	20/04/2021	68 - 76	1X - 1XX	54,4	13,7	1,45	9,4	3,3	T1 - 4
Sirio	CC	Out of Net	17/05/2021	64 - 74	1 - 1XX	55,6	13,6	1,35	10,1	1,8	T1 - 2
Sirio	CC	Out of Net	09/06/2021	68 - 77	1X - 1XX	54,3	15,4	1,15	13,4	1,9	T1 - 2
Sugar Belle	CC	Net	20/04/2021	60 - 69	2 - 1X	54,3	14,5	3,50	4,1	1,5	T3 - 5
Sugar Belle	CC	Net	17/05/2021	59 - 64	2 - 1	58,7	15,4	3,00	5,1	0,0	T1 - 2
Sugar Belle	CC	Net	09/06/2021	62 - 74	2 - 1XX	57,1	15,1	2,50	6,0	0,0	T1 - 2
Sugar Belle	CC	Net	20/07/2021	61 - 65	2 - 1	56,7	17,3	2,30	7,5	0,0	T1 - 2
Sugar Belle	CC	Net	10/08/2021	63 - 71	2 - 1X	63,8	16,4	1,60	10,3	0,0	T1 - 2
Tambor 1	CC	Net	29/06/2021	84 - 95	1XXX	46,5	13,7	1,70	8,1	4,3	T1 - 2
Tambor 1	CC	Net	10/08/2021	73 - 91	1XX - 1XXX	59,2	14,2	1,45	9,8	6,3	T1 - 2
Tambor 1	CC	Net	10/08/2021	80 - 88	1XXX	60,4	13,1	1,50	8,7	5,5	T1 - 2

Tambor 1	CC	Out of Net	09/06/2021	77 - 96	1XX - 1XXX	60,1	13,0	1,55	8,4	8,2	T1 - 3
Tambor 1	CC	Out of Net	20/07/2021	76 - 91	1XX - 1XXX	58,2	14,3	1,60	8,9	2,3	T1
Tambor 2	CC	Net	09/06/2021	75 - 89	1XX - 1XXX	60,4	12,6	1,85	6,8	7,4	T1 - 2
Tambor 2	CC	Net	29/06/2021	76 - 92	1XX - 1XXX	58,5	13,0	1,75	7,4	0,8	T1 - 2
Tambor 2	CC	Net	20/07/2021	76 - 89	1XX - 1XXX	59,2	13,1	1,70	7,7	2,8	T1
Tambor 2	CC	Out of Net	09/06/2021	75 - 84	1XX - 1XXX	64,0	14,0	1,80	7,8	4,0	T1 - 3
Tambor 2	CC	Out of Net	29/06/2021	76 - 85	1XX - 1XXX	47,1	13,5	1,60	8,4	1,9	T1 - 2
Tambor 2	CC	Out of Net	20/07/2021	79 - 95	1XXX	59,4	13,2	1,40	9,4	2,7	T1 - 2
Tambor 2	CC	Out of Net	10/8/2021	83 - 89	1XXX	60,0	14,9	1,50	9,9	5,3	T1
Tanor late	CC	Net	17/05/2021	69 - 106	1X - 1XXX	56,1	12,0	1,90	6,3	0,0	T1 - 3
Tanor late	CC	Net	09/06/2021	75 - 91	1XX - 1XXX	53,1	13,7	1,80	7,6	0,0	T1 - 3
Tanor late	CC	Net	29/06/2021	75 - 86	1XX - 1XXX	39,4	12,7	1,65	7,7	0,0	T1 - 2
Tanor late	CC	Net	20/07/2021	83 - 96	1XXX	54,8	13,1	1,55	8,5	0,0	T1
Tanor late	CC	Net	10/08/2021	81 - 90	1XXX	53,2	14,4	1,30	11,1	0,0	T1

<b>Ryton</b>											
<b>Cultivar</b>	<b>Root-stock</b>	<b>Date harvested</b>	<b>Fruit size (mm)</b>	<b>Count</b>	<b>Juice (%)</b>	<b>Brix °</b>	<b>Acid (%)</b>	<b>Ratio</b>	<b>Avg seed</b>	<b>Fruit external colour</b>	
ARCCIT 9 LS	CC	12/04/2021	53 - 65	4 - 1	51,8	10,5	1,25	8,4	1,3	T4 - 5	
ARCCIT 9 LS	CC	30/04/2021	59 - 66	2 - 1	55,0	12,3	1,20	10,3	2,5	T1 - 4	
ARCCIT 9 LS	CC	13/05/2021	55 - 66	3 - 1	56,0	11,9	1,10	10,8	0,0	T1 - 2	
ARCCIT 9 LS	CC	07/06/2021	68 - 76	1X - 1XX	56,5	12,1	1,00	12,1	1,5	T1 - 2	
Etna	CC	24/03/2021	65 - 74	1 - 1XX	58,4	9,0	1,10	8,2	8,7	T5 - 6	
Etna	CC	30/04/2021	61 - 76	2 - 1XX	59,1	11,5	0,90	12,8	8,5	T1 - 3	
Etna	CC	13/05/2021	65 - 80	1 - 1XXX	52,5	11,9	0,80	14,9	2,7	T1 - 2	
Etna	CC	07/06/2021	67 - 81	1 - 1XXX	50,0	11,3	0,85	13,3	5,7	T1 - 2	
Furr	CC	03/04/2021	68 - 76	1X - 1XX	57,9	12,1	1,25	9,7	15,2	T2 - 3	
Furr	CC	13/05/2021	68 - 77	1X - 1XX	58,0	12,6	1,20	10,5	16,1	T1 - 3	
Furr	CC	07/06/2021	72 - 76	1XX	60,6	13,4	1,10	12,2	12,8	T1 - 2	
Gold UP	CC	08/03/2021	45 - 51	5 - 4	55,7	9,7	0,95	10,2	5,2	T5 - 7	
Gold UP	CC	29/03/2021	50 - 57	5 - 3	51,3	10,5	0,85	12,4	2,7	T3 - 5	
Gold UP	CC	12/04/2021	46 - 67	5 - 1	54,1	11,3	1,00	11,3	0,0	T2 - 4	

IRM1	CC	13/05/2021	60 - 75	2 - 1XX	58,1	12,3	1,40	8,8	3,6	T1 - 3
IRM1	CC	07/06/2021	59 - 72	2 - 1XX	60,8	12,8	1,30	9,8	3,3	T1 - 3
IRM1	CC	24/06/2021	60 - 74	2 - 1XX	68,8	14,0	1,25	11,2	5,7	T1 - 2
IRM1	CC	11/08/2021	65 - 73	1 - 1XX	63,2	15,8	1,20	13,2	2,7	T - 1
IRM2	CC	07/06/2021	61 - 72	2 - 1XX	60,7	14,3	1,45	9,9	6,0	T1 - 2
IRM2	CC	24/06/2021	60 - 71	2 - 1X	58,6	13,5	1,15	11,7	4,8	T1 - 2
IRM2	CC	11/08/2021	61 - 70	2 - 1X	63,5	16,2	1,00	16,2	3,5	T - 1
Leanri	CC	29/03/2021	59 - 70	2 - 1X	54,4	11,7	1,40	8,4	3,0	T5 - 7
Leanri	CC	12/04/2021	57 - 74	3 - 1XX	58,8	13,5	1,30	10,4	2,0	T5 - 6
Leanri	CC	13/05/2021	55 - 67	3 - 1	61,5	14,7	1,35	10,9	2,8	T1 - 2
Ma'ayana	CC	12/04/2021	56 - 69	3 - 1X	61,9	12,2	1,10	11,1	3,8	T4 - 5
Ma'ayana	CC	30/04/2021	61 - 73	2 - 1XX	60,5	12,9	1,10	11,7	1,3	T1 - 3
Ma'ayana	CC	15/05/2021	56 - 70	3 - 1X	60,1	13,0	1,15	11,3	1,9	T1 - 3
Ma'ayana	CC	07/06/2021	65 - 75	1 - 1XX	55,4	13,9	1,15	12,1	2,2	T - 1
Nadorcott	CC	30/04/2021	53 - 70	4 - 1X	54,8	11,8	1,30	9,1	7,0	T2 - 5
Nadorcott	CC	13/05/2021	55 - 68	3 - 1X	53,2	14,6	1,35	10,8	5,7	T1 - 2
Nadorcott	CC	07/06/2021	68 - 78	1X - 1XX	50,3	13,2	1,05	12,6	6,9	T1 - 2
Nadorcott	CC	24/06/2021	62 - 77	2 - 1XX	50,9	14,4	1,05	13,7	8,0	T - 1
Nova	CC	29/03/2021	59 - 63	2	55,6	10,7	1,15	9,3	3,8	T5 - 6
Nova	CC	12/04/2021	61 - 66	2 - 1	61,9	11,0	1,00	11,0	2,5	T5 - 6
Nova	CC	30/04/2021	66 - 77	1 - 1XX	57,8	12,3	1,05	11,7	1,5	T1 - 4
Nova	CC	13/05/2021	65 - 72	1 - 1XX	58,3	12,0	0,95	12,6	4,0	T1 - 2
Nova	CC	07/06/2021	66 - 77	1 - 1XX	57,1	11,7	0,90	13,0	1,3	T - 1
Nova ARC	CC	29/03/2021	69 - 72	1X - 1XX	58,8	11,3	1,20	9,4	1,7	T5 - 6
Nova ARC	CC	12/04/2021	65 - 72	1 - 1XX	61,0	12,1	1,00	12,1	3,0	T4 - 5
Nova ARC	CC	13/05/2021	63 - 69	2 - 1X	57,9	14,0	1,10	12,7	0,0	T1 - 2
Nova ARC	CC	30/05/2021	59 - 72	2 - 1XX	58,3	12,8	1,05	12,2	2,7	T1 - 3
Nova ARC	CC	07/06/2021	71 - 80	1X - 1XXX	57,9	13,1	1,00	13,1	0,0	T - 1
Orr 1	CC	13/05/2021	60 - 75	2 - 1XX	54,5	13,3	1,75	7,6	2,0	T1 - 2
Orr 1	CC	7/06/2021	63 - 75	2 - 1XX	57,5	13,7	1,55	8,8	1,7	T1 - 2
Orr 1	CC	24/06/2021	64 - 78	1 - 1XXX	57,6	14,3	1,30	11,0	2,4	T1 - 2
Orr 4	CC	13/05/2021	56 - 72	3 - 1XX	54,2	13,1	1,30	10,1	1,6	T1 - 2
Orr 4	CC	07/06/2021	64 - 74	1 - 1XX	55,9	13,2	1,10	12,0	1,2	T1 - 2
Orr 4	CC	24/06/2021	60 - 74	2 - 1XX	55,6	13,6	1,00	13,6	3,4	T - 1
Page	CC	29/03/2021	62 - 65	2 - 1	56,7	9,7	1,10	8,8	4,0	T5 - 6
Page	CC	12/04/2021	55 - 70	3 - 1X	55,0	12,0	1,10	10,9	2,7	T3 - 5
Page	CC	30/04/2021	59 - 79	2 - 1XXX	58,2	13,0	1,00	13,0	3,3	T1 - 3
Page	CC	13/05/2021	54 - 67	4 - 1	58,3	13,6	0,90	15,1	2,3	T1 - 2
RHM	CC	12/04/2021	59 - 63	2 - 2	61,1	11,7	0,90	13,0	7,0	T5 - 6
RHM	CC	30/04/2021	58 - 64	3 - 1	59,8	13,5	0,95	14,2	4,8	T1 - 3
RHM	CC	13/05/2021	50 - 60	5 - 2	63,2	14,5	0,90	16,1	6,2	T1 - 2
RHM	CC	07/06/2021	64 - 69	1 - 1X	62,5	14,3	0,95	15,1	5,2	T - 1
Saint André	CC	29/03/2021	64 - 70	1 - 1XX	54,1	10,5	0,85	12,4	9,0	T5 - 7

Saint André	CC	12/04/2021	65 - 74	1 - 1XX	58,8	11,1	0,95	11,7	3,6	T5 - 6
Saint André	CC	30/04/2021	62 - 78	2 - 1XXX	56,2	11,7	0,95	12,3	3,2	T1 - 3
Saint André	CC	13/05/2021	62 - 75	2 - 1XX	57,8	12,4	0,95	13,1	4,3	T1 - 3
Samba	CC	08/03/2021	50 - 59	5 - 2	53,3	9,9	1,30	7,6	4,0	T5 - 6
Samba	CC	29/03/2021	54 - 63	4 - 2	57,4	11,0	1,20	9,2	3,2	T4 - 6
Samba	CC	12/04/2021	55 - 63	3 - 2	60,5	11,8	1,15	10,3	3,9	T3 - 5
Samba	CC	30/04/2021	60 - 71	2 - 1X	59,7	12,2	1,00	12,2	2,7	T1 - 3
Samba	CC	13/05/2021	54 - 64	4 - 1	60,7	13,1	1,00	13,1	1,5	T1 - 2
Shani SL	CC	30/04/2021	54 - 69	4 - 1X	55,6	13,3	1,75	7,6	1,8	T1 - 3
Shani SL	CC	13/05/2021	55 - 63	3 - 2	56,6	13,2	1,50	8,8	0,0	T1 - 3
Shani SL	CC	07/06/2021	61 - 68	2 - 1X	58,7	14,6	1,70	8,6	0,0	T1 - 2
Shani SL	CC	24/06/2021	63 - 71	2 - 1X	55,8	12,9	1,10	11,7	0,0	T - 1
Sirio	CC	12/04/2021	63 - 72	2 - 1XX	54,2	11,6	1,10	10,5	3,7	T3 - 5
Sirio	CC	30/04/2021	63 - 75	2 - 1XX	52,4	11,7	1,10	10,6	9,3	T1 - 4
Sirio	CC	30/05/2021	65 - 77	1 - 1XX	52,0	12,4	0,95	13,1	5,4	T1 - 3
Sirio	CC	07/06/2021	62 - 77	2 - 1XX	50,8	12,2	0,95	12,8	12,5	T - 1
Tambor 1	CC	07/06/2021	74 - 84	1XX - 1XXX	54,8	11,6	1,40	8,3	10,2	T1 - 3
Tambor 1	CC	11/08/2021	72 - 89	1XX - 1XXX	54,6	12,8	1,15	11,1	4,8	T - 1
Tambor 2	CC	07/06/2021	80 - 91	1XXX	56,6	13,0	1,25	10,4	8,0	T2 - 3
Tambor 2	CC	24/06/2021	81 - 84	1XXX	58,7	11,3	1,10	10,3	16,3	T1 - 2
Tambor 2	CC	11/08/2021	77 - 90	1XX - 1XXX	53,5	12,2	1,10	11,1	4,8	T1 - 3
Tanor Late	CC	07/06/2021	69 - 81	1X - 1XXX	53,2	12,2	1,85	6,6	0,0	T1 - 3
Tanor Late	CC	24/06/2021	74 - 83	1XX - 1XXX	39,9	12,6	1,40	9,0	1,9	T - 1
Tanor Late	CC	11/08/2021	72 - 78	1XX - 1XXX	58,8	13,4	1,00	13,4	0,0	T - 1
Tasty 1	CC	13/05/2021	66 - 76	1 - 1XX	44,4	11,9	0,85	14,0	6,9	T1 - 3
Tasty 1	CC	07/06/2021	71 - 80	1X - 1XXX	48,1	11,9	0,85	14,0	7,0	T1 - 2
Tasty 1	CC	24/06/2021	76 - 90	1XX - 1XXX	43,7	12,4	0,70	17,7	7,4	T - 1
Taylor Lee	CC	30/04/2021	69 - 76	1X - 1XX	58,7	13,2	1,30	10,2	10,8	T1 - 3
Taylor Lee	CC	13/05/2021	67 - 75	1 - 1XX	59,8	13,5	1,25	10,8	4,0	T1 - 2
Taylor Lee	CC	07/06/2021	61 - 75	2 - 1XX	61,0	14,1	1,20	11,8	8,2	T1 - 2
Taylor Lee	CC	24/06/2021	70 - 78	1X - 1XXX	62,7	14,4	1,10	13,1	10,3	T - 1

#### 5.4.11 PROGRESS REPORT: Evaluation of Lemon selections in the hot dry production areas (Letsitele and Hoedspruit)

Project 75D by J Joubert (CRI)

##### Summary

The 2021 season produced the fourth crop on the trees for the Lemon trial at Letsitele and first crop at Hoedspruit. Willowtree Long bore their third fruit set at this trial site for the northern areas due to high temperatures and the typical lemon growth rate (aggressive). Lisbon and Eureka had a better fruit set than the remaining lemon selections at the trial site. Lisbon Yen Ben developed the smallest fruit on the trees (count 138 to 100) compared to the other selections. High temperatures during the flowering periods induced poor fruit set on some of the selections. The selections were planted in November 2013 and 2017 on MxT, X639, US-812 and RL rootstock, due to the compatibility with the Eureka-type lemons.

##### Opsomming

Die 2021 seisoen was die vierde drag op die bome gewees vir die suurlemoen proef by Letsitele, en eerste drag in Hoedspruit. Dit was Willowtree Long se derde vrugset by hierdie proef blok vir die noordelike areas as gevolg van die hoë temperature en tipiese suurlemoen groeitempo (groeikragtig). Lisbon en Eureka het 'n beter vrugset gehad as die res van die suurlemoen seleksies op hierdie proef perseel. Lisbon Yen Ben ontwikkel die kleinste vruggrootte op die bome (telling 138 tot 100) in vergelyking met die ander seleksies. Hoë temperature gedurende blom periodes het swak vrugset tot gevolg gehad vir sekere van die seleksies. Die seleksies was geplant in November 2013 en 2017 op MxT, X639, US-812 and RL, vir die verenigbaarheid met Eureka-tipe suurlemoene.

##### Objectives

- To find Lemon selections suitable for the intermediate production area.
- To produce lemon selections with Eureka like fruit shape (elongated), high juice content, everbearing characteristics, low seed content and high rind oil for processing purposes.

##### Materials and methods

Field evaluations were conducted at Bosveld Citrus (Letsitele) and Unifrutti (Hoedspruit).

**Table 5.4.11.1.** List of lemon selections evaluated at Bosveld Citrus (Letsitele) during the 2021 season.

Selection	Rootstock	Planted
Eureka	X639	2013
Feminello	X639	2013
Genoa	X639	2013
Limoneira	X639	2013
Lisbon	X639	2013
Lisbon Yen Ben	X639	2013
Plaat B	X639	2013
Willowtree Long	X639	2013

**Table 5.4.11.2.** List of lemon selections evaluated at Unifrutti (Hoedspruit) during the 2021 season.

Selection	Rootstock	Planted
Eureka	MxT/X639	2017
2PH SL Eureka	MxT/US-812/X639	2017
Willowtree Long	MxT/RL/US-812/X639	2017

## Results and discussion

The lemon trial at Unfrutti cropped their first fruit on the trees this season to start of the evaluation process in Hoedspruit.

Plaat B (36.7%), Willowtree Long (37.8%) and Lisbon (38.7%) developed the lowest juice percentages for the season at the first evaluation, but Lisbon Yen Ben had the highest juice percentage of 60.7%, followed by Lisbon (second evaluation) with 50.0%. Genoa produced the biggest fruit size at Letsitele and peaked from count 113 to count 64 through the season, but 2 PH SL peaked at count 64 in Hoedspruit. The highest seed content per fruit was also on Lisbon (up to 17.4 seeds per fruit), followed by Limoneira 8A (up to 13.8 seeds per fruit). The external colour ranged from T4 to T6 at both trial sites.

## Conclusion

Eureka produced elongated fruit; Willowtree Long and Plaat B was the only other selections with a more elongated type fruit on the trees, the rest were fairly round.

For the fourth season a good to very good crop was produced on the trees at Bosveld, the yield will increase at Unifrutti due to younger tree age. The lemon selections were not that vigorous and tree canopy was less dense. High temperatures can affect the fruit set and as well as the juice percentages. The four commercial Lemon selections; Eureka, Lisbon, Limoneira and Genoa performed well and seems more suitable for the hot production areas than the other experimental selections, except for 2PH SL Eureka also looking promising.

**Table 5.4.11.3.** Internal fruit quality data for Lemon selections at Bosveld Citrus (Letsitele) during the 2021 season.

<b>Bosveld</b>							
<b>Cultivar</b>	<b>Root-stock</b>	<b>Date harvested</b>	<b>Fruit size (mm)</b>	<b>Count</b>	<b>Juice (%)</b>	<b>Avg seed</b>	<b>Fruit external colour</b>
Eureka	X639	22/02/2021	55 - 70	162 - 75	44,5	6,5	T5 - 6
Eureka	X639	24/03/2021	61 - 72	113 - 64	46,6	9,1	T3 - 5
Feminele	X639	22/02/2021	57 - 68	138 - 88	41,7	1,3	T5 - 6
Feminele	X639	24/03/2021	61 - 74	113 - 64	46,4	3,3	T4 - 6
Genoa	X639	22/02/2021	61 - 76	113 - 64	41,8	7,7	T5 - 6
Genoa	X639	24/03/2021	64 - 73	100 - 64	47,5	13,1	T3 - 6
Lisbon	X639	22/02/2021	60 - 70	113 - 75	38,7	15,7	T5 - 6
Lisbon	X639	24/03/2021	67 - 74	88 - 64	50,0	17,4	T4 - 6
Lisbon Yen Ben	X639	22/02/2021	58 - 60	138 - 113	45,5	1,1	T5 - 6
Lisbon Yen Ben	X639	24/03/2021	56 - 65	138 - 100	60,7	0,9	T3 - 5
Limoneira 8A	X639	22/02/2021	62 - 71	113 - 75	40,3	7,0	T5 - 7
Limoneira 8A	X639	24/03/2021	64 - 73	100 - 64	49,8	13,8	T3 - 5
Plaat B	X639	22/02/2021	55 - 68	162 - 88	36,7	2,7	T5 - 6
Plaat B	X639	24/03/2021	57 - 68	138 - 88	43,1	6,0	T4 - 6
Willowtree Long	X639	22/02/2021	63 - 75	100 - 64	47,3	10,2	T5 - 6
Willowtree Long	X639	24/03/2021	64 - 75	100 - 64	48,2	0,1	T4 - 6

Unifrutti							
Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Avg seed	Fruit external colour
Eureka	X639	12/02/2021	62 - 72	113 - 64	43,7	9,0	T3 - 6
Eureka	MxT	12/02/2021	57 - 65	138 - 100	46,2	7,8	T5 - 6
2PH SL Eureka	X639	12/02/2021	63 - 82	100 - 64	40,7	0,0	T3 - 5
2PH SL Eureka	MxT	12/02/2021	63 - 74	100 - 64	46,8	0,0	T4 - 6
2PH SL Eureka	US-812	12/02/2021	64 - 75	100 - 64	43,0	0,0	T5 - 6
Willowtree Long	X639	12/02/2021	59 - 76	113 - 64	46,3	6,2	T5 - 6
Willowtree Long	RL	12/02/2021	65 - 74	100 - 64	37,8	5,2	T4 - 6
Willowtree Long	MxT	12/02/2021	59 - 76	113 - 64	44,3	7,0	T5 - 6
Willowtree Long	US-812	12/02/2021	54 - 74	162 - 64	46,5	8,1	T5 - 6

#### 5.4.12 PROGRESS REPORT: Evaluation of Valencia selections in the intermediate production areas (Nelspruit)

Project 963 A by J Joubert (CRI)

##### Summary

Selections that performed well in this season, in this intermediate production area, according to optimal maturity from early to late, were as follows. Val early, one of the new early maturing (internal quality) cultivars that mature before Turkey. There was a better colour development on the fruit by the time of optimum maturity with Valearly, but deeper orange colour compared to the more yellow of Turkey when fully coloured. Turkey will follow, but bear in mind that this selection has a sensitive rind. Do not allow the fruit to hang for too long because the optimal picking period is no longer than 4-6 weeks.

Bely would follow, with good internal quality, production and fruit size, followed by the Midnight range. McClean SL and Gusocora represent this area's middle of the Valencia season. Alpha will be next in line, maturing later in this area than other areas (Letsitele), but keep the younger tree age in mind. The later selections can broaden the list of choices to extend the season, commencing with Henrietta, Kobus du Toit Late, Jasi (optimum fruit size distribution) and Louisa; followed by Lavalley with higher acid levels an ultra-late possibility.

##### Opsomming

Seleksies wat hierdie seisoen, volgens optimum rypheid van vroeg tot laat goed presteer het vir hierdie intermediere produksie area, is soos volg. Valearly is een van die nuwe vroeë Valencia opsies (vroeg intern ryp) wat voor Turkey inpas. Daar was 'n beter kleurontwikkeling op die vrugte gewees met optimum rypheid by Valearly, maar wel dieper orange kleur wanneer opgekleur in vergelyking met meer geel by Turkey. Turkey sal dan volg, wees net versigtig om nie die seleksie te lank te hang nie. Baie skil probleme kan ontwikkel, want die optimum oes tydperk strek oor gemiddeld vier weke, ses weke maksimum dan moet die vrugte af wees.

Bely kan dan volg wat goeie interne kwaliteit, produksie en vruggroote lewer, gevolg deur die Midnight reeks. McClean SL en Gusocora wat dan die middel van die Valencia seisoen vir hierdie area verteenwoordig. Alpha pas dan in, wat later in hierdie area rypword in vergelyking met ander areas (Letsitele), maar hou die jong boom ouderdom in gedagte. Die later seleksies wat kan bydra tot die keuse om die seisoen te verleng, kan bestaan uit Henrietta, Kobus du Toit Laat, Jasi (optimum vruggroote verspreiding) en Louisa; gevolg deur Lavalley met hoër suurvlakke as die ultra-laat opsie.

##### Objective

- To find suitable Valencia selections with superior characteristics for the intermediate citrus production areas.

## Materials and methods

Field evaluations and laboratory analyses were conducted at Karino-koöp in Nelspruit, Mpumalanga.

**Table 5.4.12.1.** Internal fruit quality minimum export requirements for Valencia types.

Variety	Juice %	Brix °	Min Acids	Max Acids	Ratio	Colour
Valencia EU	48	8.5	0.6	1.8%	7.5:1	Colour plate 3 of set no. 34
Midnight	52	9.5	0.85	1.8%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

\*Interim internal fruit quality standards.

**Table 5.4.12.2.** List of Valencia selections evaluated at Karino-koöp (Nelspruit) during 2021.

Selection	Rootstock	Planted
Alpha	CC	2017
Beli	CC	2017
Gusocora	CC	2017
Henrietta	CC	2017
Jasi	CC	2017
Kobus du Toit Late	CC	2017
Lavalle	CC	2017
Louisa	CC	2017
Midnight (control)	CC	2017
Midnight 1 (I15)	CC	2017
Midnight 2 (H14)	CC	2017
Midnight 3 (F17)	CC	2017
McClellan SL	CC	2017
Turkey	CC	2017
Valearly	CC	2017

## Results and discussion

This project is ongoing – all evaluations and tasks have been completed to date. Trees were visually evaluated at Karino-koöp during the 2021 season.

Alpha, Gusocora and Lavalle bore a good crop for the second time this season and evaluations were possible, all planted onto Carrizo rootstocks. All three selections were completely seedless. This year Gusocora matured first; end of June to the middle of July with good acid levels and good Brix, ranging from 10.8 to 11.4. The highest acid level between the three cultivars was on Lavalle; also developing very good Brix and juice content. Colour development remained very similar between T1 and T3 up to peak maturity. Fruit size peaked on two of the three selections, except for Lavalle with bigger fruit, between count 88/72 and 56/48; very good for Valencia production and export.

Jasi were bearing another good crop this year; The fruit size was ideal and varied from medium to large, count 72 to 56 (average). The rind texture improved this season, becoming smoother with time. Seed count per fruit was similar (low pollination) this season and varied from 2.5 to 4.2 seeds per fruit. Internal quality improved with tree age and produced better juice levels (above 56% at peak maturity), good Brix (above 10 at maturity) and higher acids (above 1.2). External colour development improved and peaked between T1 and 2 with the final evaluations.

Kobus du Toit Late was evaluated at the Karino site outside Nelspruit on Carrizo and produced medium to large fruit size (count 88 to 56) on the trees due to a better crop, with 2.8 seeds average. The colour development improved and peaked from T1 to 2. The internal quality was good, juice levels above 52%, Brix up to 11.1, and high acids earlier in the season (up to 1.25% on CC) for the later maturing selection.

McClellan SL produced fairly round fruit with soft fibre strength that peeled easily, containing low rind oil levels. All the fruit evaluated remained 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 (GA<sub>3</sub> sprays are recommended). The fruit size increased and peaked at medium-large to large (count 88/72-56). The internal quality improved to very good with high juice levels for the trial site up to 58%, Brix up to 11 and higher acid levels (above 1.05%). There was an improvement on external colour ranging from T1-2 compared to a delay the previous season.

Fruit size on Turkey was smaller this season, ranging from count 88 to 56 average, with medium to large fruit size. Fruit characteristics for Turkey were round fruit shape, smooth rind texture, very good flavour, soft rag, fairly thin rind, easy fruit peeling, and lower seed count per fruit (seedless). The internal colour was light yellow, and externally the fruit remained yellow up to over-matured fruit. It should be borne in mind that this selection is not a true Valencia and has the qualities of a mid-season orange; for instance, the exceptionally soft rag of the fruit, and the soft rind result in rind problems if managed incorrectly. Turkey should not be harvested over more than four weeks as extending the harvesting season can lead to rind disorders

Valearly, bearing a light crop on the trees, developed seedless fruit) this season. The internal quality of the fruit was good early in the season with medium-high juice (up to 53%), Brix above 10 and acid above 0.8 on Carrizo. Valearly seems to be at least two weeks earlier than the other early maturing selections, with good internal quality but slightly delayed external colour ranging from T2-3.

Henrietta performed well; juice levels peaked above 57%, with higher Brix (above 10) and acids 1.40. The external colour development improved and peaked between T1 and T3 for the season. Average seeds per fruit increased to 2.0 seeds per fruit (0.1 seeds for 2020). Louisa cropped a lighter crop, fruit was completely seedless and internal quality was good this season with higher juice (53.6% vs 50.6% in 2020) and good Brix levels (up to 11.1). The fruit colour was fairly yellow by the time of peak maturity between T1 and T2. Fruit size peaked from medium to large, count 72 to count 56.

#### Midnight (control), 1, 2 and 3

All the Midnight selections bore a fairly light crop, except 3 with a good crop on the trees. The fruit size this year was very uniform and ranged between count 88/72 and 56, juice content was around 53%, Brix levels better around 10.6 (average) and acids around 1.0% (higher on Midnight 3). All the fruit evaluated this season was seedless. The characteristic Midnight die-back was more visible on Midnight 1 compared to Midnight 2. Fruit shape was round, rind texture was fairly smooth, and fruit was raggy with a medium rind thickness which peeled moderately. Bely compared with Midnight at the trial site; juice up to 57%, Brix 10 and lower acids (avg. 1.0%).

### **Conclusions**

The internal quality for all the selections evaluated complied with the export standards at peak maturity of the fruit. These acid levels will decrease towards the end of the season, indicating extended shelf-life of the selection for example, Jasi Kobus du Toit Late and Laval. Jasi also indicated low chimera fruit numbers on the trees, providing another good late maturing Valencia option to be included in future plantings. There was no Brix: acid ratio below 7.5:1 at peak maturity this season, which is often associated with later maturing selections having higher acid levels, but rather the opposite scenario with lower acids in general on most of the fruit samples being evaluated. When the acid levels decrease, the ratio increases. There was a better colour development with all of the selections towards peak maturity time, even in the case of Valearly, where the colour development was slightly delayed even after peak quality (T2-3). The average seed count for this season remained fairly low, including Henrietta, Kobus du Toit Late and Jasi (average 2.0, 2.8 and 3.1 seeds per fruit), indicating lower cross-pollination in the mixed trial block. McClellan SL remained completely seedless.

Jasi and Kobus du Toit Late will be future possibilities to include in new Valencia plantings (optimum Valencia fruit size distribution, high juice levels, low seed counts and late maturing).

**Table 5.4.12.3.** Internal fruit quality data for Valencia selections at Karino-koöp (Nelspruit) during the 2021 season.

Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
Alpha	CC	03/06/2021	78 - 89	64 - 48	54,4	10,6	1,50	7,1	0,0	T1 - 2
Alpha	CC	24/06/2021	74 - 80	72 - 64	55,5	11,1	1,45	7,7	0,0	T1
Alpha	CC	14/07/2021	80 - 82	64 - 56	56,4	11,1	1,30	8,5	0,0	T1 - 2
Beli	CC	25/05/2021	73 - 80	72 - 64	52,4	10,4	0,95	10,9	0,0	T2 - 3
Beli	CC	03/06/2021	70 - 83	88 - 56	57,1	9,8	1,20	8,2	0,0	T1 - 2
Beli	CC	16/06/2021	72 - 81	88 - 64	55,7	10,6	0,95	11,2	0,0	T1
Beli	CC	24/06/2021	74 - 84	72 - 56	51,1	11,1	0,85	13,1	0,0	T1 - 2
Beli	CC	07/07/2021	78 - 82	64 - 56	55,0	10,4	0,80	13,0	0,0	T1
Gusocora	CC	03/06/2021	74 - 78	72 - 64	55,8	10,8	1,25	8,6	0,0	T2 - 3
Gusocora	CC	24/06/2021	72 - 84	88 - 56	55,9	11,4	1,10	10,4	0,0	T1 - 2
Gusocora	CC	19/07/2021	75 - 81	72 - 64	56,3	11,4	1,00	11,4	0,0	T1 - 2
Henrietta	CC	03/06/2021	78 - 85	64 - 56	57,3	9,2	1,50	6,1	1,8	T1 - 3
Henrietta	CC	24/06/2021	75 - 84	72 - 56	55,2	9,8	1,55	6,3	1,3	T1 - 3
Henrietta	CC	19/07/2021	75 - 79	72 - 64	60,3	10,5	1,25	8,4	1,7	T1 - 3
Henrietta	CC	13/08/2021	81 - 86	64 - 48	56,9	10,1	1,25	8,1	3,3	T1 - 3
Jasi	CC	03/06/2021	76 - 80	72 - 64	57,3	10,0	1,60	6,3	4,2	T2 - 3
Jasi	CC	24/06/2021	76 - 82	72 - 56	52,6	10,6	1,55	6,8	2,5	T1 - 3
Jasi	CC	19/07/2021	75 - 79	72 - 64	55,8	10,7	1,15	9,3	2,5	T1
Jasi	CC	13/8/2021	77 - 84	72 - 56	54,4	10,7	1,25	8,6	3,3	T1 - 2
K du Toit Late	CC	24/06/2021	69 - 81	88 - 64	54,6	10,5	1,65	6,4	2,2	T2 - 3
K du Toit Late	CC	19/07/2021	80 - 84	64 - 56	50,7	11,1	1,25	8,9	2,5	T1 - 2
K du Toit Late	CC	13/08/2021	79 - 93	64 - 40	51,8	10,8	1,00	10,8	3,7	T1 - 2
Lavalle	CC	03/06/2021	80 - 92	64 - 40	57,4	10,1	2,15	4,7	0,0	T2 - 3
Lavalle	CC	24/06/2021	77 - 85	72 - 56	57,0	11,0	2,10	5,2	0,0	T1 - 3
Lavalle	CC	19/07/2021	79 - 88	64 - 48	57,9	11,4	1,80	6,3	0,0	T1 - 3
Lavalle	CC	13/08/2021	89 - 90	64 - 40	55,8	11,3	1,75	6,5	0,0	T1 - 3
Louisa	CC	24/06/2021	74 - 80	72 - 64	51,5	10,5	1,45	7,2	0,0	T1 - 3
Louisa	CC	19/07/2021	73 - 81	72 - 64	55,6	10,9	1,35	8,1	0,0	T1 - 2
Louisa	CC	13/08/2021	79 - 83	64 - 56	53,5	11,1	1,05	10,6	0,0	T1 - 2
Midnight (Control)	CC	13/05/2021	73 - 82	72 - 56	51,5	10,8	1,25	8,6	0,0	T3 - 5

Midnight (Control)	CC	03/06/2021	75 - 80	72 - 64	63,1	10,2	1,05	9,7	0,0	T1 - 3
Midnight (Control)	CC	24/06/2021	73 - 87	72 - 48	49,2	10,9	1,00	10,9	0,0	T1 - 2
Midnight (Control)	CC	19/07/2021	73 - 83	72 - 56	50,4	11,7	0,85	13,8	0,0	T1 - 2
Midnight 1 (I15)	CC	03/05/2021	72 - 80	88 - 64	52,3	9,4	1,05	9,0	0,0	T2 - 4
Midnight 1 (I15)	CC	03/06/2021	75 - 78	72 - 64	55,6	9,5	0,85	11,2	0,0	T2 - 3
Midnight 1 (I15)	CC	24/06/2021	74 - 84	72 - 56	52,5	10,0	0,90	11,1	0,0	T1 - 2
Midnight 1 (I15)	CC	19/07/2021	75 - 82	72 - 64	54,4	10,7	0,85	12,6	0,0	T1 - 2
Midnight 2 (H14)	CC	13/05/2021	77 - 83	72 - 56	47,8	10,0	0,95	10,5	0,0	T2 - 4
Midnight 2 (H14)	CC	03/06/2021	73 - 87	72 - 48	52,7	11,0	0,90	12,2	0,0	T1 - 2
Midnight 2 (H14)	CC	24/06/2021	74 - 83	72 - 56	58,2	10,2	0,85	12,0	0,0	T1 - 2
Midnight 2 (H14)	CC	19/07/2021	74 - 82	72 - 64	46,3	12,2	0,75	16,3	0,0	T1 - 2
Midnight 3 (F17)	CC	13/05/2021	73 - 83	72 - 56	51,9	10,1	1,35	7,5	0,0	T2 - 5
Midnight 3 (F17)	CC	03/06/2021	72 - 83	88 - 56	55,1	10,9	1,05	10,4	0,0	T1 - 2
Midnight 3 (F17)	CC	24/06/2021	72 - 84	88 - 56	55,5	10,9	0,90	12,1	0,0	T1 - 3
Midnight 3 (F17)	CC	19/07/2021	75 - 80	72 - 64	54,9	11,4	0,95	12,0	0,0	T1 - 2
McClellan SL	CC	03/06/2021	73 - 86	72 - 68	57,7	10,0	1,30	7,7	0,0	T1 - 3
McClellan SL	CC	24/06/2021	72 - 80	88 - 64	54,7	10,6	1,25	8,5	0,0	T1
McClellan SL	CC	19/07/2021	75 - 80	72 - 64	58,0	11,0	1,05	10,5	0,0	T1 - 2
McClellan SL	CC	13/08/2021	74 - 89	72 - 48	56,1	10,7	1,05	10,2	0,0	T1
Turkey	CC	13/05/2021	75 - 84	72 - 56	54,8	9,9	1,15	8,6	0,0	T2 - 3
Turkey	CC	03/06/2021	74 - 87	72 - 48	58,8	11,1	0,95	11,7	0,0	T1
Turkey	CC	24/06/2021	76 - 82	72 - 56	56,2	10,9	0,95	11,5	0,0	T1
Valearly	CC	13/05/2021	77 - 82	72 - 56	53,0	10,4	0,85	12,2	0,0	T2 - 3
Valearly	CC	03/06/2021	75 - 87	72 - 48	53,0	10,2	0,80	12,8	0,0	T1 - 2
Valearly	CC	24/06/2021	77 - 89	72 - 48	48,9	11,9	0,75	15,9	0,0	T1

**5.4.13 PROGRESS REPORT: Evaluation of Mandarin Hybrid on different rootstocks in a cold production area (Buffeljagsrivier)**  
Project 1331 by W Swiegers (CRI)

**Summary**

The bud-unions visual evaluations indicated that the unions were still in good condition and the combinations compatible (need to confirm once the trial comes to an end). US 812 is a hybrid rootstock cross between a US mandarin and Beneke trifoliolate (US812). The tree size of this combination is described as medium (similar to

Carrizo tree size and growth rate). In combination with Tanor Late, the tree was bigger compared to Citrange 35 (C35) (dwarfing rootstocks) and smaller Carrizo citrange (CC). Yield production was good for a first crop. Tanor Late had the highest yield in combination with Swingle Citrumelo (SC), Rough Lemon (RL) and C35. Tanor Late, combined with Mxt, CC and SC had the best internal quality. Fruit was low seeded. The following combinations; RL, MxT, US 812 and X639 fruit size ranged peaked with counts 1x – 1xxx and SC, CC and C35 fruit size count ranged peaked between counts 1 – 1xxx . Colour development on all the rootstock combinations was good. Future evaluations will determine the adaptability of these rootstocks bearing in mind the soils and constant wetness during winter.

## Opsomming

Visuele evaluasies van die entlas, dui op 'n gesonde entlas verbinding, dit bewys dit is verenigbaar met die kombinasies (moet bevestig word wanner die proef tot 'n einde kom). US 812 is 'n hibried onderstamkruising tussen US mandaryn en Beneke trifoliaat (US 812). Die boomgrootte van hierdie kombinasie word as medium beskou (vergeelyk met Carrizo boomgrootte en groeikragtigheid). In kombinasie met Tanor Late was die boomgrootte bietjie groter as Citrange 35 (verdwergde onderstamme) en kleiner as Carrizo citrange (CC). Die produksie was goed aangesien dit die boom se eerste drag was. Tanor Late het die hoogste produksie in kombinasie met Swingle Citrumelo (SC), Rough Lemon (RL) en C35. Tanor Late in kombinasie Mxt, CC en SC het die beste interne kwaliteit. Die vrugte het lae saad gehad. Die volgende kombinasies; RL, MxT, US 812 en X639 het tussen vruggrootte 1x – 1xxx gepiek. SC, CC en C35 het tussen 1 – 1xxx vruggrootte gepiek. Kleurontwikkeling op al die onderstam kombinasies was goed. Verdere evaluasies sal die aanpasbaarheid van hierdie onderstamme bevestig, gegewe die grond en die konstante natheid gedurende die wintermaande.

## Objectives

- To investigate the performance of Tanor Late Mandarin Hybrid on suitable rootstocks in a cold citrus growing area on virgin soils.
- To improve production, internal quality, rind colour and fruit size count distributions.

## Materials and methods

Seeds of Citrange 35, Carrizo Citrange, Rough Lemon, US 812, Swingle Citrumelo, MxT and X639 were propagated by Cederberg Nursery, a CIS accredited nursery in the Citrusdal region of the Western Cape.

Tanor Late Mandarin hybrid were budded onto the following rootstocks at Cederberg nursery in 2014: Citrange 35, Carrizo Citrange, Rough Lemon, US 812, Swingle Citrumelo, MxT and X639. The trees were planted at Buffeljagsrivier in 2018.

**Table 5.4.13.1.** The number of trees per rootstock in the Tanor Late mandarin hybrid trial at Buffeljagsrivier.

Selection	Rootstock	No. of trees
Tanor Late	MxT	10
Tanor Late	C35	10
Tanor Late	CC	10
Tanor Late	RL	10
Tanor Late	US 812	7
Tanor Late	SC	10
Tanor Late	X639	9

## Results and discussion

The trial was harvested for the first time this season with a good crop on the trees. All the combinations reached T1 on the colour plate before reaching peak maturity. Juice content with all the rootstock combinations was very good above 55%. Tanor Late in combination with US 812 had the lowest crop, yielding 44,5 kg/tree with

avg. tree volume 3.5 m<sup>3</sup>. On the time of harvest the internal quality was delayed compared to RL, SC, CC and MxT. The combination had a good Brix but still with high acid 1.57%; fruit size peaked count 1xx. The combinations with the highest crop was SC (67 kg/tree; tree volume 5.3 m<sup>3</sup>) and RL (66,4 kg/tree; tree volume 7.7 m<sup>3</sup>). RL combination did have the biggest tree canopy. Tanor Late with SC as rootstock had a very good internal quality and better than RL. SC and RL combination fruit size peaked count 1xx. Tanor Late on C35 had the third highest crop and the smallest tree volume, with the highest acid and lowest ratio.

## Conclusion

The seed content in Tanor Late fruit remained significantly low. Fruit size distribution from all the rootstock combinations mostly peaked count 1xx. There was no problem with color development being T1 on the color plate. All the rootstock combination's juice content was very good above 55%. The best performing rootstock taking internal quality, yield and tree volume into account, was SC, CC and MxT. The most important combination of the above-mentioned was US 812 (US mandarin x Beneke trifoliolate). US 812 was selected for a specific high pH and calcareous soils for replanting conditions. The evaluations and harvests indicated that the other rootstocks outperformed US 812, future evaluations will be crucial to determine the best combination in these conditions.

**Table 5.4.13.2.** Internal fruit quality of Tanor Late mandarin hybrid on different rootstocks at Tangelo (Buffeljagsrivier) on 1 September 2021.

Cultivar	Rootstock	Size (mm)	Size (Count)	Juice %	Brix	Acid %	Ratio:	Seeds	Colour
Tanor Late	RL	74,3	1xx	56,3	11,1	0,91	12,2	1,2	T1
Tanor Late	SC	70,2	1x	59,2	12,9	1,31	9,9	1,0	T1
Tanor Late	CC	70,8	1x	59,3	13,8	1,31	10,5	1,8	T1
Tanor Late	US 812	73,3	1xx	61,6	13,3	1,57	8,5	1,0	T1
Tanor Late	X639	69,9	1x	58,5	13,2	1,48	8,9	0,5	T1
Tanor Late	C35	71,0	1x	58,5	13,6	1,66	8,2	1,2	T1
Tanor Late	MxT	74,5	1xx	59,0	13,2	1,22	10,9	1,2	T1

**Table 5.4.13.3.** Fruit size distribution at Tangelo during the 2021 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Tanor Late	RL	1xxx	20,16	Tanor Late	US 812	1xxx	21,53
Tanor Late	RL	1xx	30,31	Tanor Late	US 812	1xx	37,42
Tanor Late	RL	1x	16,41	Tanor Late	US 812	1x	16,61
Tanor Late	RL	1	7,84	Tanor Late	US 812	1	6,45
Tanor Late	RL	2	2,48	Tanor Late	US 812	2	2,18
Tanor Late	RL	3	1,62	Tanor Late	US 812	3	1,54

Tanor Late	RL	4	0,53	Tanor Late	US 812	4	0,26
Tanor Late	RL	5	0,08	Tanor Late	US 812	5	0,04
<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
Tanor Late	SC	1xxx	12,21	Tanor Late	X639	1xxx	19,55
Tanor Late	SC	1xx	31,69	Tanor Late	X639	1xx	36,94
Tanor Late	SC	1x	23,59	Tanor Late	X639	1x	19,69
Tanor Late	SC	1	14,9	Tanor Late	X639	1	8,59
Tanor Late	SC	2	6,14	Tanor Late	X639	2	2,54
Tanor Late	SC	3	3,67	Tanor Late	X639	3	1,7
Tanor Late	SC	4	1,43	Tanor Late	X639	4	0,38
Tanor Late	SC	5	0,1	Tanor Late	X639	5	0,02
<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
Tanor Late	MxT	1xxx	19,76	Tanor Late	C35	1xxx	17,03
Tanor Late	MxT	1xx	34,66	Tanor Late	C35	1xx	36,35
Tanor Late	MxT	1x	19,74	Tanor Late	C35	1x	22,42
Tanor Late	MxT	1	8,27	Tanor Late	C35	1	10,44
Tanor Late	MxT	2	2,72	Tanor Late	C35	2	2,45
Tanor Late	MxT	3	1,28	Tanor Late	C35	3	1,24
Tanor Late	MxT	4	0,5	Tanor Late	C35	4	0,39
Tanor Late	MxT	5	0,04	Tanor Late	C35	5	0,03
<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>				
Tanor Late	CC	1xxx	15,22				
Tanor Late	CC	1xx	33,02				
Tanor Late	CC	1x	22,45				
Tanor Late	CC	1	12,59				
Tanor Late	CC	2	4,74				
Tanor Late	CC	3	2,84				
Tanor Late	CC	4	0,87				

Tanor Late	CC	5	0,1
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**Table 5.4.13.4.** Production per tree of Tanor Late mandarin hybrid trees on different rootstocks at Tangelo (Buffeljagsrivier) during the 2021 season.

Cultivar	Rootstock	Kg/tree (2021)	Tree size m <sup>3</sup>
Tanor Late	RL	66,4	7,7
Tanor Late	SC	67,0	5,3
Tanor Late	CC	55,7	4,6
Tanor Late	US 812	44,5	3,5
Tanor Late	X639	57,1	4,0
Tanor Late	C35	64,5	3,0
Tanor Late	MxT	56,7	4,5

#### 5.4.14 PROGRESS REPORT: Evaluation of Grapefruit on different rootstocks in a semi-desert production area (Kakamas)

Project 922 by J Joubert and W Swiegers (CRI)

##### Summary

Visual evaluations of Star Ruby and Nelruby bud-unions indicated that the unions were still in good condition and the combinations compatible (need to confirm once the trial comes to an end). US 812 is a hybrid rootstock cross between a US mandarin and Beneke trifoliolate (US812). The tree size of this combination is described as medium (similar to Carrizo tree size and growth rate). In combination with Star Ruby, the tree was bigger compared to Citrange 35 (C35) and Benton citrange (BC) (dwarfing rootstocks). Nelruby tree size in combination with US812 was also the biggest compared to C35 and BC. Yield production was down this season for Nelruby and Star Ruby, while certain combinations was up in production. Nelruby outperformed Star Ruby during the season. Both selections' fruit size ranged with counts 56 – 27.

Seed counts on the Nelruby fruit were higher than Star Ruby, being virtually seedless. Colour development on both selections and all the rootstock combinations was good. Bearing in mind the impact of the high pH and calcareous soil. The following rootstocks combinations perform constant over the last five years: For Nelruby it was Citrange 35, Swingle Citrumelo (SC) and Terra Bella (TB). For Star Ruby it was Carrizo Citrange (CC), Swingle Citrumelo (SC) and Terra Bella (TB). Future evaluations will determine the adaptability of these rootstocks.

##### Opsomming

Visuele evaluasies van die Star Ruby en Nelruby entlas, met 'n gesonde entlas verbinding, het bewys dit is verenigbaar met die kombinasies (moet bevestig word wanner die proef tot 'n einde kom). US 812 is 'n hibried onderstam kruising tussen US mandaryn en Beneke trifoliaat (US 812). Die boomgrootte van hierdie kombinasie word as medium beskou (vergelyk met Carrizo boomgrootte en groeikragtigheid). In kombinasie met Star Ruby was die boomgrootte bietjie groter as Citrange 35 en Benton citrange (verdwergde onderstamme). Nelruby in kombinasie met US812 was die grootste boom in vergelyking met C 35 en BC (verdwergde onderstamme). Die oes produksie het afgeneem hierdie seisoen vir Nelruby en Star Ruby, terwyl net sekere kombinasies verbeter het met hulle produksie. Nelruby het beter presteer hierdie seisoen as Star Ruby in hierdie proef. Albei seleksies se vrugtegrootte het gepeik tussen grootte 56 – 27.

Saad tellings op die Nelruby vrugte was hoër in vergelyking met Star Ruby wat feitlik saadloos toets. Kleur ontwikkeling op albei seleksies en al die onderstam kombinasies was goed. Die volgende kombinasies het baie goed presteer oor die laaste 5 jaar wanneer die impak van die hoë pH vlakke en kalkagtigheid van die grond in ag geneem word. Nelruby in kombinasie met Citrange 35, Swingle Citrumelo (SC) en Terra Bella (TB) en

Star Ruby in kombinasie met Carrizo Citrange (CC), Swingle Citrumelo (SC) and Terra Bella (TB). Verdere evaluasies sal die aanpasbaarheid van hierdie onderstamme bevestig.

## Objectives

- To investigate the performance of Star Ruby and Nelruby Grapefruit on suitable rootstocks in a hot citrus growing area on replant soils.
- To improve production, internal quality, rind colour and fruit size count distributions.

## Materials and methods

Seeds of Benton Citrange, Citrange 35, Carrizo Citrange, Rough Lemon, US 812, Swingle Citrumelo, Terrabella and X639 were propagated by OSK Nursery, a CIS accredited nursery in the Kakamas region of the Western Cape.

Star Ruby and Nelruby grapefruit were budded onto the following rootstocks at OSK nursery in 2010: Benton Citrange, Citrange 35, Carrizo Citrange, Rough Lemon, US 812, Swingle Citrumelo, Terra Bella and X639. The trees were planted at Karsten in March 2012.

**Table 5.4.14.1.** The number of trees per rootstock in the Star Ruby and Nelruby Grapefruit trial at Kakamas.

Selection	Rootstock	No. of trees
Star Ruby	BC	6
Star Ruby	C35	6
Star Ruby	CC	6
Star Ruby	RL	3
Star Ruby	US 812	6
Star Ruby	SC	6
Star Ruby	TB	6
Star Ruby	X639	5
Nelruby	BC	6
Nelruby	C35	5
Nelruby	US 812	5
Nelruby	SC	6
Nelruby	TB	6
Nelruby	X639	6

## Results and discussion

The Grapefruit trial was harvested for the sixth time this season with a poor to good crop on the trees.

### Star Ruby

The lowest crop production for the 2021 season was in combination with BC yielding 2.7 kg/tree (2020; CC – 46.2 kg/tree) and the best on CC, yielding 162.7 kg/tree with avg. tree volume 23.5 m<sup>3</sup> (2020; TB – 139.8 kg/tree), (Table 5.4.12.4). The second highest crop was produced on SC with 126.6 kg/tree with avg. tree volume 21.7 m<sup>3</sup> and the average yield for the Star Ruby trial was 75.5 kg/tree (2020 – 89.7 kg). Internally fruit quality was good with Brix ranging from 9.6 with BC and RL up to 11.2 with SC; (average 10.15) and juice levels around 45% except for in combination with BC 19.6% (Table 5.4.12.2).

The acid content remained fairly high this season at 1.38% on average (slightly lower than 2020), The Brix:acid ratio ranged between 6.49 (TB) – 8.21 (SC). Fruit size count on CC, C35, RL, US 812, SC and X639 peaked between 36 – 27, on BC, TB counts peaked between 40 – 27.

### Nelruby

Nelruby juice content was low, below 45%, highest juice content was in combination with TB 49.3%. The lowest Brix:acid ratio of 7.24 was in combination with TB. The highest Brix:acid ratio 8.86 was on X639. All ratios are higher than 2020 season ratios, (Table 5.4.12.2). BC had the highest Brix 11.5 followed by US 812 and C35. The external colour development on all 6 rootstock combinations peaked at T1. Most of the combinations peaked at count 40 – 27 except US 812 peaking count 36 – 27. The best crop on the Nelruby trees was in combination with SC (169.7 kg/tree) with tree volume of 33m<sup>3</sup>, followed by TB (135.6 kg/tree) and its tree volume of 23 m<sup>3</sup>.

## Conclusions

The seed content in the Star Ruby fruit remained significantly lower in comparison with the Nelruby fruit. Fruit size distribution from Star Ruby mostly peaked count 36 – 27, and Nelruby fruit size count peaked between (counts 40 - 27). Nelruby cropped a better yield on the trees (average 104.2 kg/tree versus 75.5 kg) compared to Star Ruby this season. Star Ruby had improved colour development (deeper red blush on rind) whereas Nelruby was more “yellowish” although both were T1 on the colour plate.

Star Ruby and Nelruby were evaluated on eight and six rootstocks, respectively, the most important combination of the above mentioned was US 812 (US mandarin x Beneke trifoliolate). US 812 was selected for replanting conditions, very specific high pH and calcareous soils. The evaluations and harvests indicated that the other rootstocks outperformed US 812, future evaluations will be crucial to determine the best combination in these semi-desert conditions.

**Table 5.4.14.2.** Internal fruit quality of Star Ruby and Nelruby Grapefruit on different rootstocks at Karsten Boerdery (Kakamas) on 30 June 2021.

Cultivar	Root-stock	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Nelruby	BC	43,0	11,5	1,36	8,46	1,8	T 1
Nelruby	C35	45,8	11,1	1,36	8,16	1,9	T 1
Nelruby	SC	42,2	9,5	1,22	7,79	2,5	T 1
Nelruby	US 812	42,3	11,4	1,45	7,89	0,5	T 1
Nelruby	TB	49,3	10,6	1,47	7,24	0,8	T 1
Nelruby	X639	42,5	10,9	1,23	8,86	0,9	T 1
Star Ruby	BC	19,6	9,6	1,39	6,91	0,0	T 1
Star Ruby	C35	43,5	10	1,31	7,63	0,0	T 1
Star Ruby	CC	43,2	10,7	1,34	8,01	0,0	T 1
Star Ruby	RL	45,6	9,6	1,33	7,22	0,0	T 1
Star Ruby	SC	46,8	11,2	1,37	8,21	0,0	T 1
Star Ruby	US 812	42,3	10,5	1,57	6,71	0,0	T 1
Star Ruby	TB	44,5	9,8	1,51	6,49	0,0	T 1
Star Ruby	X639	44,2	9,8	1,27	7,75	0,0	T 1

**Table 5.4.14.3.** Fruit size distribution at Karsten Boerdery during the 2021 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	BC	27	55,56	Nelruby	BC	27	30,96
Star Ruby	BC	32	11,11	Nelruby	BC	32	19,83
Star Ruby	BC	36	16,67	Nelruby	BC	36	31,36
Star Ruby	BC	40	16,67	Nelruby	BC	40	14,22
Star Ruby	BC	48	0,00	Nelruby	BC	48	2,05

Star Ruby	BC	56	0,00	Nelruby	BC	56	1,58
<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
Star Ruby	C35	27	58,86	Nelruby	C35	27	17,48
Star Ruby	C35	32	17,72	Nelruby	C35	32	14,47
Star Ruby	C35	36	17,09	Nelruby	C35	36	27,27
Star Ruby	C35	40	4,43	Nelruby	C35	40	24,27
Star Ruby	C35	48	1,90	Nelruby	C35	48	9,80
Star Ruby	C35	56	0,00	Nelruby	C35	56	6,71
<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
Star Ruby	CC	27	27,65	Nelruby	US 812	27	40,47
Star Ruby	CC	32	21,45	Nelruby	US 812	32	21,40
Star Ruby	CC	36	27,65	Nelruby	US 812	36	26,05
Star Ruby	CC	40	15,90	Nelruby	US 812	40	9,77
Star Ruby	CC	48	4,81	Nelruby	US 812	48	1,86
Star Ruby	CC	56	2,54	Nelruby	US 812	56	0,47
<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
Star Ruby	RL	27	37,52	Nelruby	SC	27	26,61
Star Ruby	RL	32	19,76	Nelruby	SC	32	18,17
Star Ruby	RL	36	21,16	Nelruby	SC	36	28,49
Star Ruby	RL	40	14,37	Nelruby	SC	40	18,13
Star Ruby	RL	48	4,19	Nelruby	SC	48	5,30
Star Ruby	RL	56	2,99	Nelruby	SC	56	3,30
<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
Star Ruby	US 812	27	37,78	Nelruby	TB	27	22,08
Star Ruby	US 812	32	17,78	Nelruby	TB	32	17,60
Star Ruby	US 812	36	20,00	Nelruby	TB	36	30,85
Star Ruby	US 812	40	11,11	Nelruby	TB	40	18,79
Star Ruby	US 812	48	4,44	Nelruby	TB	48	7,73
Star Ruby	US 812	56	8,89	Nelruby	TB	56	2,96
<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
Star Ruby	SC	27	31,89	Nelruby	X639	27	22,85
Star Ruby	SC	32	21,89	Nelruby	X639	32	15,85
Star Ruby	SC	36	27,02	Nelruby	X639	36	30,90
Star Ruby	SC	40	12,82	Nelruby	X639	40	20,45
Star Ruby	SC	48	4,25	Nelruby	X639	48	6,14
Star Ruby	SC	56	2,13	Nelruby	X639	56	3,81
<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>				
Star Ruby	TB	27	36,15				
Star Ruby	TB	32	16,89				
Star Ruby	TB	36	24,21				
Star Ruby	TB	40	15,09				
Star Ruby	TB	48	4,73				
Star Ruby	TB	56	2,93				
<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>				
Star Ruby	X639	27	29,26				
Star Ruby	X639	32	20,70				
Star Ruby	X639	36	26,68				
Star Ruby	X639	40	16,04				
Star Ruby	X639	48	4,57				
Star Ruby	X639	56	2,74				

**Table 5.4.14.4.** Production per tree of Star Ruby and Nelruby Grapefruit trees on different rootstocks at Karsten Boerdery (Kakamas) during the 2021 season.

Cultivar	Rootstock	Kg/tree (2017)	Kg/tree (2018)	Kg/tree (2019)	Kg/tree (2020)	Kg/tree (2021)	AVG 5 YRS	Tree size m <sup>3</sup>
Nelruby	BC	75,3	28,1	86,8	116,6	97,5	80,9	17,8
Nelruby	C35	80,2	74,4	73,4	105,2	87,1	84,0	16,3
Nelruby	US 812	51,8	31,7	76,6	116,0	27,5	60,7	21,1
Nelruby	SC	83,9	48,7	114,6	123,2	169,7	108,0	33
Nelruby	TB	62,3	7,9	92,2	119,4	135,6	83,5	23
Nelruby	X639	62,1	35,1	74,1	93,4	107,8	74,5	21,5
Star Ruby	BC	49,9	34,3	73,0	99,2	2,7	51,8	13,4
Star Ruby	C35	58,4	45,2	86,8	114,8	23,5	65,7	12,7
Star Ruby	CC	61,7	30,7	99,9	46,2	162,7	80,2	23,5
Star Ruby	RL	62,0	66,5	105,9	47,8	87,2	73,9	21,4
Star Ruby	US 812	58,5	40,8	95,9	95,3	5,3	59,2	14,5
Star Ruby	SC	67,8	32,7	89,0	95,5	126,6	82,3	21,7
Star Ruby	TB	58,2	20,9	96,4	139,8	81,5	79,4	16,5
Star Ruby	X639	58,6	39,4	80,0	78,8	114,7	74,3	17,1

#### 5.4.15 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region (Sundays River Valley)

Project 57B by W Swiegers and Z Zondi (CRI)

##### Summary

The trees were topworked in 2012 to the following selections which were also the order of ripening: Miho Wase, Aoshima, Ueno, Dobashi Beni and the season was finished off with Imamura. 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.

##### Opsomming

Die bome was in 2012 getopwerk na die volgende seleksies toe, wat ook dien as die volgorde van rypwording; Miho Wase, Aoshima, Ueno, Dobashi Beni en die seisoen was afgesluit met Imamura. Pluk periodes vir Satsumas sal strek van 2-3 weke aangesien vrugte se sure vinnig daal en die skil powwerig raak. Vrugte se kleur is laat teenoor die interne kwaliteit en ontgroening sal moet gedoen word.

##### Objectives

- To select Satsuma cultivars with improved and consistent productivity, fruit size, rind colour, and internal fruit quality (Brix, acidity and ratio).
- To extend the harvest period (both earlier and later maturity).
- To describe the characteristics of new Satsuma cultivars and determine the climatic suitability of these cultivars in cold production regions.

## Materials and methods

Field evaluations and laboratory analyses were conducted on early to late Satsuma selections from the Sundays River Valley part of the Eastern Cape. The following selections were evaluated: Miho Wase, Ueno, Aoshima, Imamura, and Dobashi Beni.

For Satsuma mandarins, a ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which the fruit are considered over mature. This process is approximately three weeks long. Fruit harvested before and after this period would result in higher instances of quality and rind issues.

**Table 5.4.15.1.** List of Satsuma selections evaluated at Invercloy (Kirkwood) during 2021.

Selection	Rootstock	Topworked
Aoshima	Carrizo	2012
Miho Wase	Carrizo	2012
Ueno	Carrizo	2012
Dobashi Beni	Carrizo	2012
Imamura	Carrizo	2012

## Results and discussion

### Aoshima

Aoshima was the second selection to reach peak maturity. Aoshima's fruit size count was 1x - 1xx (big fruit). Aoshima juice percentage was just around 55% (low). Brix was around 9° towards peak maturity peaking at 9.3° when over mature. The selection started with a low acid %. The fruit was virtually seedless. The external colour development of the Aoshima was not very good, with a T6 - T7 on the colour plate. T 6 on the colour plate while the fruit was over mature and very pebbly.

### Miho Wase

Miho Wase was the first selection to mature to peak maturity this year. Miho Wase is also used as the control. The selection had a medium - big fruit size count of 1 - 1xx. Juice percentage for Miho Wase was higher with 56.7% this season compared to last season's 54.1% juice. Miho Wase had a Brix° of 9.7° and acid percentage of 0.82% with a ratio of 11.8 (over mature). The colour was T6 on the colour plate. The fruit had no seeds and the external colour development was once again behind the internal development. The internal colour was a deep orange.

### Ueno

Ueno is a mid to late maturing selection. Ueno's fruit size count was big, ranging from count 1x to 1xxx. Count 1x was towards build-up to peak maturity and count 1xxx was when the fruit was over mature. The juice percentage this season is around 53%. Ueno Brix° was 8.5° and the acid percentage was 0.80% at a ratio of 10.6. Ueno had no seeds and the colour of Ueno on the colour plate was a T5 at peak maturity. The fruit was flat and peelability was easy.

### Imamura

Imamura is one of the late-maturing selections for this Satsuma trial site, and it was the last selection to reach peak maturity. Imamura normally reaches peak maturity beginning to end of May in cool production regions. The juice percentage for Imamura was 54.1%. Imamura Brix° was 11.6° the highest of all the selections and a very good acid of 0.91% at a ratio of 12.7. Seed count was seedless. The colour development was T1 on the colour plate, one of the best compared to the other selections. The internal colour was deep orange and the fruit rind varied from smooth to coarse.

### Dobashi Beni

Dobashi Beni are the control selection for the mid to late maturing Satsuma selections. Dobashi Beni was the second last selection to reach peak maturity. Fruit size count was very good with a count 1 – 1x. The juice percentage at peak maturity was 62%. Brix° was 8.6° and acid % was 0.89% at peak maturity. Internal colour is deep orange, rind is smooth and peelability is easy. Dobashi Beni was seedless. This selection had a delayed external colour development being T6 on the colour plate with at 9.7 ratio being mature.

## Conclusion

All the selections had a big fruit size i.e., count 1x and up. Dobashi Beni had a good fruit size count ranging from 1 to 1x. Dobashi Beni had the best juice percentage being 62 % followed by Mihowase 56.7%. Imamura had the highest Brix° at peak maturity being 11.6 and Ueno had the lowest Brix° being 8.5° of all the Satsuma selections. All the selections were seedless or virtually seedless.

**Table 5.4.15.2.** Internal fruit quality data for Satsuma selections in the Addo and Kirkwood region of the Eastern Cape during the 2021 season.

Date	Cultivar	Root-stock	Avg fruit size (mm)	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2021-03-23	Aoshima	CC	70	1x	54.7	9.0	1.34	6.7	0.4	T7
2021-04-12	Aoshima	CC	75	1xx	55.6	9.3	0.78	11.9	0.1	T6
2021-03-23	Dobashi Beni	CC	62	2	60.1	8.6	1.27	6.8	0.0	T7
2021-04-29	Dobashi Beni	CC	65	1	62.0	8.6	0.89	9.7	0.0	T6
2021-05-11	Dobashi Beni	CC	70	1x	54.4	8.4	0.88	9.5	0.0	T4
2021-05-24	Dobashi Beni	CC	70	1x	52.7	9.6	0.77	12.5	0.0	T1
2021-05-11	Imamura	CC	70	1x	52.3	9.6	1.15	8.3	0.4	T4
2021-05-24	Imamura	CC	75	1xx	54.1	11.6	0.91	12.7	0.0	T1
2021-06-08	Imamura	CC	75	1xx	52.2	12.3	0.96	12.3	0.0	T1
2021-03-16	Mihowase	CC	65	1	58.3	9.8	1.04	9.4	0.0	T6
2021-03-23	Mihowase	CC	65	1	56.7	9.7	0.82	11.8	0.0	T6
2021-04-12	Mihowase	CC	75	1xx	56.5	9.5	0.70	13.6	0.0	T3
2021-03-23	Ueno	CC	70	1x	55.2	8.5	1.21	7.0	0.1	T7
2021-04-29	Ueno	CC	75	1xx	53.9	8.5	0.80	10.6	0.0	T5
2021-05-11	Ueno	CC	82	1xxx	49.5	8.6	0.64	13.4	0.0	T3-4

### 5.4.16 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Sundays River Valley) Project 997B by W Swiegers and Z Zondi (CRI)

#### Summary

The mandarin trial is in Dunbrody region of Kirkwood in the Sundays River Valley. Some of the selections have an interstock and some is directly on the rootstock. The selections order of ripening: Etna, Saint Andre, Samba, RHM, Tasty 1, Sirio, Leanri, Tango and IRM 2.

#### Opsomming

Die mandaryn proef is in die Dunbrody omgewing in Kirkwood in die Sondagsrivier Vallei. Van die seleksies is op 'n tussenstam getopwerk en van hulle direk op die onderstam. Die volgorde van ryppwording by die perseel was as volg: Etna, Saint Andre, Samba, RHM, Tasty 1, Sirio, Leanri, Tango en IRM 2.

## Objectives

- To select mandarin hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new mandarin hybrid cultivars and to determine the climatic suitability of these cultivars in 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 has been added to this area. The following varieties were evaluated: Etna, Saint Andre, Samba, RHM, Tasty 1, Sirio, Leanri, Tango and IRM 2

A ratio of 11:1 for mandarin hybrids is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, which 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 quality and rind issues.

**Table 5.4.16.1.** List of Mandarin hybrid selections from Kirkwood region of the Sundays River Valley (Dunbrody) during the 2021 season.

Selection	Rootstock	Topwork
Saint Andre	Carrizo	2013
Leanri	Carrizo with Midnight interstock	2015
RHM	Carrizo with Midnight interstock	2015
Samba	Carrizo with Midnight interstock	2015
Tango	Carrizo	2013
Tasty 1	Carrizo with Midnight interstock	2015
Etna	CC	2012
IRM 2	CC	
Sirio	CC	2012

## Results and discussion

### RHM

RHM had a very good fruit size count of 1. Acids start low and drop quickly before it stabilise, the acid percentage at peak maturity was 0.96% at ratio 12.5. The external colour development was T2-T3 on the colour plate at peak maturity. The selection has good Brix° above 12°. Juice percentage for RHM was very high around 56%. This selection seed count peaked at 2.4 seeds per fruit. The fruit is firm with a smooth rind and a deep orange internal colour, with a thin rind and good flavour.

### Saint Andre

Saint Andre was the second selection to reach peak maturity. At peak maturity the Saint Andre has a very good juice percentage peaking at 60%. Fruit size of Saint Andre was good fruit size count 1. As expected, the sugar was good at peak maturity with the acid percentage stabilizing around 0.95%. Brix° was 12.5° at peak maturity. During the evaluations, there were no seeds. The Saint Andre had an external colour of T2 on the colour plate at peak maturity. The fruit did reach T1 on the colour plate when they were left to hang and the acids were still around 0.93% with the Brix also going up to 13.1. Rind is slightly pebbly and flesh is deep orange. The fruit is flat to round, peelability is easy, and the flavour is very good. The trees had a good crop

on them year after year.

### Tango

Tango is mid to late maturing, seedless selection. Tango was T1 on the colour plate towards peak maturity. Juice percentages (around 60.0%) towards peak maturity. The fruit size for Tango was excellent count 1. Tango had moderate Brix° and acid percentage this season at peak maturity. Brix° was 10.1° and acid percentage was 0.83%. The rind is shiny and smooth and the flesh has a deep orange colour.

### Etna & Sirio

Etna & Sirio is early maturing mandarin hybrids. Etna reached peak maturity first on this trial site and in the early maturing window. External colour development was delayed, T3 on the colour plate at peak maturity. Etna also didn't have good internal quality at peak maturity Brix 8.2° and acid content 0.66%. Juice was good 57.7%. Fruit size count is 1x – 1xxx. Sirio reached peak maturity 2 weeks after Etna. Internal quality for Sirio was better compared to Etna. Brix 10.5° with acid content 0.88% at peak maturity with 50.6% juice. Fruit size count was extra large (count 1xxx); Rind colour development was good with a T1 at the colour plate (peak maturity). Peelability was not easy, but the fruit flavour is good.

### Samba

Samba is an early to mid-maturing mandarin hybrid selection. Samba on Carrizo rootstock with Midnight interstock produced a good crop with good internal quality. Trees are fast growing and thornless. Colour development was early in the season (deep orange) with a smooth rind texture on the fruit. T3 on the colour plate towards peak maturity and T1 at peak maturity. Fruit were seedless this season in the combined trial block and fruit size peaked (count 2 to 1x) very good fruit size. Juice percentage at peak maturity was also very good around 60%. Brix was at 12° at peak maturity and acid 0.95%. This good internal quality give Samba its unique and good flavour.

### Leanri

Leanri is also an early to mid-maturing mandarin hybrid. It had a fruit size count ranging from 1xx. Leanri has a very good juice percentage. Juice percentage was at 65.7% towards peak maturity. The sugars and acid percentage was one of the highest at peak maturity. The Brix° was 13.2° and acid percentage of 1.17% when the ratio was 11.3. This internal quality contributes to the good fruit flavour. The selection was virtually seedless during the evaluations with seed count of 0.1 – 0.5 seeds per fruit. Towards peak maturity the colour was already a T1 on the colour plate. The rind colour was deep orange as well as the internal colour.

### Tasty 1

Tasty 1 is a mid-maturing selection. Tasty 1 fruit size count was 1xx (big fruit). Juice percentage at peak maturity was below 50% (very low). Brix 10.0° and acid percentage was 0.84% at peak maturity. Colour development was delayed for Tasty 1 being T5 on the colour plate. The selection seed count was low.

### IRM 2

IRM 2 has a deep orange rind colour, and IRM 2 reached T1 on the colour plate before peak maturity. The selection produced very good internal quality fruit. The juice percentage was close to 60% towards peak maturity, Brix was 15 and acids % was 1.32%. IRM 2 was virtually seedless. The rind is very thin. Excellent eating fruit with good flavour.

## **Conclusion**

The following selections had the largest fruit size count with a 1xxx count: Tasty 1, Etna and Sirio. Most of the other selections peaked at count 1. The selections with the highest juice percentage above 60% were Saint Andre, Leanri and Samba. The selections with the highest °Brix above 13 at peak maturity was IRM 2 and Leanri. Tasty 1 and RHM were the selections that had the most seeds per fruit. IRM 2, Leanri, Samba, Tango and Sirio reached a colour T1 on the colour plate at peak maturity.

**Table 5.4.16.2.** Internal fruit quality data for Mandarin hybrid selections from Kirkwood region of the Sundays River Valley (Dunbrody) during the 2021 season.

Date	Selection	Root-stock	Avg fruit (mm)	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2021-04-29	Etna	CC	70	1x	60.6	9.1	0.93	9.8	0.2	T6
2021-05-11	Etna	CC	82	1xxx	57.7	8.2	0.66	12.4	0.1	T3
2021-05-24	Etna	CC	75	1xx	55.1	9.6	0.63	15.2	0.7	T2-3
2021-07-27	IRM 2	CC	65	1	55.0	14.6	1.45	10.1	1.5	T1
2021-08-10	IRM 2	CC	66	1	58.1	15.0	1.32	11.4	0.7	T1
2021-05-24	Leanri	CC	75	1xx	58.1	12.6	1.24	10.2	0.1	T1
2021-06-08	Leanri	CC	75	1xx	65.7	13.2	1.17	11.3	0.5	T1
2021-05-11	RHM	CC	66	1	55.8	9.9	0.90	11.0	1.9	T5
2021-05-24	RHM	CC	66	1	56.6	12.0	0.96	12.5	0.8	T2-3
2021-06-08	RHM	CC	66	1	59.3	12.4	0.88	14.1	2.4	T1
2021-06-24	RHM	CC	65	1	63.0	13.3	0.91	14.6	0.5	T1
2021-05-11	Saint Andre	CC	66	1	56.4	10.8	0.99	10.9	0.0	T4
2021-05-24	Saint Andre	CC	66	1	60.0	12.5	0.95	12.9	0.0	T2
2021-06-08	Saint Andre	CC	66	1	60.8	13.1	0.93	14.1	0.0	T1
2021-05-11	Samba	CC	62	2	56.9	9.5	0.90	10.6	0.0	T3
2021-05-24	Samba	CC	66	1	61.3	12.0	0.95	12.6	0.0	T1
2021-06-08	Samba	CC	70	1x	69.4	12.6	0.99	12.7	0.0	T1
2021-05-11	Sirio	CC	82	1xxx	52.3	8.8	0.89	9.9	0.0	T2
2021-05-24	Sirio	CC	82	1xxx	50.6	10.5	0.88	11.9	0.5	T1
2021-06-08	Sirio	CC	82	1xxx	55.4	10.9	0.88	12.4	0.0	T1
2021-07-15	Tango	CC	66	1	59.1	11.1	1.05	10.6	0.0	T1
2021-07-27	Tango	CC	67	1	59.2	10.1	0.83	12.1	0.0	T1
2021-05-11	Tasty 1	CC	82	1xxx	42.4	7.9	0.86	9.2	2.7	T6
2021-05-24	Tasty 1	CC	82	1xxx	49.1	10.0	0.84	11.9	0.8	T5
2021-06-08	Tasty 1	CC	82	1xxx	51.9	9.3	0.74	12.6	0.0	T4-5

**5.4.17 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Gamtoos River Valley)**

Project 997C by W Swiegers and Z Zondi (CRI)

**Summary**

Loerie is the main trial site in the Gamtoos River Valley area, but there is a new site on the way with all of the latest selections in Patensie. Unfortunately, the area is currently going thru a drought. The first evaluations for the new site will start as soon as the drought break. Both trial sites will form part of the Gamtoos River Valley. At Loerie the season started off with Samba and was followed up by Sirio, St Andre, Etna and the season ended with Tasty 1.

**Opsomming**

Loerie is die hoof perseel in die Gamtoos Rivier Valleie, maar daar is 'n nuwe perseel in Patensie wat al die nuwe seleksies gaan bevat. Ongelukkig gaan die area deur erge droogte. Die eerste evaluasies van die nuwe

perseel begin sodra die droogte breuk. Albei persele maak deel uit van die Gamtoos Rivier Vallei. By Loerie het die seisoen begin met Samba gevolg deur Sirio, St Andre, Etna en die seisoen het geëindig met Tasty 1.

## Objectives

- To select mandarin hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new mandarin hybrid cultivars and to determine the climatic suitability of these cultivars in cold production regions.

## Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections from the Gamtoos River Valley. A range of new mandarin hybrids had been added to this area. The following cultivars were evaluated: Saint Andre, Etna, Sirio, Tasty 1 and Samba.

A ratio of 11:1 for mandarin hybrids is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

**Table 5.4.17.1** List of experimental mandarin hybrid selections evaluated in the Loerie (N. Ferreira) region of the Gamtoos River Valley during the 2021 season.

Selection	Rootstock	Topwork
Etna	Carrizo	2012
Sirio	Carrizo	2012
Saint Andre	Carrizo	2012
Tasty 1	Carrizo	2012
Samba	Carrizo	2017

## Results and discussion

### Saint Andre

Saint Andre was the third selection to reach peak maturity in this production region. The fruit size count was count 1. The juice percentage this season was good just around 55%. At peak maturity the colour development was very good T1 on the colour plate. The acids and sugars remained stable during the production season for Saint Andre. At peak maturity Brix 12.8° and Acid percentage was 1.10%. Internal quality was good and it contributes to the good flavour. During the evaluations, the selection had a seed count peaking at 2.4 seeds per fruit.

### Etna & Sirio

Etna & Sirio is an experimental early maturing mandarin hybrids that reached peak maturity more towards the mid maturing time. Sirio reached peak maturity before Etna. The fruit size count for the Etna this season was the smaller count 1 compared to last season, count 1x. Sirio had the same fruit size count as last season 1xx. The juice percentage for Etna was good, above 60% juice at peak maturity Sirio had a slightly lower juice content. Sirio's Brix and Acid levels were slightly lower than Etna towards peak maturity. Sirio had a T1 on the colour plate range at peak maturity compared to Etna's T2. Sirio had a slightly higher seed count per fruit.

### Tasty 1

Tasty 1 developed an extra-large fruit size and peaked at count 1xx. The external colour development were not delayed being a T1 on the colour plate at peak maturity. The juice percentages were moderate for Tasty 1, being 53%. Seed count peaked 1.6 seeds per fruit. At peak maturity the Brix was 13.4° with acid percentage

of 1.07%.

### Samba

Samba produced a good crop with good internal quality. Colour development was good with early colour break, reaching T 1 on the colour plate at peak maturity. The rind colour is a deep orange. Fruit size for Samba was also favourable with medium-large fruit size count 2 – 1. Internal quality was very good with juice percentage of around 60% at peak maturity, with Brix of 12.2° and acid percentage of 1.02%. Brix:Acid ratio was 12.0. Seed count peaked at 0.9 seeds per fruit. Peelability is easier than Nova, with a good flavour fruit.

### Conclusion

All of the selections had very good external colour development (T1) at peak maturity except Etna. Sirio and Tasty 1 had the largest fruit size (count 1xx); Etna and Samba cropped the smallest fruit size (count 2 - 1). Etna, Samba and St Andre developed juice percentages above 60%. Tasty 1 had the lowest juice percentage 53%. St Andre, Samba and Tasty 1 had the highest Brix level above 13°. The selection with the highest seed count was Sirio.

**Table 5.4.17.2.** Internal fruit quality data for experimental mandarin hybrid selections from the Loerie (N. Ferreira) region of the Gamtoos River Valley region during the 2021 season.

Date	Selection	Root-stock	Avg fruit size (mm)	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2021-05-19	Etna	CC	66	1	59.4	11.9	0.95	10.7	1.2	T2-3
2021-06-01	Etna	CC	66	1	61.6	10.9	0.93	11.7	0.7	T1
2021-06-14	Etna	CC	62	2	61.0	12.1	1.01	12.0	0.3	T2
2021-05-19	Saint Andre	CC	66	1	56.4	12.2	1.23	9.9	1.3	T2
2021-06-01	Saint Andre	CC	66	1	52.7	12.8	1.10	11.6	2.4	T1
2021-06-14	Saint Andre	CC	66	1	61.2	13.2	0.99	13.3	0.3	T1
2021-04-26	Samba	CC	62	2	60.3	11.3	1.11	10.2	0.9	T2
2021-05-19	Samba	CC	66	1	57.0	12.2	1.02	12.0	0.6	T1
2021-06-01	Samba	CC	66	1	57.8	13.2	0.98	13.5	0.0	T1
2021-05-19	Sirio	CC	76	1xx	55.7	11.1	1.02	10.9	2.8	T1-2
2021-06-01	Sirio	CC	76	1xx	57.5	11.2	0.94	11.9	0.0	T1
2021-06-14	Sirio	CC	76	1xx	59.3	12.1	0.88	13.8	0.0	T1
2021-06-14	Tasty 1	CC	76	1xx	58.2	12.3	1.48	8.3	1.6	T4
2021-07-06	Tasty 1	CC	71	1x	53.0	13.4	1.07	12.5	0.0	T1

#### 5.4.18 PROGRESS REPORT: Cultivar characteristics and climatic suitability of mandarin hybrids in a cold production region (Western Cape)

Project 997D by W Swiegers (CRI)

### Summary

The trial site in Citrusdal consists of a cultivar block with a selection of all the new experimental cultivars from early-maturing to late maturing selections. Cross pollination was high in this block due to all the different selections present. The season started with Goldup, Tami 2/65 and then RHM followed by, Mayana, Samba, Etna, Leanri, Furr, Sirio, Gold Nugget, Or 4, Or 1, Nadorcott ARC, Nadorcott and ended with Tango.

### Opsomming

Die proef perseël in Citrusdal bevat meeste van die nuwe eksperimentele seleksies van vroeg tot laat rypwordend. Die kruisbestuiwing in hierdie proef perseël is baie hoog weens al die verskillende seleksies teenwoordig. Die orde van rypwording was as volg gewees Goldup, Tami 2/65 gevolg deur RHM, Mayana, Samba, Etna, Leanri, Furr, Sirio, Gold Nugget, Or 4, Or 1, Nadorcott ARC, Nadorcott en die seisoen het klaargemaak met Tango.

## 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 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 region of the Western Cape. The following selections were evaluated: Goldup, Tami 2/65, RHM, Mayana, Samba, Etna, Leanri, Furr, Sirio, Gold Nugget, Or 4, Or 1, Nadorcott ARC, Nadorcott and Tango.

A ratio of 11:1 for mandarin hybrids is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

**Table 5.4.18.1.** List of experimental mandarin hybrid selections evaluated in the Citrusdal region of the Western Cape during the 2021 season.

Selection	Rootstock	Topwork	Planted
Furr (Clemcott)	CC	2011	
Gold Nugget	CC	2010	
ARC Nadorcott	CC	2010	
Nadorcott	CC		2009
RHM	CC		2013
Tango	CC	2010	
Leanri	CC	2015	
Sirio	CC	2012	
Etna	CC	2012	
Samba	CC	2012	
Mayana	CC	2010	
Goldup	CC	2015	
Or 1	CC	2014	
Or 4	CC		Unknown
Tami 2/65	CC	2010	

## Results and discussion

### Tami 2/65

Tami 2/65 is an early maturing mandarin hybrid. Tami 2/65 was the second selection to reach peak maturity at the Citrusdal site. The fruit size for Tami 2/65 was good with a fruit size count range between 1 – 1x. Internal juice percentage was good above (55%). Internal colour is a deep orange. The fruit peels easily. The selection was virtually seedless. Rind colour development was good with T3 – T4 on the colour plate towards peak maturity. The selection doesn't have high acid and tends to drop quickly, leaving you with a short harvesting period. Brix was just below 11°. Crop on the trees looked good. Further test need to be done to determine the

potential of the cultivar.

#### Furr (Clemcott) & Leanri

Furr is used as a control for the mid-maturing mandarin selections and for Leanri. The juice content was very good for Furr, just above 60% (towards peak maturity) while Leanri juice content decreased towards peak maturity to below 50%. The fruit size count for both selections peaked at 1x. Furr and Leanri has a very good eating quality. Due to the high cross pollination in the mixed trial block, both selections produced a number of seeds per fruit; Furr up to 7.8 and Leanri up to 0.3. Leanri's external colour development was very good this season; T1 on the colour plate range at peak maturity compared to Furss's T1 – T4. Brix: Acid ratio for Furr and Leanri was very good. High sugars and good acid give Furr & Leanri its good flavour. Leanri had slightly higher sugars. Furr did have better crop compared to Leanri, Leanri had smoother fruit.

#### Etna & Sirio

Etna & Sirio is two experimental early mandarin hybrids. Etna & Sirio reached peak maturity more to the mid-maturing range. Sirio reached peak maturity after after Etna. The fruit size count for the Etna this season was count 3 compared to Sirio's fruit size count 1x. Etna had the better crop, which could be the smaller fruit size. Etna had a juice percentage above 65% towards peak maturity and Sirio was below 55% towards peak maturity. Internal quality towards peak maturity for both selections was good. Brix was around 11 and acid percentage around 1.0%. Etna and Sirio were a T1 on the colour plate at peak maturity. Both has a deep orange internal colour. Etna seed count was between 0.2 – 2 seeds per fruit while Sirio peaked at 1.1 seeds per fruit. Sirio also has better flavour than Etna. Etna's peelability is easier compared to Sirio.

#### Mayana

Fruit size for Mayana was small – medium with count 5 – 3. The juice percentage for Mayana was very good, 56.1% juice percentage at peak maturity. Rind colour development was good to reach T1 on the colour plate at peak maturity. The seed count was 1.9 – 3.4 seeds per fruit. Internal quality was good at peak maturity Brix° above 12° and acid percentage was just around 1.0%. This good Brix: Acid ratio will contribute to good eating fruit with good flavour and shelf life.

#### Or 1 & 4

The size count for both selections was medium count 1. The fruit is round to oblate. Juice percentage for both varieties at peak maturity was very good, around 60.0%. Internal quality for both Or's is very good. Brix is high, around 14° and the acids were just around 1.0% even when the fruit was over mature. This good Brix: Acid ratio will contribute to excellent eating fruit with great flavour and shelf life. Peelability is easy and oily. Or 1 & Or 4 reached T1 on the colour plate at peak maturity. Or 4 had a slightly higher seed count per fruit.

#### Gold Nugget

Tree manipulation is necessary to control the strong vegetative and upright growth habit. Gold Nugget developed good tasting fruit with a high Brix: acid ratio. The fruit peaked internally with Brix above 12°. Due to the good quality of the fruit, it will be possible to hang the fruit longer on the trees with an extended shelf life. Gold Nugget's fruit size was at count 1xx. At peak maturity and fully coloured (T1). The juice percentage for this selection was around 50%. Gold Nugget is virtually seedless.

#### Nadorcott & Nadorcott ARC

Nadorcott ARC is an induced Nadorcott selection to minimise the average seeds per fruit. Nadorcott ARC has the same growth habit and characteristics as the Nadorcott. Fruit size for Nadorcott ARC counts 1 and for Nadorcott it ranged between 2 – 1. The internal juice percentages were above 55% at peak maturity for both selections. The Nadorcott selections developed good Brix above 13° with acids around 1.0%, ensuring a good balance and eating quality as well as shelf life. The fruit was fully coloured (T1) before peak maturity. Both selections were seedless. Fruit have a natural shine on them. Nadorcott ARC reached peak maturity first, followed by Nadorcott.

#### Samba

Samba is an early – mid maturing experimental low seeded mandarin hybrid. The selection was seedless during the evaluation. Samba has a favourable fruit size count that ranges between counts 3 – 2. Crop was

good. Fruit is round to oblate with halo. Peelability is easier for Samba than Nova and Samba rind can be oily. Before peak maturity was achieved, Samba was a T 1 on the colour plate. Samba has an exceptional deep orange external colour and a very deep internal colour. At peak maturity; Samba has high Brix and good acids and these give Samba its unique and excellent flavour. The juice percentage at peak maturity for this selection was 62.6%.

#### Tango

Tango developed a very smooth rind texture, similar to Nadorcott, with a natural shine. The fruit had a very good colour development in the cooler areas (colour plate T1) at peak maturity. Tango was seedless. The fruit size peaked at count 3 – 2 in Citrusdal. Internally the average juice percentage for Tango was around 62.9%. At the trial site the Brix: acid ratio was good, with Brix 13.9° and acid 1.04%.

#### Goldup

Goldup is an experimental early maturing Mandarin hybrid and it was the first selection to reach peak maturity. It had a delayed colour development T6 on the colour plate. Size count was small, count 4 at peak maturity. Internal quality was moderate juice just below 50%, Brix just above 10 and acids percentage around 0.85%.

#### RHM

RHM was third to reach peak maturity at the trial site. The fruit size (count 3 – 1). Acid dropped quickly but stayed stable around 0.80% at peak maturity and when the fruit was over mature in cold production regions and with the good sugars it contributed to good flavour and eating quality. The external colour development was delayed with T6 on the colour plate when the fruit was towards peak maturity. The selection has good Brix° above 12.0° and acid percentage of 1.00%. Juice percentage for RHM was very good, above 60%. There were seed for this selection during evaluations with an avg. seed count of 4.2 seeds per fruit. The fruit is firm with a smooth rind and a deep orange internal colour. Some of the fruit tends to split.

#### **Conclusion**

Gold Nugget had the largest fruit size (1xx). Mayana had the smallest fruit size with a count 5 – 3. Furr had the most seeds per fruit on average (7.3 seeds per fruit). Samba, Tango, Nadorcott and Nadorcott ARC were the only selections that were completely seedless. The following selections had a juice percentage over 60% at peak maturity Furr, Tami 2/65, Or 1, Tango, RHM, Etna, Samba and Leanri. Most of the selections were a T1 on the colour plate range (good colour development) before or at peak maturity.

**Table 5.4.18.2.** Internal fruit quality data for experimental mandarin hybrid selections from the Citrusdal region of the Western Cape during the 2021 season.

Date	Cultivar	Rootstock	Avg fruit size (mm)	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2021-05-18	Mayana	CC	56,6	3	58,2	13,2	1,23	10,7	1,9	T4 - T5
2021-06-07	Mayana	CC	50,7	5	56,1	12,5	0,85	14,7	3,4	T1
2021-05-18	Etna	CC	58,7	3	65,2	11,2	1,11	10,1	2,0	T1
2021-06-07	Etna	CC	56,5	3	65,7	11,0	0,90	12,2	0,2	T1
2021-08-05	Gold Nugget	CC	78,0	1xx	49,9	12,7	0,80	15,8	0,3	T1
2021-03-25	Gold up	CC	52,8	4	48,9	10,4	0,85	12,3	0,0	T6
2021-04-13	Gold up	CC	58,0	3	68,7	10,2	0,73	14,0	0,2	T6 - T7
2021-05-18	Leanri	CC	60,9	2	59,6	12,5	1,14	11,0	0,3	T5
2021-06-07	Leanri	CC	70,4	1x	48,0	12,8	1,10	11,7	0,1	T1
2021-05-18	Furr	CC	66,2	1	60,7	12,7	1,33	9,6	6,8	T6
2021-06-07	Furr	CC	68,9	1x	60,7	11,8	1,06	11,1	7,8	T1 - T4

2021-08-05	Nadorcott	CC	66,1	1	56,7	12,4	1,09	11,4	0,0	T1
2021-09-08	Nadorcott	CC	63,1	2	56,3	14,9	1,07	13,9	0,0	T1
2021-08-05	Nadorcott ARC	CC	67,8	1	57,2	11,8	1,09	10,9	0,0	T1
2021-09-08	Nadorcott ARC	CC	67,7	1	58,0	13,4	0,96	14,0	0,0	T1
2021-08-05	Or 1	CC	65,8	1	61,1	14,0	1,05	13,3	0,6	T1
2021-08-05	Or 4	CC	67,7	1	58,7	13,7	0,96	14,3	1,2	T1
2021-05-06	RHM	CC	57,8	3	59,0	12,2	1,09	11,2	5,2	T6
2021-05-18	RHM	CC	56,3	3	62,5	12,1	1,10	11,0	1,2	T6
2021-06-07	RHM	CC	64,2	1	65,7	13,0	0,85	15,3	6,3	T1
2021-05-18	Samba	CC	59,1	2	56,7	12,2	1,08	11,3	0,0	T2
2021-06-07	Samba	CC	58,5	3	62,6	12,7	1,02	12,4	0,0	T1
2021-06-07	Sirio	CC	69,4	1x	54,1	11,1	1,09	10,2	1,1	T1
2021-08-05	Tango	CC	62,1	2	59,4	13,3	1,20	11,1	0,0	T1
2021-09-08	Tango	CC	59,3	3	62,9	13,9	1,04	13,4	0,0	T1
2021-05-18	Tami 2/65	CC	64,2	1	61,3	10,9	0,98	11,2	0,0	T3 - T4
2021-06-09	Tami 2/65	CC	68,6	1x	58,8	10,9	0,70	15,6	0,1	T1

#### 5.4.19 PROGRESS REPORT: Cultivar characteristics and climatic suitability of mandarin hybrids in a cold production region (South West Cape)

Project 997E by W Swiegers (CRI)

#### Summary

This is a fairly new trial site in South West Cape. It's a cultivar block with a selection of all the new experimental cultivars from early maturing to late maturing selections. South West Cape is well suited for soft citrus. There is cross pollination in this block due to all the different selections that are present. A new site is going to be added to this site to cover more of the new selections. The order of ripening was as follows: Goldup, RHM, Leanri, Mayana, Or 4, Taylor Lee LS, Or 1, IRM 2 and the season ended with IRM 1.

#### Opsomming

Dit is 'n nuwe proef perseël in die Suid Wes Kaap. Die meeste van die nuwe eksperimentele seleksies van vroeg tot laat rypwordend kom in die perseël voor. Die Suid Wes Kaap is goed geskik vir sagte sitrus verbouing. Die kruisbestuiwing in hierdie proef perseël is hoog weens al die verskillende seleksies teenwoordig. Daar gaan nog 'n perseël bykom wat ook van die nuutste seleksies sal bevat. Die orde van rypwording was as volg gewees: Goldup, RHM, Leanri, Mayana, Or 4, Taylor Lee LS, Or 1, IRM 2 en IRM 1 wat die seisoen afgesluit het.

#### 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 determine the climatic suitability of these cultivars in cold production regions.

#### Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections from Buffeljagsrivier region of the South West Cape. The following selections were evaluated: Goldup, RHM, Leanri, Mayana, Or 4, Taylor Lee LS, Or 1, IRM 2 and IRM 1

A ratio of 11:1 for mandarin hybrids is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater quality and rind issues.

**Table 5.4.19.1.** List of experimental mandarin hybrid selections evaluated in the Buffeljagsrivier region of the South West Cape during the 2021 season.

Selection	Rootstock	Topwork
Mayana	CC	2014
IRM 1	CC	2014
IRM 2	CC	2014
Taylor Lee LS	CC	2014
Goldup	CC	2014
Or 1	CC	2014
Or 4	CC	2014
RHM	CC	2014
Leanri	CC	2014

## Results and discussion

### Mayana

Mayana is an early to mid-maturing experimental mandarin hybrid. Mayana had good Brix: Acid ratio towards peak maturity, Brix° was 12.1° and acid percentage was 1.14% at ratio 10.6. This will give Mayana its good flavour. The seed count peaked at 0.4 seeds per fruit. Mayana were fully coloured at peak maturity. Fruit size for Mayana was favourable with 3 count.

### IRM 1 & 2

The IRM 1 & 2 are late-maturing experimental mandarin hybrids. IRM 2 reached peak maturity before IRM 1. IRM 1 & 2 are prone to ribbing and alternate bearing. The fruit size count for IRM 1 was good (count 1x), IRM 2 was slightly smaller (count 1 – 1x). Internally the juice content for both selections increased towards peak maturity to above 60%, IRM 1 was slightly higher. Brix: Acid ratio at peak maturity was very similar for IRM 1 & 2, Brix was high above 14° and acid was good above 1.2%, IRM 2 had Brix close to 16. Seed count peaked around 1.3 seeds per fruit. IRM 1 & 2 were a T1 on the colour plate before peak maturity. The rind was smooth and peelability easy.

### Taylor Lee LS

Taylor Lee LS is mid to late maturing experimental mandarin hybrid. The trees bore medium fruit on the trees with count 1, and at peak maturity the juice content was just below 70%. Brix: Acid ratio at 13.5 the selection had Brix 13.4° and acid of 1.00%. The selection seed counts were on average 4.9 seeds per fruit. Taylor Lee LS reached T1 colour on the colour plate before peak maturity.

### Leanri

Leanri is an experimental early – mid maturing mandarin hybrid. The average seed count for Leanri ranged between 2.2 – 3.3 seeds per fruit. Leanri had a good Brix° and acid ratio towards peak maturity (ratio 11.0), the Brix was 13.7° and acid was 1.25%. This gives Leanri its good flavour. The juice percentage for Leanri was very good around 60%. The fruit was fully coloured up before peak maturity. Fruit size count was (count 2).

### RHM

The fruit size range ranged from medium (count 2 – 1) and the acid dropped quickly towards peak maturity to around 0.93% but stayed stable at around 0.85% until over mature. The external colour development was good with T1 on the colour plate when the fruit was at peak maturity. At peak maturity the selection had good Brix° around 11 and acid percentage around 0.90%. Juice percentage for RHM was good above 60%. There

were seeds in this selection during evaluations with an average count of 1.2 seeds per fruit. The fruit is firm with a smooth rind and a deep orange internal colour.

#### Or 1 & 4

Or 1 & 4 is a mid - late maturing mandarin hybrids. The size count for Or 1 & 4 ranged from count 2 – 1. Juice percentage increase towards peak maturity to above 60%, with Or 1 being slightly higher. Internal quality for the two Or selections is very good. Brix is high above 15° and the acids was still above 1.00% at peak maturity. This good Brix: Acid ratio will contribute to excellent eating fruit with great flavour and shelf life. Peelability is easy but oily. Or 1 & 4 reached T1 on the colour plate towards peak maturity. Or 1 had a slightly higher seed count.

#### Goldup

Goldup is an early maturing experimental mandarin hybrid. It reached peak maturity first in the trial block. Ratio of 12.4 was reached on 04 May. Internal quality was fair. The juice percentage was the lowest in the trial block with 43.4%, with 10.5° Brix and an acid percentage of 0.85%. This selection does tend to have a low acid to start with and it tends to drop quickly, but stabilises. Colour development was slightly delayed at peak maturity of T1 - T2 on the colour plate. Fruit size ranged between 5 – 4 (small). Goldup seed per fruit peaked at 3.8 during evaluations. Fruit shape is flattish and has a natural shine. Peelability is easy and the rind has a prominent aroma when it gets peeled. Rind oil is high.

#### **Conclusion**

IRM 1 & 2 had the largest fruit size peaking at count (1x) and Goldup had the smallest fruit size count (5 - 4). Taylor Lee LS had the most seeds per fruit on average (4.8). None of the selections were completely seedless. Most of the selections had a juice percentage above 60% at peak maturity and all of them were above 55% except Goldup. All the selections reach T 1 on the colour plate before or at peak maturity. Selections that didn't reach Brix above 12° towards peak maturity and at peak maturity were Goldup and RHM.

**Table 5.4.19.2.** Internal fruit quality data for experimental mandarin hybrid selections from the Buffeljagsrivier region of the Western Cape during the 2021 season.

Date	Cultivar	Rootstock	Avg Fruit size (mm)	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2021-05-19	Edit x Nova	CC	57,4	3	58,7	11,6	1,42	8,1	0,1	T5
2021-06-09	Edit x Nova	CC	56,3	3	49,1	12,1	1,14	10,6	0,4	T1 - T3
2021-04-14	Gold up	CC	48,2	5	48,6	10,5	1,11	9,4	3,8	T6 - T7
2021-05-04	Gold up	CC	54,5	4	43,4	10,5	0,85	12,4	2,2	T1 - T2
2021-06-09	Goldup	CC	53,7	4	60,5	10,7	0,62	17,1	0,6	T1
2021-08-06	IRM 1	CC	68,7	1x	62,2	14,0	1,52	9,2	1,4	T1
2021-09-09	IRM 1	CC	72,2	1x	68,5	14,2	1,25	11,4	1,2	T1 - T2
2021-08-06	IRM 2	CC	69,1	1x	62,9	14,2	1,29	11,0	1,2	T1
2021-09-09	IRM 2	CC	65,4	1	63,7	15,9	1,37	11,6	1,3	T1
2021-05-19	Leanri	CC	61,0	2	57,5	12,0	1,48	8,1	2,2	T1 - T5
2021-06-09	Leanri	CC	60,8	2	61,9	13,7	1,25	11,0	3,3	T1
2021-08-06	Mor 26	CC	67,0	1	57,6	15,4	1,40	11,0	0,8	T1
2021-09-09	Mor 26	CC	60,7	2	62,5	17,9	1,36	13,1	1,6	T1
2021-08-06	Or 1	CC	65,0	1	61,2	15,9	1,26	12,6	0,6	T1
2021-09-09	Or 1	CC	62,0	2	65,9	17,3	1,14	15,2	0,6	T1
2021-08-06	Or 4	CC	65,3	1	58,7	15,8	1,06	14,9	0,5	T1
2021-09-09	Or 4	CC	62,8	2	65,1	15,9	1,11	14,4	0,5	T1

2021-05-19	RHM	CC	64,3	1	61,3	10,0	0,93	10,7	0,0	T5 - T6
2021-06-09	RHM	CC	62,8	2	64,4	11,9	0,85	14,0	1,2	T1
2021-06-09	Taylor Lee LS	CC	66,3	1	64,5	13,6	1,44	9,4	5,4	T1 - T3
2021-08-06	Taylor Lee LS	CC	70,8	1	66,5	13,4	1,00	13,5	4,9	T1
2021-09-09	Taylor Lee LS	CC	66,5	1	67,8	14,0	1,04	13,5	4,0	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 W Swiegers and Z Zondi (CRI)

#### Summary

There were 3 trial sites in the Sundays River Valley from next season there will be only 2. They are all between Kirkwood and Addo. The trial site at Endulini is a site with early to late maturing navel selections. This is the main Navel trial site for the region. Next season the second trial site will be an early navel trial site at Dunbrody. In the region the season started with the following early selections: Sunrise Early, Fukumoto, Rosalina, Lina, Lethaba Early, Tibsreahny Early and Trosky Early the mid-maturing selections ripening were as follows Washington, Cara Cara, De Wet 1, Fischer, Addo Early, KS Early, Painter Early 2, Kirkwood Red and Navelina. The season ended with the following late maturing selections Suitangi, Autumn Gold, Lazyboy, Hutton, Cal Lane Late, Clarke, Witkrans 3, Caloma, Gloudi, Glen Ora Late 2, Summer Gold Suitangi, Barnfield Summer, Cambria 3, Glen Ora Late and Carninka Late.

#### Opsomming

Daar was 3 proef persele in die Sondagsrivier valley en die volgende seisoen gaan daar slegs 2 wees. Hulle is tussen Kirkwood and Addo geleë. Die proef by Endulini bevat die meeste vroeë tot laat rypwordende navel seleksies. Dit is die hoof perseel vir die area. Volgende seisoen gaan daar slegs 2 proef persele wees. Die 2de een gaan 'n vroeë navel proef wees. Die seisoen het begin met Sunrise Early, Fukumoto, Rosalina, Lina, Lethaba Early, Tibsreahny Early en Trosky Early gevolg deur die mid rypwording seleksies Washington, Cara Cara, De Wet 1, Fischer, Addo Early, KS Early, Painter Early 2, Kirkwood Red en Navelina. Die laat rypwordings seleksies was as volg Suitangi, Autumn Gold, Lazyboy, Hutton, Cal Lane Late, Clarke, Witkrans 3, Caloma, Gloudi, Glen Ora Late 2, Summer Gold Suitangi, Barnfield Summer, Cambria 3, Glen Ora Late en Carninka Late.

#### Objectives

- To select navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new navel cultivars and to determine the climatic suitability of these cultivars in cold production regions.

#### Materials and methods

Field evaluations and laboratory analyses were conducted on navel selections from the Sundays River Valley region of the Eastern Cape. The following early to late maturing selections were evaluated: Sunrise Early, Fukumoto, Rosalina, Lina, Lethaba Early, Tibsreahny Early, Trosky Early, Washington, Cara Cara, De Wet 1, Fischer, Addo Early, KS Early, Painter Early 2, Kirkwood Red, Navelina, Suitangi, Autumn Gold, Lazyboy, Hutton, Cal Lane Late, Clarke, Witkrans 3, Caloma, Gloudi, Glen Ora Late 2, Summer Gold, Barnfield Summer, Cambria 3, Glen Ora Late and Carninka Late.

For navels, a ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which it is considered over mature. This process from start to the end

of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

**Table 5.4.20.1.** List of navel selections evaluated at Sundays River Valley (Endulini) during 2021.

<b>Selection</b>	<b>Rootstock</b>	<b>Planted</b>
Cara Cara	CC	2015
Carninka Late	CC	2015
Clarke	CC	2015
De Wet 1	CC	2015
Glen Ora Late	CC	2015
Glen Ora Late 2	CC	2015
Gloudi	CC	2015
Cambria 3	CC	1997
Kirkwood Red	CC	2015
KS Early	CC	2015
Caloma	CC	2015
Letaba Early	CC	2015
Lazyboy	CC	2015
Navelina	CC	2015
Painter Early 2	CC	2015
Suitangi	CC	2015
Sunrise Early	CC	2015
Trosky Early	CC	2015
Washington	CC	2015
Witkrans 3	CC	2015
Fukumoto	CC	2015
Fischer	CC	2012
Rosalina	CC	2015
Lina	CC	2015
Tibsreahny Early	CC	2015
Addo Early	CC	2015
Autumn Gold	CC	1997
Hutton	CC	1997
Cal Lane Late	CC	2015
Summer Gold	CC	1997
Barnfield Summer	CC	1997

## **Results and discussion**

### Early maturing selections

Sunrise Early were the first selection to reach peak maturity beginning – mid April, and Trosky Early were the last selection to reach peak maturity of the early maturing selections. Trosky peak maturity were reached end April. Lina was used as control for the early maturing selections. All the trees had a good crop on them especially for early maturing navels. All the early selections had a low juice content. Tibsreahny Early was the selection with highest juice content peaking at 54.3%, followed by Trosky Early 52.1%. The selection bore favorably sized fruit and peaked at count 56, Lina and Sunrise Early also bore fruit size counts 72 and 64. Lina, Tibsreahny Early en Trosky Early had the highest Brix at peak maturity above 10° but below 11°. The acid content for all the selctions kept well and the fruit were able to hang to improve flavour and colour development. Tibsreahny Early had the best colour development being T1 – T2 on the colour plate, all of the other selections peaked at T5 – T6.

### Mid maturing selections

Washington was the first of the mid-maturing selections having reached peak maturity mid May, it was also the control for the mid-maturing selections. Navelina were the last of the mid-maturing selections to have reached peak maturity, at end May Navelina ratio were 9.3:1 (towards peak maturity). All the selections did bore a good crop. Washington, Navelina, De Wet 1 and Painter Early were the only selections to have reached

juice content just above 50%. All the selections peaked at count 56 except Fischer Navel count 48 and Addo Early count 40. Washington and Fischer had the best Brix between 10.5° - 11.5°. The acids content of all the selections stayed stable even after peak maturity, it did help with colour development. The only selection to reached T1 on the colour plate were Fischer.

#### Late maturing selections

On the late maturing selections, Suitangi reached peak maturity in the beginning of June. Carninka Late which is a very late maturing navel did finish off the season, having reached peak maturity end of July. The control for these selections were Cal Lane Late. The crop was very good for all the selections with a favourable fruit size count 56. Only Barnfield Summer had a bigger fruit size count 48, and Cambria had slightly smaller fruit peaking at count 64. The late maturing selections with the highest and a moderate juice content were Caloma, Glen Ora Late selctions, Clarke, Carninka Late, Cal Lane Late and Suitangi, they juice content were around 55%. Selections with the highest Brix above 12° were Summer Gold, Cambria and Carninka Late. The acid content also stayed stable after the selections reached peak maturity and improved with colour development. The flavour also improved as the Brix went up with the stable acids. Selections that reach T1 on the colour plate were the following Autumn Gold, Barnfield Summer, Cambria, Carninka Late and Glen Ora Late selections.

#### Pigmented selections

The first pigmented variety to reach peak maturity was Rosalina at mid April with Brix:acid content 10:1 the Brix were 8.8 and acid were 0.88% with a juice content 53.3% and fruit size count 56 and T7 on the colour plate. Fruit did have an intermediate red internal colour, unfortunately, rind colour did not colour up enough to see the blush on the rind. Cara Cara is the control for pigmented navels. Cara Cara were the second pigmented variety to reach peak maturity and it reached peak maturity mid May with ratio 10.2. the Brix was 9.8 with acid content 0.96%. Juice percentage was low 49.8% wat the acids were stable for Cara Cara and the fruit were left to hang and the juice content increased and peaked at 61.5%. No creasing was found. The variety was a T5 on the colour plate. . Fruit size is uniform large, (count 56). Navel ends are small - medium. Internal flesh colour is an intermediate red, and flavour is very good. Kirkwood Red finished the season for the pigmented navels, when peak maturity was reached end May. Brix was 10, with 1.01% acid to support the Brix and give Kirkwood red a good flavour. Juice content was above 55%, due to the stable acids the colour improved from T6 to T3 on the colour plate. Fruit size was good with a count 56. Internal colour pigment is red. The tree is more compact compared to Cara Cara.

#### **Conclusion**

The Addo area is well suited for navel production in South Africa. Most of the selections had a very good fruit size and peaked at count 56. Most of the selections had delayed colour development. As the season went on, the juice content did start to improve slightly and the Brix also got higher. All the selections had very good stable acids. All the navel selections were seedless.

**Table 5.4.20.2.** Internal fruit quality data for early to late Navel selections from the Addo/Kirkwood region of the Sundays River Valley during the 2021 season.

Date	Selection	Root stock	Avg fruit size (mm)	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg Seed	Colour
2021-04-26	Addo Early	CC	92	40	41.8	8.2	0.95	8.6	0.0	T6
2021-05-27	Addo Early	CC	83	56	47.5	8.4	0.81	10.4	0.0	T4
2021-06-08	Autumn Gold	CC	83	56	50.6	9.9	0.91	10.9	0.0	T6
2021-07-07	Autumn Gold	CC	85	56	50.9	11.2	0.84	13.3	0.0	T1
2021-06-08	Barnfield	CC	83	56	45.8	8.7	1.08	8.1	0.0	T6
2021-06-23	Barnfield	CC	88	48	49.0	8.8	0.88	10.0	0.0	T5-6

2021-07-07	Barnfield	CC	89	48	47.1	9.1	0.90	10.1	0.0	T1
2021-06-08	Cal Lane Late	CC	83	56	54.9	10.8	1.06	9.6	0.0	T4-5
2021-06-23	Cal Lane Late	CC	84	56	51.7	10.5	0.88	11.9	0.0	T4
2021-07-07	Cal Lane Late	CC	83	56	53.7	11.1	0.89	12.5	0.0	T4
2021-06-23	Cambria 3	CC	79	64	50.8	11.6	1.23	9.4	0.0	T3
2021-07-07	Cambria 3	CC	76	72	46.8	13.1	1.20	10.9	0.0	T1
2021-05-11	Cara Cara	CC	83	56	49.8	9.8	0.96	10.2	0.0	T6
2021-05-27	Cara Cara	CC	83	56	53.5	10.2	0.84	12.1	0.0	T5
2021-06-08	Cara Cara	CC	83	56	61.5	10.0	0.86	11.6	0.0	T5
2021-07-07	Carninka Late	CC	82	56	51.6	10.7	1.14	9.4	0.0	T4
2021-07-27	Carninka Late	CC	81	56	56.7	11.2	1.08	10.4	0.0	T1
2021-08-16	Carninka Late	CC	80	64	54.2	12.2	1.03	11.8	0.0	T1
2021-06-08	Clark	CC	83	56	54.3	9.9	1.03	9.6	0.0	T6
2021-06-23	Clark	CC	85	56	53.2	10.3	0.89	11.6	0.0	T5
2021-07-07	Clark	CC	86	48	52.5	10.8	0.81	13.3	0.0	T3
2021-04-26	De Wet 1	CC	83	56	47.0	8.8	0.96	9.2	0.0	T7
2021-05-11	De Wet 1	CC	83	56	50.6	9.3	0.92	10.1	0.0	T6
2021-05-27	De Wet 1	CC	83	56	51.5	9.4	0.86	10.9	0.0	T5-6
2021-05-24	Fischer Navel	CC	88	48	46.5	10.9	1.16	9.4	0.0	T3
2021-06-08	Fischer Navel	CC	79	64	44.1	11.1	1.03	10.8	0.0	T1
2021-03-29	Fukumoto	CC	83	56	48.4	9.6	1.13	8.5	0.0	T7
2021-04-12	Fukumoto	CC	83	56	49.7	9.3	0.87	10.7	0.0	T6
2021-04-26	Fukumoto	CC	83	56	47.3	9.8	0.93	10.5	0.0	T6
2021-05-11	Fukumoto	CC	83	56	43.5	9.7	0.85	11.4	0.0	T5
2021-06-23	Glen Ora Late	CC	85	56	53.5	10.3	1.13	9.1	0.0	T5
2021-07-07	Glen Ora Late	CC	83	56	54.9	11.3	1.13	10.0	0.0	T1
2021-07-27	Glen Ora Late	CC	83	56	53.8	11.9	0.97	12.3	0.0	T1
2021-06-23	Glen Ora Late 2	CC	83	56	55.8	10.6	1.04	10.2	0.0	T5
2021-07-07	Glen ora Late 2	CC	83	56	55.1	11.8	1.05	11.2	0.0	T1
2021-07-27	Glen Ora Late 2	CC	83	56	53.9	11.5	0.92	12.5	0.0	T1
2021-06-08	Gloudi	CC	83	56	52.1	10.1	1.08	9.0	0.0	T5-6
2021-06-23	Gloudi	CC	81	56	51.9	10.4	1.02	10.2	0.0	T5
2021-07-07	Gloudi	CC	83	56	51.0	11.1	0.86	12.9	0.0	T4
2021-06-08	Hutton	CC	83	56	52.0	10.4	1.01	10.3	0.0	T5
2021-06-23	Hutton	CC	86	48	52.6	10.0	0.90	11.1	0.0	T6
2021-07-07	Hutton	CC	85	56	51.9	11.1	0.81	13.7	0.0	T3
2021-05-11	Kirkwood Red	CC	83	56	52.1	9.8	1.04	9.4	0.0	T6
2021-05-27	Kirkwood Red	CC	83	56	58.5	10.0	1.01	9.9	0.0	T6
2021-06-08	Kirkwood Red	CC	83	56	55.5	10.1	0.95	10.6	0.0	T5
2021-06-23	Kirkwood Red	CC	85	56	54.1	10.5	0.80	13.1	0.0	T3
2021-05-11	KS Early	CC	83	56	47.4	9.2	1.13	8.1	0.0	T6
2021-05-27	KS Early	CC	83	56	49.5	9.8	0.95	10.3	0.0	T5
2021-06-08	KS Late	CC	83	56	50.1	10.4	1.06	9.8	0.0	T6
2021-06-23	KS Late	CC	82	56	50.4	10.4	0.98	10.6	0.0	T5
2021-07-07	KS Late	CC	83	56	54.4	11.2	0.86	13.0	0.0	T4
2021-06-08	Lazyboy	CC	78	64	46.8	10.4	0.80	10.4	0.0	T6-7

2021-06-23	Lazyboy	CC	82	56	47.6	11.0	0.76	14.5	0.0	T6
2021-04-12	Letaba Early	CC	83	56	49.0	9.2	1.00	9.2	0.0	T7
2021-04-26	Letaba Early	CC	83	56	49.2	9.8	0.91	10.8	0.0	T5
2021-05-27	Letaba Early	CC	83	56	51.5	10.0	0.78	12.8	0.0	T3-4
2021-03-29	LF Early	CC	75	72	43.6	9.5	0.99	9.6	0.0	T7
2021-04-12	LF Early	CC	78	64	47.4	9.7	0.90	10.8	0.0	T7
2021-04-26	LF Early	CC	83	56	43.5	10.4	0.78	13.3	0.0	T5
2021-03-29	Lina	CC	74	72	46.0	10.3	1.25	8.2	0.0	T8
2021-04-12	Lina	CC	79	64	49.0	10.1	1.06	9.5	0.0	T7
2021-04-26	Lina	CC	83	56	46.1	10.7	0.92	11.6	0.0	T6
2021-05-11	Lina	CC	83	56	48.0	10.3	0.82	12.6	0.0	T6
2021-05-11	Navelina	CC	83	56	54.1	9.8	1.14	8.6	0.0	T6
2021-05-27	Navelina	CC	79	64	52.5	10.1	1.09	9.3	0.0	T5
2021-05-11	Painter Early 2	CC	83	56	55.0	9.8	1.14	8.6	0.0	T6
2021-05-27	Painter Early 2	CC	83	56	50.0	10.4	1.04	10.0	0.0	T5-6
2021-03-29	Red Lina	CC	83	56	50.2	8.5	0.96	8.9	0.0	T7
2021-04-12	Red Lina	CC	83	56	53.3	8.8	0.88	10.0	0.0	T7
2021-04-26	Red Lina	CC	83	56	48.4	9.2	0.68	13.5	0.0	T6
2021-06-08	Suitangi	CC	83	56	55.2	9.9	0.90	11.0	0.0	T5
2021-06-23	Suitangi	CC	85	56	53.5	10.1	0.80	12.6	0.0	T5
2021-06-08	Summer Gold	CC	83	56	45.6	11.6	1.34	8.7	0.0	T6
2021-06-23	Summer Gold	CC	84	56	53.5	12.4	1.21	10.2	0.0	T4
2021-07-07	Summer Gold	CC	83	56	48.7	12.9	1.15	11.2	0.0	T3
2021-04-12	Tibs Early	CC	83	56	53.4	9.6	1.03	9.3	0.0	T6
2021-04-26	Tibs Early	CC	83	56	51.5	10.1	0.95	10.6	0.0	T6
2021-05-24	Tibs Early	CC	83	56	54.3	10.6	0.86	12.3	0.0	T1-2
2021-04-12	Trosky Early	CC	83	56	50.3	10.0	1.14	8.8	0.0	T7
2021-04-26	Trosky Early	CC	83	56	49.0	10.7	1.09	9.8	0.0	T6
2021-05-27	Trosky Early	CC	83	56	52.1	10.9	0.94	11.6	0.0	T4
2021-05-11	Washington	CC	83	56	46.2	9.9	0.93	10.6	0.0	T6
2021-05-27	Washington	CC	83	56	50.2	10.7	0.88	12.2	0.0	T5
2021-06-08	Washington	CC	83	56	54.0	10.3	0.88	11.7	0.0	T2
2021-06-08	Witkrans 3	CC	83	56	52.6	9.8	1.04	9.4	0.0	T6
2021-06-23	Witkrans 3	CC	83	56	49.9	10.1	0.90	11.2	0.0	T3
2021-07-27	Witkrans 3	CC	81	56	52.5	11.0	0.83	13.3	0.0	T3

#### 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 W Swiegers and Z Zondi (CRI)

##### Summary

The trial consists of a few experimental early, mid and late navel selections. Sunrise Early and Painter Early 2 started the season followed by Addo Early, Lazyboy and the season ended with Suitangi. This region is going through severe drought some varieties did not reach peak maturity.

##### Opsomming

Hierdie proef bestaan uit 'n paar eksperimentele vroeë-, middel- en laat nawel seleksies. Orde van rypwording was Sunrise Early en Painter Early 2 gevolg deur Addo Early, Lazyboy en die seisoen is afgesluit deur Suitangi. Die streek gaan gebuk onder erge droogte en van die seleksies het nie optimale rypheid bereik nie.

## Objectives

- To select navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new navel cultivars and to determine the climatic suitability of these cultivars in cold production regions.

## Materials and methods

Field evaluations and laboratory analyses were conducted on navel selections from regions of the Gamtoos River Valley. The following selections were evaluated: Sunrise Early, Lazyboy, Painter Early 2, Addo Early and Suitangi

A ratio of 9:1 is considered to be the build-up towards peak maturity for selections. When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. After reaching the peak, the ratio increases to 11:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in higher instances of quality and rind issues.

**Table 5.4.21.1.** List of navel selections evaluated at Loerie site in the Gamtoos River Valley, Eastern Cape during the 2021 season.

Selection	Rootstock	Topworked	Planted
Sunrise Early	Carrizo	2012	
Addo Early	Carrizo		2016
Lazyboy	Carrizo	2013	
Painter Early 2	Carrizo	2012	
Suitangi	Carrizo		2016

## Results and discussion

### Sunrise Early

Sunrise Early is an early-maturing navel and were also the first selection to reach peak maturity. The selection had good fruit size count 64 - 56; perfect for navel production and export. The internal quality was moderate with juice content around 50%. At peak maturity, the external colour peaked at colour plate T5. The Brix remained around 9° and acid percentage around 0.9%. The acids stayed stable long after peak maturity and Brix and juice content went up and T1 were reached on the colour plate.

### Addo Early

Addo Early was the third selection at peak maturity in this trial site. It is an experimental early maturing Navel. The navel end on the fruit was small. This selection had smaller fruit count 64 compared to 2020 season fruit size of counts 56 - 48 at peak maturity in the trial site. The external colour development was very good T1 (colour plate). Internal quality was moderate, it improves as the tree gets older with Brix just below 11 and juice content just below 55%.

### Painter Early 2

Painter Early 2 was the second selection to mature for this navel trial. Medium sized fruit was on the trees with count 72 - 64. Painter Early 2 was at T4 on the colour plate at peak maturity. The juice percentage of Painter Early 2 increased towards peak maturity to 52.3%, as the fruit hang due to the stable acids, juice peaked just

below 60%. Painter Early 2 had fair Brix and acid levels at peak maturity. Fruit shape is round with smooth rind and small navel ends.

### Suitangi

Suitangi is one of the late maturing experimental navel selections evaluated. It was the third crop on the trees. The external colour development was delayed; T4 on the standard colour plate at peak maturity. The selection had a small navel end. Suitangi peaked at count 64. Suitangi internal quality was moderate to good at peak maturity with juice content just above 50%. Brix levels around 12° with acids of 0.95% assured good tasting fruit with good flavour. Dark orange external colour.

### Lazyboy

Lazyboy is a late maturing navel with moderate - good internal quality. Brix was 12.7° and the fruit hangs well on the tree with acid percentage of 1.19%. Even when the fruit is over mature the acid is still stable before it drops. Fruit size peaked at count 64. Navel ends are mainly small to closed with occasional fruit having more open navels. Fruit shape is round and rinds are smooth. Externally the colour development was delayed; T4 at peak maturity. The flavour is very good.

### **Conclusion**

The fruit size of all the navel selections peaked at count 64 at peak maturity, except Sunrise Early peaking count 56. The Navel selection with the highest juice percentage was Painter Early 2 (59.2%). All of the selections had a good external colour development on the colour plate at peak maturity. Suitangi and Lazyboy developed the highest Brix values for this trial.

**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 2021 season.

Date	Selection	Rootstock	Avg fruit size (mm)	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2021-06-01	Addo Early	CC	78	64	46.4	10.1	1.15	8.8	0.0	T2
2021-06-14	Addo Early	CC	78	64	53.6	10.8	1.07	10.1	0.0	T1
2021-06-14	Lazyboy	CC	74	72	48.8	11.2	1.60	7.0	0.0	T6
2021-07-06	Lazyboy	CC	79	64	50.3	12.7	1.19	10.7	0.0	T4
2021-04-20	LF Early	CC	82	56	49.6	9.6	1.09	8.8	0.0	T6
2021-05-03	LF Early	CC	82	56	49.8	8.7	0.90	9.7	0.0	T5
2021-05-19	LF Early	CC	82	56	53.6	10.4	0.92	11.3	0.0	T2
2021-06-01	LF Early	CC	79	64	49.2	11.0	0.90	12.0	0.0	T1
2021-05-03	Painter Early 2	CC	75	72	49.6	9.5	1.15	8.3	0.0	T6
2021-05-19	Painter Early 2	CC	79	64	52.3	10.4	0.91	11.4	0.0	T4
2021-06-01	Painter Early 2	CC	79	64	50.8	10.9	0.88	12.4	0.0	T1
2021-06-14	Painter Early 2	CC	79	64	59.2	11.5	0.86	13.4	0.0	T1
2021-06-01	Suitangi	CC	79	64	51.7	10.8	1.18	9.2	0.0	<b>T6</b>
2021-07-06	Suitangi	CC	79	64	51.3	11.6	0.95	12.2	0.0	T4
2021-07-21	Suitangi	CC	79	64	52.6	12.4	0.95	13.1	0.0	T1

## 5.4.22 PROGRESS REPORT: Cultivar characteristics and climatic suitability of experimental navel oranges in a cold production region (Western Cape)

Project 998D by W Swiegers (CRI)

### Summary

Citrusdal is probably one of the country's best regions to farm Navels. The trial consists of most of the recent selections and a few newer ones will be added. The trial consists of a few experimental early, mid and late navel selections in two trial sites. Fukumoto and Lina are the controls for early maturing navels. Fischer was used as control for the early-mid selections, Washington as control for the mid-maturing navel selections and Lane Late for the late navel sections. Cara Cara was used as the control for the pigmented navels. Most of the trees are older and have big tree volumes. Tibshreany Early, Lina, Rayno Early, Navelina, Fischer and Palmer, started the season as the early navel selections for evaluation. The mid navel selections that were evaluated in order of ripening were as follows: Cara Cara, Kirkwood Red, Gerhard Early and Washington. The late selections that were evaluated and were last to reach peak maturity were Cambria, Witkrans, Carninka, and Lane Late.

### Opsomming

Citrusdal is seker een van die beste streke in die land vir Navels. Die proef het die meeste van die nuwe seleksies. Daar gaan nog uitgebrei word op hulle. Hierdie spesifieke proef bestaan uit 'n paar eksperimentele vroe-, middel- en laat nawel seleksies in 2 proef persele. Fukumoto en Lina is as kontrole gebruik vir die vroe seleksies. Fischer is as kontrole gebruik vir die vroe - mid seleksies, Washington word as kontrole gebruik vir die middel seleksies en Lane Late dien as kontrole vir die laat nawel seleksies. Cara Cara word as kontrole gebruik vir die gepigmenteerde seleksies. Die meeste van die bome is al ouer en die bome het 'n groot boom volume. Die volgorde van rypwording was beginnende met die vroe seleksies Tibshreany Early, Lina, Rayno Early, Navelina, Fischer en Palmer gevolg deur die middel seleksies se volgorde Cara Cara, Kirkwood Red, Gerhard Early en Washington. Die seisoen was afgesluit met die laat seleksies se orde van rypwording Cambria, Witkrans, Carninka, en Lane Late.

### Objectives

- To select navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new navel cultivars and to determine the climatic suitability of these cultivars in cold production regions.

### Materials and methods

Field evaluations and laboratory analyses were conducted on navel selections from regions of the Citrusdal Valley. The following selections were evaluated: Tibshreany Early, Lina, Rayno Early, Navelina, Fischer, Palmer, Cara Cara, Kirkwood Red, Gerhard Early, Washington, Cambria, Witkrans, Carninka, and Lane Late.

A ratio of 9:1 is considered to be the build-up towards peak maturity for selections. When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. After reaching the peak, the ratio increases to 11:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

**Table 5.4.22.1.** List of navel selections evaluated at various sites in the Citrusdal, Western Cape during the 2021 season.

Selection	Rootstock	Planted	Topworked
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Cara Cara	Carrizo	2009	
Fischer	Carrizo	2009	
Gerhard Early	Carrizo	2009	
Kirkwood Red	Carrizo	2009	
Lina	Carrizo		2016
Lane Late	Carrizo	2009	
Washington	Carrizo	2009	
Carninka	Carrizo		2010
Navelina	Carrizo		2016
Rayno Early	Carrizo	2009	
Tibs Early	Carrizo		2014
Witkrans	Carrizo		2011
Cambria	Carrizo		2011
Palmer	Carrizo		2011

## Results and discussion

### Gerhard Early

Gerhard Early is an experimental early maturing navel. Gerhard Early trees bore medium to large sized fruit that peaked at count 64. Gerhard Early had delayed color development (color plate T2 - 3) when the fruit was at peak maturity. Sugars and acids were good at peak maturity, Brix around 10.7° and acid around 1.09%.

### Fischer

Fischer (control) had a delayed colour development with colour plate;T6 at peak maturity. Fischer had a good fruit size which peaked at count 56. Fischer internal quality was very good at peak maturity, Brix were between 9 - 10° and acid around 0.90% - 1%. The flavour was very good. The navel end for Fischer was small to closed and the fruit had a smooth rind. The crop was very good.

### Washington

Washington was used as control for the mid-maturing navel. The external colour development was behind the internal quality of the fruit (T1 – T4) on the colour plate at peak maturity. Washington fruit size peaked at count 56. Brix levels around 10.3° with acids of 1.09%. Navel ends were medium for Washington.

### Cara Cara

Cara Cara is the control for pigmented navels. Cara Cara is a mid-maturing pigmented navel. Compared to last season's colour development of T5 on the colour plate at peak maturity, this season the colour development was T1 – T3 at peak maturity. The fruit size peaked count 64. Fruit shape was round with smooth rind. The navel ends were small. The flavour was good. Internal colour was an intermediate red in the beginning of the season and as the season went on the red flesh became a bit deeper red. At peak maturity with Brix:Acid ratio of 10.1 the Brix was 10.9 with 1,08% acid.

### Kirkwood Red

Kirkwood Red is a mid-maturing pigmented navel. Peak maturity was reached later than Cara Cara navel. The colour development for Kirkwood Red was better than last season T3 – T4, being T1 – T3 this season. The good acids allowed us to keep the fruit longer on the trees and it did colour up to T1 on the colour plate. Internal quality for Kirkwood Red was good. The flavour was excellent. Fruit size for Kirkwood Red was medium (count 72 - 64). Flesh colour was deep red; even the fruit stem was red.

### Carninka Late

Carninka Late is a late maturing experimental navel. Peak maturity was reached in July. Carninka late had fruit size, count ranged between (count 64 – 56). The internal fruit quality at 11.7 ratio, was good, Brix 10.5° and good acid percentage (0.90%). Fruit is firm and has a smooth peel. Colour development was T1–T2 on the colour plate at peak maturity. Fruit can hang on the trees for longer periods. Navel end is close to small and flavour very good.

### Navelina

Navelina is a mid-maturing navel. It was the selection's third crop. The tree is not as vigorous as the Washington. Fruit size peaked at large fruit (counts 56 with a very small to closed navel). The fruit had a delayed colour (T6 – T7) at peak maturity. Fruit shape is slightly elongated, and rinds are smooth. Internal quality was moderate.

### Lina

Lina was the second to reach peak maturity at this trial site and also one of the early maturing controls. The selection had a delayed colour development with a colour plate of T6 – T7 when it was at peak maturity. The selection had a good fruit size and peaked at count 64. The fruit shape was more elongated with a large navel-end (fairly open). Internal quality at peak maturity for Lina was good; Brix above 11.2° and acids around 1.09%.

### Lane Late

Lane Late is the control for the late maturing selections. It reached peak maturity last in the trial block. Fruit size was medium to large, fruit size count were count 64 – 56. The internal quality at peak maturity was good with juice percentage just above 55%, Brix 10° and acid percentage 0.91%. The colour development were optimum being T1 on the colour plate.

### Rayno Early

Rayno Early is a very early maturing experimental navel. It was the third selection to reach peak maturity at the trial site. Fruit size was medium with counts 88. For an early maturing navel, it managed to get very good Brix above 11° at peak maturity with stable and good acids around 1.02%. T5 – T6 was the colour on the colour plate when the fruit was at peak maturity. Crop was fair - good.

### Tibshreany Early

Tibshreany Early is also an experimental early maturing navel. The crop was fair. It was the first selection to reach peak maturity at the trial site. Peak maturity was reached at the end of March – beginning April, Tibshreany Early fruit size count ranged from 88 - 72 (medium fruit). At over maturity the Brix was around 11.4° with acid percentage of 0.97%. Tibshreany Early also kept its acid stable. Colour development was delayed with T6 – T8 at a ratio of 11:7.

### Witkrans

Witkrans is a very promising late maturing navel. The trees also had a good crop on them. The external colour development was delayed with a T2 - T4 on the colour plate at peak maturity. The fruit size count for Witkrans was good, peaking at a count of 64 which is great for navel production and export. Witkrans acids were around 0.89% at peak maturity. Brix was good and along with the good acid it will give Witkrans great flavour. It has a closed to small navel end.

### Cambria

Cambria is a well-known late navel selection with very good internal quality. Cambria had small navel end openings. The fruit shape was more elongated compared to the other navel selections. Small to medium sized fruit and peaked at count 64. The Brix was 11.4° with acids, 0.81% and Brix:acid ratio 14. Colour development was delayed.

### Palmer

The external colour development of the selection was delayed (colour plate T5) at peak maturity. The selection had a good fruit size and peaked at count 56. The acids and **rix** were good at peak maturity but and Juice percentage were low.

## **Conclusion**

Most of the fruit were bigger in 2021 season compared to the 2020 season. The following selections (Carninka, Fischer, Kirkwood Red, Lane Late, Navelina, Palmer and Washington) were the only selections that peaked at fruit size count 56. The selections were seedless. The best colour development was from Witkrans, Lane Late,

Kirkwood Red, and Carninka Late. The selections with Brix above 11.0° at peak maturity were Cambria, Carninka Tibsreany Early, Kirkwood Red, Lina, Lina, Navelina, Rayno Early and Lane Late.

**Table 5.4.22.2.** Internal fruit quality data for Experimental Navel selections from the Citrusdal region of the Western Cape during the 2021 season.

Date	Selection	Rootstock	Avg fruit size (mm)	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2021-06-07	Cambria	CC	71,4	88	59,5	9,5	1,16	8,2	0	T5 - T6
2021-08-05	Cambria	CC	66,5	105	47,7	11,4	0,81	14,0	0	T2 - T4
2021-05-18	Cara Cara	CC	76,6	64	54,7	10,5	1,11	9,5	0	T4
2021-06-07	Cara Cara	CC	78,3	64	45,9	10,9	1,08	10,1	0	T1 - T3
2021-06-07	Carninka	CC	80,9	56	52,6	9,6	1,12	8,5	0	T5 - T6
2021-08-05	Carninka	CC	81,8	56	53,4	10,5	0,90	11,7	0	T1 - T2
2021-09-08	Carninka	CC	78,2	64	54,1	11,5	0,90	12,8	0	T1 - T3
2021-04-13	Fischer	CC	77,4	64	42,2	9,3	1,07	8,7	0	T7 - T8
2021-05-06	Fischer	CC	81,5	56	49,6	9,1	0,94	9,6	0	T6
2021-05-18	Fischer	CC	83,3	56	49,3	10,9	1,00	10,9	0	T4
2021-05-06	Gerhard Early	CC	78,1	64	48,8	10,1	1,16	8,7	0	T5 - T6
2021-05-18	Gerhard Early	CC	79,9	64	51,1	10,1	1,08	9,4	0	T5
2021-06-07	Gerhard Early	CC	76,3	72	42,3	10,7	1,09	9,8	0	T2 - T3
2021-05-18	Kirkwood Red	CC	73,0	72	49,5	11,3	1,26	9,0	0	T5
2021-06-07	Kirkwood Red	CC	78,3	64	49,6	12	1,22	9,9	0	T1 - T3
2021-08-05	Kirkwood Red	CC	84,5	56	49,8	12,6	0,88	14,3	0	T1
2021-06-07	Lane Late	CC	76,5	64	54,3	8,8	1,17	7,5	0	T4 - T6
2021-08-05	Lane Late	CC	80,4	64	55,9	10	0,91	11,0	0	T1
2021-09-08	Lane Late	CC	82,2	56	54,5	11,3	0,90	12,6	0	T1 - T2
2021-04-13	Lina	CC	75,2	72	45,8	11,2	1,09	10,2	0	T6 - T7
2021-05-06	Lina	CC	76,4	72	53,5	12,4	1,10	11,2	0	T4 - T6
2021-05-18	Lina	CC	77,0	64	50,0	12,8	1,06	12,1	0	T3
2021-04-13	Navelina	CC	73,0	72	43,4	10	1,12	8,9	0	T7 - T8
2021-05-06	Navelina	CC	80,6	56	51,3	10,4	1,01	10,3	0	T6 - T7
2021-06-07	Navelina	CC	79,2	64	47,2	12	1,22	9,9	0	T1 - T3
2021-05-06	Palmer	CC	79,4	64	47,7	9,8	1,00	9,8	0	T6 - T7
2021-05-18	Palmer	CC	79,4	64	47,0	10,3	0,98	10,5	0	T5
2021-06-07	Palmer	CC	80,6	56	50,1	10,8	0,96	11,2	0	T3 - T4
2021-04-13	Rayno Early	CC	68,5	88	45,9	10,6	1,08	9,8	0	T6 - T7
2021-05-06	Rayno Early	CC	71,4	88	53,9	11	1,02	10,7	0	T5 - T6
2021-05-18	Rayno Early	CC	72,2	88	54,4	12,3	1,06	11,6	0	T2
2021-03-25	Tibsreany Early	CC	69,7	88	45,6	11,1	1,16	9,6	0	T6 - T7
2021-04-13	Tibsreany Early	CC	72,7	72	41,8	11,4	0,97	11,7	0	T6 - T8
2021-05-18	Tibsreany Early	CC	73,1	72	43,7	13	0,95	13,7	0	T3
2021-05-06	Washington	CC	79,7	64	50,7	10,5	1,16	9,1	0	T6 - T7
2021-05-18	Washington	CC	73,9	72	50,6	9,8	1,02	9,6	0	T5
2021-06-07	Washington	CC	81,8	56	44,2	10,3	1,09	9,4	0	T1 - T4
2021-06-07	Witkrans	CC	75,3	72	44,6	9,6	1,15	8,3	0	T5 - T6

2021-08-05	Witkrans	CC	74,0	72	54,1	10,6	0,89	11,9	0	T2 - T4
2021-09-08	Witkrans	CC	77,2	64	54,3	10,9	0,80	13,6	0	T1 - T2

#### 5.4.23 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Valencia oranges in a cold production region (Sundays River Valley)

Project 1097A by W Swiegers and Z Zondi (CRI)

#### Summary

Valencia discussed in this trial were top worked in the 2011 season. The trees produced their first crop in the 2015 season. There is a new trial site. The early maturing selection for the trial site is Turkey with Midnight as control. The mid-maturing Valencia selections are Alpha, Gusocora, Bennie 2, Henrietta, Louisa and Midnight 1-3. The late maturing Valencia selections will be McClean SL. At this trial site the season started with Turkey, followed by Midnight 1, Mc Clean SL, Bennie 2, Midnight F17, Midnight 2, Gusocora, Louisa, Henrietta, Alpha, and the season ended off with Midnight.

#### Opsomming

Die Valencia wat bespreek word in hierdie proef was in die 2011 seisoen getopwerk. Die bome het hulle eerste drag in die 2015 seisoen gehad. Daar is met 'n perseel begin. Die vroeë seleksie vir die proef perseel bestaan uit Turkey en Midnight wat as kontrole dien. Die mid seleksies is Alpha, Gusocora, Bennie 2, Henrietta, Louisa, en Midnight 1-3. Die laat rypwordende Valencia seleksies was as volg; McClean SL. Die proef perseel se seisoen het begin met Turkey, gevolg deur Midnight 1, Mc Clean SL, Bennie 2, Midnight F17, Midnight 2, Gusocora, Louisa, Henrietta, Alpha, en die seisoen het afgeëindig met Midnight. Die volgorde van rypwording kan beïnvloed word deur die area wat 'n intermediere area is vir Valencia.

#### Objective

Select Valencia cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (seedlessness, ratio), and extended harvest period (both earlier and later maturity).

To describe the cultivar characteristics of new Valencia cultivars and to determine the climatic suitability of these cultivars in a cold production region.

#### Materials and methods

Field evaluations and laboratory analyses were conducted on Turkey, Midnight, Midnight F17, Midnight 2, Midnight 1, Alpha, Bennie 2, Louisa, Henrietta, Gusocora and Mc Clean SL.

**Table 5.4.23.1.** Internal fruit quality minimum export requirements for Valencia types.

Cultivar	Juice %	Brix	Min Acid	Max Acids	Ratio	Colour
Valencia EU	48	8.5	0.6	1.8%	7.5:1	Colour plate 3 of set no. 34
Midnight	52	9.5	0.85	1.8%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

\*Interim internal fruit quality standards.

**Table 5.4.23.2.** List of Valencia selections evaluated at Panzi (Kirkwood) during 2021 season.

Selection	Rootstock	Topwork
Alpha	CC	2011
Bennie 2	CC	2011
Gusocora G5	CC	2011
Henrietta	CC	2011

Louisa	CC	2011
McClellan SL	CC	2011
Midnight	CC	2011
Midnight 1	CC	2011
Midnight 2	CC	2011
Midnight F17	CC	2011
Turkey	CC	2015

## Results and discussion

### Alpha

Alpha bore medium size fruit this season on the trees, peaking at count 72. Alpha Valencia were virtually seedless and the fruit shape remained fairly round with a slightly pebbly rind. The external colour development peaked at T1 with good internal quality, high Brix just around 11° supported with slightly higher acids by the time of maturity. Juice content peaked at 59.3% at peak maturity.

### Bennie 2

The fruit size peaked from count 64 this season, a good Valencia export fruit size. Bennie 2 has good acids for the fruit to hang on the trees longer to harvest later, resulting in fewer rind problems (pitting). The selection had a seed count 2.3 – 3.2. There was no delay in external colour development (T1) at peak maturity. The rind colour was deep orange with a smooth to coarse rind. The flesh was orange and the fibre strength was soft compared to the other Valencia selections. Bennie 2 developed a very good internal quality juice percentage just below 60%. The crop was very good.

### Gusocora

There were no delay in external colour development on the fruit (T1). When the selection reached a T1 the Brix was around 10.9 with an acid of 1.45%. Gusocora was virtually seedless and will be regarded as a seedless selection. The juice content of Gusocora this season was high 60.1% and the fruit size ranged between counts 88 – 72. The fruit was firm with a round shape and a smooth rind.

### Midnight, Midnight 1, 2 & F17

Midnight was used as control in this trial site. All the Midnight selections that were evaluated had no seeds except Midnight 2. The fruit size count for all the selections peaked at count 72. The juice content for all the Midnight selections met the minimum export standards and were good between 55 – 60%. All the Midnight selections reached T1 on the colour plate at peak maturity. Comparing Brix and acid content between Midnight (control) and the Midnight selections. The Midnight 2 & 3 had a slightly higher Brix and Brix and acid content was very good.

### McClellan SL

Fruit shape for McClellan SL is a fairly round fruit with a soft fibre strength that peels easily, containing low rind oil levels. All the fruit evaluated remained completely seedless. The trees bore medium sized fruit (count 72). The internal quality was good with high juice levels for this trial site (61.5%), Brix 11.6 and acid around 1.28%. Juice content increased as the fruit hung but not by much. Before peak maturity, there was no delay in external colour development being a T1.

### Turkey

Fruit size for Turkey was perfect for export with fruit size peaking at count 72. Turkey was the first selection to reach peak maturity. Turkey juice content were around 52% moderate juice content. Brix was above the 10° for export and peaked at 11.5. The external colour of this selection was T1 at peak maturity. Turkey seed count peaked at 1.5 seeds per fruit during the evaluations. Fruit shape was round with coarse rind. The rind colour was deep orange.

### Henrietta

Henrietta juice levels peaked above 60% with Brix (just below 11°) and acids 1.42% at peak maturity. The external colour development was T1 for the season. The average seeds per fruit were 0.5 seeds per fruit. Fruit size peaked at count 72.

#### Louisa

The fruit was virtually seedless and internal quality was good with juice (58.6%) and good Brix levels (11°). The rind colour was yellow by the time of peak maturity between T1. Fruit size peaked at medium size fruit, count 88 to count 72.

#### **Conclusions**

None of the Valencia selections had a problem with external colour development, all of them reached T1 on the colour plate at peak maturity. All of the selections' internal and external qualities complied with the minimum export requirement for Valencia types. Bennie 2 had the highest count of 3.2 seeds per fruit followed by Turkey and Midnight 2 with 1.5 seeds per fruit. All the other selections were virtually seedless. All of the selections had a fruit size count ranging from 88 - 64. The following selections developed a juice content above 60% at peak maturity; Gusocora, Henrietta, Mc Clean SL, Midnight 1 and Midnight 2.

**Table 5.4.23.3.** Internal fruit quality data for Valencia selections at Panzi (Sundays River Valley) during the 2021 season.

Date	Cultivar	Root stock	Avg Fruit size (mm)	Count	Juice %	Brix°	Acid %	Ratio	Avg. Seed	Colour
2021-07-15	Alpha	CC	76	72	59.4	10.5	1.76	6.0	0.1	T1
2021-07-27	Alpha	CC	74	72	59.3	11.0	1.60	6.9	0.0	T1
2021-08-10	Alpha	CC	76	72	59.8	11.0	1.35	8.1	0.0	T1
2021-07-15	Benny 2	CC	78	64	59.3	9.9	1.50	6.6	3.2	T1
2021-07-27	Benny 2	CC	78	64	59.5	10.2	1.51	6.8	2.3	T1
2021-08-10	Benny 2	CC	77	64	59.8	11.5	1.59	7.2	0.0	T1
2021-07-15	Gusocora(G5)	CC	75	72	56.1	10.0	1.56	6.4	0.5	T2
2021-07-27	Gusocora(G5)	CC	72	88	60.1	10.9	1.45	7.5	0.0	T1
2021-08-10	Gusocora(G5)	CC	75	72	61.0	10.7	1.23	8.7	0.0	T1
2021-07-15	Henrietta	CC	75	72	59.0	10.0	1.63	6.1	0.0	T1
2021-07-27	Henrietta	CC	71	88	56.8	10.2	1.56	6.9	0.5	T1
2021-08-10	Henrietta	CC	74	72	60.4	10.7	1.42	7.5	0.5	T1
2021-07-15	Louisa	CC	74	72	53.7	10.1	1.38	7.3	0.0	T4
2021-07-27	Louisa	CC	71	88	58.6	10.9	1.45	7.5	0.3	T1
2021-08-10	Louisa	CC	72	88	57.1	11.0	1.23	8.9	0.0	T1
2021-07-15	McClean SL	CC	74	72	59.0	10.8	1.31	8.2	0.0	T1
2021-08-10	McClean SL	CC	73	72	61.5	11.6	1.28	9.1	0.0	T1
2021-07-15	Midnight	CC	75	72	59.4	9.7	1.20	8.1	0.0	T1
2021-07-27	Midnight	CC	75	72	55.2	10.0	1.06	9.4	0.2	T1
2021-08-10	Midnight	CC	76	72	59.1	10.8	1.04	10.4	0.0	T1
2021-07-15	Midnight 1	CC	75	72	60.3	10.1	1.15	8.8	0.0	T1
2021-07-27	Midnight 1	CC	73	72	54.8	10.1	1.08	9.4	0.0	T1
2021-08-10	Midnight 1	CC	74	72	60.9	10.8	0.96	11.3	0.0	T1
2021-07-15	Midnight 2 H14	CC	71	88	56.2	10.5	1.36	7.7	0.4	T1

2021-07-27	Midnight 2 H14	CC	70	88	61.9	10.4	1.27	8.2	1.2	T1
2021-08-10	Midnight 2 H14	CC	69	88	57.4	11.8	1.34	8.8	1.5	T1
2021-07-15	Midnight F17	CC	74	72	53.6	9.4	1.14	8.2	0.0	T1
2021-07-27	Midnight F17	CC	71	88	57.2	9.7	1.08	9.0	0.0	T1
2021-08-10	Midnight F17	CC	72	88	56.7	11.2	0.95	11.8	0.0	T1
2021-06-08	Turkey	CC		72	53.7	10.8	1.44	7.5	1.5	T2
2021-06-24	Turkey	CC	78	64	52.8	10.9	1.23	8.9	1.5	T1
2021-07-15	Turkey	CC	78	72	52.5	11.5	1.15	10.0	0.0	T1

#### 5.4.24 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Valencia oranges in a cold production region (Citrusdal)

Project 1097B by W Swiegers (CRI)

##### Summary

The climate and the soil make this region an intermediate region to farm Valencia oranges. The Valencia fruit tends to get high sugars, but acids also stay high in this region. It gives fruit with good flavour and shelf life. Most of the trees were planted in 2009 and consist of early-, mid- and late maturing selections. The ripening order was based on ratio, starting with Midnight H14, Turkey, McClean SL, Midnight F17, Bennie, Alpha 2, Alpha, Midnight, Val Late and the season finished off with Bennie 3. All the selections met the export standards 05.08.2021

##### Opsomming

Die klimaat en die grond maak die verbouing van Valencia 'n intermediere area. Hoë suikers word verkry, maar die suur bly hoog. Dit maak vrugte met goeie geure en hou vermoë. Die meeste bome is in 2009 geplant en bestaan uit vroeë-, mid- en laat rypwordende seleksies. Die orde van rypwording gebaseer op "ratio" was as volg, with Midnight H14, Turkey, McClean SL, Midnight F17, Bennie, Alpha 2, Alpha, Midnight, Val Late en die seisoen is afgesluit met Bennie 3. Al die seleksies het aan die uitvoer standaard voldoen op 05.08.2021

##### Objective

- Select Valencia cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (seedlessness, ratio), and extended harvest period (both earlier and later maturity).
- To describe the cultivar characteristics of new Valencia cultivars and to determine the climatic suitability of these cultivars in a cold production region.

##### Materials and methods

Field evaluations and laboratory analyses were conducted on Midnight H14, Turkey, McClean SL, Midnight F17, Bennie, Alpha 2, Alpha, Midnight, Val Late, Bennie 3.

**Table 5.4.24.1.** Internal fruit quality minimum export requirements for Valencia types.

Cultivar	Juice %	Brix	Min Acid	Max Acids	Ratio	Colour
Valencia EU	48	8.5	0.6	1.8%	7.5:1	Colour plate 3 of set no. 34
Midnight	52	9.5	0.85	1.8%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

\*Interim internal fruit quality standards.

**Table 5.4.24.2.** List of Valencia selections evaluated at Kweekkraal (Citrusdal) during 2021 season.

Selection	Rootstock	Topwork
Alpha	CC	2009
Alpha 2	CC	2016
Bennie	CC	2009
Bennie 3	CC	2016
Turkey	CC	2009
Valencia Late	CC	2009
McClellan SL	CC	2009
Midnight	CC	2009
Midnight H14	CC	2011
Midnight F17	CC	2016

## Results and discussion

### Alpha & Alpha 2

The internal quality for Alpha was better than Alpha 2, this could be that Alpha is an older tree. Alpha internal quality at peak maturity were very good with juice content 59.8%, Brix 12.2° and acid 1.41% compared to Alpha 2 internal quality that was good with juice content 56.9%, Brix 11.4° and acid 1.36%. Fruit size for Alpha varied from count 88 to 72 compared to Alpha 2 count 72 to 64, but still good for Valencia production and export. External colour peaked on T1 for both selections. As the fruit hung on the trees the internal quality improved for export fruit of higher standards. Both selections were seedless during the evaluations.

### Bennie & Bennie 3

The fruit size count peaked for Bennie & Bennie 3 at count 72 to 64 and a good Valencia export size. But due to the high acid the fruit was left to hang so that the acids can come down and Brix rise to above 11.5°. This made the selection mature much later but also gave it a good flavour. The selection had a much better internal quality after the fruit was left to hang a bit. Seed count for Bennie ranged between 0.0 – 0.1 seeds per fruit, while Bennie 3 was seedless. There was no delay in external colour development peaking at (T1) at peak maturity. Bennie and Bennie 3 developed a good juice content above 55% as the fruit matured and was left on the tree a bit longer.

### Valencia Late

Valencia Late was the control for the late maturing selections. The Valencia Late produced size fruit count 88 - 72. The internal and external quality was good, the juice content was around 55%, and Brix started off good at 11° with acid content bit high 1.66%, but did drop. Colour development was good T1 – T2. Seed counts were between 0.0 – 0.8 seeds per fruit.

### McClellan SL

McClellan SL tree bore fruit with fruit size count ranging from count 88 - 72. The trees bore a good crop. The selection was seedless. External colour development were T1 – T2 on the colour plate at peak maturity. Brix was good (above 11.5°) peaking at 12.5:1 and acids remained stable towards the end of the season around 1.2%, resulting in a very good Brix: Acid ratio. The fruit is firm with a round to elongated fruit shape with a smooth rind. Externally as well as internally the colour is deep orange.

### Turkey

Fruit size did vary for Turkey with count ranging between 72 – 64. Brix was around 11.5° and acids around 1.20%. This will meet the export standards as well as the external colour that was at T1 – T2 on the colour plate. Turkey was virtually seedless. Juice content for Turkey was good. Fruit characteristics for Turkey were round fruit shape, with a very good flavour, soft rag, fairly thin rind and easy peeling. The internal colour was light yellow, and externally the fruit remained yellow. This selection has the qualities of a mid-season orange; for instance, the exceptionally soft fruit, and the soft rind that can result in rind problems if managed incorrectly.

### Midnight, Midnight F17 and Midnight H14

Midnight was used as control for this trial site but also as control for the other two Midnight selections. The fruit size development for Midnight and Midnight H14 peaked at count 72 followed by Midnight F17 count 88. The smallest fruit size count was for Midnight F17 was count 105. All the Midnight selections bore round fruit with a medium to coarse rind, fibre strength was fairly soft and the fruit peeled easily. The Midnight selections were seedless. The colour development at peak maturity was T1 – T2 for all the selections. The trees produced well. All the Midnight selections had a good Brix of more or less the same above 11.5°. Juice content was above 55% while Midnight F17 was close to 60%.

### **Conclusions**

All of the selections external colour development were T1 – T2, on the colour plate when the fruit was left to hang. All the selections met the minimum export standards. Valencia Late were the selection with the highest number of seeds per fruit. All the other selections were completely/virtually seedless. The fruit size ranged between count 88 - 64 but all of them were good enough for export. All the selections produced a Brix around 11°. Acid content for the selections varied around 1.0 – 1.4%. Midnight F17 had the highest juice content at 61%.

**Table 5.4.24.3.** Internal fruit quality data for Valencia selections at Kweekkraal (Citrusdal) during the 2021 season.

Date	Cultivar	Root stock	Avg fruit size (mm)	Count	Juice %	Brix °	Acid %	Ratio	Avg. Seed	Colour
2021-08-05	Alpha	CC	75,1	72	56,6	11,9	1,73	6,9	0,0	T1
2021-09-15	Alpha	CC	75,0	72	58,7	11,9	1,56	7,6	0,0	T1 - T2
2021-10-05	Alpha	CC	70,7	88	59,8	12,2	1,41	8,6	0,0	T1 - T2
2021-08-05	Alpha 2	CC	77,8	64	56,9	10,4	1,50	6,9	0,0	T1 - T3
2021-09-15	Alpha 2	CC	74,4	72	60,2	10,7	1,36	7,9	0,0	T1 - T2
2021-10-05	Alpha 2	CC	75,2	72	56,9	11,4	1,36	8,4	0,0	T1
2021-08-05	Bennie	CC	78,1	64	56,4	10,3	1,43	7,2	0,1	T1 - T3
2021-09-15	Bennie	CC	76,0	72	58,9	10,9	1,37	8,0	0,0	T1
2021-10-05	Bennie	CC	75,4	72	57,1	12,1	1,41	8,6	0,0	T1
2021-08-05	Bennie 3	CC	77,3	64	58,0	10,4	1,74	6,0	0,0	T1
2021-09-15	Bennie 3	CC	76,4	72	60,5	10,6	1,28	8,3	0,0	T1
2021-10-05	Bennie 3	CC	77,3	64	58,1	11,5	1,33	8,7	0,0	T1
2021-08-05	Mc Clean SL	CC	73,6	72	57,4	11,3	1,50	7,5	0,0	T1 - T2
2021-09-15	Mc Clean SL	CC	72,5	88	60,3	11,5	1,33	8,7	0,0	T1 - T2
2021-10-05	Mc Clean SL	CC	70,8	88	56,4	12,5	1,20	10,4	0,0	T1
2021-08-05	Midnight	CC	71,3	88	55,9	10,5	1,54	6,8	0,0	T2 - T3
2021-09-15	Midnight	CC	73,8	72	55,3	10,6	1,28	8,3	0,0	T1 - T2
2021-10-05	Midnight	CC	72,5	88	55,0	11,7	1,24	9,4	0,0	T1
2021-08-05	Midnight 2 H14	CC	71,2	88	58,4	10,3	1,14	9,1	0,0	T2 - T4
2021-09-15	Midnight 2 H14	CC	70,9	88	57,6	11	1,17	9,4	0,0	T3
2021-10-05	Midnight 2 H14	CC	73,8	72	53,4	11,5	0,93	12,3	0,0	T1 - T2

2021-08-05	Midnight 3 F17	CC	72,2	88	56,1	10	1,40	7,2	0,0	T2 - T3
2021-09-15	Midnight 3 F17	CC	69,9	88	61,0	10,9	1,14	9,6	0,0	T2 - T3
2021-10-05	Midnight 3 F17	CC	68,6	105	58,7	11,9	1,14	10,4	0,0	T1 - T2
2021-08-05	Turkey	CC	73,3	72	54,5	10,1	1,35	7,5	0,0	T1 - T2
2021-09-08	Turkey	CC	77,3	64	57,8	11,6	1,25	9,3	0,0	T1 - T2
2021-10-05	Turkey	CC	76,2	72	56,3	12,5	1,21	10,3	0,2	T1
2021-08-05	Valencia Late	CC	74,8	72	55,8	11	1,66	6,6	0,7	T1 - T3
2021-09-08	Valencia Late	CC	72,0	88	56,4	11,7	1,42	8,2	0,0	T1
2021-10-05	Valencia Late	CC	69,4	88	56,2	11,8	1,39	8,5	0,8	T1 - T2

#### 5.4.25 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Clementine mandarins in a cold production region (Sundays River Valley) Project 1000B by W Swiegers and Z Zondi (CRI)

##### Summary

Only some of the selections were evaluated. The open selections will be used as controls for the new selections in the future. For the Sundays River Valley there are two Clementine sites with most of the selections. A new exciting site will come into production in a few years with all the latest selections. Some of the selections are on interstock. The season started with Basol, Early Esbal followed by Nules, Large Esbal and ended with Esbal.

##### Opsomming

Die seleksies wat geëvalueer was die seisoen is net 'n paar van die seleksies. Die oop seleksies dien as kontroles vir die nuwe seleksies. Vir die Sondags Rivier Vallei is daar 2 Clementine persele wat die meeste seleksies bevat. Daar kom 'n nuwe opwindende perseel by met al die nuwe seleksies. Van die seleksies is op 'n tussen stam. Die seisoen het begin met Basol, Early Esbal gevolg deur Nules, Large Esbal en ge-eindig met Esbal.

##### Objectives

- To select Clementine cultivars with improved and consistent productivity, fruit size, rind colour, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the cultivar characteristics of new Clementine cultivars and to determine the climatic suitability of these cultivars in cold production regions.

##### Materials and methods

Field evaluations and laboratory analyses were conducted on Clementine selections from the Sundays River Valley region of the Eastern Cape; planted 2012. The following cultivars were evaluated: Basol, Early Esbal, Large Esbal, Esbal and Nules.

A ratio of 11:1 Clementines is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater quality and rind issues.

**Table 5.4.25.1.** List of Clementine selections evaluated at Invercloy (Kirkwood) during 2020.

Selection	Rootstock	Planted
Esbal	Carrizo	2012
Nules	Carrizo	2012

**Table 5.4.25.2.** List of Clementine selections evaluated at Dunbrody (Kirkwood) during 2020.

Selection	Rootstock	Topworked
Basol	Carrizo with Midnight as interstock	2015
Early Esbal	Carrizo with Midnight as interstock	2015
Large Esbal	Carrizo with Midnight as interstock	2015

## Results and discussion

### Basol

Basol is the earliest maturing Clementine selection. Basol trees tend to develop galls on the trunk. The Basol trees with the navel interstock don't show galls on the trees yet. This one is with an interstock. Fruit size for Basol was small with count 5 - 4. Internal quality was excellent for Basol at ratio 11.8:1 the juice content was 57.3%, Brix 13° and acid 1.10%. This selection was virtually seedless. External colour break was delayed, T5 on the colour plate. The fruit peels easily. Basol tends to have a very short harvest period before the fruit is over mature and starts to granulate and get puffy.

### Esbal & Early Esbal & Large Esbal

Esbal was used as control for the two experimental Esbal selections. Early Esbal is selected to mature before Esbal and Large Esbal is selected to crop bigger fruit than Esbal. The order of ripening for the 3 selections was Early Esbal first, then Large Esbal and Esbal finished the season. Early Esbal had slightly smaller fruit size ranging count 5 - 4 while Esbal were count 2 and Esbal Large fruit size count was also count 2. Early Esbal had the best internal quality, highest juice content, and the best Brix:acid ratio towards peak maturity. Early Esbal were completely seedless, Large Esbal seed count peaked at 1.3 seeds per fruit and Esbal seed count peaked at 0.8 seeds per fruit. All three Esbal selections had a delayed colour development at peak maturity. Fruit was round to oblate with a smooth to pebbly rind

### Nules

Nules were the third selection to reach peak maturity. The selection had a fair juice percentage (54.3%) at peak maturity. Nules had a favourable fruit size count 3 - 1. Internal quality for Nules at peak maturity was good; Brix (12.1°) and acid (0.94%). Nules also kept its acids well. This contributes to Nules good flavour. Nules average seed count was 1.5 seeds per fruit. Rind colour development was fair with T4 on the colour plate at peak maturity. Peelability is easy and the internal colour is orange.

## Conclusion

On average Nules had the highest seed count of all the selections that were evaluated, around 1.5 seeds per fruit. All of the selections had delayed colour development at peak maturity. Degreening practices will be essential after harvesting to ensure optimal colour development. Basol, Large Esbal and Nules had the highest Brix (12 - 13°). Basol and Early Esbal had the smallest fruit size count 4 - 5. Early Esbal had the highest juice percentage 64.3%.

**Table 5.4.25.3.** Internal fruit quality data for Clementine selections in the Sundays River Valley region of the Eastern Cape during the 2021 season.

Date	Cultivar	Root-stock		Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg-Seed	Colour
2021-03-18	Basol	CC	53	4	57.0	12.5	1.08	11.5	0.0	T6
2021-03-23	Basol	CC	53	4	57.3	13.0	1.10	11.8	0.0	T5
2021-04-29	Basol	CC	51	5	58.9	12.3	0.94	13.1	0.1	T1
2021-03-23	Early Esbal	CC	53	4	59.8	10.3	0.91	11.3	0.0	T6
2021-04-12	Early Esbal	CC	51	5	61.4	10.9	0.99	11.0	0.0	T6
2021-04-26	Early Esbal	CC	53	4	64.3	11.4	0.91	12.5	0.0	T 2-3
2021-05-11	Large Esbal	CC	62	2	52.0	10.5	1.15	9.1	1.3	T5-6
2021-05-24	Large Esbal	CC	62	2	52.6	12.0	1.18	10.2	0.0	T4
2021-05-11	Esbal	CC	62	2	51.8	9.8	1.30	7.5	0.7	T6
2021-05-24	Esbal	CC	62	2	56.1	11.6	1.25	9.3	0.8	T3
2021-04-12	Nules	CC	58	3	51.4	11.4	1.30	8.8	2.5	T6
2021-05-11	Nules	CC	67	1	52.3	11.1	1.00	11.1	1.3	T5-6
2021-05-24	Nules	CC	62	2	54.3	12.1	0.94	12.9	0.8	T4

5.4.26 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of Clementine mandarins in a cold production region (Western Cape)**  
Project 1000D by W Swiegers (CRI)

### Summary

There were two trial sites in Citrusdal where we evaluated Clementines this season. The open selections are used as controls in the trial sites. The selections that were evaluated were early-, mid selections. The early selections will play an important role as they start to overlap the Satsumas. Octubrina started the season in Citrusdal followed Clemenluz and Nules finished the season.

### Opsomming

Daar is 2 proef persele in Citrusdal waar Clementines gevalueer was die seisoen. Die oop seleksies dien as kontroles vir die proef persele. Die seleksies wat gevalueer word is vroe-, mid seleksies. Die vroe seleksies gaan nog 'n belangrike rol speel in die toekoms soos wat dit begin oorvleul met Satsumas. Octubrina het die seisoen in Citrusdal begin, gevolg deur Octubrina en Nules het die seisoen afgesluit.

### Objectives

- To select Clementine cultivars with improved and consistent productivity, fruit size, rind colour, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the cultivar characteristics of new Clementine cultivars and to determine the climatic suitability of these cultivars in cold production regions.

### Materials and methods

Field evaluations and laboratory analyses were conducted on Clementine selections from various regions in the Western Cape. The following varieties were evaluated: Nules, Octubrina, and Clemenluz.

A ratio of 11:1 Clementines is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over mature. This process from start to the end

of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

**Table 5.4.26.1.** List of Clementine selections evaluated at Kweekkraal and Stargrow (Citrusdal) during 2021.

Selection	Rootstock	Planted	Topworked
Nules	Carrizo	2009	
Octubrina	Carrizo		2016
Clemenluz	Carrizo		2009

## Results and discussion

### Nules

Nules was used as control. At peak maturity fruit size count was 2 (medium). The internal quality was excellent with Brix (13.2°) and acid (1.09%) at peak maturity ratio 12:1. Acids stayed stable at 1% even when the fruit was over mature. Internal colour development was delayed, being T4 on colour plate at peak maturity. Those acids will give the fruit good shelf life and the high sugars with acids give Nules its good flavour. The rind is smooth and thin and it peels easily. The seed count was 0.6 – 3.7 seeds per fruit.

### Octubrina

Octubrina is a new experimental early maturing Clementine. It must have sweet orange as an interstock. It reaches peak maturity about 2 – 3 weeks before Nules. Crop was good. Fruit size range was small for Clementine production, with fruit size count 4, due to the good crop. Crop thinning practices must be applied to improve fruit size. At peak maturity (ratio 12:3), the Brix was 12.2 and acid percentage was 0.99%. Acids stayed stable after peak maturity, which is very good. Seeds were present during evaluations due to strong cross pollination in trial block. External colour development very good at peak maturity, being T1 on the colour plate. The selection does degreen very well. The fruit is round to flattish and peelability is easy.

### Clemenluz

Clemenluz is an early maturing Clementine selection. Nules was used as a control for this section. The Clemenluz reached peak maturity before Nules according to the ratio. Compared to Nules, Nules internal quality was slightly better than Clemenluz. Clemenluz seed count peaked at one seeds per fruit. Colour development was delayed at peak maturity. Rind colour is a yellow-orange and the peelability of the fruit is easy.

## Conclusion

None of the selections were completely seedless, because they were planted in mixed trial blocks. Octubrina had the highest seed count 2.1 – 4.3 seeds per fruit. Most selections had delayed colour development but reached T1 on the colour plate before the fruit was overmature. Degreening practices will be essential after harvesting to ensure optimal colour development. Octubrina had the highest Brix (14.4°) and acid (1.13%) towards over maturity. Octubrina had the smallest fruit size and peaked at count 4.

**Table 5.4.26.2.** Internal fruit quality data for Clementine selections in the Citrusdal region (Kweekkraal and Stargrow) of the Western Cape during the 2021 season.

Date	Cultivar	Root-stock	Avg. size (mm)	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2021-04-13	Nules	CC	56	3	64,9	12,2	1,16	10,5	3,7	T7 - T8
2021-05-18	Nules	CC	63	2	58,0	13,2	1,09	12,1	1,7	T4
2021-06-07	Nules	CC	61	2	59,1	13,2	1,02	12,9	0,6	T1
2021-05-06	Octubrina	CC	52	4	57,6	12,2	0,99	12,3	4,3	T4 - T5
2021-05-18	Octubrina	CC	51	4	50,5	14,4	1,13	12,7	2,1	T1

2021-05-06	Clemenluz	CC	59	3	54,1	10,7	0,93	11,5	1,0	T5 - T6
2021-05-18	Clemenluz	CC	57	3	59,0	11,5	1,01	11,4	0,8	T1 - T3

#### 5.4.27 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Clementine mandarins in a cold production region (South West Cape)

Project 1000E by W Swiegers (CRI)

#### Summary

The trial site doesn't have a wide range of selections at the moment. Buffeljagsrivier region will be one of the biggest Clementine trial sites in the future with another site bearing its first crop in 2022. The season started with Basol, followed by Nules, Early Esbal and ended with Large Esbal.

#### Opsomming

Die proef perseel het nie op die oomblik 'n wye verskeidenheid van seleksies nie. Buffeljagsrivier area gaan in die toekoms een van die grootste Clementine proef persele word. Die nuwe perseel gaan sy eerste vrugte in 2022 produseer. Die seisoen het begin met Basol, gevolg deur Nules, Early Esbal en ge-eindig met Large Esbal.

#### Objectives

- To select Clementine cultivars with improved and consistent productivity, fruit size, rind colour, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the cultivar characteristics of new Clementine cultivars and to determine the climatic suitability of these cultivars in cold production regions.

#### Materials and methods

Field evaluations and laboratory analyses were conducted on Clementine selections from the Buffeljagsrivier region of the South West Cape; the planting date was 2014. The following cultivars were evaluated: Basol, Early Esbal, Large Esbal and Nules.

A ratio of 11:1 Clementines is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater quality and rind issues.

**Table 5.4.27.1.** List of Clementine selections evaluated at Olivedale (Buffeljagsrivier) during 2021 season.

Selection	Rootstock	Planted
Basol	Carrizo	2014
Nules	Carrizo	2014
Early Esbal	Carrizo	2014
Large Esbal	Carrizo	2014

#### Results and discussion

##### Basol

Basol is an early maturing Clementine selection. Fruit size count for Basol was 4 - 3. Basol juice percentage at peak maturity started with 64.8%. There were barely seeds in this selection. This selection reached T5 – T6 at peak maturity. Basol had the Brix at 11.0° and acid at 0.85% at peak maturity. The fruit peels easily. Basol's

rind colour is deep orange. Basol has a very short harvest period before the fruit is over mature and starts to granulate.

#### Esbal & Early Esbal & Large Esbal

Esbal was used as control but did not bore any crop. Experimental varieties were Early Esbal & Large Esbal Clementine. Early Esbal was selected to reach peak maturity earlier than Esbal, while Large Esbal was selected for bigger fruit size. In this trial site Early Esbal reached peak maturity before Large Esbal. Fruit size count for Early Esbal count 5 – 4 and Large Esbal fruit size count 4 – 2. Internal quality for Early Esbal and Large Esbal were very good with juice content above 55%, Brix above 11° and acid around 1%. Rind colour development was slightly better for Early Esbal T1 – T3 compared to Large Esbal T5 on (colour plate) when the fruit was at peak maturity. Fruit is round to oblate. Rind is smooth to pebbly. Peelability is easy but rind oil a bother. The seed count for Early Esbal peaked at 0.6 seeds per fruit and the average Large Esbal seed count was 2 seeds per fruit.

#### Nules

Nules was the second selection to reach peak maturity. The selection's juice percentage decreased towards peak maturity. Internal quality for Nules at peak maturity was fair - good. Brix (13°) and acid (0.88%) and juice content 47.8%. Nules seed count ranged from 1.1 to 5.1 seeds per fruit. Rind colour development was not good for Nules with a T7 on the colour plate at peak maturity. Nules had a good fruit size at count 3 – 2 (peak maturity). Yields were fair for Nules. Peelability is easy and internal colour is orange.

#### **Conclusion**

Early Esbal had the lowest seed count and Nules had the highest seed count. Early Esbal were the only selections to reach T1 on the colour plates. Degreening practices will be essential after harvesting to ensure optimal colour development for Nules. Nules had the highest Brix (13°). Early Esbal had the smallest fruit size count 5 - 4. Nules had the biggest fruit size count 3 – 2. Basol & Early Esbal had the best juice percentage.

**Table 5.4.27.2.** Internal fruit quality data for Clementine selections in the Buffeljagsrivier region (Olivedale) of the South West Cape during the 2021 season.

Date	Cultivar	Root-stock	Avg size (mm)	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2021-03-26	Basol	CC	53	4	64,8	11	0,85	12,9	0,0	T5 - T6
2021-04-14	Basol	cc	52	4	62,4	11,4	0,79	14,5	1,2	T4 - T7
2021-04-14	Early Esbal	CC	49	5	52,7	10,9	1,18	9,2	0,6	T6
2021-05-04	Early Esbal	CC	51	5	61,6	11,1	1,06	10,5	0,1	T1 - T3
2021-05-19	Early Esbal	CC	54	4	64,8	11,4	0,83	13,8	0,2	T1
2021-04-14	Large Esbal	CC	51	4	43,2	10,5	1,74	6,0	2,0	T7
2021-05-19	Large Esbal	CC	64	2	55,8	11,7	1,01	11,6	1,7	T5
2021-04-14	Nules	CC	58	3	48,6	10,7	1,21	8,8	5,1	T8
2021-05-04	Nules	CC	60	2	47,8	13	0,88	14,8	1,1	T 7

#### 5.4.28 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region (Western Cape)

Project 57D by W Swiegers (CRI)

##### Summary

The trial location is in an area well suited for Satsuma production. Most of the trees were planted in 2012. The trees look fair with medium to large tree canopies. The order of ripening was as follows; Miyagawa Wase started the season, followed by, Miho Wase, Sugiyama, Ueno, and Imamura was the selection to finish the season. The fruit is under strong cross pollination due to the mix trial block otherwise the fruit would be seedless.

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.

##### Opsomming

Die proef se ligging is goed geskik vir Satsuma produksie. Die meeste bome is geplant in 2012. Die bome lyk gematig met goeie boom volume. Die orde van rypwording was as volg: Miyagawa Wase het die seisoen begin gevolg deur Miho Wase, Sugiyama, Ueno, en Imamura het die seisoen klaargemaak. Die vrugte is onder sterk kruisbestuiwing a.g.v gemengde proefblok. As dit nie vir dit was nie sou die seleksies saadloos gewees het.

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.

##### Objectives

- To select Satsuma cultivars with improved and consistent productivity, fruit size, rind colour, and internal fruit quality (Brix, acidity and ratio).
- To extend the harvest period (both earlier and later maturity).
- To describe the characteristics of new Satsuma cultivars and determine the climatic suitability of these cultivars in cold production regions.

##### Materials and methods

Field evaluations and laboratory analyses were conducted on Satsuma selections from the Citrusdal region of the Western Cape. The following selections were evaluated: Imamura, Miho Wase, Miyagawa Wase, Sugiyama, and Ueno.

For Satsuma mandarins, a ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which the fruit is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

**Table 5.4.28.1.** List of Satsuma selections evaluated at Stargrow (Citrusdal) during 2021.

Selection	Rootstock	Topworked
Imamura	Carrizo	2012
Miho Wase	Carrizo	2011
Miyagawa Wase	Carrizo	2012
Sugiyama	Carrizo	2012
Ueno	Carrizo	2012

## Results and discussion

### Miho Wase

Miho Wase was the second selection to mature in this trial site. The rind was smooth, and the fruit peeled easily. Fruit size for Miho Wase was mostly count 2 – 1. The selection was seedless. Fruit colour on the colour plate was T5 – T7 at peak maturity. Fruit matured internally, rind colour development was delayed. Sugar at peak maturity was around 9.5° with an acid percentage of around 0.87%. Juice content decreased towards peak maturity.

### Imamura

Imamura is a late maturing Satsuma. In this cold production region, it reached peak maturity from end-May. It was the last selection to reach peak maturity. For a Satsuma, Imamura had a good Brix: Acid ratio, 10.3° and 0.96% respectably (ratio 10.7 at peak maturity). One of the selections with a good juice percentage. Seed count was 5 – 10 seeds per fruit. External colour development was T3 - T5 on the colour plate. Internal colour was deep orange.

### Miyagawa Wase

Miyagawa Wase was the first selection to reach peak maturity. The fruit size of Miyagawa Wase at peak maturity was count 2 – 1. Brix: acid ratio 11.3:1; Brix was 9.7° and acid was 0.85%. Colour development was not good and delayed compared to internal maturity. The colour on the colour plate at peak maturity was T6 – T7. This selection was low seeded and the juice percentage decreased towards peak maturity. The fruit was smooth and flat and the internal colour was deep orange.

### Ueno

This selection is a mid to late maturing selection for this trial site. It had a low highest juice content medium to extra-large fruit size count with a 1 – 1xx count. The Brix° and acid percentage for Ueno at peak maturity were very good, 10.1° and 1.01% respectively. There were 6 – 15 seeds per fruit and Ueno colour on the colour plate at peak maturity was T6 – T7. Fruit peeled easily.

### Sugiyama

Sugiyama is a mid to late maturing Satsuma. At this trial site it reached peak maturity in mid-April. Sugiyama fruit size count 1. The Brix° and acid percentage of Sugiyama were around 9.6° and 0.95% respectively at peak maturity. Sugiyama had the highest juice percentage. Seed count for this selection ranged 0 – 14 seeds per fruit. There was also a delay in colour development with a T6 – T8 on the colour plate towards peak maturity.

## Conclusion

Most of the selections peaked with a medium to extra-large fruit size (count 1 - 1xx). Ueno had the biggest fruit size count peaking at count 1xx. Imamura had the highest Brix° of all the Satsuma selections above (10°). Ueno had the highest seed count with 15 seeds per fruit. Rind colour development was not good for any of the selections at peak maturity. Sugiyama had the highest juice percentage.

**Table 5.4.28.2.** Internal fruit quality data for Satsuma selections in the Citrusdal region of the Western Cape during the 2021 season.

Date	Cultivar	Root-stock	Avg. size (mm)	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2021-05-18	Imamura	CC	63,1	2	55,2	9,3	1,20	7,7	5,0	T5
2021-06-07	Imamura	CC	70,6	1xx	55,1	10,3	0,96	10,7	10,0	T3 - T5
2021-03-25	Miho Wase	CC	65,2	1	54,0	9,3	1,04	8,9	0,0	T6
2021-04-13	Miho Wase	CC	65,2	2	57,5	9,9	0,87	11,4	0,0	T6 - T8

2021-05-06	Miho Wase	CC	64,5	1	44,5	9,4	0,85	11,1	0,0	T5 - T7
2021-03-25	Miyagawa Wase	CC	62,8	2	56,3	9	1,11	8,1	0,0	T6 - T7
2021-04-13	Miyagawa Wase	CC	63,5	2	55,2	9,7	0,85	11,3	0,0	T6 - T7
2021-05-06	Miyagawa Wase	CC	67,0	1	51,2	9,9	0,82	12,1	2,0	T5 - T6
2021-04-13	Sugiyama	CC	60,6	2	56,9	9,6	0,95	10,1	0,0	T6 - T8
2021-05-06	Sugiyama	CC	65,1	1	45,9	9,5	0,87	11,0	8,0	T5 - T6
2021-05-18	Sugiyama	CC	64,3	1	58,1	9,2	0,74	12,5	14,0	T2 - T4
2021-04-13	Ueno	CC	66,3	1	51,2	9,7	1,13	8,6	10,0	T7 - T8
2021-05-06	Ueno	CC	75,2	1xx	43,2	10,1	1,01	10,0	6,0	T6 - T7
2021-05-18	Ueno	CC	72,2	1xx	53,9	9,9	0,88	11,2	15,0	T4 - T5

#### 5.4.29 PROGRESS REPORT: Studies into the high incidence of chimeras of Valencia orange cultivars, specifically Valencia Late

Project: 1185 by P Cronje, J Joubert, W Swiegers, H Maree, R Bester and V White (CRI)

##### Summary

Chimeric fruit were sampled from a 'Valencia Late' commercial orchard in Malelane. Three oranges with clear chimeric borders were selected and DNA was extracted from the non-chimeric and chimeric segments from chimeric fruit as well as the leaves behind the fruit. DNA was also extracted from Carrizo citrange trifoliolate leaf material as a control. DNA from the seven samples were sent to MacroGen for high-throughput sequencing (HTS). Variant detection analyses were performed to identify the difference between samples 1-6 and all samples were compared to sample 7 to identify potential scion/rootstock species chimeras. Analyses to date indicate that the chimera expression on fruit is not due to a species chimera admixture and can potentially be the result of single nucleotide polymorphisms (SNPs). However, no SNPs was identified to be common to only the non-chimeric or chimeric segments. To identify combinations of SNPs, more detailed analysis will be required. The data can also further be mined for a focussed analysis in specific genomic regions that may be identified in other experiments. It is also not excluded that the differences can be on an epigenetic level however, epigenetic analyses will require additional resources. Total RNA was also extracted from leaf material from a 'Valencia Late' tree with chimeric fruit as well as from the nucleus material used to establish these 'Valencia Late' plant material (one sample from ARC, Nelspruit and one sample from CRC, Nelspruit). The total RNA extracted was sent to MacroGen for HTS however, no viruses and viroids were detected in any of these RNA samples. The orchard level analysis of chimeras continued and indicated that the 'Valencia Late' orchard in Malelane had constantly higher incidence and severity compared to Letsitele and Hoedspruit. Furthermore, the % chimera indicated varied significantly between seasons, with a general decrease observed across the last three seasons. Canopy position did not significantly affect incidence however the western side were generally numerically higher. On the shoot level there is evidence that chimera incidence does vary across seasons, which could indicate that chimera incidence is not localized to a shoot and can occur randomly throughout the canopy. These preliminary results in this challenging project are slowly adding information to this poorly understood genetic event in citriculture and will be continued for the foreseeable future.

##### Opsomming

Chimeriese vrugte is vanuit 'n 'Valencia Late' kommersiële boord in Malelane versamel. Drie lemoene met duidelike chimeriese grense is geselekteer en DNS is uit die nie-chimeriese en chimeriese segmente van chimeriese vrugte, sowel as die blare agter die vrugte, geëkstraheer. DNS is ook as kontrole uit *Carrizo citrange trifoliaat* blaarmateriaal geëkstraheer. DNS van die sewe monsters is na MacroGen gestuur vir hoë-deurset-volgordebepaling (HTS). Variant-deteksie-analises is uitgevoer om die verskil tussen monsters 1-6 te

identifiseer en alle monsters is met monster 7 vergelyk om potensiële vermenging van okulering/onderstam spesies as chimeras te identifiseer. Ontledings tot op datum dui daarop dat die uitdrukking van chimera op vrugte nie die gevolg is van 'n spesie-chimera-byvoeging (admixture) nie, en kan moontlik die gevolg wees van enkelnukleotiedpolimorfismes (SNP's). Geen SNP's is egter geïdentifiseer wat algemeen is vir slegs die nie-chimeriese of chimeriese segmente nie. Ten einde kombinasies van SNP's te identifiseer, sal meer gedetailleerde ontleding vereis word. Die data kan ook verder ontgin word vir 'n gefokusde analise in spesifieke genomiese streke wat in ander eksperimente geïdentifiseer kan word. Dit is ook nie uitgesluit dat die verskille op 'n epigenetiese vlak kan wees nie, maar epigenetiese ontledings sal bykomende hulpbronne vereis. Totale RNS is ook vanuit blaarmateriaal van 'n 'Valencia Late' boom met chimeriese vrugte geëkstraheer, sowel as van die moeder materiaal wat gebruik is om hierdie 'Valencia Late' plantmateriaal te vestig (een monster van LNR, Nelspruit en een monster van CRC, Nelspruit). Die totale RNS wat onttrek is, is na MacroGen gestuur vir HTS, maar geen virusse en viroïede is in enige van hierdie RNS-monsters opgespoor nie. Die boordvlak ontleding van chimeras is voortgesit en dui daarop dat die 'Valencia Late'-boord in Malelane voortdurend hoër voorkoms en intensiteit het in vergelyking met Letsitele en Hoedspruit. Verder het die aangeduide % chimera betekenisvol tussen seisoene gevarieer, met 'n algemene afname waargeneem oor die laaste drie seisoene. Blaredakposisie het nie die voorkoms betekenisvol beïnvloed nie, hoewel die westelike kant oor die algemeen numeries hoër was. Op lootvlak is daar bewyse dat die voorkoms van chimera wel oor seisoene verskil, wat daarop kan dui dat die voorkoms van chimera nie tot 'n loot beperk is nie, en kan lukraak enige plek deur die blaredak voorkom. Hierdie voorlopige resultate in hierdie uitdagende projek voeg stadigaan inligting tot hierdie swak verstaande genetiese gebeurtenis in *citriculture*, en sal vir die afsienbare toekoms voortgesit word.

#### 5.4.30 **PROGRESS REPORT: Evaluation of new University of Florida (UF) rootstocks**

Project 1246 (2019/20 – 2022/3) by P Cronje, J Niemann and J Joubert (CRI)

##### **Summary**

The UF/CREC rootstock programme has been breeding rootstocks for 30+ years to address various problems in this production area. Recently, due to the massive natural screening as the HLB epidemic spread in Florida, some potential tolerant/resistant rootstocks, with commercial potential, were identified (rescue trees). After successfully importing the seeds of 20 new selections from this breeding programme, it was successfully submitted to DALRDD/Plant Health for pathogen screening in 2021. Thereafter the seeds were delivered to Du Roi nursery at Letsitele for germination to develop seedlings. At this stage, each rootstock selection was assessed to select uniform seedlings. For each selection, a standard photo ID chart of distinguishing characteristics i.e., leaf types, growth pattern and root development, were made to assist in future propagation from seeds in commercial nurseries. It was observed that even at this early stage of the project, differences in the selections are evident and are expected to result in significant changes in nursery practices, canopy development and fruiting potential. After selecting seedlings based on the selection criteria, they were budded with 'Nadorcott LS' mandarin and 'Midnight' Valencia to allow planting in March 2023 as part of two new orchards at Crocodile Valley Estate in Nelspruit.

##### **Opsomming**

Die UF/CREC teel en ontwikkel al vir die laaste 30+ jaar onderstamme om verskillende probleme in hierdie area aan te spreek. As gevolg van die massiewe natuurlike sifting wat plaasgevind het soos die HLB-epidemie in Florida versprei het, is enkele potensiële verdraagsame/weerstandige onderstamme geïdentifiseer (reddingsbome). Ná saad van 20 onderstam seleksies ingevoer is, is die sade aan DALRDD/Plant Gesondheid vir patogeen-onderzoek gelewer en daarna vrygestel. Die saad is aan Du Roi kwekery in Letsitele verskaf vir ontkiëming en saailing vestiging. Hierna is in 'n poging om vir elke seleksie 'n standaard tipe te identifiseer vir latere plantvermeerdering, 'n foto ID kaart gemaak wat identifiseerbare aspekte soos blaartipes, groeiwyse en wortelontwikkeling insluit. In hierdie vroeë stadium van die projek kan alreeds groot verskille tussen die seleksies gesien word wat vermoedelik 'n impak sal maak op kwekerypraktyke, boomontwikkeling en potensiaal om vrugte te produseer. Die geselekteerde saailinge is met 'Nadorcott LS' mandaryn en 'Midnight' Valencia geokuleer om gereed te wees vir plant in Maart 2023 as deel van twee nuwe boorde op Crocodile Valley Estate in Nelspruit.

## 6 CITRUS IMPROVEMENT SCHEME (CIS)

By P.H. Fourie, J.B. Meyer, M. le Roux, M.J. Nell, G. Cook (CRI) and E. Jooste (ARC-TSC)

### Summary

The South African Citrus Improvement Scheme (CIS) strives to ensure a profitable citrus industry that is established with high quality citrus trees that are free from diseases and horticulturally true to type. Certified rootstock seed and budwood are supplied from the Citrus Foundation Block (CFB) outside Uitenhage. Budwood supply declined in the 2021/22 season (5.62-million buds) from the all-time high levels in the 5 years prior (average of 6.53-million). Mandarin (30.8%) was the most popular citrus type, followed by Valencia (25.4%), lemon (13.3%), navel (11.1%), Clementine (7.3%) and grapefruit (6.9%). Budwood stock of the 465 cultivar lines at CFB must be constantly managed to meet demand of sought-after varieties. CFB's ability for primary supply was 76%, with the remainder of budwood authorized for supply from certified trees in certified nurseries. CFB is the primary supplier of rootstock seed in SA, and supplied around 82.4% of certified seed in 2021/22, with the remainder being seed produced in certified nurseries. The new rootstock seed orchards of high demand rootstock cultivars contributed to the seed harvest and 2020 yielded a record seed harvest of >8000 L, of which 5857 L was supplied. Seed orders from the 2021 harvest declined with only 4746 L supplied. The nursery star-grading system was implemented since the November 2021 audit round, and nurseries mostly showed an improved score in the May 2022 audit round: average score improved from 69.6% to 71.8% with 10 and 11 nurseries graded with 4 and 5 stars, respectively.

### Opsomming

Die doelwit van die Suid-Afrikaanse Sitrus Verbeteringskema (SVS) is om die winsgewendheid van die suider-Afrikaanse sitrusbedryf te verbeter deur te verseker dat die industrie gevestig word met hoë kwaliteit, siektevrye kwekerybome wat tuinboukundig tipe-eg is. Gesertifiseerde okuleerhout en saad word voorsien vanaf die in Sitrus Grondvesblok buite Uitenhage. Okuleerhout verskaffing het in die 2021/22-seisoen (5.62 miljoen ogies) afgeneem vanaf die hoogste vlakke van alle tye in die 5 jaar tevore (gemiddeld van 6.53 miljoen). Mandaryne (30.8%) was die gewildste sitrustipe, gevolg deur Valencia (25.4%), suurlemoen (13.3%), nawels (11.1%), Clementine (7.3%) en pomelo (6.9%). Okuleerhout-voorraad van die 465 kultivarlyne by CFB moet voortdurend bestuur word om in die vraag na gesogte variëteite te voorsien. CFB se vermoë vir primêre voorsiening was 76%, met die res van die ogies wat gemagtig vir voorsiening van gesertifiseerde bome in gesertifiseerde kwekerye. CFB is die primêre verskaffer van onderstamsaad in SA, en het 82.4% van gesertifiseerde saad in 2021/22 verskaf; die res is saad wat in gesertifiseerde kwekerye geproduseer word. Die nuwe onderstam-saadboorde van hoë-aanvraag kultivars het tot die saadoes bygedra en 2020 het 'n rekordsaadoes van >8000 L gelewer, waarvan 5857 L verskaf is. Saadbestellings van die 2021-oes het afgeneem met slegs 4746 L wat verskaf is. Die kwekery-stergraderingstelsel is sedert die ouditronde van November 2021 geïmplementeer, en kwekerye het meestal 'n verbeterde telling in die Mei 2022-ouditronde getoon: gemiddelde telling het van 69.6% tot 71.8% verbeter met 10 en 11 kwekerye wat met onderskeidelik 4 en 5 sterre gegradeer is.

### 6.1 Introduction

The purpose of the CIS is to enhance the standard of the South African citrus industry by ensuring that only horticulturally superior plants, which are free of viruses, diseases and pests, are supplied to growers and certified. The Citrus Growers Association of southern Africa (CGA) is responsible for the CIS and delegated its authority to CRI. In order to achieve this objective, close co-operation is required between CRI, the Agricultural Research Council - Tropical and Subtropical Crops (ARC-TSC), DALRRD's Directorate of Plant Health (DPH) and citrus nurseries represented by the South African Citrus Nurserymen's Association (SACNA). 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. Additionally, Cultivar and Pathology sub-committees co-ordinate the respective CIS activities.

Budwood and rootstock seed is produced and supplied from the CRI Citrus Foundation Block (CFB) outside Kariega. The phytosanitary status of propagation material certified by the CIS is ensured by virus-elimination and diagnostic services of cultivars prior to CIS introduction and routinely confirmed through re-indexing of mother trees as well as multiplication blocks. This report summarises budwood and rootstock seed production and supply, nursery and tree certification services, and the pathogen elimination and diagnostic support services by ARC-TSC and CRI's CRC in Nelspruit.

## 6.2 Budwood

This report summarises the seasonal supply of budwood from 1 July 2021 to 30 June 2022. Certified budwood supply declined in the 2021/22 season (5.62-million buds) from the all-time high levels in the 5 years prior (5-year average of 6.53-million); 16.6% lower than in 2020/21. Budwood demand was mostly from Limpopo (39.0%), Western Cape (30.1%), followed by the Eastern Cape (14.0%), Mpumalanga (10.7%) and the other provinces ranging from 2.3% to 0.6% (Table 6.2.1).

Mandarin (30.8%) was the most popular citrus type, followed by Valencia (25.4%), lemon (13.3%), navel (11.1%), Clementine (7.3%) and grapefruit (6.9%); in 2020/21 this proportion was 36.8%, 24.8%, 11.7%, 9.0%, 7.2% and 7.9%, respectively (Table 6.2.2). All other variety types accounted for 4.0% of the total budwood supply (2.4% in 2020/21). A break-down of buds per variety type to nurseries in different provinces are given in Table 6.2.3.

Mandarin supply remained high at 1.73 million buds, but was down from the 2.48-million in 2020/21 and 2.58 million in 2019/20 (Fig. 6.2.1). Valencia demand was stable from 2014/15 – 2016/17 with a 3-year average of 560 thousand buds; however, in the past five seasons, supply increased to 1.3 million buds supplied per year for 2017/18, 2018/19, 2019/20, to 1.6 million in 2020/21 (18.8% increase), with a slight decrease to 1.43 million in 2021/22 (Fig. 6.2.2), and surpassed lemon demand. Supply of lemon budwood increased from 435 thousand buds in 2018/19 to around 760 thousand buds per season in 2019/20, 2020/21 and 2021/22 (Fig. 6.2.3), with Eureka ranking as the second most popular cultivar in 2019/20, 2020/21 and fourth in 2021/22 (Table 6.2.4). The demand for 2PH Eureka SL, currently in the ninth position, has increased and material is supplied from an approved interim source, as the source supplied to CFB appeared to be unstable. Navel demand increased from 508 thousand buds in 2019/20 to 607 thousand buds in 2020/21, to 621 thousand in 2021/22 (Fig. 6.2.4). The high demand for Clementine buds of approximately 500 thousand buds per season 2019/20 and 2020/21, decreased slightly to 410 thousand in 2021/22 (Fig. 6.2.5). Grapefruit demand has been low for a number of years and increased from a low base of 45-, 77-, and 144 thousand buds in 2014/15, 2015/16 and 2016/17, respectively, to 336-, 360-, 362- and 531 thousand buds supplied in 2017/18, 2018/19, 2019/20 and 2020/21, respectively, with a decreased to 386 thousand in 2021/22 (Fig. 6.2.6). This high demand is significant and recent supply figures are comparative to the 10-year average of 535 thousand buds during the 1990's grapefruit boom.

The top 30 varieties comprised 88.6% of total number of buds supplied. RHM was the most popular cultivar, followed by Midnight, ARC Nadorcott LS, Eureka, Tango, Star Ruby, Turkey, Octubrina, 2PH Eureka SL, Jasi, Bennie 2, Witkrans, Cara Cara and Nova (Table 6.2.4). RHM supply dropped from 418 thousand buds in 2019/20 to 95 thousand buds in 2020/21, and increased to 531 thousand buds in 2021/22. Midnight Valencia supply remained high at 509 thousand buds. ARC Nadorcott LS supply levels have decreased from 903 749 in 2020/21 to 424 124 in 2021/22. The protected mandarin varieties in the top 10 (RHM, ARC Nadorcott LS (ARCCIT9) and Tango contributed to 23.7% to the total budwood supply, with a large proportion (38.7%) of these buds BCIN supplied in 2021/22: RHM (49.0%), ARC Nadorcott LS (13.7%) and Tango (52.2%). 2PH Eureka SL was supplied from an approved interim source and these are reported as BCIN figures (100%). The top 10 cultivars comprised 62.6% (3.5 million) of all total budwood supplied, of which 68.4% were supplied from the CFB (Table 6.2.4).

Primary CFB supply significantly improved from 67.9% and 61.8% in 2017/18 and 2018/19, respectively, to 75.6%, 76.4 and 76.0 in 2019/20, 2020/21 and 2021/22, respectively (Fig. 6.2.7). BCIN proportion per variety type: mandarins (39.6% of which RHM, ARC Nadorcott LS, and Tango comprised of 96.5%), Valencia's (25.2% of which Midnight, Turkey, Jasi, Bennie 2, and Delta comprised 96.0%), lemon (14.6%, of which 2PH Eureka

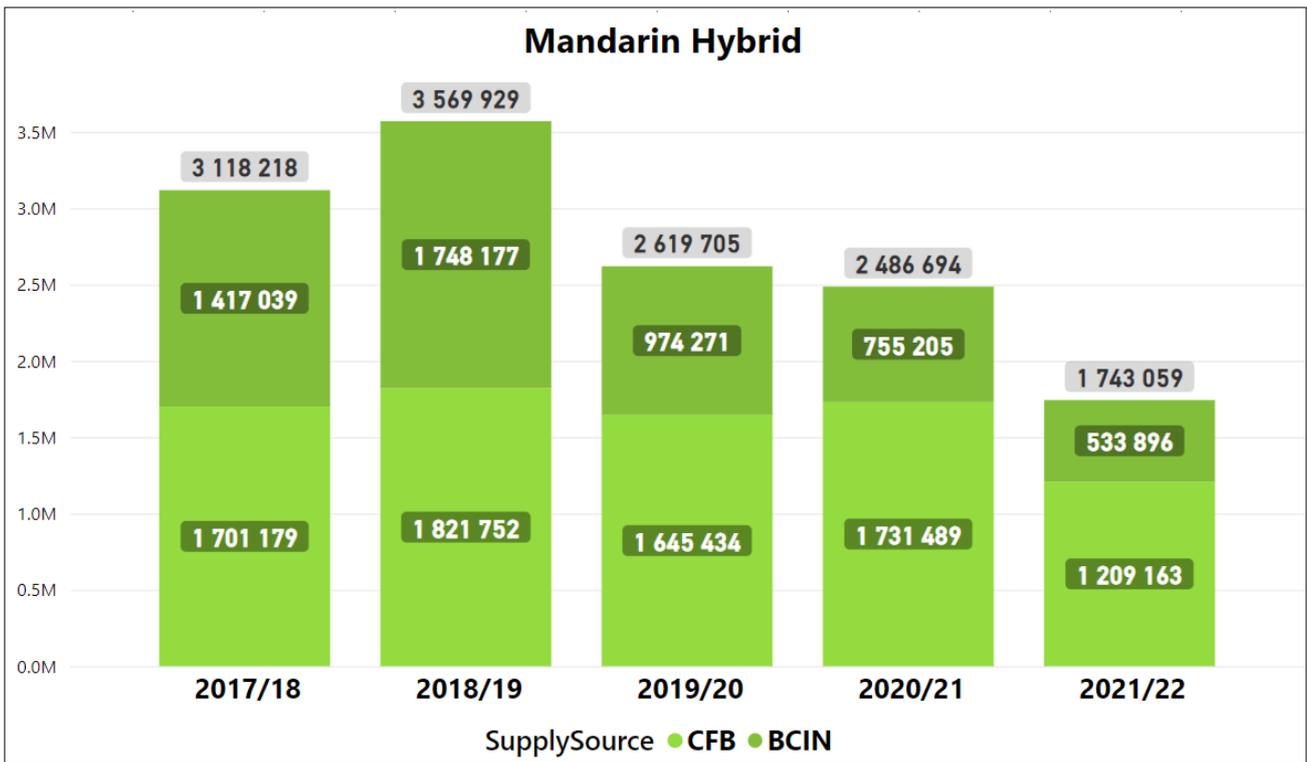
lemon comprised 96.8%), Clementine (7.3%, comprising of Octubrina 97.5%), navel (5.9%, mostly Cara Cara and Witkrans 77.9%) and other variety types (7.3%, satsuma 3.1%, grapefruit 2.6%, mostly Star Ruby, lime, midseason and diverse 1.7%).

**Table 6.2.1.** Buds supplied during the period July to June 2019/20 – 2021/22.

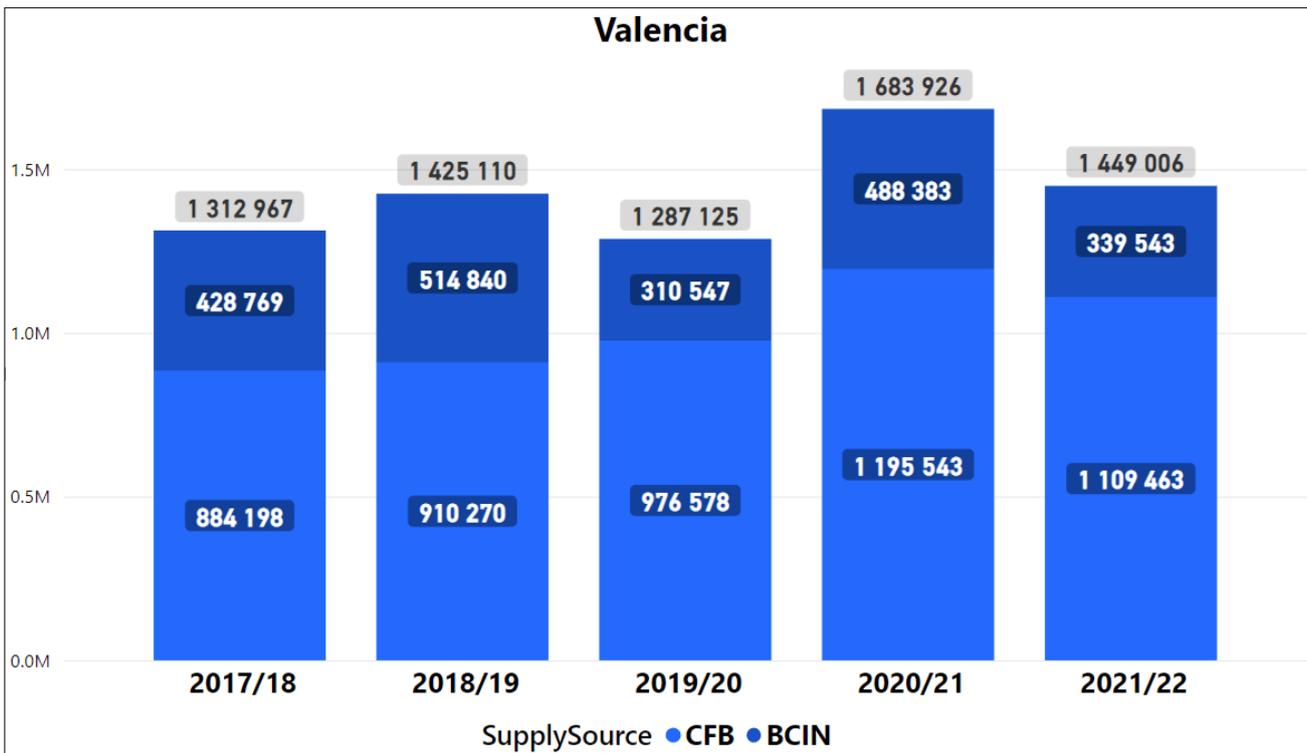
Area	2019/20	Dist %	2020/21	Dist %	2021/22	Dist %
<b>Local</b>	<b>6 102 566</b>	<b>99.3%</b>	<b>6 725 551</b>	<b>99.7%</b>	<b>5 547 876</b>	<b>98.7%</b>
Eastern Cape	1 037 927	16.9%	933 039	13.8%	786 731	14.0%
Gauteng	174 475	2.8%	99 574	1.5%	49 608	0.9%
KwaZulu natal	58 100	0.9%	57 500	0.9%	34 230	0.6%
Limpopo	1 906 388	31.0%	2 101 203	31.2%	2 191 350	39.0%
Mpumalanga	614 506	10.0%	652 232	9.7%	599 076	10.7%
North West	142 410	2.3%	98 070	1.5%	125 464	2.2%
Northern Cape	76 200	1.2%	75 710	1.1%	68 863	1.2%
Western Cape	2 092 560	34.1%	2 708 223	40.2%	1 692 554	30.1%
<b>International</b>	<b>40 190</b>	<b>0.7%</b>	<b>19 035</b>	<b>0.3%</b>	<b>74 119</b>	<b>1.3%</b>
India	1 250	0.0%		0.0%		0.0%
Swaziland		0.0%	320	0.0%		0.0%
USA	200	0.0%		0.0%		0.0%
Zambia	1 100	0.0%		0.0%		0.0%
Zimbabwe	37 640	0.6%	18 715	0.3%	74 119	1.3%
<b>Total</b>	<b>6 142 756</b>	<b>100.0%</b>	<b>6 744 586</b>	<b>100.0%</b>	<b>5 621 995</b>	<b>100.0%</b>

**Table 6.2.2.** Buds supplied during the period July to June 2019/20 – 2021/22.

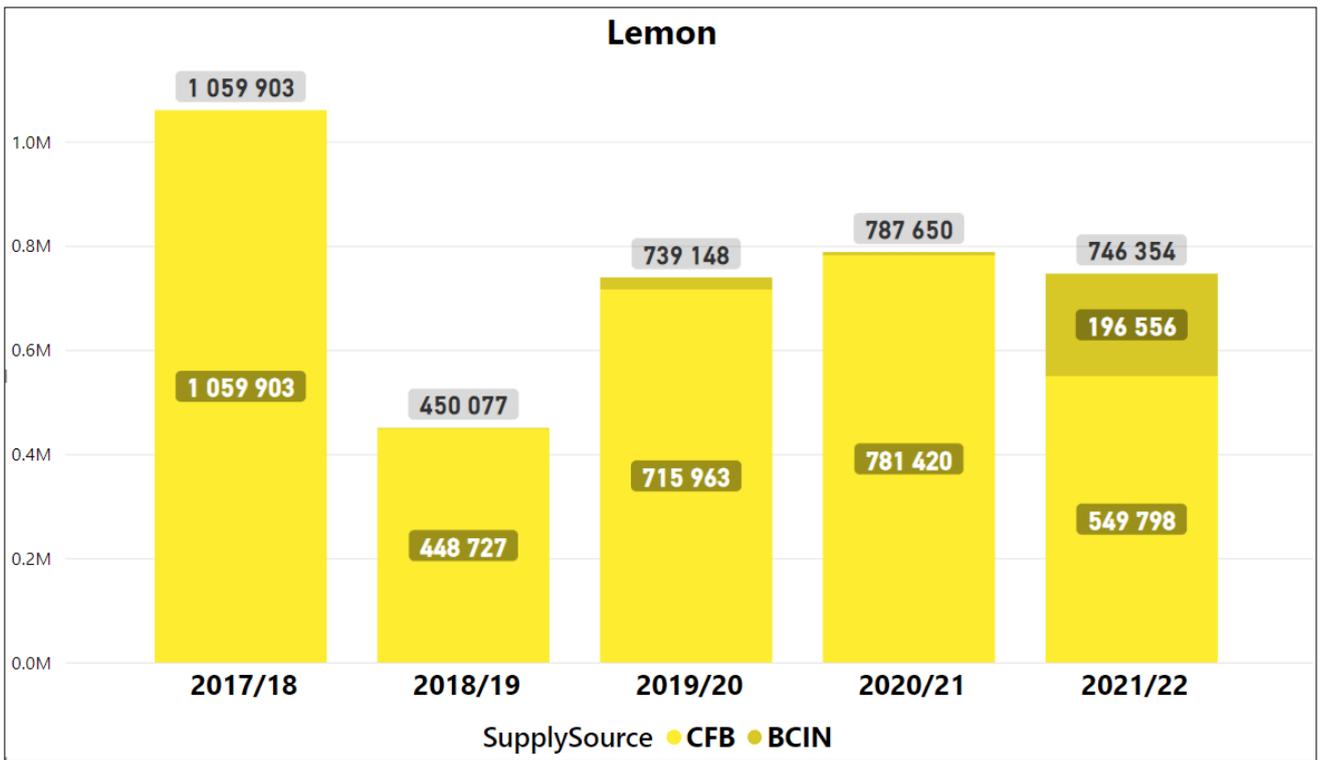
Variety Type	2019/20	Dist %	2020/21	Dist %	2021/22	Dist %
<b>Local</b>	<b>6 102 566</b>	<b>99.3%</b>	<b>6 725 551</b>	<b>99.7%</b>	<b>5 547 876</b>	<b>98.7%</b>
Clementine	495 190	8.1%	485 346	7.2%	410 136	7.3%
Diverse	8 970	0.1%	12 737	0.2%	35 413	0.6%
Ellendale	100	0.0%		0.0%		0.0%
Grapefruit	361 610	5.9%	531 584	7.9%	385 553	6.9%
Kumquat	14 590	0.2%	16 890	0.3%	20 970	0.4%
Lemon	737 798	12.0%	787 350	11.7%	745 404	13.3%
Lime	36 560	0.6%	65 026	1.0%	59 670	1.1%
Mandarin Hybrid	2 581 965	42.0%	2 479 594	36.8%	1 729 059	30.8%
Midseason	18 419	0.3%	20 066	0.3%	35 682	0.6%
Navel	508 286	8.3%	607 830	9.0%	621 278	11.1%
Pummelo	7 685	0.1%	3 476	0.1%	3 775	0.1%
Rootstock	20	0.0%	1 480	0.0%	50	0.0%
Satsuma	44 248	0.7%	40 866	0.6%	71 890	1.3%
Valencia	1 287 125	21.0%	1 673 306	24.8%	1 428 996	25.4%
<b>International</b>	<b>40 190</b>	<b>0.7%</b>	<b>19 035</b>	<b>0.3%</b>	<b>74 119</b>	<b>1.3%</b>
Clementine		0.0%	540	0.0%	75	0.0%
Diverse		0.0%	100	0.0%	4 500	0.1%
Lemon	1 350	0.0%	300	0.0%	1 000	0.0%
Lime		0.0%	25	0.0%	25	0.0%
Mandarin Hybrid	37 740	0.6%	7 100	0.1%	14 000	0.2%
Midseason		0.0%	150	0.0%		0.0%
Navel	1 100	0.0%	200	0.0%	34 519	0.6%
Valencia		0.0%	10 620	0.2%	20 000	0.4%
<b>Total</b>	<b>6 142 756</b>	<b>100.0%</b>	<b>6 744 586</b>	<b>100.0%</b>	<b>5 621 995</b>	<b>100.0%</b>



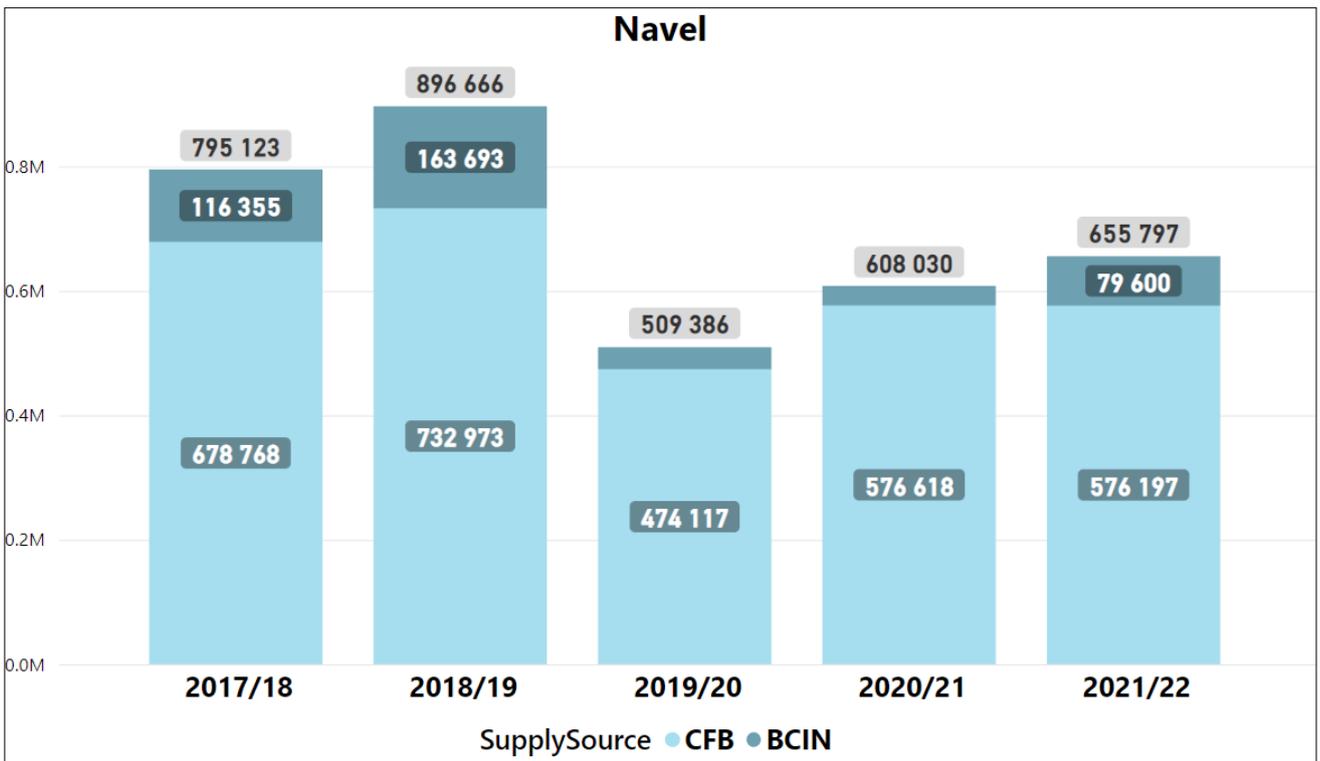
**Figure 6.2.1.** Mandarin hybrid budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2017/18 – 2021/22.



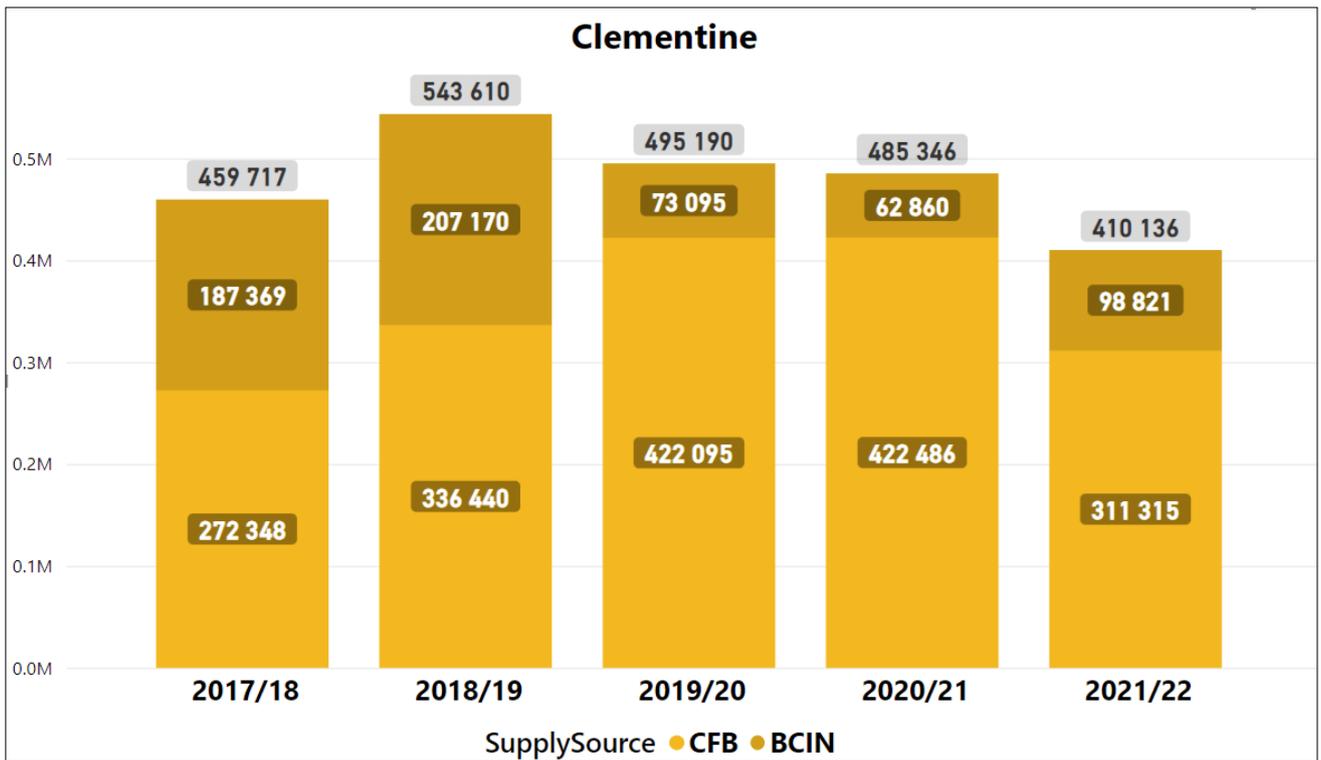
**Figure 6.2.2.** Valencia budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2017/18 – 2021/22.



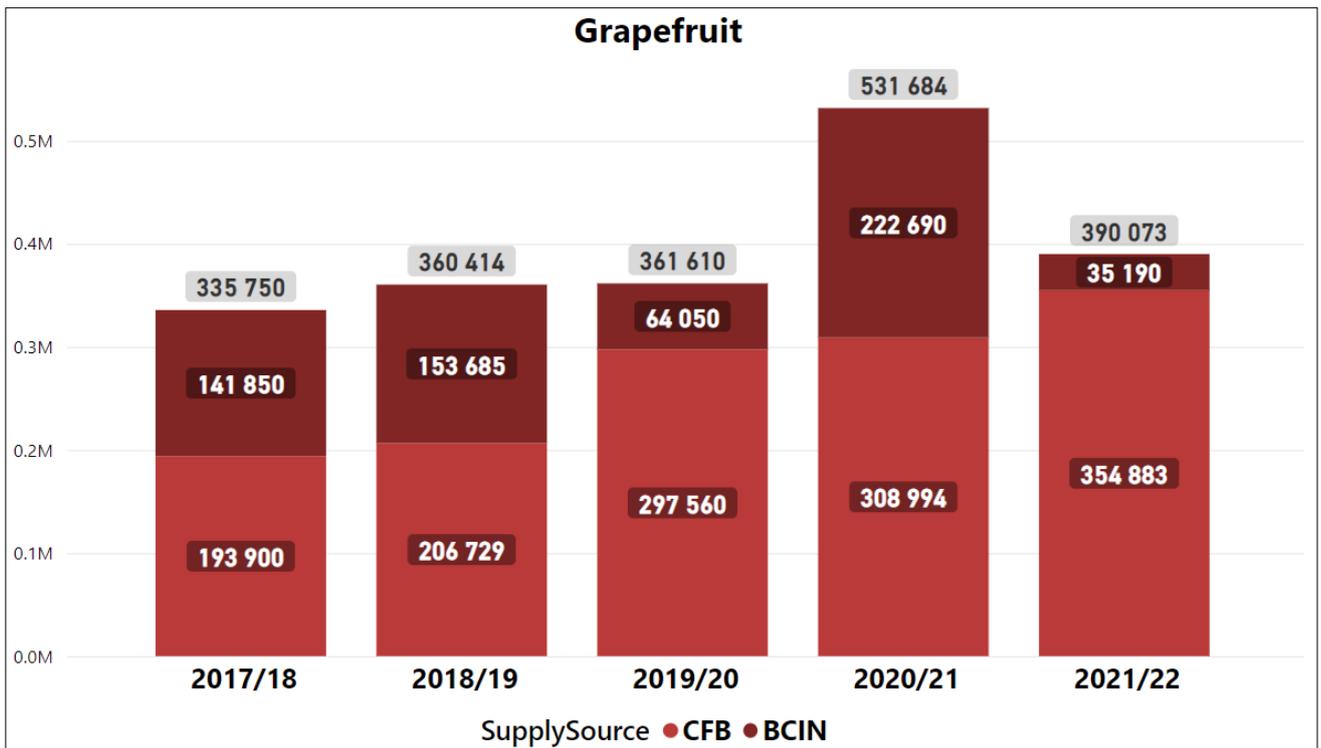
**Figure 6.2.3.** Lemon budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2017/18 – 2021/22.



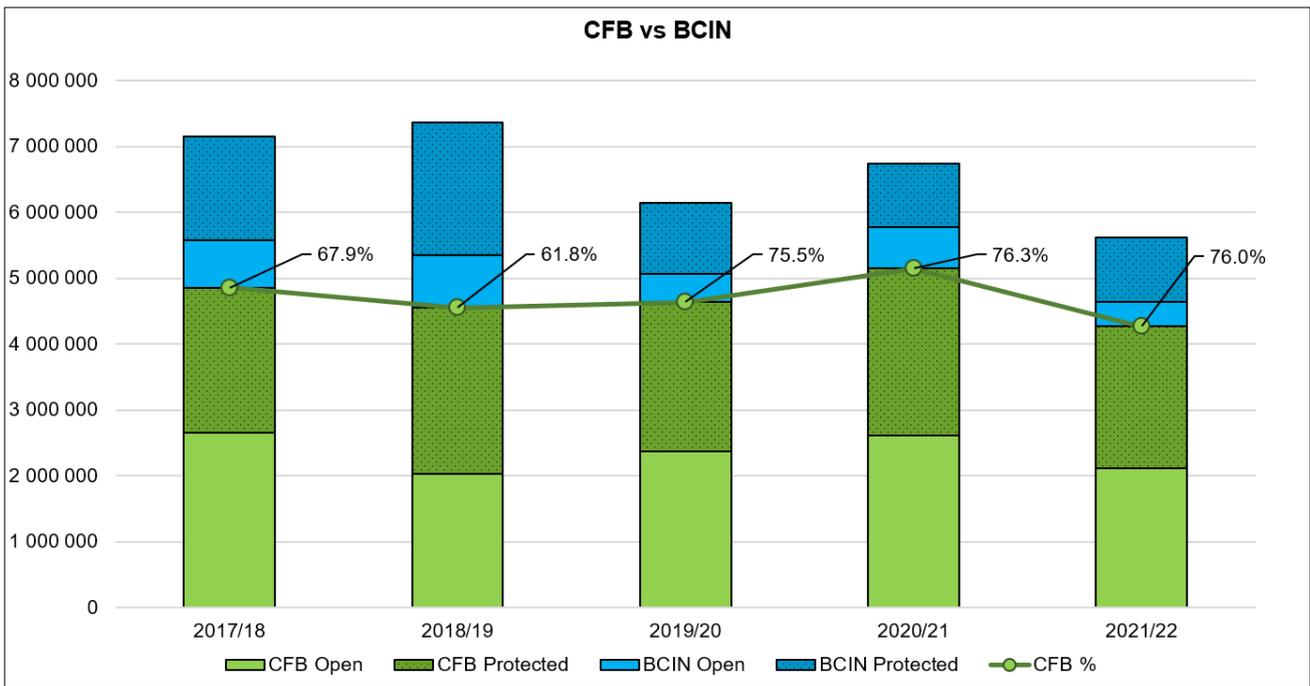
**Figure 6.2.4.** Navel budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2017/18 – 2021/22.



**Figure 6.2.5.** Clementine budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2017/18 – 2021/22.



**Figure 6.2.6.** Grapefruit budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2017/18 – 2021/22.



**Figure 6.2.7.** Budwood of open and protected cultivars (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2017/18 – 2021/22.

**Table 6.2.3.** Buds supplied per variety type per area (total number of buds per season) during the periods July to June from 2019/20 – 2021/22.

Variety Type	Season	EC	GP	KZN	LP	MP	NW	NC	WC	Exported	Total
Clementine	2019/20	43 644	25 500		18 600	6 150	2 000		399 296		495 190
	2020/21	26 795	200		27 380	7 006			423 965		485 346
	2021/22	113 221			1 595	1 265			294 055		410 136
Diverse	2019/20	400	5 430		2 380	40			720		8 970
	2020/21	130	7 230		1 330	1 114	150	10	2 773	540	13 277
	2021/22	3 557	6 170		10 836	700	500		13 650	75	35 488
Ellendale	2019/20								100		100
Grapefruit	2019/20	2 800	3 000	2 000	196 760	78 550	9 900	2 900	65 700		361 610
	2020/21	15 396	13 300		345 033	77 120	17 470	38 010	25 255	100	531 684
	2021/22	4 812	800	1 940	224 578	77 765	10 000	22 520	43 138	4 500	390 053
Kumquat	2019/20	900	5 050		1 730		3 000	500	3 410		14 590
	2020/21	20	5 700		2 750	2 000	1 130		5 290		16 890
	2021/22	300	2 100		8 800	1 400			8 370		20 970
Lemon	2019/20	243 982	35 820	29 500	264 500	13 817	29 700	8 000	112 479	1 350	739 148
	2020/21	212 599	20 550	13 000	255 043	94 458	18 250	6 800	166 650	300	787 650
	2021/22	124 908	26 813	7 000	228 908	63 550	18 900	8 000	267 325	1 000	746 404
Lime	2019/20	2 210	6 515		4 550	14 500	2 075	500	6 210		36 560
	2020/21	4 970	5 900		6 600	25 386	2 360	10	19 800	25	65 051
	2021/22	1 390	1 910	3 000	20 350	4 900	1 000		27 120	25	59 695
Mandarin Hybrid	2019/20	425 227	21 300		684 435	343 058	43 450	10 000	1 054 495	37 740	2 619 705
	2020/21	303 419	5 040	5 500	497 341	175 267	18 420	8 000	1 466 607	7 100	2 486 694
	2021/22	88 253	5 980	3 600	875 407	117 160	24 400	4 500	609 759	14 000	1 743 059
Midseason	2019/20	550					400		17 469		18 419
	2020/21	9 160			600	66	500	20	9 720	150	20 216
	2021/22	21 104				100	1 200	18	13 260		35 682
Navel	2019/20	87 776	31 260	2 500	144 040	13 597	24 900	39 800	164 413	1 100	509 386
	2020/21	86 711	23 750	11 300	77 620	45 817	19 620	7 750	335 262	200	608 030
	2021/22	95 518	5 030	9 690	199 440	65 516	34 924	11 300	199 860	34 519	655 797
Pummelo	2019/20				7 600				85		7 685
	2020/21	18	100		300	2 408			650		3 476
	2021/22				1 800	750			1 225		3 775
Rootstock	2019/20								20		20
	2020/21	720	200						560		1 480
	2021/22	50									50
Satsuma	2019/20	17 203		1 000	6 500		2 000	3 500	14 045		44 248
	2020/21	20 910		8 500	3 220	4 006	2 000		2 230		40 866

	2021/22	52 600		3 000	800	1 200			14 290		71 890
<b>Valencia</b>	2019/20	213 235	40 600	23 100	575 293	144 794	24 985	11 000	254 118		1 287 125
	2020/21	252 191	17 604	19 200	883 986	217 584	18 170	15 110	249 461	10 620	1 683 926
	2021/22	281 018	805	6 000	618 836	264 770	34 540	22 525	200 502	20 000	1 448 996

**Table 6.2.4.** Top 30 cultivars based on total number of buds supplied for seasons July to June from 2019/20 – 2021/22.

2019/20			2020/21			2021/22		
Cultivar	BCIN	TOTAL	Cultivar	BCIN	TOTAL	Cultivar	BCIN	TOTAL
ARC Nadorcott LS MAN	460 547	920 281	ARC Nadorcott LS MAN	375 233	974 008	RHM MAN	260 770	531 672
Eureka LEM	18 605	632 344	Eureka LEM	3 700	652 281	Midknight VAL	161 411	509 776
Tango MAN	141 100	474 842	Midknight VAL	141 770	588 724	ARC Nadorcott LS MAN	58 270	424 124
Midknight VAL	106 348	470 085	Star Ruby GFT	221 010	491 156	Eureka LEM	6 000	423 194
RHM MAN	292 893	418 104	Tango MAN	166 915	425 483	Tango MAN	196 310	375 888
Star Ruby GFT	64 050	332 210	Jasi VAL	173 566	359 759	Star Ruby GFT	34 190	324 873
Nules CLE	58 065	281 954	Turkey VAL	60 370	251 305	Turkey VAL	90 560	285 705
Jasi VAL	97 274	202 229	Bennie 2 VAL	105 607	235 789	Octubrina CLE	96 321	270 758
Nova MAN	16 200	176 361	Nules CLE	11 800	217 095	2PH Eureka SL LEM	190 236	190 236
Leanri MAN	8 545	171 148	Leanri MAN	12 000	210 659	Jasi VAL	18 072	182 314
Witkrans NAV	14 419	152 402	Nova MAN	21 400	182 667	Bennie 2 VAL	38 780	164 525
Turkey VAL	19 000	143 545	Octubrina CLE	48 110	176 940	Witkrans NAV	24 730	163 783
Bennie 2 VAL	20 360	122 078	Witkrans NAV	8 412	145 783	Cara Cara NAV	37 250	137 047
Octubrina CLE		120 602	Cara Cara NAV	22 000	126 183	Nova MAN	2 900	112 724
Cara Cara NAV	19 750	96 035	Ma'ayana MAN	61 471	110 992	Cambria NAV	680	81 106
Or 4 MAN		95 014	Sigal MAN	33 388	108 288	Leanri MAN		80 051
Cambria NAV	1 100	94 747	Cambria NAV		97 645	Delta VAL	17 220	69 877
Kobus du Toit Late VAL	47 203	82 203	RHM MAN	24 000	95 158	Miho Wase SAT	41 200	66 415
Nadorcott 1 MAN		73 185	Nadorcott 1 MAN	18 200	91 143	Nules CLE		65 211
Sigal MAN	17 846	64 840	PE 1 MAN	30 798	86 059	Late VAL	1 650	58 537
Bahianinha NAV		62 350	Bahianinha NAV		73 459	Bearss LIM	4 850	56 050
Late VAL		59 970	Florida C4-15-19 MAN	9 000	70 875	Bahianinha NAV	800	54 582
Andes 1 Clemenluz CLE	4 700	55 300	Red Lina NAV		62 984	Lisbon LEM		53 645
Gusocora (G5) VAL	6 000	53 400	Limoneira 8A LEM		61 675	Gusocora (G5) VAL	1 200	53 364
Delta VAL		49 490	Bearss LIM	18 176	57 470	Limoneira 8A LEM		44 620
Du Roi VAL	9 362	47 300	Late VAL		38 259	Washington NAV	500	42 898
Furr (Clem x Murcott) MAN		43 175	Marsh GFT	1 200	37 419	Kobus du Toit Late VAL		42 354
Washington NAV		39 510	Du Roi VAL	1 700	32 634	Nadorcott 1 MAN		41 554
PE 1 MAN	10 880	36 590	Furr (Clem x Murcott) MAN	2 800	31 571	Red Lina NAV		39 957

Genoa LEM		36 354	Delta VAL		31 288	Clemensoon CLE		36 912
<b>Top 30</b>	<b>1 434 247</b>	<b>5 607 648</b>	<b>Top 30</b>	<b>1 572 626</b>	<b>6 124 751</b>	<b>Top 30</b>	<b>1 283 900</b>	<b>4 983 752</b>
<b>&gt; Top 30</b>	<b>67 880</b>	<b>535 108</b>	<b>&gt; Top 30</b>	<b>23 030</b>	<b>619 835</b>	<b>&gt; Top 30</b>	<b>63 156</b>	<b>638 243</b>
<b>Total</b>	<b>1 502 127</b>	<b>6 142 756</b>	<b>Total</b>	<b>1 595 656</b>	<b>6 744 586</b>	<b>Total</b>	<b>1 347 056</b>	<b>5 621 995</b>
	<b>24.5%</b>			<b>23.7%</b>			<b>24.0%</b>	

### 6.3 Seed

CFB is the primary supplier of rootstock seed in SA, supplied around 82.4% of certified seed. In 2021/22, yields were in line with the estimated yield potential. The harvest in 2020/21 was a new record at the CFB for all seed varieties with >8000 L seed harvested, of which 5857 L was supplied; the remainder was kept in cold storage at CFB. However, in 2021/22 a decline in yield was seen for Carrizo citrange (11% lower than 2020/21), MXT, Swingle citrumelo and X639 (50%, 18% and 75%, respectively). C35 citrange was 17.3% higher than 2020/21, as well as rough lemon (28.1%) and US-812 (SXB) (155.8%) for the same period.

During May to April 2022, 4746 litres of seed were supplied locally (Table 6.3.1) and 48 litres of seed were exported (Table 6.3.1). Carrizo citrange remains the most popular rootstock (39.7%), followed by Swingle citrumelo (14.2%), X639 (7.0%), Rough lemon (6.4%) and C35 citrange (6.1%) and other rootstock cultivars (9.1%) (Table 6.3.2).

**Table 6.3.1.** Seed (litres) supplied by the CFB during the periods May to April 2019/20 – 2021/22.

Area	2019/20	Dist %	2020/21	Dist %	2021/22	Dist %
<b>Local</b>	6 972	99.3%	6 355	98.3%	4 746	99.0%
Eastern Cape	738	10.5%	887	13.7%	1 015	21.2%
Gauteng	157	2.2%	57	0.9%	54	1.1%
KwaZulu Natal	95	1.4%	25	0.4%	68	1.4%
Limpopo	2 594	37.0%	2 606	40.3%	1 592	33.2%
Mpumalanga	495	7.1%	630	9.7%	487	10.1%
North West	228	3.2%	114	1.8%	143	3.0%
Northern Cape	562	8.0%	125	1.9%	104	2.2%
Western Cape	2 104	30.0%	1 912	29.6%	1 285	26.8%
<b>International</b>	<b>46</b>	<b>0.7%</b>	<b>110</b>	<b>1.7%</b>	<b>48</b>	<b>1.2%</b>
Australia		0.0%		0.0%	43	0.9%
Botswana		0.0%	3	0.0%		0.0%
Democratic Republic of the Congo	5	0.1%	7	0.1%	4	0.1%
Namibia		0.0%		0.0%	1	0.0%
Swaziland		0.0%	12	0.2%		0.0%
Zimbabwe	41	0.6%	88	1.4%		0.0%
<b>Total</b>	<b>7 018</b>	<b>100.0%</b>	<b>6 464</b>	<b>100.0%</b>	<b>4 794</b>	<b>100.0%</b>

**Table 6.3.2.** Seed (litres) supplied by the CFB during the periods May to April 2019/20 – 2021/22.

Rootstock cultivar	2019/20	Dist %	2020/21	Dist %	2021/22	Dist %
<b>CFB</b>	<b>6 175</b>	<b>88.0%</b>	<b>5 857</b>	<b>90.6%</b>	<b>3 951</b>	<b>82.4%</b>
Benton Citrange	21	0.3%	8	0.1%	67	1.4%
C35 Citrange	888	12.7%	427	6.6%	292	6.1%
Carrizo Citrange	2 845	40.5%	2 374	36.7%	1 904	39.7%
Flying Dragon	20	0.3%	23	0.4%	41	0.9%
Minneola X Trifoliata	60	0.9%	202	3.1%	100	2.1%
Rough Lemon	294	4.2%	358	5.5%	305	6.4%
Swingle Citrumelo	1 178	16.8%	942	14.6%	682	14.2%
Troyer Citrange	327	4.7%	152	2.4%	29	0.6%
US-812 (Sunki x Benecke)	47	0.7%	57	0.9%	117	2.4%
Volckameriana	125	1.8%	74	1.1%	63	1.3%
X639	361	5.1%	1 227	19.0%	334	7.0%
Yuma Citrange	6	0.1%	10	0.2%	12	0.3%
Other	4	0.1%	5	0.1%	5	0.1%
<b>Imported</b>	<b>452</b>	<b>6.4%</b>	<b>21</b>	<b>0.3%</b>	<b>0</b>	<b>0.0%</b>

<b>SPIN**</b>	<b>391</b>	<b>5.6%</b>	<b>587</b>	<b>9.1%</b>	<b>844</b>	<b>17.6%</b>
<b>Total</b>	<b>7018</b>	<b>100.0%</b>	<b>6 464</b>	<b>100.0%</b>	<b>4 794</b>	<b>100.0%</b>

\*\*Seed produced in nurseries

#### 6.4 Production

##### Budwood:

*Cultivar introduction:* The STG facilities at CRI-Nelspruit released 6 new cultivars to the CFB and ARC-TSC introduced 27 (Table 6.4.1). Introduced cultivars are budded to rootstock seedlings in CFB's rapid multiplication tunnels, 970 new multiplication trees were made from these releases.

**Table 6.4.1.** Cultivar introductions from 2017/18 – 2021/22.

<b>Source</b>	<b>2017/18</b>	<b>2018/19</b>	<b>2019/20</b>	<b>2020/21</b>	<b>2021/22</b>
ARC: New introductions	13	9	10	21	27
ARC: Re-introductions	15	5	6	5	3
CRI: New introductions	6	7	22	10	6
CRI: Re-introductions	22	0	2	10	1

*Multiplication blocks:* CFB presently maintains more than 118 thousand multiplication trees of 465 cultivar lines with a potential annual budwood stock of >8.4 million buds.

In order to timeously address budwood demand, CIS obtains budwood demand estimates from the CIS Cultivar Committee, private cultivar owners/agents and nurseries. This feedback is considered with historical supply and BCIN records, and multiplication tree stocks are managed accordingly. In 2021/22, 11 606 new multiplication trees, of 44 cultivar lines, were made. To address space constraints, 13 072 multiplication trees from less popular cultivars were removed. There was a big effort made to remove trees of categories 5 and 4L. Redundant trees for category 4-L and 5 cultivars were destroyed.

**Table 6.4.2.** Multiplication of existing cultivars from 2017/18 – 2021/22.

<b>Source</b>	<b>2017/18</b>	<b>2018/19</b>	<b>2019/20</b>	<b>2020/21</b>	<b>2021/22</b>
Re-multiplication of existing cultivar lines	84	57	51	65	44

*Mother block:* The mother trees were replanted in greenhouse 5 in 2011-2019, with the majority planted in 2015. These trees were made from the original mother trees in greenhouse 1 or planted out from the first release. Of the 135 cultivars planted out as mother trees, a further 21 were confirmed as true-to-type (TtT) in 2021, bringing the total number of TtT cultivars in the mother block to 79. Thus far, 58.5% of these cultivars were confirmed as true-to-type.

**Table 6.4.3.** Cultivars established in the mother block and confirmed true-to-type

<b>Mother trees</b>	<b>No of Cultivars</b>	<b>2017/18</b>	<b>2018/19</b>	<b>2019/20</b>	<b>2020/21</b>	<b>2021/22</b>	<b>Not Yet TtT</b>
Planted 2011	16	1	1	2		3	9
Planted 2014	2		2				0
Planted 2015	93	3	30	14		15	31
Planted 2017	22	-	-	5		2	15
Planted 2019	2	-	-			1	1
<b>Total</b>	<b>135</b>	<b>4</b>	<b>33</b>	<b>21</b>	<b>0</b>	<b>21</b>	<b>56</b>

*Evaluation block:* In total, 487 cultivars are currently established in the evaluation block (two trees per cultivar line) for true-to-type evaluation purposes, of which 252 were confirmed as true-to-type (51.7%). This initiative is very successful and allowed the CIS to confirm the true-to-type status for cultivars of which CFB did not have mother trees (category 4).

**Table 6.4.4.** Cultivars established in the evaluation block and confirmed as true-to-type

Evaluation block trees	No of Cultivars	2017/18	2018/19	2019/20	2020/21	2021/22	Not Yet TtT
Planted 2016	290		42	70	2	75	101
Planted 2017	13		10	2			1
Planted 2018	51			2	3	24	22
Planted 2019	37			7		15	15
Planted 2020	1						1
Planted 2021	61						61
Planted 2022	34						34
<b>Total</b>	<b>487</b>		<b>52</b>	<b>81</b>	<b>5</b>	<b>114</b>	<b>235</b>

*Pre-immunisation block:* To optimise the greenhouse space, category 4 cultivars were sub-divided: 4-high (annual budwood demand of more than 144 buds, but less than 5550) and 4-low (annual budwood demand of +/-144 buds per annum). For the latter only two trees of each cultivar are maintained in the pre-immunised block. This will ensure that pre-immunised budwood for these low demand cultivars is available at CFB, but that surplus increase trees can be significantly reduced. This was done for 156 cultivars.

*Nucleus block:* CFB duplicated 407 accessions in two bays of the polycarbonate nucleus block facility from the CRI's nucleus block facility and 179 accessions from the ARC nucleus block.

**Table 6.4.5** Duplication of the nucleus block at the CFB

Source	2021/22
ARC: Duplication of the nucleus block	179
CRI: Duplication of the nucleus block	407

Seed: During 2021/22 surplus seed for C35, Carrizo, Swingle and Troyer was available after all orders were supplied. Unfortunately, the full demand for RL and US-812 (SXB) could not be met.

CFB continued with selective light pruning after the harvest season. Two rounds of gibberellic acid were applied, one after two-thirds petal fall (10 ppm) and another 1 week later (10 ppm) to X639 and Carizzo. A lot of natural bee activity was again observed during flowering. This may be the result of delaying application of imidacloprid until after flowering.

Removal of all trees outside insect-secure structures from CFB is a necessary measure to ensure the sustained biosecurity of the CFB budwood sources, considering the threat of exotic pests and diseases and the potential quarantine implications. Close to 6000 rootstock trees were budded on X639 for planting at the seed farm when acquired. A suitable farm was identified and an offer to purchase accepted. CRI is busy with the due diligence investigation to meet the OTP's suspensive conditions.

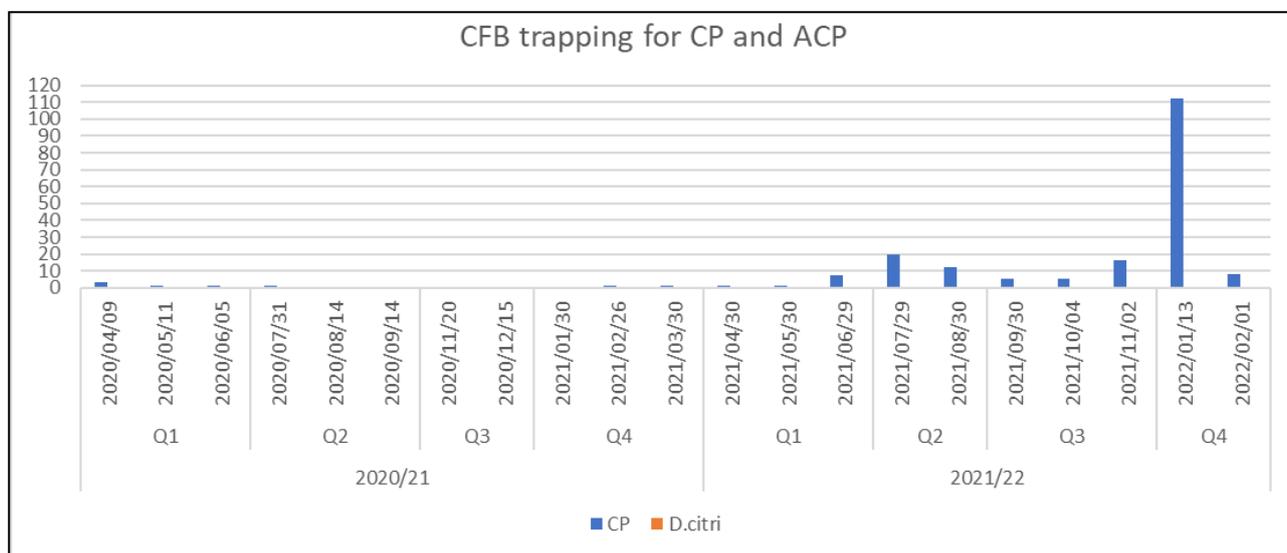
CFB pest monitoring: Pest monitoring at the Citrus Foundation Block (CFB) includes weekly scouting and the use of yellow sticky traps in insect-secure structures and seed orchards. Monitoring includes pests such as aphids (AP), Asian (ACP, *Diaphorina citri*) and African (*Trioza erytraeae*, CP) citrus psyllids, and other citrus production pests. Both psyllid species are vectors of Citrus Greening bacteria while many aphid species are vectors for *citrus tristeza virus*. Trapping was implemented from 2017 and the CFB was examining the yellow sticky traps until September 2019, whereafter trap reading was conducted by CRI's biosecurity division at the

CRC in Nelspruit. Monitoring of traps on the 5-km perimeter of the buffer zone surrounding the CFB was implemented since April 2020. Thus far, 11 locations are routinely monitored.

No citrus psylla (CP) or Asian Citrus Psylla (ACP) species were detected in any of the insect-secure structures, during scouting and trapping for Quarter 1 to 4 in 2020, 2021 and 2022. Furthermore, no sign of CP damage or life stages were detected in the insect-secure structures.

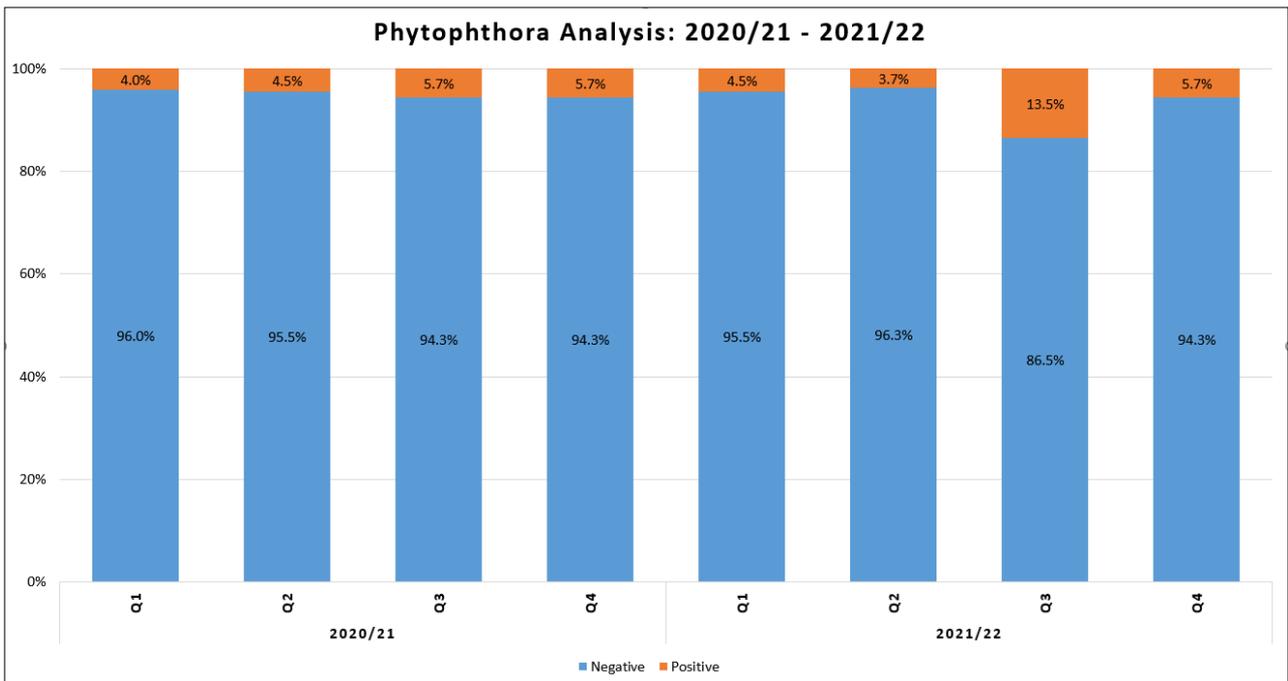
With trapping in orchards, CP were recorded in higher than expected numbers (Fig. 6.3.1) with the highest of 40 insects per trap recorded in GVB 6. Alice (acetamiprid (neonicotinoid)) was applied, which resulted in a significant reduction in insects per trap (2-3 per trap). Minor feeding damage was observed on the flush in most orchards. Application of chemical products was carefully timed to limit the effects on pollinators in seed source orchards.

On the 5-km perimeter monitoring traps, no CP was trapped. ACP was never trapped at the CFB or in the surrounding buffer zone. Some aphids were recorded in the greenhouses but it was eradicated as soon as the pest was found. CFB continued with inspections for any breaches in of structures on a weekly basis. Only minor breaches occurred and immediate action was taken to rectify the breach.



**Figure 6.4.1** Total number of *Trioza erytrae* (CP) and *Diaphorina citri* (D. citri) trapped on a total of 45 traps from all seed orchards and 9 traps from the 5-km perimeter.

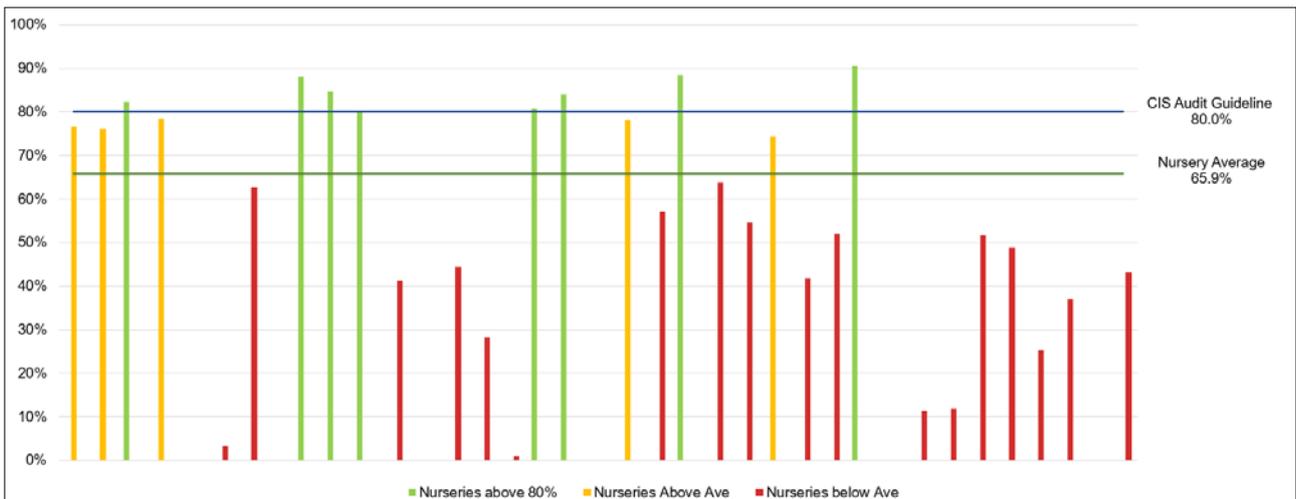
*Phytophthora* monitoring: Quarterly samples from increase trees, the evaluation block and mother trees were taken for analysis of the soil- and water-borne *Phytophthora* species and submitted to CRI’s Diagnostic Centre at the CRC in Nelspruit. *Phytophthora citrophthora* was the only species that was detected. Ridomil resistance was confirmed in some of these isolates. CFB routinely uses captan to control *Phytophthora* in pots, and has also implemented the use of phosphonates to strengthen plant resistance. Generally, treatment with captan is effective as shown in re-tests after treatment (Figure 6.4.2).



**Figure 6.4.2** Quarterly results for *Phytophthora* detected in all potted trees at the CFB.

### 6.5 Tree Certification

In total, 4 094 024 trees were certified during April 2021 to March 2022; 152 113 of the trees presented for certification, did not meet the certification criteria. This was similar to the numbers of trees certified in previous two annual periods: 4 048 594 and 4 394 063 trees certified, with 54 885 and 151 147 trees not meeting the certification criteria. Figure 6.5.1 indicates the nursery tree certification criterion of trees certified as a percentage of budwood supplied during the 2-year period prior to the maximum age certifiable age of trees in the nursery (30 months after budding). Of the 36 certified nurseries, 8 nurseries scored above the 80% benchmark, 5 nurseries above the nursery average of 65.9%, and 24 nurseries below this average (note that new nurseries might not have certified trees in this period yet). This criterion was included in the updated nursery certification guideline and nurseries were penalised if they did not meet the 80% benchmark.



**Figure 6.5.1** Nursery tree certification as a percentage of budwood supplied during the 2-year period prior to the maximum age certifiable age of trees in the nursery (30 months after budding). The updated CIS audit guidelines require a tree certification percentage of >80%.

## 6.6 Nursery Certification

After phasing in the new audit criteria and star-grading system since May 2020, the results from November 2021 audits were published. This system distinguishes between good to excellent nurseries (3-, 4-, and 5-star nurseries) and nurseries participating adequately in the scheme but needing to work actively to improve certain fundamental criteria (1- and 2-star nurseries). The highlights of this audit round were to audit and provisionally certify the very first HLB-Safe Nursery in South Africa and to have the extension managers join the audits in their regions. The inputs from the extension managers were invaluable. Thirty-five nurseries were certified and the average audit percentage was 69.6%, with 6 and 9 nurseries graded with 4 and 5 stars (Table 6.6.1).

It was clear after the May 2022 audit round that most nurseries worked hard to improve their star-grading. The average audit score increased 71.8%. There were a number of improvements in star-gradings since the previous audit, with 10 and 11 nurseries graded with 4 and 5 stars (Table 6.6.1). Two new nurseries obtained provisional certification.

Overall a distinct trend can be witnessed in the 3-, 4-, and 5-star nurseries to retain their gradings and a strong desire to improve in the 1- and 2-star nurseries. The star-grading system has brought positive change to the CIS certified nurseries of southern Africa.

A summary of the number of nurseries that fall under each star rating are shown in Table 6.6.1, and the updated nursery list in Table 6.6.2.

**Table 6.6.1.** Star-rated CIS Certified following the November 2021 and May 2022 audits.

Nursery star rating	Final audit score	Audit round	
		Nov-21	May-22
<b>5-star</b>	>80%	9	11
<b>4-star</b>	70-79%	6	10
<b>3-star</b>	60-69%	17	12
<b>2-star</b>	50-59%	2	2
<b>1-star</b>	<50%	1	0
<b>New Nursery</b>	-	-	2
<b>Average audit %</b>		69.6%	71.8%
<b>Nurseries certified</b>		<b>36</b>	<b>37</b>

**Table 6.6.2.** CIS Certified Nurseries in May 2022

Nursery	Star-Grading	Town / Province		Contact Person	Cell	Email
Apapanzi	5-star	Kirkwood	EC	Nellis Meiring	082 550 6210	nellis@srvalley.co.za
Attwell Citrus	5-star	Kirkwood	EC	Wayne Attwell	072 463 7118	mandy@attwellcitrus.co.za
Augsburg	4-star	Clanwilliam	WC	Alta Laing	079 527 0316	admin@augsburnursery.co.za
BF Joubert	4-star	Kirkwood	EC	Francois Joubert	084 951 1922	bfjkweek@srvalley.co.za
Cape Grow	3-star	Kraaifontein	WC	Eugene Nepgen	084 416 0184	eugene@cgrow.co.za
Casmar	2-star	Mooiooi	NW	Neville Wenhold Jnr	082 881 4189	casmarnursery@absamail.co.za
Cederberg Tree	5-star	Citrusdal	WC	Patricia Willemse	076 622 7007	trish@cederbergreenursery.co.za
Dodhill	4-star	Chegutu	ZIM	Pete Breitenstein	+263 77 222 1046	dodhill@iwayafrica.co.zw
Du Roi	5-star	Letsitele	LP	Zylon McGaffin	076 227 6704	zylon@duroi.co.za
Du Roi Halls	5-star	Nelspruit	MPU	Arve Grindstad	071 411 0131	arve@duroi-halls.co.za
Esselen	4-star	Malelane	MPU	Louis Esselen	078 803 7010	esselenkwekery@gmail.com
Gamtoos	3-star	Patensie	EC	Keuler Engela	072 260 9813	keuler@rikusld.co.za
Groep 91	NEW NURSERY	Letsitele	LP	Jaco Lindeque	084 802 4708	jaco@groep91.co.za
Groot Patrysvlei	4-star	Clanwilliam	WC	EP van Niekerk	082 456 0135	epvn@capespanfarms.co.za

H J Joubert	3-star	Montagu	WC	Herman Joubert	082 578 5747	hopewell@breede.co.za
Henley Citrus	4-star	Letsitele	LP	André Swanepoel	084 513 8649	productionmanager@bigday.co.za
Heuers Wholesale	3-star	Brits	NW	David Seewald	082 887 4269	david@heuers.co.za
Hoedspruit	3-star	Hoedspruit	LP	Lafras Tremper	083 652 2167	tremper@hdsnursery.com
Komati Fruit	5-star	Malelane	MPU	Milanie vd Merwe	082 418 7693	milanie@riversidefarm.co.za
Lafranette	4-star	Kirkwood	EC	Francois Nel	082 412 1739	conel@srvalley.co.za
Letsitele	3-star	Letsitele	LP	Elzanne Engelbrecht	074 472 2919	elzanne@mahela.co.za
Mistkraal	5-star	Kirkwood	EC	Tyna Ferreira	082 789 5150	beans@srvalley.co.za
Moorland Seedlings	5-star	Loerie	EC	Rian Moore	082 2860 604	rian@moorland.co.za
Ngwenya	3-star	Malelane	MPU	Milanie vd Merwe	082 418 7693	milanie@riversidefarm.co.za
Nouvelle la Cotte	3-star	Letsitele	LP	Riaan Lemmer	083 253 1586	riaan@nouvellecotte.co.za
Oranjerivier Citrus	3-star	Kakamas	NC	Blom Rossouw	083 306 0622	osk@vodamail.co.za
Paksaam	4-star	Patensie	EC	Adri Ferreira	082 923 4412	paksaam@gamtoos.co.za
Parma (Hoedspruit)	5-star	Hoedspruit	LP	Albert Horn	072 022 4356	parma@global.co.za
Parma (Lutzville)	4-star	Lutzville	WC	Boetie Mouton	082 896 5066	parma@namaquanet.co.za
Rietvlei	4-star	Tzaneen	LP	Lucas McLean	083 630 3236	rietvlei@global.co.za
Sondagsrivier Hillside	2-star	Kirkwood	EC	Willem Truter	083 227 6655	willem@srvalley.co.za
Stargrow	5-star	Citrusdal	WC	Andries vd Westhuizen	082 873 3336	andries@stargrow.co.za
Tulbagh	3-star	Tulbagh	WC	Bredell Roux	082 214 2520	admin@tulbaghnursery.co.za
Tweeling	3-star	Kirkwood	EC	Jan Potgieter	082 560 2179	tweeling@srvalley.co.za
Waterfall	3-star	Adelaide	EC	Rudy van der Meulen	082 695 3433	rudyvdmeulen@gmail.com
West Coast Fruit Tree	NEW NURSERY	Yzerfontein	WC	Neels van Rooyen	084 524 7417	Vanrooyen15@yahoo.com
Witkrans	5-star	Boshoek	NW	Linda Grobler	082 414 4739	Witkrans1@mweb.co.za

## 6.7 Statutory Improvement Scheme

The new Plant Improvement Act (PIA) came into force in 2018, which stipulates that public Schemes must be converted to statutory schemes. The Citrus Improvement Scheme schedule was updated accordingly. A Memorandum of Understanding between the Minister of DALRRD and the designated authority, CGA, was approved by stakeholders at CISAC-2019. In the face of imminent biosecurity challenges, the urgency in proceeding with promulgation was stressed at CISAC-2019. All stakeholders finally agreed on 19 June 2020 that the CIS be promulgated as a compulsory statutory CIS. The Registrar of the Plant Improvement Act was requested to proceed with the necessary steps toward promulgation. Some final (minor) changes were made to the Scheme schedule to bring it in line with the legislation and required formats, as well as the application and certification options of the Scheme (use of certified budwood and seed is compulsory, but nursery and tree certification are voluntary). This updated draft was approved by CISAC in 2021. Joan Sadie retired as PIA-Registrar in July 2021 and was thanked for her constructive contributions to the Scheme and the compulsory citrus scheme proposal.

Since Mrs Sadie's retirement, the CIS Manager has engaged with the PIA-Director, Mr Thabo Ramashala. The Citrus Improvement Scheme schedule was submitted to DALRRD legal services for inputs, and minor issues are attended to (requirement for Minister of Finance to approve will be removed). It is expected that the schedule will be presented for approval by the Minister of Agriculture, whereafter the Schedule will be Gazetted. Thereafter, a virtual information session will be arranged to address the public participation process requirement including briefing of the provincial governments.

An acting Registrar for PIA, Herman Mootane, was appointed, and reported that DALRRD Legal Services approved the documentation (scheme schedule and MoU between CGA and Minister), and that submission to the Minister was imminent.

## 6.8 Protective zone surrounding the Citrus Foundation Block

The legislation, declaring a radius of 5 km around the CFB as a citrus free area, was published in the Government Gazette on 21 January 2011. Orders to remove all citrus trees were issued by DALRRD. Most residents have removed their citrus trees. DALRRD and CRI-CIS have made several follow-up visits to one owner refusing to remove trees. DALRRD has laid a case at the Uitenhage Police station, and the owner eventually agreed to remove the citrus trees. CFB assisted with the removal. CRI's Biosecurity Division and DALRRD will repeat surveys and follow up on orders issued for tree removal at other properties with citrus trees.

## 6.9 FINAL REPORT: Shoot tip grafting and CIS diagnostic services at CRI-Nelspruit Project 1144 by J. H. J. Breytenbach, C. Steyn, R. de Bruyn, M Rikhotso and G. Cook (CRI)

### Summary

The success of the Citrus Improvement Scheme (CIS) relies on the diagnostic detection of pathogens, their eradication and the maintenance and distribution of healthy propagation material. Shoot tip grafting (STG) is used to eliminate graft transmissible pathogens from citrus material before release to the Citrus Foundation Block (CFB) and introduction into the Nucleus Block (NB). Biological and molecular indexing is done on new introductions, prior to release to the CFB, as well as on accessions maintained at the CFB to establish whether graft transmissible disease agents might have been inadvertently introduced. Seventeen new selections were received for STG and 6 were released to the CFB and added to the gene source. The gene source maintained at CRI currently comprises 431 accessions. The screening of the CFB multiplication blocks for citrus viroids in greenhouse structures 4, 4C, 4D and 5 was completed. No viroids were detected in 584 samples. Mother trees in rows 1-9 and rows 20-21 (274 samples) were PCR indexed for all scheme pathogens including citrus viroids, CPsV, CTLV, CiVA and other citrus coguviruses as well as CTV. No pathogens were detected. Biological indexing of CFB mother trees in GH5, rows 1-7 commenced for CPsV and citrus coguvirus detection according to a 10-year schedule. Evaluation is ongoing and is a 12-month process. General diagnostics and investigations of *ad hoc* problems, relating to graft transmissible diseases, were additionally conducted and these activities are reported. Additionally, refurbishment of the two old glasshouses at the CRI Nelspruit facility was completed and commissioned. The upgrade was done to meet the requirements of the 'HLB-Safe' standards to ensure the safety of new cultivars, processed through the STG facility.

### Introduction

As with any commercial tree crop, citrus species are susceptible to various graft transmissible diseases (GTD) caused by viruses, viroids, bacteria, phytoplasmas and unidentified pathogens. The overall objective of the southern African Citrus Improvement Scheme (CIS) is to enhance the productivity of the industry by ensuring supply of the highest quality propagation material. Graft transmissible diseases have detrimental effects on the growth and production of citrus trees and are responsible for stunting, decline, small fruit and a range of other harmful effects. The framework of disease-free planting material is a phytosanitary programme based on detection and elimination of causal agents and maintenance and distribution of healthy propagation material. Shoot tip grafting (STG) is used to eliminate these diseases (Navarro, 1976) and has been used in South Africa since 1977 (de Lange *et al.*, 1981). The STG technique was developed by Murashige *et al.* (1972) and improved by Navarro *et al.* (1975) and de Lange (1978). STG facilities at CRI are used to introduce new cultivars and selections which are added to the gene source after STG and indexing. Some cultivars and selections of the virus-free gene source, maintained at the ARC-TSC, were duplicated in part at CRI Nelspruit as back-up sources.

Indexing, or establishing whether GTD disease agents are present, is done by inoculating indicator host plants that are sensitive to various graft transmissible pathogens. Molecular detection techniques such as Reverse-Transcription Polymerase Chain Reaction (RT-PCR) and PCR are used to confirm biological indexing results.

Since CTV and its aphid vector, *Toxoptera citricida*, is endemic in South Africa, virus-free material is pre-immunised with a suitable cross-protection source to mitigate the effects of severe CTV strains (Müller and

Costa, 1987). Cross-protection is a function of the CIS, where specific 'pre-immunising' CTV sources are applied to all citrus cultivars apart from lemons and limes, before supply to the CFB at Uitenhage. Currently, three CTV sources are used for cross-protection in the CIS depending on the citrus type (von Broembsen and Lee, 1988; van Vuuren *et al.*, 1993a; van Vuuren *et al.*, 1993b; van Vuuren *et al.*, 2000) and pre-immunisation procedures have been adapted to suit South African conditions (Fourie and van Vuuren, 1993).

Re-indexing of the mother trees at the CFB is done to ensure these trees remain free of graft transmissible pathogens. Indexing for CTV and citrus viroids (CVds) are done biennially. Screening for other GTD such as citrus psorosis virus (CPsV), citrus tatter leaf virus (CTLV), Citrus Impetratura disease (CID) which is associated with citrus virus A (CiVA), and other coguviruses such as concave gum-associated virus (CCGaV) is done every 10 years.

## Objectives

- A. Cultivar introduction (administration, establishment, STG, diagnostics, pre-immunisation, submission to the CFB and Nucleus Block)
- B. Maintenance of the virus-free gene source
- C. Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB
- D. Collaboration and duplicate indexing with ARC-TSC laboratory
- E. *Ad hoc* diagnostics for GTDs for growers and external institutions
- F. *Ad hoc* investigations as required by CIS
- G. Facility management

## Materials and methods

### A. Cultivar introduction (administration, establishment, STG, diagnostics, pre-immunisation, submission to CFB and Nucleus Block)

*In vitro* cultured rootstocks: The standard method used for *in vitro* cultured rootstocks is to expose the cotyledons by removing the seed coat of Troyer citrange or Rough lemon seed and surface sterilise in a 20% solution of household bleach, which contains 3.5% sodium hypochlorite (NaOCl), for 10 minutes followed by three rinses in sterile distilled water. Three to four seeds are planted in growth tubes containing sterile Murashige and Skoog (MS) agar medium (Murashige and Skoog, 1962). Germination takes place in an incubator at a constant temperature of 28°C in continuous darkness. When the seedlings have reached a height of 30 to 40 mm, they are stored at 4°C in darkness.

Scion preparation: Method 1: Buds of the source plant are budded on a standard rootstock in the glasshouse. After bud growth and maturation (approximately 3 to 4 months) the source plant is defoliated by hand to induce flushing. Ten to 14 days later the new shoots are harvested and surface sterilised on a flow bench for 5 minutes in a 7.5% solution of household bleach and then rinsed three times in sterile distilled water.

Method 2: If sufficient budwood of the source plant is supplied, numerous buds are budded to a few rootstocks each with 2 buds. As shoots develop from the buds they are used directly for STG and only one bud is left to grow as the reference source plant. The time from budding to STG is reduced by at least 3 months. Shoot harvesting and preparation for STG is done as above.

STG: The rootstock seedling is aseptically decapitated about 50 mm above the cotyledons. The cotyledons and their auxiliary buds are removed and a balcony incision is made, 1 mm horizontally and 1 – 2 mm diagonally approximately 10 mm from the top. The cuts are made through the cortex to reach the cambium. A shoot tip consisting of the apical meristem and 2 to 3 leaf primordia is excised from the growth point of the collected shoots under a stereo microscope. The growth tip is placed on the horizontal cut surface 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 hours of light per day.

**STG plant propagation:** The shoot tip normally starts growing 3 to 4 weeks after STG. The 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 covered with a plastic bag for 8 days to prevent desiccation. Once the graft has sufficiently grown, the plant is screened for GTD pathogens.

**Virus indexing:** The micro-graft is pre-screened for CTV and CVds by RT-PCR. If the plant is negative, buds are taken for biological indexing, but if the plant is positive, it is the new source plant from which further STGs are done.

Elimination of GTD pathogens is confirmed by biological indexing on sensitive indicators as described by van Vuuren and Collins (1990). Biological indexing results are thereafter verified using molecular diagnostics to detect CVd, CTV, CPsV, CTLV and Liberibacter spp that cause Citrus Greening and Huanglongbing.

On average, it takes 24 to 30 months to obtain a virus-free accession, which includes the biological indexing to confirm the virus-free status of the cultivar. However, delays can occur with elimination of some pathogens. The reason for these “difficult to remove” cases is unknown.

#### **B. Maintenance of the virus-free gene source**

Virus-free STG plants are established on virus-free rootstocks and maintained in an insect-free tunnel. Material derived from the gene source is pre-immunised with suitable CTV cross-protection sources (van Vuuren and Collins, 1990), prior to release to the CFB at Uitenhage. Two trees of each selection are maintained in the gene source and trees have to be re-budded to new rootstocks every 5 years as part of the routine maintenance. Photo records of fruit from each cultivar/selection are kept on the database to confirm cultivar identifications. The purpose is to ensure that the correct citrus fruit type is produced from each accession and as an additional confirmation that no mix-ups have occurred. Cultivar identification of the gene source accessions at the ARC are also done with the assistance of a CRI cultivar evaluator.

#### **C. Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB**

All CFB mother trees are re-indexed every second year to establish if the CTV pre-immunized source is maintained. Re-indexing of CFB mother trees for CVds is done simultaneously every second year. All CFB mother trees and seed source trees are inspected annually for symptoms of citrus greening disease by ARC-TSC and CRI virologists. PCR and/or biological indexing are conducted on plants showing suspicious symptoms. Most other citrus viruses are transmitted by infected budwood only, minimizing the infection potential at the CFB. Re-indexing of CFB mother trees for CTLV, CPsV and coguviruses including CiVA and CCGaV is done every 10 years.

Screening of CFB multiplication trees for CVds is done yearly on a third of the multiplication trees. Therefore, all the CFB multiplication trees are screened every third year. The screening is done by direct RT-PCR of pooled samples of each cultivar. Each pooled sample consists of 20 leaves. A leaf is taken from every third tree and a pooled sample is therefore representative of a block of 60 trees. Each cultivar is sampled separately and the number of sub-samples of a cultivar is proportional to the size of the block. Each sample is tested with viroid-specific tests for citrus bent leaf viroid (CBLVd), hop stunt viroid (HSVd), citrus dwarfing viroid (CDVd), citrus bark cracking viroid (CBCVd), citrus viroid V (CVd V) and citrus exocortis viroid (CEVd).

#### **D. Collaboration and duplicate indexing with ARC-TSC laboratory**

Shoot tip grafting for the CIS is done at both CRI and ARC-TSC laboratories. To confirm the pathogen-free status of new accessions prior to release to the CFB, duplicate molecular testing is done by both laboratories.

#### **E. Ad hoc diagnostics for GTDs for growers and external institutions**

Field material received for diagnostics is generally budded to 3 indicator host plants. The plants are cut back to force new growth and maintained in glasshouses at various temperatures required for symptom expression depending on the suspected disease being indexed. The indicators are monitored for symptoms for a minimum of 3 months post inoculation. Direct molecular tests are also done, depending on the diagnostic requirement.

#### **F. Ad hoc investigations as required by CIS**

Diseases of unknown aetiology or outbreaks of graft transmissible pathogens are occasionally encountered and require investigation. Investigations may include biological and molecular indexing for the presence of graft transmissible diseases, surveys, trials or other analyses.

### G. Facility management

Routine maintenance and improvements at the CIS Nelspruit facilities are done to ensure the safekeeping of accessions.

### Results and discussion

Task table

Objective / Milestone	Achievement
A. Cultivar introduction and STG pipeline	<ul style="list-style-type: none"> <li>• 19 accessions in STG pipeline</li> <li>• 17 new accessions received</li> <li>• 6 accessions released to the CFB</li> </ul>
B. Maintenance of the virus-free gene source	<ul style="list-style-type: none"> <li>• 431 cultivars maintained</li> <li>• Renewal of gene source is completed</li> <li>• Citrus type verification of fruiting trees confirmed 53 cultivars TtT this season</li> </ul>
C. Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB	<ul style="list-style-type: none"> <li>• Biological indexing of the CFB mother trees, row 1-7 for CPsV and citrus coguviruses</li> <li>• Screening multiplication blocks for viroids: growth structures 4, 4C, 4D and 5 completed (584 samples)</li> <li>• CFB mother trees in GH5 rows 1-9 and 20-21 were indexed for CVds, CTV, CTLV, CPsV and citrus coguviruses (274 samples).</li> </ul>
D. Collaboration and duplicate indexing with ARC-TSC laboratory	<ul style="list-style-type: none"> <li>• All accessions sent to the CFB were duplicate tested prior to release.</li> </ul>
E. <i>Ad hoc</i> diagnostics for GTDs for growers and external institutions	<ul style="list-style-type: none"> <li>• Approx. 300 <i>ad hoc</i> samples analysed</li> </ul>
F. <i>Ad hoc</i> investigations as required by CIS	<ul style="list-style-type: none"> <li>• Biological and molecular indexing of trees of a cultivar, at two sites, done to confirm pathogen free status for approval as interim supply budwood sources.</li> </ul>
G. Facility management	<ul style="list-style-type: none"> <li>• Routine maintenance and internal audits were done on a weekly basis. An external audit was conducted by the CIS manager in May and November 2021.</li> <li>• Insect monitoring in all CRI growth rooms and tunnels is ongoing as stipulated in the action plan for HLB and ACP.</li> <li>• Physical breaches in tunnels and glasshouses were monitored and sealed as detected</li> <li>• Refurbishment of the two old glasshouses were completed and commissioned.</li> <li>• A dedicated steam room was built for soil sterilisation.</li> <li>• The STG lab laminar flow was serviced.</li> <li>• The large tunnel's water pump was replaced.</li> <li>• A fan motor of one of the gene source tunnels was replaced.</li> <li>• The water distiller was serviced.</li> <li>• An annual change of batteries for all temperature loggers was done.</li> </ul>

**A. Cultivar introduction (administration, establishment, STG, diagnostics, pre-immunisation, submission to CFB and Nucleus Block)**

Introductions for STG and subsequent release to the CFB (phase 6) from 2017 to date are summarised in Table 6.9.1. Seventeen new selections of three variety types were submitted for STG in this report period. Nineteen accessions are at various stages in the STG pipeline including long term biological indexing.

A total of 232 STGs were done within this period, including failed grafts. Seventy-seven STGs were successfully micro-grafted, which is a 33% STG success rate.

In the 2017 annual cycle, nine cultivars in the nucleus block (NB) tested positive for GTD pathogens and were re-introduced to the STG pipeline for pathogen removal. These cultivars went through STG and of these, 6 were completed and re-introduced to the gene source. The three remaining cultivars are in the final stage of biological indexing.

To facilitate a faster turn-around with the STG process, new introductions are tested directly via RT-PCR, prior to STG, to determine the original pathogen status and then again directly after STG as soon as sufficient material is available for testing. These additional steps allow quicker detection of pathogens not eliminated by the initial STG step. Re-STG can therefore commence quicker rather than waiting for completion of the biological indexing. This process does, however, not replace the final biological indexing and PCR to confirm the pathogen-free status prior to final release of the accession. These additional tests are routinely done and samples processed are not reported.

Biological indexing of ten successful STGs for CTV, CTLV, CVds, CPsV and coguviruses were finalised and confirmed negative by RT-PCR. Biological indexing of 11 STGs for CTV, CTLV, CVds CPsV and coguviruses are ongoing. Six cultivars were interim-released to the CFB.

**Table 6.9.1.** STG submissions in the pipeline for graft transmissible disease elimination and indexing (phases 1 to 5) (see footnote). Interim release (phase 6) is reported as released to CFB.

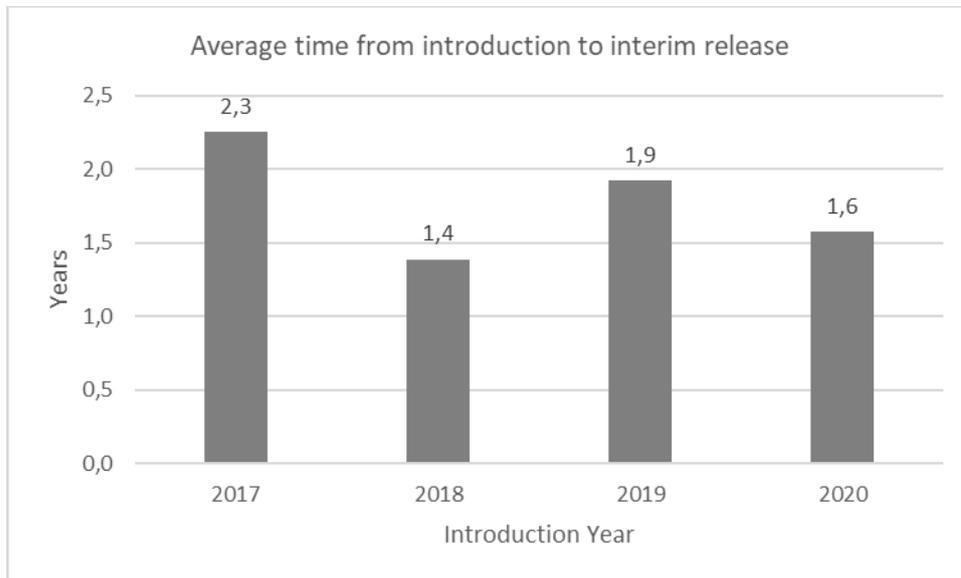
Variety type <sup>2</sup>	STG introductions and releases 2017 to 2021 <sup>1</sup>																	
	2017/18			2018/19			2019/20			2020/21			2021/22					
	Bf	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	Withdrawn	Bf	New Introductions	Releases to CFB	Withdrawn	Balance
C	2	1	1	2	2	1	3	0	2	1	0	0	0	1	1	0	0	2
G	1	0	0	1	0	0	1	0	1	0	1	0	0	1	2	1	0	2
L	1	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	0	0
Li	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	0	0	1
Mi	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0
Ma	2	3	3	2	1	1	2	3	1	4	0	2	0	2	0	2	0	0
N	10	3	1	12	2	3	11	1	9	3	1	2	1	1	8	0	1	8
V	9	2	1	10	1	2	9	4	7	6	0	3	1	2	5	2	0	5
Or	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rs	0	2	0	2	0	0	2	1	2	1	1	0	0	2	0	1	0	1
<b>Total</b>	<b>25</b>	<b>11</b>	<b>6</b>	<b>30</b>	<b>6</b>	<b>7</b>	<b>29</b>	<b>11</b>	<b>22</b>	<b>18</b>	<b>3</b>	<b>10</b>	<b>2</b>	<b>9</b>	<b>17</b>	<b>6</b>	<b>1</b>	<b>19</b>

Phases: 1: Introduction, 2: STG phase, 3: Successful STG micrografted to rootstock seedling, 4: Biological indexing phase, 5: Line tested virus-free and CTV pre-immunisation commences, 6: Interim release to CFB, 7: 12-month biological indexing, and 8: Final release

<sup>1</sup> Bf = Brought forward from previous year; Balance = Balance for the current reporting year.

<sup>2</sup> Variety type: C = Clementine; G = Grapefruit; L = Lemon; Li = Lime; Mi = Midseason; Ma = Mandarin; N = Navel; R = Reticulata; V = Valencia; Or = Ornamental; Rs = Rootstock.

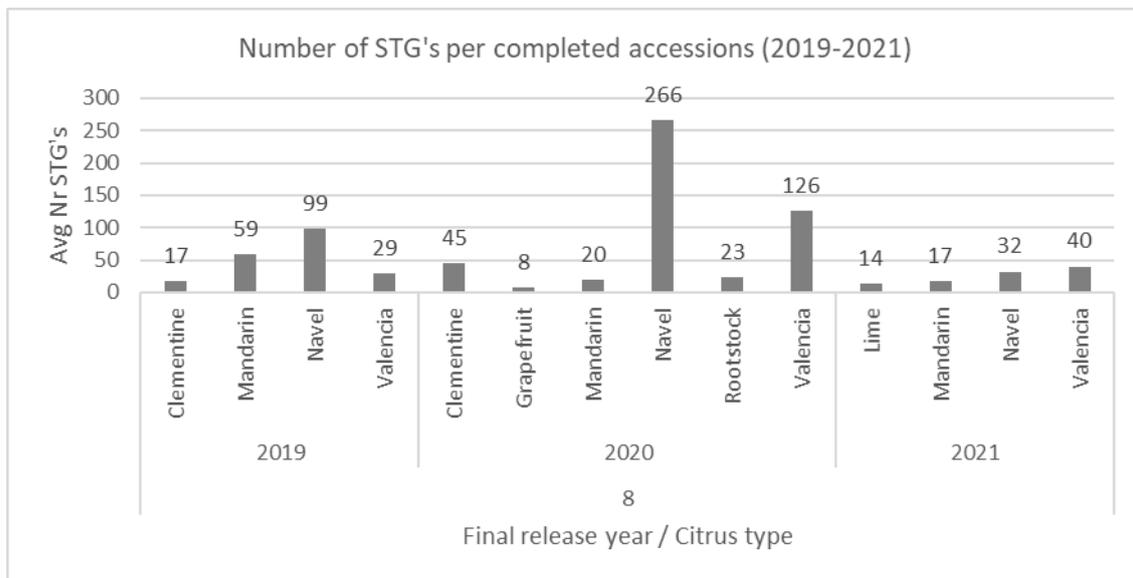
To gauge the efficiency of the STG progress over time, some parameters are graphically presented in Figures 6.9.1 to 6.9.4, which provide additional information to the numbers provided in Table 6.9.1.



**Figure 6.9.1.** The average time (years) that accessions were in the STG pipeline from introduction to interim release is shown per introduction year for the period 2017-2020.

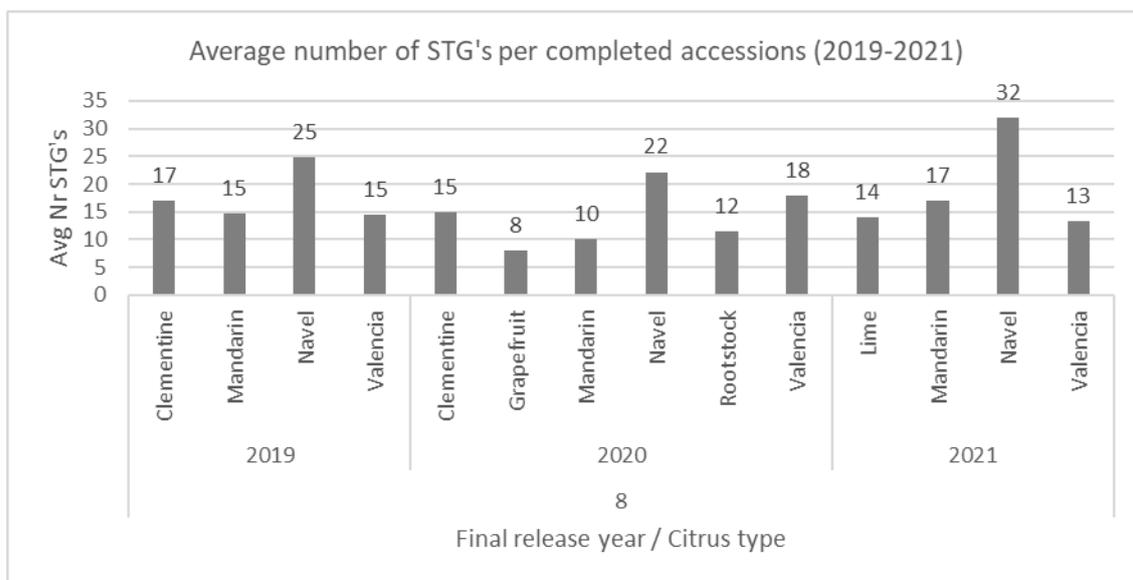
The supply of new cultivars allows the industry to remain competitive in a global market. The STG process was recognised as a hindrance as some cultivars remained in the STG pipeline for lengthy periods. Improvements to the STG process are assessed in how quickly accessions are released to the CFB. Figure 6.9.1 shows the average time from introduction of cultivars to interim release to the CFB since 2017. A significant reduction in the average STG pipeline time was achieved since 2017. These averages are for 11, 6, 11 and 3 accessions in 2017, 2018, 2019 and 2020 respectively. However, problem accessions are occasionally encountered for which pathogen elimination is problematic and therefore remain in the STG pipeline for lengthy periods. The reasons for these failures are not always clear. The 4 accessions that were previously delayed in the STG pipeline were successfully completed and released to the CFB.

As a measure of productivity, the number of STGs performed on finalised accessions, per citrus type and per year finalised, is shown in Figure 6.9.2. A total of 232 STGs were done for the period April 2020 - March 2021. The graph indicates the year, not restricted to this report period, but shows a high output of STGs.



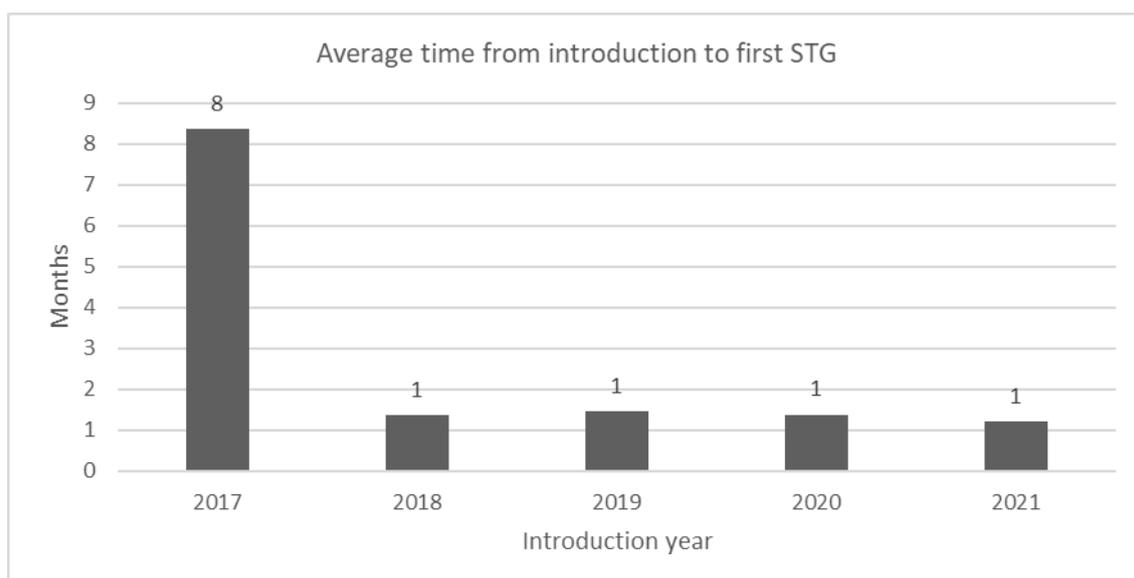
**Figure 6.9.2.** The number of STGs done for finalized accessions per citrus type and per year in which the accessions were final released to the CFB.

The efficiency and unpredictability of the STG process can be assessed by the number of STG's that were performed to obtain a pathogen-free accession. Figure 6.9.3 shows the average number of STGs per citrus type for accessions released per year. The values indicate that the STG efficiency has remained constant and the number of STGs required to obtain a successful plant is within the expected range.



**Figure 6.9.3.** The average number of STGs performed per citrus type for accessions released per year.

An improvement to the STG system, implemented since 2018, was the use of all additional buds. Extra buds are budded to available rootstocks and the bud-shoots are used directly for STG. This eliminates the time required for a nurse plant to grow sufficiently before defoliation can occur. Figure 6.9.4 shows that since implementing this approach the average time before the first STG can be performed was reduced to 1 month.



**Figure 6.9.4.** The average period (months) from receipt of accession to the first STG.

### B. Maintenance of the virus-free gene source

The nine accessions, which tested positive for CVds and/or CTV during the routine indexing in 2017, are currently in the STG pipeline for pathogen elimination. The processing of 6 accessions were completed and re-introduced to the gene source, the remaining 3 are in the final stage of biological indexing.

The CRI gene source currently comprises 431 accessions and the number of selections per variety type is shown in Table 2. A *Phytophthora* outbreak in the CRI Nucleus Block was addressed by a total renewal of all accessions. As a result of the *Phytophthora* root rot and losses during re-budding, some accessions were lost and were replaced from the ARC nucleus block (20 cultivars). Twelve cultivars were re-STGd from CFB lines and tested negative for CTV and CVds after biological indexing, whereafter they were successfully re-introduced in the gene source. The CRI Nucleus Block is completely renewed and the plants are in good condition, already bearing fruit.

A photo record is kept of fruit produced on the NB trees each year and kept in a database. The database is used to confirm the citrus fruit type of each accession to ensure that no potential mix-ups have occurred. Fifty-three additional accessions were verified during the 2021 season. One accession was not true to type and was resubmitted from the CFB for STG. The number of accessions confirmed to be the correct citrus type is presented in Table 6.9.2.

**Table 6.9.2.** The number of accessions per variety type maintained in the CRI Nucleus Block and the number of accessions confirmed to date as the correct citrus type.

Variety Type	No. of cultivars at CRI	Citrus type confirmed by fruit
Clementine	39	23
Diverse (Citron, Sour orange, etc.)	2	1
Ellendale	3	-
Grapefruit	27	21
Kumquat	2	2
Lemon	26	24
Lime	3	2
Mandarin hybrid	73	48
Midseason	37	20
Navel	107	59
Ornamental	5	5
Pumelo	8	6
Rootstock	24	21

Satsuma	8	8
Valencia	67	39
<b>Total</b>	<b>431</b>	<b>279</b>

### C. Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB

CFB mother trees in GH5 from rows 1-17 were biologically indexed for CPsV and coguviruses, according a 10-year cycle schedule. Evaluation is a 12-month process and this batch will be concluded at the end of April 2022.

CFB mother trees in GH5 from rows 1-9 and 20-21 were indexed for CVds, CTV, CTLV, CPsV and coguviruses, according a 10-year cycle schedule. These 274 samples constituted approximately half of the mother trees. This batch tested negative for the panel of GTD pathogens. The mother trees were also screened for the presence of CTV to determine whether the pre-immunisation source is present. This indicated that either some cultivars were not pre-immunised or that the CTV was not retained over time. Seventy-four percent of sweet orange trees were positive for CTV, whereas only 33% of soft citrus trees were positive. Research is ongoing to better understand cross-protection and CTV strain interaction with the citrus hosts.

The screening of the CFB multiplication blocks for citrus viroids in greenhouse structure 4, 4C, 4D and 5 was completed. This entailed a representative sub-sampling of 46 800 trees and processing of 584 samples. No viroids were detected in these samples.

GFMS35 is the CTV source currently used for grapefruit pre-immunisation. However, field trials indicated that a single-strain CTV isolate, B390-5, was associated with improved tree health and production. The Citrus Improvement Scheme Advisory Committee (CISAC) approved B390-5 as a new pre-immunisation source for grapefruit pending comparative assessment with GFMS35 in semi-commercial trials. Swingle citrumelo rootstocks were budded with Star Ruby containing the respective CTV sources at both CRI and a commercial nursery. Comparative trials will be planted at two trial sites in the spring of 2022.

### D. Collaboration and duplicate indexing with ARC-TSC laboratory

Shoot tip grafting for the CIS is done at both the CRI and ARC-TSC laboratories. To confirm the pathogen-free status of new accessions prior to release to the CFB, duplicate molecular testing is done by both laboratories. The number of ARC accessions tested for specific pathogens are presented in Table 6.9.3.

**Table 6.9.3.** Sample numbers of duplicate tests of ARC introductions, for various pathogens.

Pathogen	ARC-TSC accessions
CTV <sup>3</sup>	58
CVd	27
CTLV	34
CPsV & coguviruses	29
'Ca' Liberibacter species	13

<sup>3</sup> Includes testing to confirm CTV pre-immunization

### E. Ad hoc diagnostics for GTDs for growers and external institutions

- Approximately 300 samples were received for various *ad hoc* analyses in this report period. Analyses were done for various pathogens including CVds, CTV, citrus Liberibacters, citrus coguviruses, citrus leprosis associated viruses, CPsV and also SSR analyses to verify cultivars and rootstocks.

### F. Ad hoc investigations as required by CIS

- Biological and molecular indexing of trees of a cultivar, at two sites, were done to confirm pathogen-free status for approval as interim supply budwood sources. Analysis was concluded and approval for interim supply was granted. According to CIS interim source requirements an annual indexing of the

propagation sources is required. Direct PCR screening of the propagation sources at the two sites was concluded and biological indexing commenced.

- SSR marker analysis was done for 12 cultivars
- CTV strain analysis was done for two cultivars.

#### G. Facility management

- Routine maintenance and internal audits were done on a weekly basis and two external audits by the CIS manager in May and November
- Insect monitoring in all CRI growth rooms and tunnels is ongoing as stipulated in the action plan for HLB and ACP.
- Physical breaches in tunnels and glasshouses were monitored and sealed as detected.
- Refurbishment of the two old glasshouses were completed and commissioned.
- A dedicated steam room was built for soil sterilisation.
- The STG lab laminar flow was serviced.
- The large tunnel's water pump was replaced.
- A fan motor of one of the gene source tunnels was replaced.
- The water distiller was serviced.
- An annual change of batteries for all temperature loggers was done.

#### Conclusion

Efficient pathogen detection and elimination enables supply of healthy bud wood to the industry and is the primary objective of this project. Successful elimination of GTDs from new selections was achieved and these were added to the gene source and supplied to the CFB. The CRI gene source maintenance is an ongoing function. Diagnostic services were provided and analysis of industry problems relating to graft transmissible diseases were addressed.

#### Technology transfer

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## 6.10 **FINAL REPORT: Diagnostic and technical services for the Citrus Improvement Scheme by the ARC-TSC**

Project 1217 by E. Jooste, N. Hlalele Z. Theledi and R. Roberts (ARC)

### **Summary**

During the 2021/2022 reporting period, the STG backlog of accessions in the PEQ pipeline (introductions from 2014-2018) was cleared. Two accessions from 2019 are currently prioritised in the STG phase to meet the minimum timeline requirement for the PEQ timeline. The average STG success rate was 25% and this contributed to accessions progressing quicker in the pipeline. Twenty-seven accessions were interim-released after 6 months of biological indexing and a further 29 were final-released after completing the long biological indexing. A decrease in the average time of accessions in the PEQ pipeline introduced in more recent years are shown over time. The average time from introduction to first STG remained between 2 to 3 months. The ARC nucleus block currently maintains 556 citrus accessions. To date, variety type verifications were done on 357 of these accessions, with 35 new verifications for the current reporting period. Thirty-four pre-immunised sources were verified with fruit identifications. Confirmation of the disease-free status of nursery plants was done using molecular detection as a pre-screening tool and diagnostic support was rendered to CRI on request.

### **Opsomming**

Gedurende die 2021/2022-verslagperiode, is die STG-agterstand van toetredings in die PEQ-pyplyn (ingebring vanaf 2014-2018) uitgewis. Twee toetredings vanaf 2019 word tans in die STG-fase geprioritiseer om aan die minimum tydlynvereiste vir die PEQ-tydlyn te voldoen. Die gemiddelde STG-sukseskoers was 25% en dit het daartoe bygedra dat toetredings vinniger in die pyplyn gevorder het. Sewe-en-twintig toetredings is tussentyds vrygestel, ná 6 maande van biologiese indeksering, en 'n verdere 29 is finaal vrygestel nadat die lang biologiese indeksering voltooi is. 'n Afname in die gemiddelde tyd van toetredings in die PEQ-pyplyn wat in meer onlangse jare ingebring is, word oor tyd getoon. Die gemiddelde tyd vanaf bekendstelling tot eerste STG het tussen 2 tot 3 maande gebly. Die LNR-kernblok onderhou tans 556 sitrustoetredings. Tot op hede is variëteitstipe-verifikasies op 357 van hierdie toetredings gedoen, met 35 nuwe verifikasies vir die huidige verslagperiode. Vier-en-dertig vooraf geïmmuniseerde bronne is met vrug-identifikasies geverifieer. Bevestiging van die siektevrye status van kwekeryplante is gedoen deur gebruik te maak van molekulêre opsporing as 'n vooraf-evalueringsinstrument, en diagnostiese ondersteuning is op versoek aan CRI gelewer.

### **Introduction**

The disease management unit at the Agricultural Research Council's Tropical and Subtropical Crops, Mbombela campus (ARC-TSC) is a role player in the mandate of the Citrus Improvement Scheme (CIS) to ensure the supply of pathogen-free propagation material to the South African citrus industry. The Post Entry Quarantine (PEQ) function, assigned by the Department of Agriculture, Land Reform and Rural Development (DALRRD) to the ARC-TSC, is critical to ensure that no foreign pathogens are introduced via imported budwood. Budwood imports are subjected to shoot tip grafting (STG), to eradicate graft transmissible pathogens, *i.e.* viruses, viroids and bacteria. Once STG is completed, the material is indexed based on

biological indicators and molecular tests are conducted to detect various pathogens. Seed imports are subjected to grow out tests and subsequent molecular diagnosis for pathogens as per the quarantine requirements.

The different phases of the STG process are summarised in Annexure 6 (CIS Procedural Guide). When a selection is cleared from Plant Quarantine Services, Stellenbosch, the budwood arrives at the ARC-TSC facility the next day. The selection is received and is then in **Phase 1**, where a unique number is assigned to a selection and source plants are established. The unique number is used in all further processes to track the accession and all relevant information is captured in a database. If a problem with the quality of the budwood is encountered, the importer is informed.

**Phase 2** is the shoot tip grafting (STG) phase where the top 0.15 mm of a meristem of a shoot tip is grafted onto an etiolated rootstock. Success of the procedure is variable and therefore a number of STG attempts are required for each selection and are reported. Many factors contribute to the STG success rate and may include the pathogen status of the plant, the size of meristem tip and various unknown biological factors that may influence the growth of the initial meristem.

Successful growth of the shoot-tip can be seen within 2 to 3 weeks and the *in vitro* plant is then micro-grafted onto a rootstock to establish a nurse plant. This is **Phase 3** of the procedure.

The nurse plant is left to grow until the bark is mature enough for biological indexing, which is **Phase 4** in the process. Prior to biological indexing, the nurse plant is pre-screened for pathogens with molecular techniques. If a nurse plant tests positive for any pathogen, the STG procedure is repeated (Phase 2). Biological indexing for various pathogens and diseases are conducted on a range of citrus hosts and evaluated over various time-frames for symptom development.

If the nurse plant indexes free of pathogens, **Phase 5** commences and includes pre-immunisation with an approved mild-CTV source depending on the citrus type. The CTV pre-immunisation is done simultaneously with the biological indexing if sufficient buds are available, to save time in the process.

Accessions progress to the interim release phase, or **Phase 6**, when both the 6-month biological indexing and duplicate molecular confirmation tests are negative and CTV pre-immunisations are confirmed. Budwood is then released to the Citrus Foundation Block (CFB) if the client wishes to introduce the accession into the CIS. At this stage, the selection can be released to the client for trial purposes.

Once interim-released, the accession is in **Phase 7** and includes a 12-month biological evaluation for Citrus Greening/HLB, Impietratura and Psorosis-like pathogens. If the biological evaluations are negative, duplicate molecular testing for the pathogens is done in the relevant diagnostic laboratories at ARC and CRI to confirm the negative status of the plants.

**Phase 8** is the final release of the accession to the client and introduction to the CIS if required.

#### **1. PEQ for citrus propagation material, including pathogen-therapy using shoot-tip-grafting (STG), conventional and molecular diagnostics**

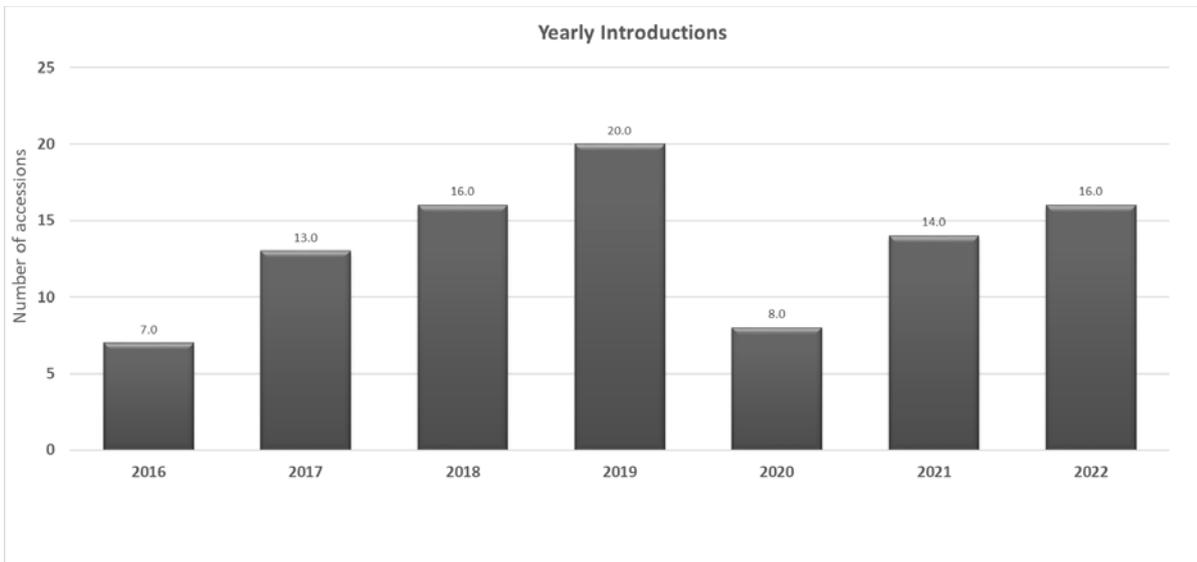
The PEQ introductions were established in the quarantine glasshouse at the ARC-TSC and the following outputs were recorded for April 2021-March 2022:

#### **Analysis of introductions, processes and timeframes relating to citrus PEQ**

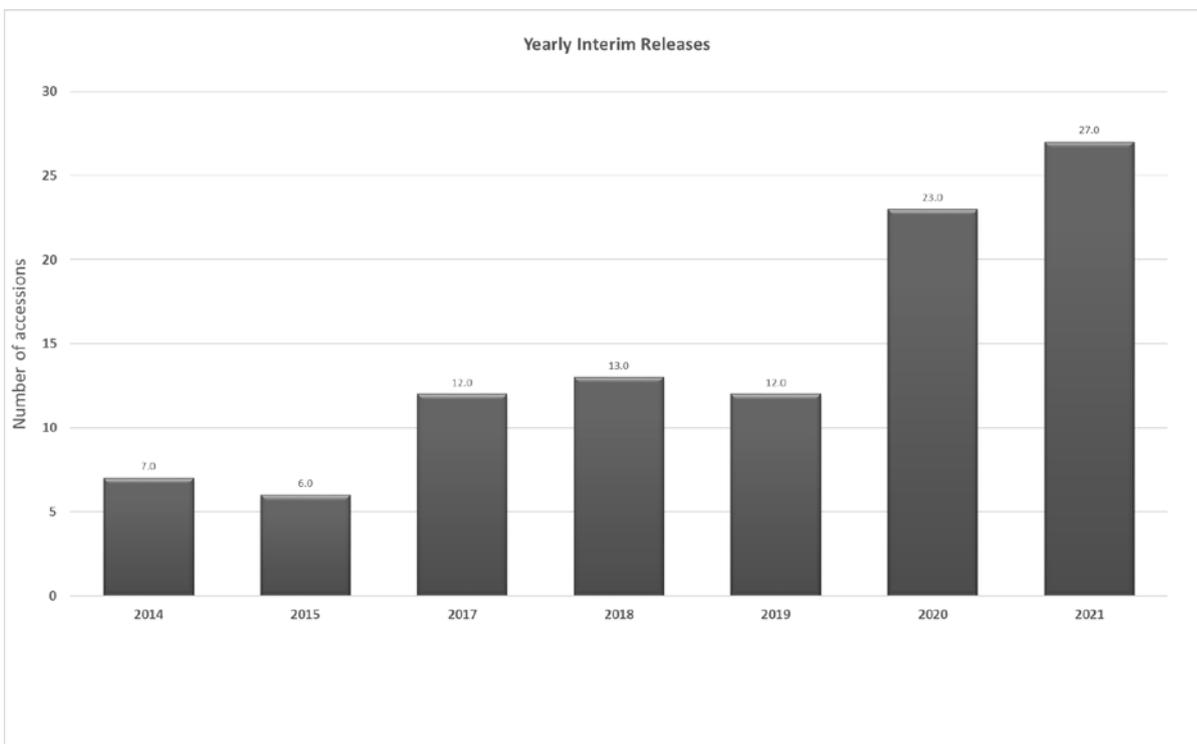
##### *Yearly introductions, interim and final releases*

The number of accessions introduced to the PEQ pipeline from 2016 to 31 March 2022 is shown in figure 6.10.1. Fourteen international budwood introductions were received from April 2021 to December 2021. Three of these introductions had to be removed from the pipeline because of duplicate imports by the agent. Sixteen

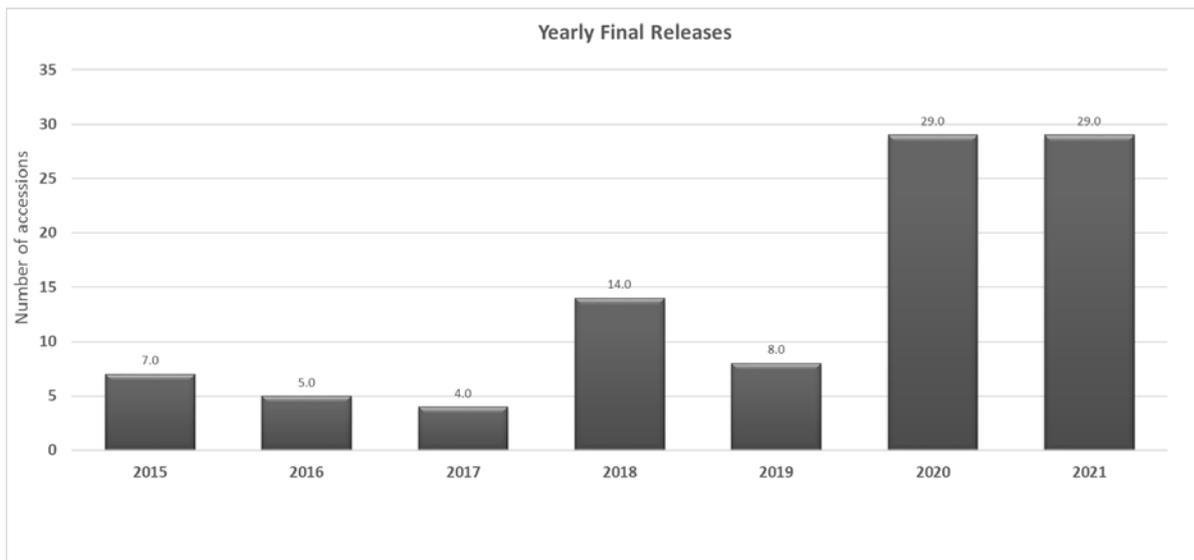
introductions were received by 31 March 2022 of which three introductions had bad quality budwood and plants were not established for these introductions. The import agent was notified about the condition of the budwood. Progress on the new introductions will be discussed later in this report. Figures 6.10.2 and 6.10.3 show the number of accessions for interim (phase 6) and final (phase 8) release. A total of 27 interim releases and 29 final releases were documented for 2021/2022.



**Figure 6.10.1.** Yearly introductions from 2016 to March 2022



**Figure 6.10.2.** Yearly interim releases from 2014 to 2021



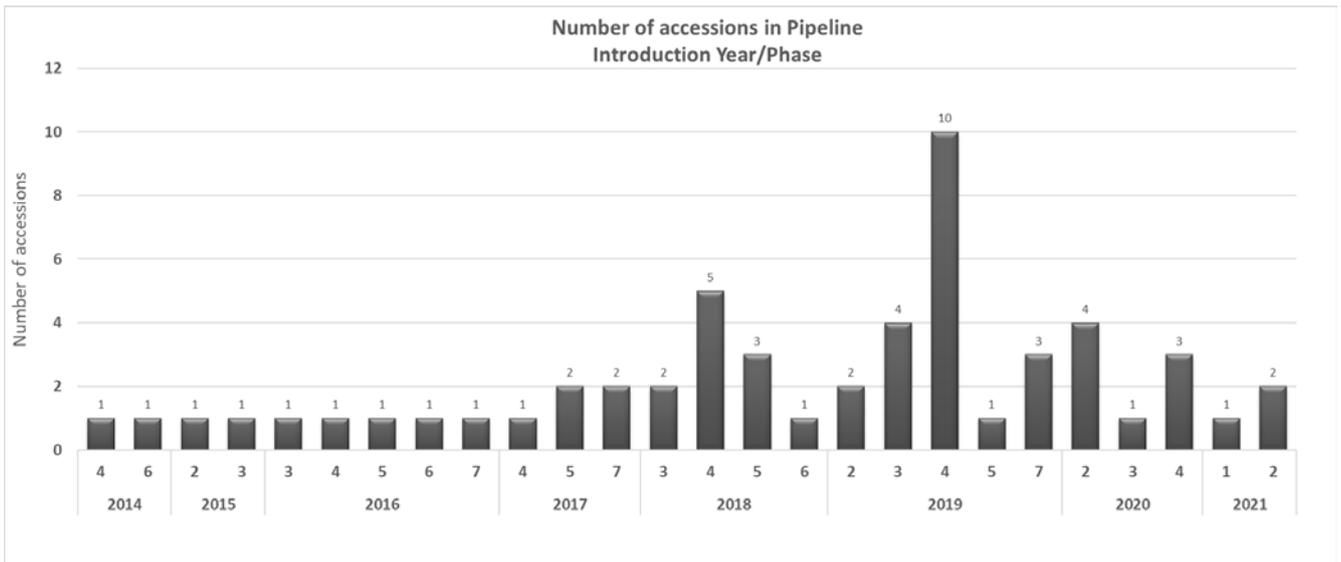
**Figure 6.10.3.** Yearly final releases from 2015 to 2021

### Accessions in the pipeline

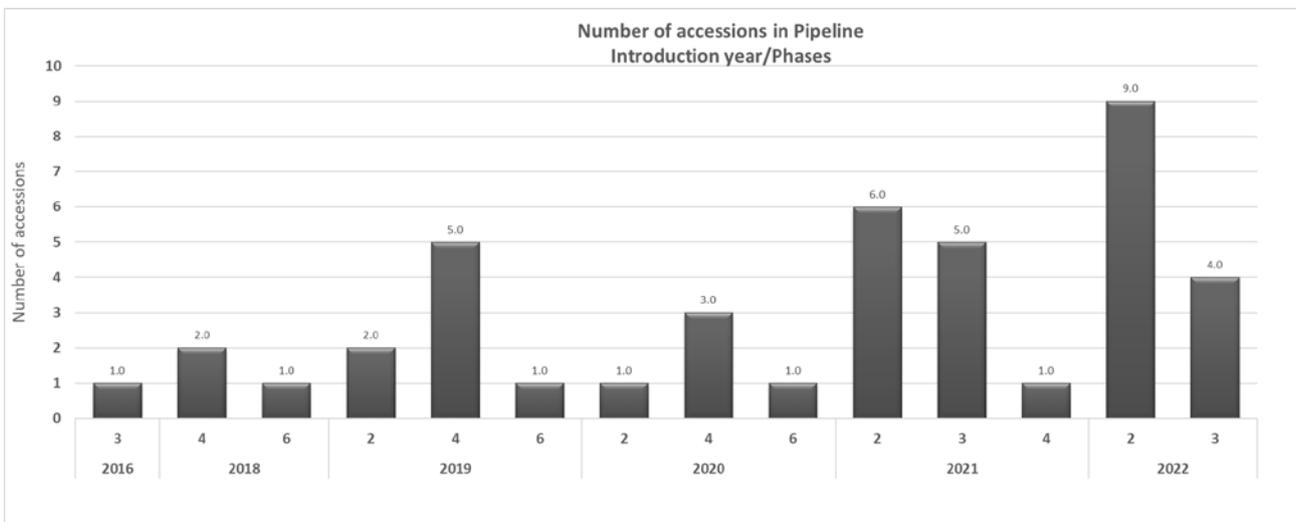
Progress in the total of 40 accessions, including one local ARC accession, which are currently in the pipeline is summarised in figure 6.10.4. The phases in the pipeline are as follows:

- Phase 1: Introduction
- Phase 2: STG phase
- Phase 3: Successful STG micro-grafted to rootstock seedling
- Phase 4: Biological indexing phase
- Phase 5: Line tested virus-free and CTV pre-immunisation commences
- Phase 6: Interim release to CFB
- Phase 7: 12-month biological indexing
- Phase 8: Final release

The progress on selections in the pipeline between the previous and current reporting period are shown in Figures 6.10.4A (2020-2021) and 6.10.4B (2021-2022). The backlog of introductions from 2014, 2015 and 2016 have all been cleared, except for one introduction that is currently micro-grafted (Phase 3). All introductions from 2017 have been released. Three introductions from 2018 are currently in the pipeline, two in the biological indexing phase (Phase 4) and one that is in long indexing, but interim released (Phase 6). Only eight introductions from the 2019 imports are left in the pipeline, two in the STG phase (Phase 2). These introductions are prioritised for STG. One accession from 2020 is currently in the STG phase (Phase 2), three are in indexing (Phase 4) and one in long indexing (Phase 7). Three introductions from 2021 were removed from the pipeline because of duplicate imports by the agent. Six established introductions remain in the STG phase (Phase 2) and they are prioritised in the STG process. Five nurse plants were micro-grafted (Phase 3) and are growing to start biological indexing and one accession is already in the biological indexing phase (Phase 4). The latter introduction is a fast-growing lemon that had successful STG after 37 days from introduction. Four of the 2022 introductions are already micro-grafted and growing to have material ready for biological indexing.

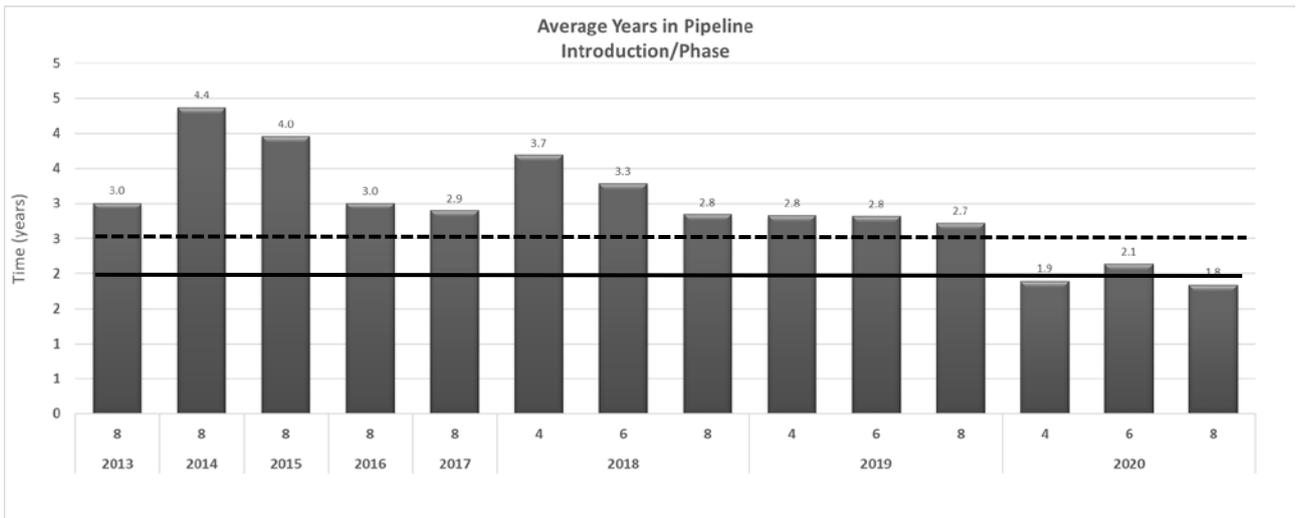


**Figure 6.10.4A.** Number of accessions, from the previous report, in the pipeline. The introduction year and number of samples in the different STG phases are indicated.



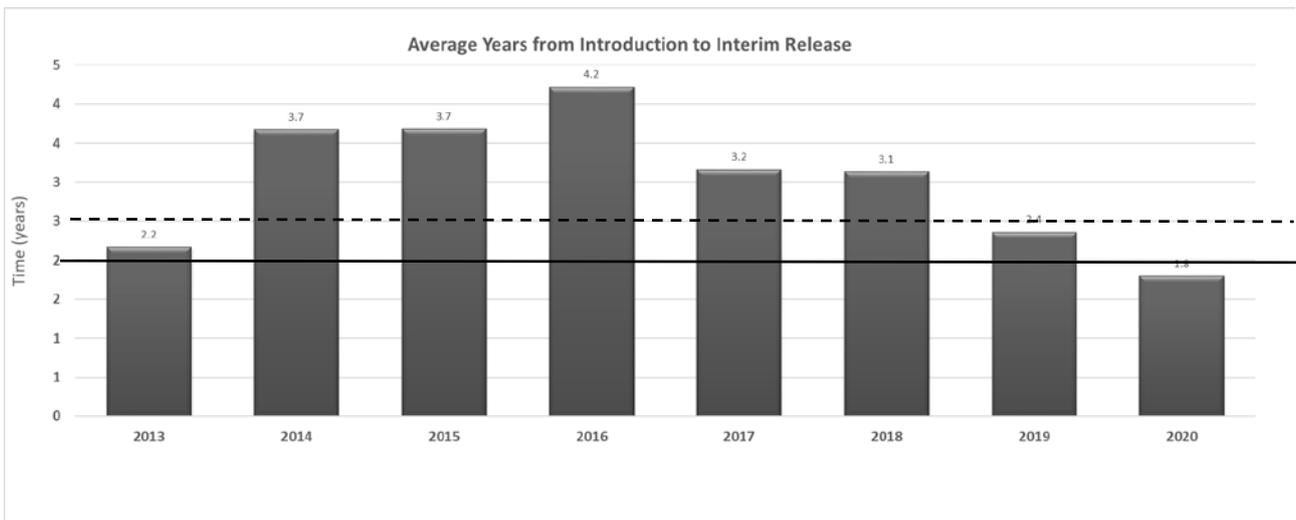
**Figure 6.10.4B.** Number of accessions, for the current reporting period, in the pipeline. The introduction year and number of samples in the different STG phases are indicated.

The average time for accessions in the pipeline is shown in figure 6.10.5. A general decrease in the time taken for accessions to transition through the pipeline is evident in recent years, i.e. from 2020 onwards. The average time to reach the biological indexing phase was reduced from 3.7 years in 2018 to 1.9 years in 2020. The time taken for introductions in 2018 to reach the interim release stage was 3.3 years that was improved to 2.8 years in 2019 and 2.1 years for the 2020 introductions.



**Figure 6.10.5.** The average time of accessions in the pipeline indicating year of introduction and the phase. The expected- and long-term average time for PEQ completion are indicated with a solid line and dashed line, respectively.

Figure 6.10.6 shows the average time for accessions to move from introduction to interim release during the 2013-2020 period. The dashed line indicates the long-term average period to release accessions from PEQ. Previously, thirty-eight accessions were used to compile a baseline average across 3 years. From the graph, it is clear that accessions introduced in 2019 proceeded quicker to the interim release phase. A clear improvement in average release times are shown from 2016 to 2020.



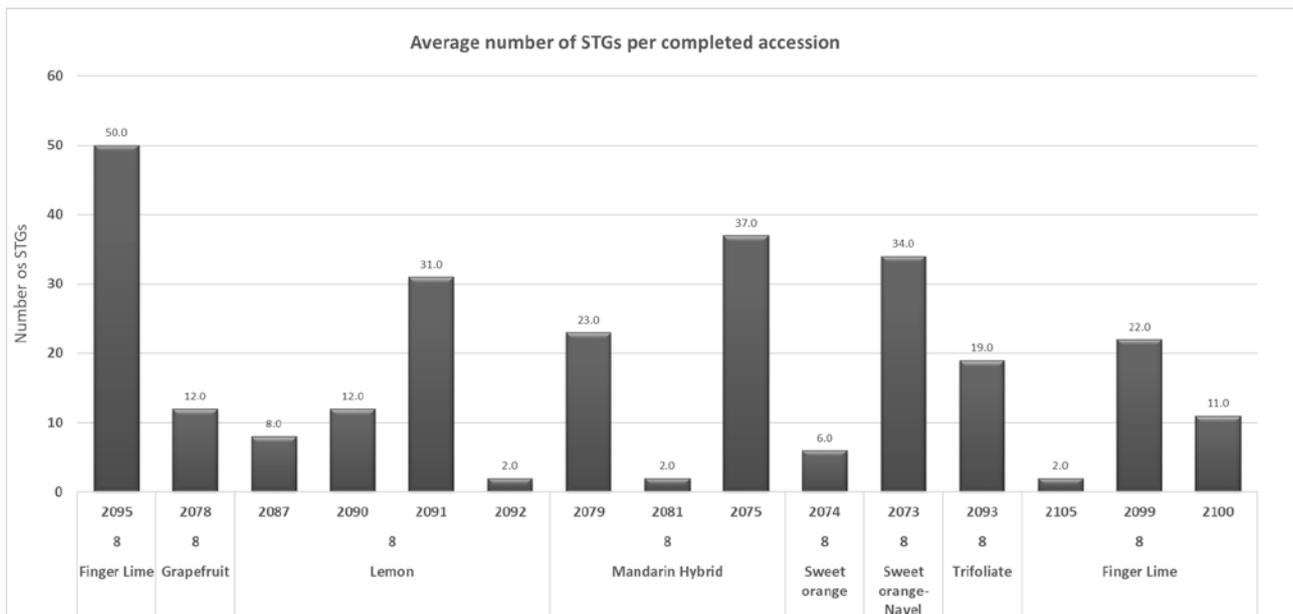
**Figure 6.10.6.** Average time (years) from introduction to interim release

**STG success rate vs. number of STGs conducted**

During the reporting period, STG was performed on 29 introductions in the pipeline. Thirty-nine successful STG's were obtained from 17 introductions for the reporting period. The STG success rates varied between 6.6% to 55% per accession, with an average success rate of 25%. For some cultivars, a higher STG success rate was recorded and included lemon, mandarin, sweet orange and rootstock varieties.

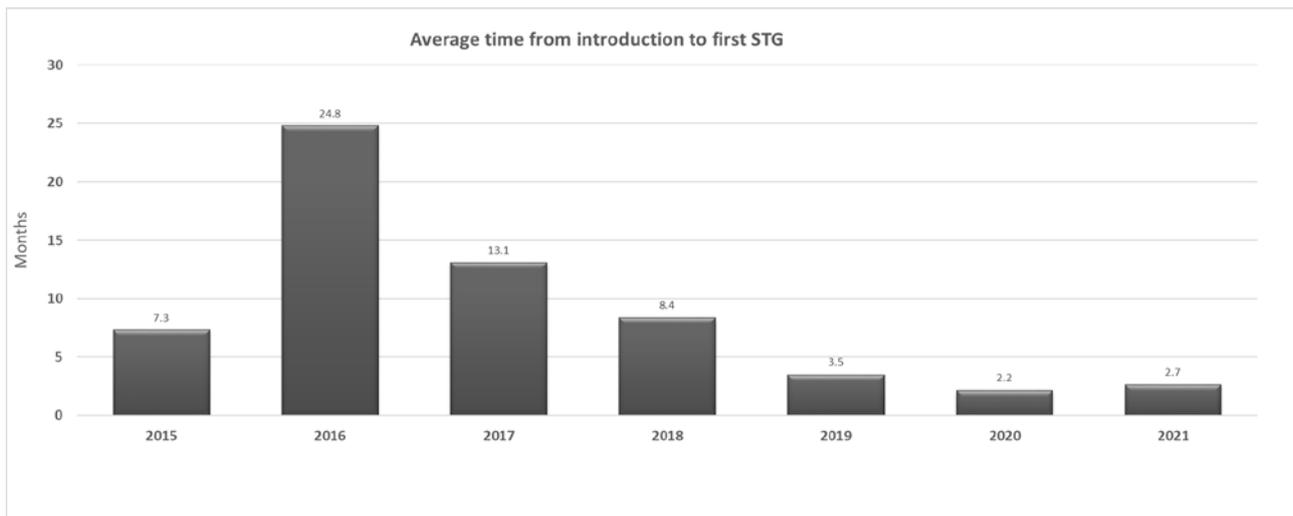
**Number of STGs required to establish a nurse plant**

The STG technique is not always successful at the first attempt. The average number of STGs per accession for completed introductions received in 2019 and 2020, are summarised in Figure 6.10.7.



**Figure 6.10.7.** Average number of STGs for completed introductions received in 2019 and 2020 per citrus group

The average time from introduction to first STG attempt is presented in Figure 6.10.8. An improvement in this time is seen from 2017 with a significant improvement visible in 2019, 2020 and 2021.



**Figure 6.10.8.** Average time from introduction to first STG

## 2. Maintenance of a virus-free nucleus block of citrus cultivars and germplasm

Currently, the ARC nucleus block is comprised of 556 accessions (Table 6.10.1). Mistakes during repotting/relabeling in the past, have led to some mix-ups, which became apparent during the trueness-to-type (TtT) evaluation of exported, field or CFB Evaluation Block trees. Citrus type verifications are done annually with the assistance of Mr Johan Joubert from CRI, with photo records for each accession. For the 2021/2022 growing season, 179 fruit identifications were done with 35 new identifications (Figure 6.10.9). In total, 357 cultivars have been confirmed as the expected citrus type, representing 64% of the nucleus block. A total of 34 pre-immunised sources were confirmed. The confirmed pre-immunised sources are mainly the newer releases. The pre-immunised sources represent the original tree of an accession and type confirmation on these accessions ensures early type verifications.

**Table 6.10.1.** Summary of cultivar type identifications from fruiting trees in the ARC nucleus block

Variety Type	ARC accessions	Citrus type confirmed by fruit 2020/2021	Additional citrus types confirmed by fruit 2021/2022	Pre-immunised sources type confirmation
Clementine	43	23	2	1
Diverse (Citron, Sour orange, etc.)	9	4	1	1
Ellendale	5	1	0	0
Grapefruit	30	24	1	0
Kumquat	3	3	0	0
Lemon	44	29	4	0
Lime	4	2	1	2
Finger lime	5	0	0	3
Mandarin hybrid	150	80	5	24
Midseason	40	18	1	0
Navel	80	42	8	0
Pumelo	17	11	1	2
Rootstock	55	31	1	0
Satsuma	17	16	1	0
Valencia	55	38	9	2
<b>Total</b>	<b>556</b>	<b>322</b>	<b>35</b>	<b>34</b>



**Figure 6.10.9.** Fruit for true-to-type identification in the ARC-TSC nucleus block

**3. Introduction of cultivars to the CIS Citrus Foundation Block following *Citrus tristeza virus* pre-immunisation**

During this period, 26 imported introductions and one local ARC introduction were interim released to the CFB after biological indexing and molecular verification (Figure 6.10.2). Plant material from 32 virus-free selections was sent to cultivar owners and researchers to use in trials and mutation breeding. Twenty-nine accessions that completed the long biological indexing phase (Phase 7) were final-released. The final releases included three local ARC selections (Figure 6.10.3). Three accessions were re-introduced to CFB on request.

A total of 179 virus-free selections were sent to CFB to establish the CFB nucleus block. These accessions from the ARC nucleus block were tested (as described in Annexure 6C) prior to duplication of the nucleus source at CFB. Confirmations on pathogen status of the ARC nucleus block is ongoing.

#### 4. CIS diagnostic support for CRI-Nelspruit, including validation of diagnostic tests and improvement of diagnostic protocols

Confirmation of the disease-free status of nurse plants was done using molecular detection as a pre-screening tool (described in Annexure 6C).

Diagnostic support of duplicate tests for ARC and CRI accessions are summarised in Tables 6.10.3 and 6.10.4. Molecular detection for quarantine pathogens, listed in Table 6.10.3, was optimised and screening of budwood on arrival was conducted. Testing of the nucleus block material for quarantine pathogens is ongoing.

**Table 6.10.3.** ARC molecular screening of PEQ accessions for release to CFB

Pathogen	Number of accessions	Number of tests
CTV-Pre-immunisations	42	42
CTV-indexed	39	39
CTLV	37	37
Liberibacter's	33	33
Viroids	36	216
CPsV, CiVA, Cogu	41	82
Visual inspection for Impietratura	41	
Citrus yellow vein clearing virus (CYVCV)	12	12
Citrus variegation virus (CVV)	12	12
Dichoroviruses	12	12
Citrus leaf blotch virus (CLBV)	12	12

**Table 6.10.4.** CRI molecular screening of accessions for release to CFB

Pathogen	Number of accessions	Number of tests
CTV-Pre-immunisations	17	17
CTV-indexed	39	39
CTLV	24	24
Liberibacter's		
Viroids	39	234
CPsV and CiVA	12	24
Visual inspection for Impietratura	12	

CIS diagnostic support was provided in the validation of PCR test results of CFB mother block material.

#### Technical CIS support through participation on CISAC and CIS-Pathology committees

- Jooste, A.E.C, Hlahlele, N, Theledi, Z and Roberts, R. Participated in the CIS Pathology meeting and the CIS facility audit, 25 May 2021
- Jooste, AEC. Participated in the 20th annual CISAC meeting, Teams meeting, July 2021

- Jooste, A.E.C, Hlahlele, N, Theledi, Z and Roberts, R. Participated in the CIS facility audit, 17 November 2021
- Jooste, A.E.C, Hlahlele, N, Theledi, Z and Roberts, R. Participated in the CIS Pathology meeting, 10 February 2022

## Acknowledgment

ARC-TSC, PEQ laboratory, thank Citrus Research International for the financial, technical and diagnostic support.

## 7 EXTENSION / VOORLIGTING

By Hannes Bester, MC Pretorius, Wayne Mommsen, André Combrink, Catherine Savage, Natasha Jackson, Jan Landman, Coenraad Fraenkel, David Groenewald, Andrew Mbedzi and Melton Mulaudzi

### 7.1 Voorligtingoorsig

#### 7.1.1 Terugblik op die 2021 seisoen

Die oorspronklike oesskatting van al die sitrustipes is reeds vroeg in die seisoen afwaarts aangepas a.g.v die feit dat vrugte oor die algemeen kleiner is as verwag. Die totale oesskatting aan die einde van die tweede kwartaal was 159.6 mil kartonne, teenoor die oorspronklike skatting van 163 miljoen kartonne. Die volumes van al die sitrustipes was uiteindelik hoër as in 2020. Die totale uitvoervolume van sitrus was 11 miljoen kartonne op teenoor 2020, met die grootste toename in sagtesitrus-volumes. Die verdienste in die markte was oor die algemeen teleurstellend. Markte was oor die algemeen vroeg onder druk, sekere markte was oorvoorsien, die gehalte van die vrugte was om verskeie redes nie altyd na wense nie en die wisselkoers was nie so gunstig soos die vorige jaar nie.

Produsente se gevoel was deurgaans dat dit 'n moeilike seisoen met baie uitdagings sou wees. Veral aan die logistieke kant is daar probleme ondervind met die beskikbaarheid van houers. Die tekort aan houers en trokke om vrugte na die hawens te vervoer was reeds vroeg 'n groot uitdaging. Die politieke onrus in KZN het sake dramaties vererger en oes- en verpakking in die noordelike gebiede is met tot drie weke vertraag. Buiten dat vrugte soms dae in vragmotors in die son moes staan, het vrugte ook in pakhuisse en boorde verouder en kon verwag word dat die raklewe en kwaliteit betekenisvol geaffekteer sou word. Hierdie vertraging in die logistieke ketting het tot hoër bederf in die markte bygedra met gevolglike gehalte-eise en laer pryse. Verder het stygende insetkoste 'n groot negatiewe effek op winsmarges gehad.

Die situasie in die hawens is 'n groot bron tot kommer, veral as die verwagte groei in suider-Afrika se sitrus-uitvoere in ag geneem word. Die toestand van die masjienerie en toerusting, kapasiteit van die hawens, beskikbaarheid van houers, korrupsie, finansies en algemene bestuur sal dringend aandag moet kry om die groei in uitvoervolumes te akkommodeer.

Marktoegang het ernstig onder skoot gekom met die hoë getalle onderskeppings van swartvlek en valskodlingmot in die EU. Hoewel dit nog onduidelik is wat die uitkoms gaan wees in terme van die maatreëls wat die EU gaan vereis vir 2022, is indringende werk plaaslik gedoen om te verhoed dat kouesterilisasië afgedwing gaan word. Een van die veranderinge wat aan die FMS (False codling moth Management System) aangebring is, behels die verpligte gebruik van 'n A15C karton met verbeterde lugvloei. As gevolg van verskeie faktore is hierdie verandering eers op 'n laat stadium aan die sitrusbedryf bekendgemaak, wat tot groot ontevredenheid onder verskeie pakhuisse gelei het, wat reeds kartonne vir volgende jaar aangekoop het voordat die volgende prysstyging in werking tree.

Die nuutste riglyne vir die gebruik van Imidacloprid was in Augustus Snykant 325 bespreek, en dit beteken die produk sal nie op die meeste produserende bome vir die 2022 seisoen gebruik kan word nie. Die moontlikheid vir 'n uitbraak van witluis, dopluis of plantluis, asook psylla, gaan 'n uitdaging wees. Met die druk op MRL's in die EU kan produsente nie bekostig om addisionele aktiewes korrektief te gebruik nie en die lys van

beskikbare chemie word al hoe korter. Die beheer van peste en plaë moet uiters fyn bestuur word, anders gaan dit groot impak hê op die keuses van markte.

### 7.1.2 **Blik op die 2022 seisoen**

Daar heers 'n groot mate van onsekerheid oor wat produsente te wagte kan wees die komende seisoen. Die markte was reeds verlede jaar onder druk, probleme met hawens wat nie effektief gefunksioneer het nie en baie lae winsmarges en selfs verliese was aan die orde van die dag, en die vooruitsig vir die komende seisoen lyk op hierdie stadium nie veel beter nie. Daarby weet niemand met sekerheid wat presies die gevolge van die oorlog in die Ukraine op die sitrus-uitvoere gaan wees nie. Die verwagting is dat Suid-Afrika slegs 50-60% van die normale volumes na Rusland sal kan verskeep.

Die oesskatting vir 2022 was einde van die eerste kwartaal van 2022 nog nie gefinaliseer nie, maar dit was duidelik dat die oes weereens beduidend groter as verlede seisoen gaan wees. Die eerste suurlemoene is reeds verskeep, maar produsente is versigtig om voorspellings oor die pryse te maak. Tydens die afgelope CRI Na-oes werksinkels, asook tydens die CMF vergadering, is produsente gemaak om met omsigtigheid te besluit oor die uitvoer van hul vrugte en nie marginale tellings en kwaliteit uit te voer nie, aangesien dit nie net druk op die markte gaan plaas nie, maar ook op die beskikbare verkoelings-kapasiteit en die wankelrige logistieke stelsel.

Met die goeie reën oor die grootste gedeelte van die land was vrugset, vruggrootte, spuitprogramme en toegang tot boorde 'n bekommernis vir meeste van die produsente.

Hier en daar het haël in sommige streke geval, Hoedspruit (lig), Komatipoort en Malelane (lig), Nelspruit area (lig) Swaziland (swaar tot medium). Ngonini is 70 % uitgeslaan.

Vroeë variëteite soos suurlemoene is die vruggroottes mooi met goeie opbrengs, pomelo's lyk baie goed, gehalte en groottes lyk mooi, pompelmoes (Jackson) lyk uitstekend, mooi drag aan die bome, min siektes, met baie mooi grotes, brix is uitstekend. Kleur op die suurlemoene is 'n probleem, kleurplaat 7 en 6 met baie Oleo a.g.v die reën, Botritus en silwermyt steek ook hier en daar sy kop uit, hoë skouer en windskade, maar nie erg nie.

Produsente is maar op hul hoede oor vervoer, politieke onrus, diesel pryse en arbeids kwessies (vakbonde begin vroeg), die nuwe karton veranderings en effekte daarvan, hawe aspekte en verkoeling, beskikbaarheid van pallette en kartonne.

Meeste van die pakhuis het hulle gradering baie opgeskerp, net sekere tellings en graderings gaan gepak word na markte met hoeveelhede wat noukeurig dopgehou word.

Oor die algemeen is daar 'n baie goeie opbrengs met mooi vrugte, moeite wat gedoen word op die boordbespuitings, chemie, waks, kwaliteit en algemene praktyke in en om die pakhuis. Aanhoudende besoeke word deur CRI aan pakhuis gebring en reëlings van forums om almal op hoogte te hou van veranderings asook die deel van inligting en nuwe verwickelinge.

### 7.1.3 **CRI Postharvest technical forum**

Daar is vroeg in 2021 melding gemaak van Morrisons se versoek dat oop vertoon-kartonne sonder "securing sheets" (SS) gepalettiseer moet word. Dit is ook genoem dat hulle in kennis gestel is dat die produsente nie bereid is om dit te doen nie, en die redes is ook aan hulle deurgegee. Morrisons het bevestig dat hulle dit so aanvaar.

Na aanleiding van Lidl se versoek dat A15C kartonne se binnestukke ten volle gedruk moet word, is 'n skrywe deur die CGA en CRI-PTF saamgestel en uitgestuur. Daarin is produsente vriendelik versoek om saam te staan en nie te voldoen aan die onredelike versoek nie. Daarna was dit weer met Lidl en sekere uitvoermaatskappye gekommunikeer. Aan die einde van die tweede kwartaal is berigte ontvang dat Lidl besluit

het om nie voort te gaan met die gedrukte A15C binnestukke nie, maar dat hulle gaan versoek dat A15C buitestukke nou met Lidl kunswerk daarop geïmplementeer moet word, wat 'n baie meer sinvolle besluit is.

Gedurende die tweede kwartaal is verneem dat sogenaamde 10 Slat en E Palette deur sommige pakhuis (hoofsaaklik in die Oos-Kaap) gebruik word. Volledige spesifikasies is van beide palette gekry. Modelle van swaar dubbelwand riffelbord met die uitleg/spasiëring van die bo-dek planke het getoon dat die vertikale lugvloei deur die 10 Slat palet nie te sleg is nie, maar die feit dat heelwat van die kartonne se hoeke nie op bo-dek planke rus nie, is 'n probleem. Wat die E Palet betref is die vertikale lugvloei 'n probleem, en dit alleen is 'n bron van kommer.

As gevolg van ernstige probleme met beurtkrag by tye, het die personeel by Sappi se laboratorium waar die toetse gedoen word, agter geraak met die kartontoetse vir die akkreditasie-stelsel. Om sake te vererger het die Innovation Hub kompleks waar die Sappi Technology Centre (STC) geleë is ook vir dae sonder water gesit. Om hulle te help is daar besluit om op 'n adhoc basis die basiese massa van sekere kartonne se papier te bepaal. Monsters (100x100mm) van die kartonne is gesny, die deklae (liners) en die riffelmedium (fluting) is van mekaar geskei en die basiese massa is deur die STC bepaal. Terselfdertyd is daar ook seker gemaak dat die gespesifiseerde papier gebruik word. Dit was tot 'n sekere mate 'n "bedekte" seën, want dit het getoon dat die inligting van sommige kartonvervaardigers op die vraelyste, wat saam met die kartonne ingestuur moet word, nie korrek is nie. In enkele gevalle is dit bevestig en dit is met die betrokke vervaardigers opgeneem. Covid-regulasies het verder bygedra om die kartontoetse uiters moeilik te maak vir die Sappi Technology Centre (STC) se laboratorium personeel. Vir etlike weke was hulle verplig om met "skeleton staff" te werk. Die laaste toetse was veronderstel om gedurende week 31 gedoen te word, maar met al bogenoemde probleme was die laaste toetse eers gedurende week 34 gedoen. In samewerking met die STC is ook besluit om meer kartonne vanaf pakhuis te kry. In uiters moeilike omstandighede het die STC personeel tog daarin geslaag om al die kartonne vir die seisoen te toets.

Produsente in Zimbabwe het navraag oor die akkreditasie van kartonvervaardigers gedoen nadat hulle deur 'n kartonvervaardiger in Zimbabwe genader is. 'n Afskrif van die CRI Pakmateriaal Spesifikasies en Palettiserings Protokolle dokument is weer vir hulle gestuur en daar is aan hulle genoem dat al die inligting rondom geakkrediteerde kartonvervaardigers, en die kartontoetse, in bogenoemde dokument vervat is. CRI-PTF het ook aangebied om hulle kartonne te toets. Hulle het laat weet dat hulle sal voortgaan om hulle kartonne by geakkrediteerde kartonvervaardigers te koop.

Verskeie produsente het advies gevra nadat 'n sekere kartonvervaardiger vir hulle kartonne teen baie aantreklike pryse aangebied het. Dis aan hulle voorgestel dat hulle vir die betrokke vervaardiger moet vra om hulle volledige spesifikasie op skrif te sit en ook vir hulle te vra vir stapelsterkte toetsverslae wat deur 'n SANAS geakkrediteerde laboratorium gedoen is. Die terugvoering was dat hulle besluit het om by hul verskaffer/s te bly, "want goedkoop koop is duur koop".

Geweldadige optrede en plundery in KZN het ernstige probleme veroorsaak. Gedurende hierdie periode was CRI-PTF op 'n baie gereelde basis in kontak met die papier-, karton- en paletvervaardigers. Daar kan met dankbaarheid gerapporteer word dat pakhuis nie met verpakking gestop het omdat daar nie genoeg pakmateriaal was nie. Al die rolspelers moet gekomplimenteer word. Hulle beplanning was uitstekend en dit beklemtoon net weer hoe belangrik goeie kommunikasie is.

Die groot verskeidenheid van kartonne (afmetings, kartonkodes en massa van verpakte kartonne) is al vir 'n geruime tyd 'n bron van groot kommer. Dit het egter nou so 'n krisis geword dat die CGA 'n taakspan aangestel het om die probleme aan te spreek en op te los. Die CGA, taakspan het begin om 'n lys op te stel van kartonne/kodes wat nie in die CRI-Pakmateriaal Spesifikasies en Palettiserings Protokolle dokument gelys is nie. Die oorgrote meerderheid van die kartonne/kodes wat by die inname punte op die stelsels geregistreer word, verskyn nie in bogenoemde dokument nie. Die Verpakkingswerkgroep is uit die aard van die saak betrek. Al die geakkrediteerde kartonvervaardigers is gekontak en versoek om volledige inligting oor al die kartonne (afmetings en kodes) wat hulle vervaardig, te verskaf. Ongelukkig het heelwat produsente gedurende die afgelope twee tot drie jaar hulle kartonvervaardigers opdrag gegee om kartonne met ander afmetings te

vervaardig en dan het hulle ook op hulle eie kodes besluit. Die CGA taakspan poog om volledige inligting by die produsente en uitvoerorganisasies te kry.

Die papiervervaardigers is op 'n voortdurende basis besig met navorsings- en ontwikkelingswerk op papier met 'n laer basiese massa en hoër tipiese waardes, met die doel om meer koste-effektiewe kartonne te vervaardig. Gedurende die jaar is semi-kommersiële proewe met A15C kartonne gedoen. Die 175g/m<sup>2</sup> "liners" is vervang met die nuwe verbeterde 170g/m<sup>2</sup> Kraftpride en die 165g/m<sup>2</sup> Ultraflute is vervang met die nuut ontwikkelde 150g/m<sup>2</sup> Ultraflute Plus. Die proewe was baie suksesvol en die nuwe papierkombinasies sal nou kommersieel geïmplementeer word.

In een geval is klagtes van oorsee ontvang oor onstabiele paletvragte en kartonne wat inmekaar gesak het. Soos met baie ander soortgelyke gevalle is die gehalte van die kartonne bevestig. Verskeie e-posse en selfoon boodskappe met foto's en video "clips", wat die probleme uitwys, is in diepte bestudeer en dit was baie duidelik dat die probleme veroorsaak is deur rowwe hantering en palettiserings protokolle wat in sekere gevalle nie gevolg is nie. Veral die rowwe hantering is 'n groot bron tot kommer. Die bevindinge is, tot bevrediging, aan die betrokke uitvoermaatskappy deurgegee.

In Maart vanjaar is 'n versoek ontvang om 'n 18kg teleskopiese karton vir Japan te ontwikkel. Daar is aan hulle gemeld dat suurlimoene in die bestaande A15C karton se bruto massa wissel van 16,5 tot so hoog as 17,8kg. Nadat die koper in Japan die bestaande A15C karton aanvanklik aanvaar het, het hulle in Augustus laat weet dat hulle nou weer vra vir 'n 18kg karton. Op versoek van die uitvoermaatskappy word voorlopige werk op 'n "eksperimentele" A15C karton, wat 60mm hoër gemaak is en waarin een ekstra laag vrugte gepak word, gedoen. Alles dui daarop dat om verskeie, baie goeie redes, dit nie suksesvol sal wees nie en die moontlikheid om die bestaande D15C karton te probeer gaan nou ondersoek word.

Al die moeilike omstandighede gedurende die laaste paar maande van 2021 het kommer laat ontstaan oor die beskikbaarheid van genoegsame papier, kartonne en palette. Gedurende die laaste kwartaal was daar baie noue kontak met papier-, karton- en paletvervaardigers. Produsente/pakhuse kon egter tot aan die einde van die seisoen pak sonder enige onderbrekings.

Gedurende November is dit amptelik aangekondig dat daar met die aanvang van die 2022 seisoen 'n A15C karton met addisionele gate vir verbeterde ventilasie geïmplementeer gaan word. Kartonvervaardigers was onder druk om dié kartonne so spoedig moontlik te vervaardig en uit die aard van die saak is die kartonne dringend by Sappi laat toets om vas te stel of die kartonne met die addisionele ventilasie-gate wel aan die vereiste stapelsterkte spesifikasies sou voldoen. Om behulpsaam te wees met die suksesvolle vervaardiging en implementering van die nuwe A15C kartonne is daar besluit om 'n "Packaging Technical Working Group" (PTWG) te stig. In samewerking met die geakkrediteerde kartonvervaardigers en CRI is die PTWG gestig en volledige inligting oor die samestelling is bekend gemaak. Wat die vou en lym van die nuwe karton betref was CRI-PTF in verbinding met verskaffers van masjienerie wat kartonne vou en lym om in diepte hierna te kyk. Verskeie produsente en koöperatiewe pakhuse het bevestig dat hulle nog steeds versoeke ontvang om "CHEP" palette vir sekere markte te gebruik. Al die verslae wat handel oor die negatiewe aspekte van "CHEP" palette is weer aan hulle gestuur en die groot probleem met vertikale lugvloei is weer aan hulle uitgewys.

Inligting oor 'n karton in Egipte met dieselfde afmetings as ons A15C karton is van 'n uitvoermaatskappy ontvang en beweer dat dié kartonne aansienlik goedkoper as A15C kartonne in SA is. CRI-PTF is gevra om kommentaar te lewer. Na 'n volledige ondersoek is aan hulle gekommunikeer dat die karton nie aanvaarbaar vir SA is nie, om die volgende redes:

- 'n Eenstuk-karton wat se stapelsterkte glad nie aan SA se vereistes sal voldoen nie
- Onaanvaarbaar vir vrugte wat met 'n "bulge" verpak word
- Ventilasië-gate totaal onaanvaarbaar.

CRI-PTF Pakmateriaal Spesifikasies en Palettiserings Protokolle dokument vir 2022 is gedurende Januarie opgedateer en onder alle rolspelers versprei.

'n Voorseisoen vergadering is gedurende Januarie 2022 met Sappi se bestuur gehou. Hierdie vergadering is 'n jaarlikse instelling. Dit gaan hoofsaaklik oor die belangrikste aspekte/verwagtinge van die 2022 seisoen.

Sluiting van hulle papiermeulens is vanjaar langer as in die verlede omdat hulle baie meer opgraderingswerk en installering van nuwe tegnologie moet doen. Sappi het nogtans die versekering gegee dat hulle die kartonvervaardigers se papierbestellings sal uitvoer en hulle kliënte nie in die steek sal laat nie. Sappi het ook 'n "Sappi Sphere" voorlegging gedoen wat handel oor die volhoubaarheid van produksie van hout en vervaardiging van papier. Tydens hierdie vergadering is die akkreditasie toetse van kartonne by hulle SANAS geakkrediteerde laboratorium ook bespreek. Hulle het ingestem om, soos in die verlede, voort te gaan met die akkreditasie-toetse en dat hulle al die kostes weer sal dra. 'n Skedule vir die akkreditasie toetse gedurende 2022 is in samewerking met die Sappi Technology Centre se personeel opgestel en versprei.

Baie navrae is hanteer oor die A15C S2 kartonne. Die kartonvervaardigers het gedurende die kwartaal in alle erns begin om dié kartonne te vervaardig. Alhoewel die vervaardigers versoek is om nie die addisionele twee klein (20mm deursnee) ventilasie-gate in te sit nie, het sommige vervaardigers besluit om proaktief op te tree en die gaatjies tog in te sit. Die eerste stapelsterkte resultate was aansienlik laer as die vereiste 600Kgf. Op grond daarvan is 'n omsendskrywe aan al die kartonvervaardigers gestuur waarin hulle versoek is om die twee gaatjies te verwyder.

Die kartontoetse vir die akkreditasiestelsel het gedurende week 7 begin. Omdat so spoedig moontlik maksimum inligting oor die stapelsterkte van die nuwe A15C kartonne verkry moes word, is die kartonvervaardigers versoek om tot verdere kennisgewing slegs nuwe A15C kartonne na die laboratorium te stuur. Die vroeë aanduidings is dat die stapelsterkte, selfs van die kartonne sonder die twee 20mm deursnee gaatjies, ietwat laer is as die vorige ontwerp A15C SV kartonne. Meer inligting sal so spoedig moontlik beskikbaar gestel word.

#### 7.1.4 Postharvest extension

The 2021 season was a challenging one. From the beginning, there were reports of rind burn, particularly in the Western Cape. Climate was very likely a role player, while the use of sanitisers in the orchard could also be a contributor.

As the season progressed, other problems came to light. The weakening of the Rand and the increase in fuel prices were some of the concerns and challenges faced by packhouses. However, the KZN unrest, Transnet cyber-attack and lack of trucks, containers and ships to, and at, the Durban port had by far the greatest negative impact on the packhouses, particularly the Northern packhouses, but the ripple effect was felt by all regions. The delays and lack of transport resulted in packhouse shutdowns, some for up to a total of five weeks, pushing the season much later than was good for the fruit. Knock-on effects included lack of space at packhouses, harvesting fruit with blossoms on the trees, re-greening of fruit on the tree due to fertilization programmes starting before harvesting, delays in pruning and spray programmes, and most seriously, low quality of arrivals. Claims came through of fruit up to ten weeks after they were packed. This compounded in much more decay seen on arrivals. Latent pathogens and green mould claims factoring at number one for decay. Soft fruit was also a great concern and directly attributed to older fruit in the market.

Covid 19 had a minimal impact early on during the packing season, with operations continuing as normal, until the third wave and harsher lockdown restrictions were imposed. As the country went into peak infection periods, packhouses restricted visitors and asked staff that can work from home to do so. However, as infection numbers decreased and vaccinations increased, operations continued normally again.

Some of the issues seen during the season were related to chemicals. A minor disruption at the beginning of the 2021 season was the shortage of Hydrochloric acid which the packhouses use to manage the pH of various solutions. A much larger and lingering concern was that of the removal of the EU import tolerance for the active propiconazole. Propiconazole residues became a concern as packhouses had to stop use, clean the line and look for alternatives as the deadline to withdraw propiconazole from the EU approached. Packhouses were noticing residue levels above the LOD despite long-term stoppages of the active. Additionally, the alternative GRAS product had a limited supply in South Africa and so many smaller packhouses had been unable to procure the product. Sour rot has been a concern this season and will likely continue to be so. Sour rot control as well as the general trend of the loss of postharvest actives was a common concern raised at the research

priority meetings and during packhouse visits. Propiconazole residues lingering on the packlines persisted into the 2022 season.

Green mould (*Penicillium digitatum*) resistance against imazalil was picked up towards the end of the packing season. It is of critical importance that packhouses monitor their retention samples and send swabs into CRI's DC for testing if they suspect a loss of sensitivity towards imazalil.

Once packing had been completed for 2021, many packhouses took a critical look at operations and tried to optimise the processes so that the consequences of possible future delays would not be so harshly felt as in 2021. There is only so much control that a packhouse has, but those with good operations fared better than those without. It is also of note that bigger packhouses and exporters coped easier with the delays than did the smaller entities.

Packhouse forum meetings had been largely suspended due to the risks of Covid but towards the end of 2021 many packhouses felt the need to meet and discuss the challenges of the year. Post-season Packhouse Forum meetings were held in Sunday's River Valley, Patensie, Citrusdal, Swellendam, Nelspruit, Hoedspruit, Letsitele, and Tshipise/Weipe with good attendance.

Generally, it is agreed upon that 2021 fruit from South Africa was of a good quality with very little decay or rind issues seen before export. However, the delays resulting in fruit not being able to be picked at optimal maturity, the stoppages of the packhouse, and the extraordinarily long delays in truck, at port, on ship, and at final destination, created quality issues due to age. Many rind conditions were reported and a fair amount of green mould decay was seen, although in many cases the decay is suspected to be secondary infections, possibly over sour rot infections for which there was no available fungicide treatment during the latter half of 2021. Each delay had a knock-on effect with ships by-passing our ports or fruit clashing in the market resulting in oversupply. All in all, 2021 was a very tough and challenging season for the South African packhouses.

The 2022 season began with almost as many challenges. Packing began early in the North but only in earnest in the South after the Easter weekend. Before packing started a lot of attention had been given to the chemical programmes for 2022. After the 2021 season of high decay due to the transport delays, many packhouses are looking at what adjustments can be made to ensure their fruit's longevity. Packhouses were undeniably concerned over a repeat season in terms of logistics and high decay.

The conflict in Ukraine has had multiple knock-on effects. Packhouses were very concerned about being unable to pack fruit for Russia and although export volumes are down to the federation, some fruit has been able to travel, starting with Satsumas on direct shipments to Russia.

As 2022 progresses, and Covid restrictions are mostly relaxed, one-on-one packhouse visits can continue. A typical visit covers the full packline from pre-packhouse drench to palletizing, with advice given as needed. Much focus is placed on the individual chemical treatments and applications (e.g. waxing). A critical look from an outside perspective is able to pick up aspects such as injury points or poor practices.

### **7.1.5 Produksiestreke**

#### **7.1.5.1 Wes-Kaap Produksiestreek**

Daar is oor al die streke in die Wes-Kaap 'n koeler lente en vroeë somer in 2021 beleef. Die vrugset vir 2022 was veral op die laat Mandaryne weens die koeler weer bogemiddeld. Die gevolg was dat die meeste vroeë en midseisoen variëteite tussen 5 en 10mm kleiner was as vergelyk word met die laaste drie jaar. Dit was veral op die Clementines waar die vruggrootte 'n baie groot uitdaging was. Die Satsumas was in sommige areas drie tot vier weke laat die seisoen in vergelyking met vorige seisoene en veral die kleur was baie erg vertraag. Die later variëteite het 'n goeie set opgelewer en waar die produsente 'n goeie uitdun strategie gevolg het, was ooste goed te wees.

Die blaaspoortjie druk in die Wes-Kaap die seisoen was minder in vergelyke met vorige jare. Daar het wel 'n groter hoeveelheid witluis voorgekom, veral in boorde onder nete. Die dopluis druk was ook hoër as in die verlede en dit mag wees weens die feit dat sekere produkte nie meer beskikbaar is vir behandeling nie. Witluis druk is ook hoër en is ook toegeskryf aan produkte wat in die verlede effektief was vir die beheer en wat nou nie meer beskikbaar is nie. Die produkte wat wel beskikbaar is se effektiwiteit word bevraagteken.

Die droogte van 2015 tot 2018 het ekstreme braktoestande in die grond veroorsaak, veral in die westelike gedeeltes van die Wes-Kaap. Die laaste twee jaar se bogemiddelde reënval het beslis die braktoestande verminder en die hergroei oor die algemeen lyk in al die streke baie beter as in die verlede. Die Overberg-area het egter geweldige nat toestande beleef, wat bespuitings bemoeilik het en die risiko vir Phytophthora verhoog het.

In die Benede-Oranjerivier is daar areas wat gedurende die winter van 2021 swaar ryptoestande beleef het. Gevolglik het daar op sekere variëteite terugsterwing plaasgevind in die laerliggende gedeeltes en veral die vrugset op suurlemoene is in 2022 ondergemiddeld. Die Star Ruby en Valencia variëteite lyk belowend. Daar is egter ook baie bewolkte toestande beleef in die lente en vroeë somer en die gevaar vir kleiner vrugte met hoër sure, asook kraakskil, is wel 'n bekommernis.

Die CGA "roadshows" in Citrusdal, Ashton en Benede-Oranjerivier is goed ontvang deur die produsente en die interaksie met CRI verteenwoordigers was baie positief. Die produsente is opgewonde oor die toename in navorsing vanaf CRI en die aanstellings in voorligting vir ondersteuning in die onderskeie areas.

Daar was verwarring oor die aangepaste FMS stelsel en die "5 data boom" vereiste vir die VSA. Dit is egter uitgesorteer en meeste produsente het die nodige stelsels in plek gekry om die data in te samel en in te voer in Phytclean.

'n Besproeiing webinar is gedurende Januarie beskikbaar gemaak, sowel as twee Youtube video's wat handel oor die trek van grond- en blaarmonsters. Die terugvoer uit die bedryf is oorweldigend positief en interaksie met produsente rakende die inligting is steeds besig.

Daar is 'n konsultante forum gestig in die Wes-Kaap wat elke twee maande ontmoet in die onderskeie produksiestreke. Die kommunikasie tussen CRI, konsultante en produsente is krities om te verseker dat goeie inligting en riglyne tydig die produsente bereik.

CRI is genader om betrokke te raak by die "Eureka Climate Smart" projek wat gedeeltelik befonds word deur Stellenbosch Universiteit, Metos SA, Terraclim, Winfield United, PESSL en TIA (Technology Innovation Agency). CRI voorligting sal hulp verleen in die plasing van 200 weerstasies oor die Wes-Kaap (slegs posisie en produsent identifisering) sowel as in die opleiding van die produsente in die gebruik van weerdata in beter produksie besluitneming. (Die opleiding sal een opleiding sessie in Citrusdal behels in Junie 2022).

#### **7.1.5.2 Oos-Kaap Produksiestreek**

Die droogte in die Gamtoosrivier vallei was 'n groot bron tot kommer. Vrugte was geneig om af te speen indien dit nie betyds gepluk is nie. Die droogte het ook 'n negatiewe effek op die hou vermoë en rակlewe gehad. Produsente was veral bekommerd oor die ontwikkeling van fisiologiese skildefekte. Wat verder hiertoe bygedrae het, is die wisselvallige weerstoestande waar koeler weer gereeld afgewissel is met skielike warm temperature en lae humiditeit op sekere dae. Hoewel 'n paar ligte reënbuie gedurende die derde kwartaal uitgesak het, het feitlik geen afloopwater die opgaardamme bereik nie. Die reën het nogtans bygedra om die ergste stremming op die bome te verlig.

"Storage mould" was 'n ernstige probleem op veral suurlemoene in die Oos-Kaap, maar het ook toenemend op ander variëteite begin voorkom, asook in ander produksiestreke. Daar sal uit 'n navorsings-oogpunt sterk fokus op hierdie probleem wees om 'n oplossing te vind. Oor die algemeen was die kwaliteit van die vrugte aanvanklik baie goed, met goeie uitpakpersentasies. Hoewel interne gehalte vanjaar goed was, is meer bederf

uit die markte gerapporteer. 'n Beduidende persentasie hiervan het wel hul oorsprong in die boorde en pakhuis, maar die vertraagde vervoer na die markte het die probleme aansienlik vererger.

Oor die algemeen lyk die oes en vrugkwaliteit baie goed in die Oos-Kaap produksiestreke vir die 2022 seisoen. Dit is grotendeels te danke aan die goeie reënval vanaf einde Oktober tot Januarie. Gemete vruggroottes was redelik klein in begin Desember en vergelykbaar met die 2021 seisoen wat gekenmerk is deur klein vrugte. Die Desember vruggroei was egter uiters goed en vruggroottes het opgevang. Nog goeie reënval gedurende die laat somer en lente sal 'n verdere positiewe impak hê op vruggroote.

Behalwe vir swakker of wisselvallige vrugset op sommige van die Novas en Clementine boorde in die Katrivier Vallei, het die oes uitstekend gelyk tot en met middel Desember. Op 13 Desember het daar ongelukkig 'n hewige haelstorm die area getref. Groot skade is aangerig aan boorde, asook infrastruktuur. Die skade was egter nie oor die hele area nie. Produksie-eenhede naby Adelaide en aan die Grahamstad-kant van Fort Beaufort, asook die dorpsgebied, is in sommige gevalle erg beskadig. Die Bo-Katrivier produsente is nie geraak nie. Terugvoer uit die area dui aan dat oesverliese wissel tussen 20% tot 50% op sommige plase. Skade aan boorde is grotendeels aan die westekant van die bome aangerig. Die totale omvang van die skade sal egter eers meer duidelik word later in die seisoen.

Blaaspoottjie getalle was oor die algemeen laag hierdie seisoen met min tot geen skade aan vrugte tot en met Februarie. Behalwe vir windskade is die vrugte redelik skoon. Windskade blyk egter minder te wees as vorige seisoene. Wat wel hierdie seisoen meer sigbaar is, veral in die Sondagsrivier-vallei (SRV) op suurlemoene en ook lemoene wat later as gewoonlik geblom het, is botritis as gevolg van die nat weerstoestande vanaf laat Oktober tot Desember.

Witluis en geassosieerde roetskimmel was verantwoordelik vir 'n redelike persentasie afgradering van vrugte of uitskot die laaste twee seisoene in die Oos-Kaap produksiestreke. Produsente het dus redelik fokus geplaas op witluis-beheer die afgelope seisoen met goeie resultate, maar daar sal sekerlik nog 'n oorvloed van witluis wees in boorde wat swaar besmet was verlede jaar. Witluis getalle piek natuurlik in Januarie, maar huidige terugvoer en observasies dui dat getalle oor die algemeen onder beheer kom, veral waar geïntegreerde plaag- en siektebeheer programme toegepas is.

Die swartvlek risiko sal ongelukkig hoër wees in die 2022 seisoen as gevolg van die nat weerstoestande gedurende laat Oktober tot Januarie. Daar is elke jaar 'n uitgerekte blom op sekere variëteite in die Oos-Kaap streke, party seisoene is dit egter net erger as ander. Meer problematies die laaste seisoen en weer hierdie seisoen in die SRV is die Valencias wat in sommige gevalle 3 tot 4 weke later deur blomblaarval gegaan het. Dit is dan soortgelyk in 'n mate soos wat gerapporteer word uit die Sentrale Produksiestreek. Klimaatsfaktore is grotendeels die oorsaak. In die SRV, veral aan die Addo kant, was daar verlede seisoen heelwat Valencia-boorde wat eers 80-100% blomblaarval bereik het teen vroeg tot mid-November. Sodanige boorde kon eers in Oktober ge-oes word as gevolg van te hoë suurvlakke. Die meeste van hierdie Valencia boorde, waar die vrugte laat gehang het, het hierdie seisoen weer laat geblom en weer 2 tot 3 weke later as gewoonlik blomblaarval bereik. Effektiewe swartvlek asook plaagbeheer is uiters moeilik in hierdie situasies.

Met bogenoemde in gedagte is dit duidelik dat al die produsente na die laaste seisoen baie meer daarop fokus en bewus is van die risiko wat Swartvlek en dan ook FCM inhou vir die bedryf. Terugvoer en ondersoek dui aan dat, ten spyte van die nat weerstoestande, die meeste produsente in staat was om hul swartvlek-strategie te volg en hul bespuitings betyds toe te dien sonder enige gapings in die program. Die hoop is dus daar vir minder swartvlek-onderskeppings die komende seisoen.

Vanaf 'n FCM oogpunt is daar gefokus en seker gemaak dat alle rolspelers die veranderinge tot die FMS verstaan en sodoende kan implimenteer. Die *Bactrocera dorsalis* (BD) status is laat verlede jaar verander na dit gevestig is in die SRV. Die beheer van BD in die SRV verander dus van een van volgehoue uitwissing na toekomstige volgehoue onderdrukking. Daar is seker gemaak dat die produsente en hulle adviseurs alles verstaan rondom die statusverandering en dan die protokol wat gevolg moet word vanaf die jaar.

Observations and feedback from the Eastern Cape production regions at the end of March 2022 still indicate generally clean fruit with very little thrips and wind damage visible on most varieties. All areas are reporting decent crops in most varieties. Reports on fruit size vary, but for the most part, fruit size either looks to be on par with last season or slightly bigger.

Some damage from the December hailstorm in the Katriver area is still visible on affected Navel and Satsuma orchards, but the majority of the damaged fruit has dropped from the trees with the remaining fruit being very clean. Fortunately, as reported earlier, only about four farms were hard hit by the hail, suffering up to 80% crop loss mainly on Satsumas and navels. The negative impact of the hail on the overall crop volumes of the Katriver area however doesn't seem to be too severe.

Thrips populations remained relatively low from mid-November to February with most of the softer/IPM thripicides (Delegate, Biomectin, Tartar-emetic) giving the desired 2-4 weeks of control. With the lower thrips levels, less of the disruptive/harsh products were generally sprayed in November and later. The result of this should be less repercussion pest (mealybug, red scale, red mite) outbreaks in the period leading up to harvest and hopefully also next season. However, with this said, there remain isolated incidences where producers were battling with mealybug into February and later in all three EC areas. In most of these cases, either harsh thripicides were applied earlier in the season or the producers did not follow an effective early preventative strategy.

By end of March, the FCM levels still seemed to be under control. Indications are that producers have worked hard to implement the changes to the False Codling Moth Management System (FMS) and a high level of orchard sanitation was being maintained by most. At the same time reports came through of massive locust swarms coming through the Karoo, heading towards the SRV area. Local efforts, in collaboration with EC-Agri and the Department of Agriculture Disaster Management division, were launched to combat the swarms in the Steytlerville area by means of helicopter aerial spraying. Although these efforts were ongoing, big swarms managed to enter both the Patensie and Kirkwood production areas in the last week of March. Expert opinion however indicated the specific species of locust (the Brown Locust) are mainly grass feeders and should not pose any significant risk to the citrus plantings. Investigations and observations indicated that was indeed the case. CRI subsequently put out a Cutting Edge on the situation in end-March stating that spraying of pesticides for the pest on citrus trees should not be necessary.

Unfortunately, at the same time as reports came through of the arrival of the locusts in Patensie, fruit piercing moth (*Serrodus partita*) were also reported in satsuma orchards. As there is very little that one can do to combat this pest, producers were advised to harvest the fruit as soon as there is enough colour break to de-green and in so doing get the fruit off the trees as quickly as possible to limit the damage from the pest.

Lastly, it is worth mentioning that increasing production cost, market uncertainty, and the various challenges around logistics are a big concern and on everyone's mind.

### 7.1.5.3 Sentrale Produksiestreek

Die oeslading in hierdie streek was bogemiddeld vir 2021, met vruggrootte in sommige kultivars wat kleiner was. Vrugkleur was ook 'n uitdaging weens die warmer winter vroeg in die seisoen. Uitpak persentasies was bogemiddeld, in die orde van meer as 85%. Vrugkwaliteit was ook baie goed, en geen noemenswaardige verliese is aangemeld nie. Die interne kwaliteit was ook goed en na-oes probleme was nie meer as vorige seisoene nie. Die grootste na-oes probleme aangemeld was saprofitiese groei op stingels en suurvrot. Bruinvrot het ook in sekere areas groter uitdagings na die goeie somerreën getoon. Selfs te midde van nog n inperking en Covid-19 gevalle het die oes en pakprosesse redelik glad verloop. Sekere produsente het kleiner vruggroottes op sekere kultivars gehad, waar ander weer 'n goeie normale grootte gehad het. Sekere areas wat bogemiddelde reënval gedurende Januarie en Februarie gekry het, het hoër swartvlekdruk ervaar in vergelyking met sekere areas wat minder reën gehad het.

Produsente het 'n uiters uitdagende seisoen t.o.v logistieke aspekte beleef. Daar moes dringend en baie vining baie keer buitengewone planne beraam word om vrugte vanaf die plaas tot die hawens en verder te kon skuif.

Buiten die logistieke nagmerrie wat trokke, houers en hawe-probleme ingesluit het, het onrus veroorsaak dat vrugte nie vervoer kon word nie en die oesproses moes gestaak word vir 'n sekere tyd. Sekere areas soos KZN het veiligheidsprobleme ervaar en produsente moes hulself en gesinne organiseer om moontlike terroristiese aanvalle op eiendom en personeel te voorkom. Daar moes dringend alternatiewe planne beraam word t.o.v plukkers en pakkers wat vir 'n tydperk nie gebruik kon word nie.

'n Uiters oneweredige en uitgerekte blom is gedurende die lente in meeste van die areas in die Sentrale produksiestreek ondervind. Die uitdagings was dat 20% van 'n boom reeds 80-100% blomblaarval stadium bereik het en die ander gedeelte van die boom eers op 80% ballonvorming stadium was en hierdie verskynsel het vir ten minste drie weke voortgeduur. Die vreemde verskynsel kan moontlik aan die klimaat toegeskryf word, aangesien die lente-temperature baie laag tot koud was en dan afgewissel is met een tot twee uiters warm dae – diep in die 30 to selfs 40 grade gestyg het en onmiddelik dan weer vir 'n week onder 20 grade. Baie bewolkte dae het in die Laeveld-streek voorgekom met bitter min sonskyndae. Blaaspootjiebeheer was 'n groot uitdaging weens die uitgerekte blom en in sekere gevalle moes daar ten minste twee keer daarvoor gespuit word.

Sekere boorde in Kwazulu-Natal was baie later geoes weens die opeenhoping by die hawens, asook die onrus situasie. Dit het veroorsaak dat van die bome selfs tot drie weke later as gewoonlik geblom het.

Die vroeë somer temperature (einde November tot Desember), was wel warmer, maar min sonskyndae is veral in Nelspruit en Onderberg gebied ondervind. Bo-normale reënvalsyfers vir November en Desember is aangemeld. Die swartvlek-risiko is uiters hoog en produsente het uitdagings gehad met die tweede swartvlekbespuiting wat moes opkom en weens aanhoudende reën en nat boorde was dit 'n uitdaging. Alternaria- en Phytophthora-risiko weens die langdurige nat toestande kan 'n groot risiko wees vir 2022. Laer insekdruk is aangemeld, maar heelwat wind het wel voorgekom en windskaade is reeds sigbaar op meeste kultivars.

Die grootste Alternaria uitbraak is in jare in die Nelspruit en Burgersfort area aangemeld. Leanri en Nova kultivars is geaffekteer. Hoër digtheid aanplantings word erger geaffekteer asook bome onder e. Die moontlikheid bestaan dat sekere produsente nie betyds al hul Swartvlek bespuitings kon afhandel nie en dat hul moontlik boorde sal moet ontrek van die EU programme.

Laat blaaspootjie in enkele boorde is waargeneem maar witluis is aansienlik laer in die streek. Geen reën het tydens Februarie en begin Maart in die streek geval nie. Nelspruit het wel in Maart weer 130 mm gekry. Vruggroei het wel in Februarie en Maart vergroot maar vrugte is oor die algemeen kleiner. Geen noemenswaardige skade is weens oorstromings of haelbuie aangemeld nie.

#### 7.1.5.4 Noordelike Produksiestreek

The impact of early rains in October 2021 with respect to CBS was observed in a number of orchards during this period. The diagnostic laboratory was inundated with samples of citrus fruit to analyse for CBS.

Many growers in the northern region were concerned early in the season about FCM numbers discovered in the packhouse. An increase in packhouse inspections for FCM saw a corresponding increase in the number of FCM larvae identified in fruit graded from the pack line. Workers were incentivised for each FCM found in some packhouses. Corresponding threshold values were calculated way below the FMS threshold level, as was the result of the pre-packhouse 800-fruit samples.

Mealybug remained a concern for grapefruit and early navels. Many orchards were withdrawn for export of grapefruit to South Korea and rejections of pallets from PPECB in the packhouse were significant for fruit destined for Japan and China.

Produksie (opbrengs en kwaliteit) op plase was oor die algemeen goed gewees. Vruggrootte asook pryse per karton was ongelukkig af gewees in hierdie uitdagende jaar en sal groot impak hê op die "bottom line" teen die einde van die seisoen.

In hoë reënval omstandighede was daar uitdagings omtrent die CBS spuitprogramme, veral omdat daar baie gevalle is waar CBS weerstand opgebou het teen Benlate. Druk van Supermarkte vereis ook dat daar geen Bezimidazole residue op vrugte is nie. In Noord-Zimbabwe het hulle groot uitdagings met Botrytis-beheer gehad. Dit is ook waar Benlate die beste opsie sou wees, maar is nie oorweeg nie as gevolg van onsekerheid.

Oesmanipulasie en produksiebestuur, veral wat snoei betref, word nie goed toegepas nie. Daarom is dit opmerkend dat baie boorde geen binne-vrugte gehad het nie en die algemene gebruik van spasië in die boom word nie benut nie. Daar kan groot verbetering plaasvind met opbrengs, vruggroote, kwaliteit en die aantal produktiewe jare van boorde. Daar was heelwat insidente waar topwerk op afgesnyde bome nie goed presteer het nie. Sodra hierdie strategie oorweeg word, moet die insette wat nodig is nie onderskat word nie.

The citrus crop in the Northern region, from Beit Bridge down to Hoedspruit, is looking promising with generally a good fruitset for the 2022 season. The climate during the fruitset period was moderate with cloudy and wet conditions across the province. Although frequent spells of rain was experienced, it did not seem to restrict CBS preventative sprays. Growers were well aware of the implications of high CBS pressure at this point because reports of CBS interceptions from the Northern region were still being received as fruit was arriving in the markets. Wet conditions have prevailed in the North during spring, so CBS risk management continued to be of high importance.

A strange belt of hail fell in the Weipe area, which will have bearing on some of the early grapefruit, but more specifically the damaged observed on about 70 hectares of Lemons means that the fruit will not be packed for export. The hail reached into southern Zimbabwe orchards, but will have much less impact on export volumes. Besides this event of nature, the citrus crops in the North have had low thrips pressure, likely to do with the wet and overcast conditions and wind speeds have been reported to be much less. Citrus fruit is very clean in all other areas. The general feeling is that fruit size is going to be good with the potential to be one size smaller than expected.

There were multiple reports, in different growing areas, of fruit lesions related to CiVA. The incidences in orchards are of a low to negligible percentage, but the frequency of occurrence should be noted. A second observation during the flowering period was the presence of saprophytic *Cladosporium spp.* around the calyx. This raised much concern, but it has been explained to be climate related and not harmful to the fruit or the cause of any fruit drop.

As expected there have been sporadic outbreaks of mealybug, likely due to the omission of Imidacloprid in most orchards. However, there is a correlation between the implementation of preventative mealybug sprays and explosions in the population. Both poor selection of active ingredients for thrips sprays and instances where no mealybug preventative sprays were applied seem to contribute. A third concern is the poor timing of releases of natural enemies. The Letsitele region is focussing a lot of energy on mealybug management. There is a strong presence of *Delottococcus aberiae* which does not have a historical natural enemy complex in the region. This mealybug prefers to hide in protected area under the calyx on citrus fruit, making it more challenging to control. Few reports of other pests being a significant problem have been received in the North.

There is a continued need for information on IPM, decision-making in IPM and training of scouts to support successful implementation of IPM strategies. Extension's coordination of the two IPM decision-making workshops, in Groblersdal and in Nelspruit is an ongoing initiative. The aim is to reach all roleplayers in different production areas to emphasise the importance of IPM practice for the future of this industry. Scout training workshops were also held in Weipe, Tshipse and in Nelspruit in November and December. The main driver behind this initiative remains the pressure of an expected reduction of pesticide MRL's on citrus fruit. Technical transfer groups in Letsitele and Hoedspruit are getting a fresh breath of air with changes in the management structures and can be validated by the increased participation by their constituent growers.

With an eye on the future, the risk of invasive citrus pests has shaped the mindset of some roleplayers to increase capacity of Citrus nursery trees. Currently this goes against the perceived trend of saturated citrus markets, yet plantings of citrus continue to increase. Soft citrus varieties are in particular demand and lemons

too. Also, vegetable farmers are investing in tree crops to diversify risk, with citrus being an attractive investment opportunity at this point in time. Citrus growth beyond our northern border is also very attractive to investors. Water resources and availability of virgin soils are likely one of the drivers besides the support of the local government to help boost the economy. Good communication between the growers and the variety focus groups is going to be crucial. And Extension will be needed in these new areas.

FCM remains a concern for many growers and has led to numerous initiatives, product development of bio-rational controls such as mating disruption and attract and kill and also the need for service delivery related to FCM monitoring and compliance with the FMS. River Bioscience is increasing their activities in the northern region, which is likely going to have a positive impact and becoming a vehicle for improved FCM control strategies.

Die 2021 uitvoer seisoen het nie so goed afgeloop in terme van opbrengs op belegging nie. Daarom sal produsente baie versigtig wees met hulle beplanning vir die toekoms en daar word baie klem gelê op die beperking van produksiekostes.

Die 2022 seisoen het afgeskop met meer suurlemoene as wat in die verlede in die noorde vroeg gepak is. Dit kan toegeskryf word aan meer hektare wat in produksie kom en die pakhuise wat belê het in ontgroeningskamers. Markte het darem positief gereageer. Maar die suurlemoene is oor die algemeen klein in die Noorde en dit wil voorkom of die totale kartonskattings tot datum laer is as verwag.

Growers were optimistic about their yields for 2022 with a hint that fruit size would be slightly on the small side. However, there was no rainfall in February and March in the Northern growing regions and this has had a major impact on fruit size which will likely be carried through to all varieties.

Very little thrips damage has been reported and mealybug pressure is generally lower than the previous season. Red-scale remains a concern for growers with sporadic outbreaks in most of the northern citrus growing regions, however, one has to consider the challenges some of the growers have with spray equipment and time it takes to complete a round of sprays on the larger production units. Fruit fly numbers are indicatively lower this season and new changes to the FMS have resulted in more enquiries from growers, which is an indication of more intensive monitoring and this will very likely be translated into better management.

However, of the many challenges the most frustrating for the growers in the Northern region is inefficiencies in the compliance systems at the start of the citrus season. This places much stress on everybody involved and the concern lies particularly with systems supporting management of phytosanitary pests. The industry cannot afford anything to slip through the cracks at such an early stage in the season.

CRI extension visited Zimbabwe as part of the CGA roadshow in March. The delegation was met by a large crowd of stakeholders in the industry. With the EU Phytosanitary measures now encompassing all Southern African citrus producing regions in the legislation, the Citrus Growers Association of Zimbabwe has sought support through the functions of the Horticultural Development Council (HDC) which plays a vital role in improving horticultural business efficiencies and competitiveness with a particular focus on exports. The number of CGA Members in the North of Zimbabwe have increased, mostly planting lemons and soft citrus with some Valencias. Support from extension was raised as a priority and much needed by the Zimbabwe growers.

#### **7.1.6 Research priority meetings**

The research needs for the various production regions for 2021-22 were determined during May 2021. The meetings with the technical committees from the respective study groups (TTGs) were chaired by the NEM, AEM (North) and AEM (Central) in the different production regions.

There is a huge overall concern regarding the availability of chemicals for effective pest and disease management, including postharvest diseases, in the near future. There is also a strong drive towards using

less chemicals and more bio-control options, based on market demands. This should be a strategic focus point for future research.

In many areas the challenge is not so much to address only research needs, but to find a suitable and effective spray program for integrated pest and disease management (IPDM) that fits the pest and disease profile of the area or production unit. The pest management has to slot into the blackspot spray program cycle, which makes it very challenging for an effective IPM program. The consequence is the need for products with a longlasting residual efficacy.

#### **7.1.7 Simposium terugvoer**

As gevolg van die feit dat die Simposium wat 2021 sou plaasvind weens Covid-regulasies weer uitgestel moes word, is 'n alternatiewe manier gebruik om terugvoer van die mees belangrike projekte van elke navorsingsprogram aan die sitrusbedryf te gee. Aanbiedinge is vooraf deur die sprekers opgeneem en op die CRI-website gelaai, vanwaar produsente dit kon aflaai en deurgaan op 'n tyd wat vir hulle geleë is. Dit is twee weke later opgevolg met 'n lewendige digitale vrae-en-antwoord sessie waartydens vrae en antwoorde hanteer is.

#### **7.1.8 CRI Geïntegreerde plaag- en siektebestuur werkswinkels**

Aanbiedinge van slegs die mees kritiese plaag en siektes is gedurende September 2021 vooraf opgeneem en op die CRI-website gelaai, vir produsente om dit op hul eie tyd deur te werk. Vrae kon op die website gelys word, waarna sprekers dit digitaal kon beantwoord. Hierdie manier van kommunikeer is beslis nie die ideale manier om voorligting effektief te doen nie. Onderwerpe moet beperk word, aanbiedinge moet heelwat verkort word en die getalle produsente wat inskakel is aansienlik minder as die bywoning van fisiese werkswinkels.

#### **7.1.9 Tegniese besoek aan Namibia**

Gedurende Oktober is 'n tegniese besoek aan Namibia gebring om hulp te verleen en inligting te verskaf oor die produksie en uitvoer van sitrus. Die voorvereiste vir hierdie besoek was dat die produsente 'n amptelike struktuur skep om samewerking met CRI/CGA en die RSA moontlik te maak. 'n Gesamentlike vergadering met die Namibia Agronomical Board (NAB) en die nuutgestigte Namibia Citrus Association (NCA) is gehou om die belangrikheid van regulering, biosekuriteit en tegniese ondersteuning vanaf CRI/CGA aan die Namibiese produsente te bespreek. Die belangrikheid om die invoer van plantmateriaal streng te reguleer en verder te verseker dat gesertifiseerde plantmateriaal gebruik word om sitrus op groot skaal in Namibia te vestig, is sterk beklemtoon. Die NCA en NAB is versoek om hul behoeftes vir tegniese- en marktoegang ondersteuning skriftelik deur te gee om te bepaal tot watter mate CRI/CGA tegniese hulp kan verleen. Geen kommunikasie in hierdie verband is nog ontvang nie.

Vergaderings met voornemende sitrusprodusente is in Mariental, Outjo en Tsumeb gehou waartydens groot fokus op biosekuriteit en die CIS geplaas is. Kultivar-opsies en die ekonomie van sitrusproduksie is ook bespreek. Die vergaderings is verbasend goed bygewoon. Die samewerking tussen die NCA en die Namibiese regering is krities belangrik as daar enigsins vordering met sitrus-ontwikkelings gemaak wil word. Die vereiste regulering moet in plek gesit word, marktoegang moet onderhandel word en infrastruktuur moet nog geskep word. Die grootste ontwikkeling waarvan gepraat word is 'n 4000 ha ontwikkeling noord-oos van Tsumeb.

Tydens 'n vergadering met 'n verteenwoordiger van die Namibia University of Science and Technology (NUST) is 'n versoek vir samewerking met CRI op navorsingsvlak gerig. Geen verdere gesprekke hieroor het intussen plaasgevind nie.

#### **7.1.10 CRI Na-oes werkswinkels**

Die na-oes werkswinkels is Gedurende Januarie en Februarie weer baie suksesvol in die ses groot produksiestreke aangebied, met rekord bywoning van meer as 1400. Daar is groot klem op veral logistieke

aangeleenthede, verkoeling, marktoegang en pakhuispraktyke geplaas. Die terugvoer was baie goed en dis duidelik dat die interaksie by hierdie werkwinkels nooit deur virtuele vergaderings vervang kan word nie.

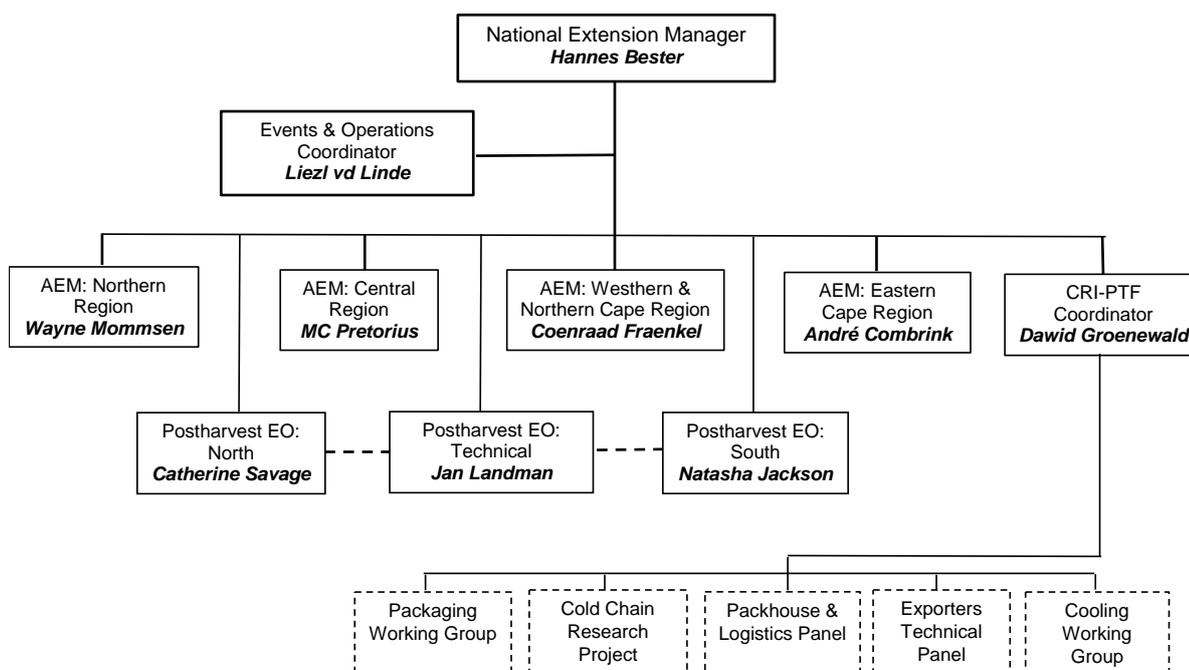
### 7.1.11 11de CRI Sitrusnavorsings Simposium

Die reëlings vir die simposium vorder uitstekend en daar is groot verwagtinge van die produsente en ander rolspelers t.o.v die navorsingsterugvoer van die afgelope vier jaar. Die simposium sal van 21-24 Augustus 2022 by Champagne Sports Resort (CSR) plaasvind. Maatskappye is gretig om te borg a.g.v die uitstekende blootstelling wat hulle in die verlede by die simposium gekry het. Bywoning van meer as 750 persone word verwag en CSR maak reg om te verseker dat hulle weereens uistaande diens sal lewer.

## 7.2 VOORLIGTING DIVISIE

Die verhoging in die statutêre heffings het dit moontlik gemaak om gedurende 2021 die kapasiteit binne die Voorligtings divisie te vergroot. Vier nuwe aanstellings is gemaak. Twee na-oes voorligters is aangestel, een wat verantwoordelikheid neem vir die pakhuise in die suide, en een wat landwyd op tegniese aangeleenthede fokus. Twee addisionele Area Voorligtingbestuurders is ook aangestel, een vir die Oos-Kaap en die ander een vir die Wes- en Noord-Kaap. Die opgedateerde organogram vir Voorligting as volg:

**Extension Organogram**



### Opsomming van aktiwiteite vir periode April – Jun 2021

Datum	Studiegroep/Aktiwiteit	Onderwerp/Aksies	Betrokkeses/ Sprekers
1 Apr	Innovation Hub Pretoria	Inligting oor papier- kombinasies vir volle reeks uitvoerkartonne	Johan Nel, Corroseal Dawid Groenewald
6 Apr	LAS/ACP Webinar – Universiteit van Florida	Beheermaatreëls: ACP, LAS. Grondgesondheid in HLB geïnfekteerde boorde	MC Pretorius Aruna Manrakhan Paul Fourie

			Internasionale kenners van die universiteit
7 Apr	1. Timac Africa 2. NSSA meeting - Zoom	1. Nuwe chemie – bemesting, vruggrootte en kleur + nuwe CBS middle – low toxic 2. NSSA kommitee agenda	MC Pretorius Timac personeel – Andries van der Walt
7 Apr	Voorligting Teams Vergadering	Simposium Besprekings en Jaarbeplanning	Voorligting Personeel
8 Apr	Staff meeting	Agenda	Voorligting personeel
	BAC meeting	Agenda	Hannes Bester Wayne Mommsen MC Pretorius
8 Apr	Hoedspruit Pakhuis besoek	Witluis infestasiemoniteering	Wayne Mommsen
9 Apr	Zoom bespreking	Grondgedraagdesiektes	MC Pretorius Jan v Niekerk
14 Apr	Zoom bespreking CIS	QMS aansoek in Oos Kaap vir ontleding/monitering van kwekery monsters	MC Pretorius Paul Fourie Jan v Niekerk Elaine Basson Jacolene Meyer Michael Nell
15 + 16 Apr	Ohrigstad studiegroep	Vergroeningsbestuur praatjie Na oes – boordpraktyke vir na oes bestuur Kultivar uitstalling Probleem boord besoeke	MC Pretorius Catherine Savage Johan Joubert
19 & 20 Apr	Grobbersdal	Moosrivier – CBS boord en boord met terugsterwing besoek. Hertzogboerdery Piet Engelbrecht	MC Pretorius
20 – 23 Apr	Brits area	Packhouse visits	Catherine Savage
21 Apr	Zoom bespreking	HLB safe sisteem vir Kwekerye	MC Pretorius Paul Fourie Solomon Gebejehu Aruna Manrakhan Tim Grout Jacolene Meyer Wayne Kirkman
21 Apr	Sappi Technology Centre	Groot probleme met beurtkrag en water en toets van kartonne erg benadeel. Pad vorentoe	Sappi se bestuur Dawid Groenewald
22 Apr	Joubert en Seuns	Boom beskerming met nete – verskillende opsies	MC Pretorius
22 Apr	Burgersfort Boordbesoeke	Tyd saam Evans Mauda Re: Witluis biologiese beheer	Wayne Mommsen Evans Mauda
23 Apr	LA Visagie	Moontlike virus afneem op Nadorcotts	MC Pretorius James Warrington

29 Apr	Landman Vars Produkte	Biosekuriteit, uithang van ACP lokvalle	Wayne Mommsen Armand Du Plessis
29 Apr	Sappi Technology Centre	BCT toetse op A11D kartonne en weeg papier	Laboratorium personeel Dawid Groenewald
30 Apr	BASF vergadering	Nuwe CBS en sistemiese insek produkte	MC Pretorius
	CSR meeting	Simposium bespreking	Hannes Bester
30 Apr	Groep 91	Besoek boorde, bespreek Navorsing Prioriteite en Letsitele Area	Wayne Mommsen Jan-Louis Pretorius Henk Van Rooyen
3 – 7 May	Fort Beaufort & Patensie	Packhouse visits	Catherine Savage
3 May	JBT: John Perold	JBT produkte	Hannes Bester
	Pieter Raath	SRCC vergadering	Hannes Bester Pieter Raath
	Burgersfort	Navorsings prioriteite	MC Pretorius
4 May	Fort Beaufort	Katrivier Navorsingsprioriteite	Hannes Bester Catherine Savage
	Ohrigstad	Navorsingsprioriteite	MC Pretorius
5 May	Addo	SRV Navorsingsprioriteite	Hannes Bester
6 May	Patensie	GRV Navorsingsprioriteite	Hannes Bester Catherine Savage
	Joubert & Vennote Zoom meeting	Werwing vir nuwe poste	Hannes Bester
	Onderberg	Navorsingsprioriteite	MC Pretorius
	Letsitele	Navorsingsprioriteite	Wayne Mommsen
7 May	Sappi Technology Centre	UltraFlute Plus voorlegging	Sappi bestuur Dawid Groenewald
7 May	Karino	CBS bespuitings proewe besoek	MC Pretorius James Warrington Chris Kellerman Tian Schutte C Kotze
10 May	Karino	Navorsingsprioriteite	MC Pretorius Catherine Savage
	Letsitele Pakhuis besoek	Witluis afkerings ondersoek	Wayne Mommsen
11 May	Ashton	Breederivier/Swellendam Navorsingsprioriteite	Hannes Bester
	Paarl	Boland/Swartland Navorsingsprioriteite	Hannes Bester
12 May	Citrusdal	Citrusdal Navorsingsprioriteite	Hannes Bester
12 May	Grobblersdal/Marble Hall	Navorsingsprioriteite	MC Pretorius Catherine Savage
13 May	Kakamas	Benede-Oranjerivier Navorsingsprioriteite	Hannes Bester
	Komati	Navorsings besoek – proefperseel bespreking	MC Pretorius Paul Cronje Johan Joubert
	Hoedspruit	Navorsingsprioriteit vergadering	Wayne Mommsen
17 May	Hartswater	Vaalharts Navorsingsprioriteite	Hannes Bester
18-21 May	Nelspruit	Postharvest EO onderhoude Een-tot-een gesprekke binne Voorligting	Onderhoude Paneel Hannes Bester Liezl vd Linde Catherine Savage

			Wayne Mommsen MC Pretorius
17 – 21 May	KZN	Packhouse visits	Catherine Savage
18 – 19 May	KZN	Navorsingsprioriteite	MC Pretorius Catherine Savage Johan Joubert
21 May	Rabek Pallets, Tzaneen	Demonstrasie van hitte- behandeling van hout en bespreking oor beskikbaarheid van hout	Allan Campbell Dawid Groenewald.
22 May	Houers Koöperatief, Letsitele	Sessie met nuwe bemarkingsbestuurder en gesprekke rondom LIDL se versoek	Wimpie Mostert Adriaan Du Buisson Dawid Groenewald
22 May	Waterberge	Navorsingsprioriteit vergadering	Wayne Mommsen
24-25 May	CSR	Simposium-bespreking	Hannes Bester
	Brits	CBS bespuitingsproewe besoek	MC Pretorius C Kellerman
25 May	Brits	Navorsingsprioriteit vergadering	Wayne Mommsen
27 May	Musina	Navorsingsprioriteit vergadering	Wayne Mommsen
31 May	NSSA zoom vergadering	NSSA sake bespreking en symposium beplanning	MC Pretorius
2 Jun	Zoom	Citrosol Webinar	Catherine Savage
2 Jun	Zoom vergadering	Artikel bespreking	MC Pretorius Mieke Daneel
	Tolwe: Wilnick Boerdery	Kwekeryboompie klagte ondersoek	Wayne Mommsen
3 Jun	Webinar	Statistiek en data ontleding met "R"	Wayne Mommsen Aruna Manrakan Evans Mauda
4 Jun	Zoom	Citrus Academy postharvest series meeting	Catherine Savage Wilma du Plooy
3 – 4 Jun	Pongola	Opening van nuwe pakhuis	MC Pretorius C Kellerman
7 Jun	Letsitele Vergadering	Noordchem Smitprogramme besprekings	Wayne Mommsen Eddie Vorster
8 Jun	SRV Zoom meeting	Bactrocera dorsalis	Hannes Bester Aruna Manrakan Vaughan Hattingh Elma Carstens
	Hoedspruit	Studiegroep: Vergroeningsbestuur Vrugte uitstalling	MC Pretorius Johan Joubert
9 Jun	Loerie: Phonnice Thalwitzer	Sitrus-aanplanting	Hannes Bester
10 Jun	CRI Zoom meeting	Communication strategy	Hannes Bester Liesl vd Linde Jon Pinker Vaughan Hattingh Other
	Groblersdal	Probleem boord besoeke en bespreking vir studiegroep vergadering; Rosle, Marble Hall sitrus	MC Pretorius

11 Jun	Research programme Zoom meeting	HLB Research programme planning	Hannes Bester MC Pretorius Wayne Mommsen Researchers
14 Jun	Stellenbosch: Pieter Raath	Bemestingskursusse bespreking	Hannes Bester Pieter Raath
15 Jun	Stellenbosch: CRI meeting	CBS research discussion	Hannes Bester MC Pretorius Researchers
	Hano Maree	HLB navorsings programme en Extension	Hannes Bester Hano Maree
	Paul Cronje	Tarl Berry / CRI-PTF rolverdeling	Hannes Bester Paul Cronje
16 Jun	Stellenbosch: Coenraad Fraenkel	Namibia produsente-besoeke en biosecurity	Hannes Bester
17 Jun	Pretoria en Evergreen Markte	Ondersoek probleme met uitvoerkartonne en palette op beide markte	Johan du Toit Subtropico Dawid Groenewald
17 Jun	Paarl: Villa – Marius Boshoff	Simposium aanbieding Chemiese produkte Villa Kortkursus	Hannes Bester
21 Jun	Karino	Vergroenings boorde besoek en monsters getrek	MC Pretorius J Warrington
22 Jun	MS Teams meeting	IPM research priority meeting	Catherine Savage Wayne Mommsen CRI entomologists
22 Jun	Vaalharts: Danie Mathewson	Boord- en pakhuisbesoeke	Hannes Bester
	1. Onderberg 2. Zoom vergadering	3. Decline boorde besoek IPM navorsings beplanning vergadering	MC Pretorius Wayne Mommsen
23 Jun	Vaalharts: Retha van Niekerk Michael van Niekerk	Boord- en pakhuisbesoeke	Hannes Bester
	Karino	4. Algemene bespreking – aanplantings en bemarking en pakhuis besoek	MC Pretorius A Muller
	Webinar	Artificial intelligence Prof. Ampetzidis	Wayne Mommsen Other CRI Staff
24 Jun	Karino	Algemene jaarvergadering	MC Pretorius
29 Jun	MS Teams meeting	Postharvest strategy meeting	Catherine Savage Wilma du Plooy CRI DM managers
29 Jun	Webinar	ESSA Conference online	Wayne Mommsen
		Entomology in SA	Other CRI Staff
30 Jun	Tom Burke	Investigate CBS interceptions	Wayne Mommsen

**Opsomming van aktiwiteite vir periode Julie – September 2021**

Datum	Studiegroep/Aktiwiteit	Onderwerp/Aksies	Betrokkeses/ Sprekers
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1 Jul	Sondagsrivier pakhuis- besoeke: SRCC: Christo Theron 2Rivers: Hennie Ehlers Johané Niemann SRCC: Meiring Bezuidenhout	Bekendstelling van Natasha Jackson	Hannes Bester Natasha Jackson
1 – 2 Jul	Groblersdal	Studiegroep beplan – Vergroenings bestuur en bemesting – uitgestel en boordbesoeke is gedoen	MC Pretorius Pieter Raath
11 Jul	Nelspruit	CIS soilborne meeting	MC Pretorius Paul Fourie Jan v Niekerk Elaine Basson Jacolene Meyer Michael Nel
12 Jul	Nelspruit	BAC meeting	MC Pretorius BAC kommitee
14 Jul	Sondagsrivier Pakhuis- forum Teams meeting	Storage mould	Natasha Jackson Catherine Savage Wilma du Plooy Hannes Bester
16 – 17 Jul	Letsitele	QMS opleiding, Mahela kwekery besoek, Produsente en boorde besoek	MC Pretorius Elaine Basson
18 Jul	Nelspruit	CGA – Teams meeting – MRL en volhoubare produksie praktyke	MC Pretorius
19 Jul	Sappi Technology Centre	Bepaling van basiese massa van Liners & Fluting	STC personeel Dawid Groenewald
19 Jul	JBT: John Perold	Opdatering oor produkte	Hannes Bester
19 Jul	Onderberg	Boordbesoeke	MC Pretorius K Grobler Melanie v d Merwe
20 Jul	Gamtoosrivier pakhuis- besoeke: CGA Direkteur: Phillip Dempsey PSB: Madeleine Ludwig Indulini: Gustav Bell en Susan Du Raan Manderyn Bdy: Warren Meyer	Bekendstelling van Natasha Jackson	Hannes Bester Natasha Jackson
	Croc Valley	CBS evaluering/ demonstrasie	MC Pretorius Providence Moyo C Kellerman J Warrington Croc Valley personeel
21 Jul	CSF Teams meeting	Agenda	Vaughan Hattingh Hannes Bester
26 Jul	Glenfair, Sake-sentrum, Pretroria	Beskikbaarheid van palette	Janus Roets Palkor

			Dawid Groenewald
29 Jul	Citrosol webinar	Na-oes Bederfbeheer	Hannes Bester
2 Aug	Simposium pre-recordings downloads	Agenda	Liezl vd Linde Hannes Bester
2 – 5 Aug	CFB Uitenhage	CFB jaarlikse audits	MC Pretorius
3 Aug	CRI-BOD meeting	Agenda	Vaughan Hattingh Tim Grout Hannes Bester Jon Pinker
4 Aug	Sappi Technology Centre	2021 Akkreditasie toets oorsig.	Craig Zorab en STC personeel Dawid Groenewald
6 Aug	Lynnwood Bridge, Pretoria	Vergadering met Houers Koöperatief. Beplanning tot einde 2021. Besikbaarheid van papier en kartonne	Wimpie Mostert - Houers Dawid Groenewald
10 – 11 Aug	Letsitele/ Weipe	Weipe ontwikkeling – besoek grondvoorbereiding en wortelgesondheids bestuur	MC Pretorius Eddie Vorster
11 Aug	Simposium live Q&A	Agenda	Liezl vd Linde Hannes Bester Sean Moore Paul Cronje Jan van Niekerk Aruna Manrakhan Pieter Raath Tarl Berry Tim Grout
12 Aug	PE: Sean Moore	IPM & DM werkswinkel beplanning	Hannes Bester Sean Moore
17 Aug	Nelspruit	BASF – nuwe produkontwikkeling bespreking	MC Pretorius Rita v d Merwe
18 Aug	Nelspruit	Na Oes beplanning tov produk effektiwiteit asook navorsings resultate afgelope seisoen	MC Pretorius Wilma du Plooy Catherine Savage
24 Aug	Kavango Sitrus Teams meeting: Paul Rossouw	Nuwe ontwikkeling in Namibia	Hannes Bester
	Alkmaar	Bemestings aanbevelings en oes resultate	MC Pretorius James Warrington
25/26 Aug	Spier Landgoed	Voorlegging by MPact Landbou Werkswinkel	Mpact Bestuur Dawid Groenewald
27 Aug 2	Irene Sake-sentrum	Premier Pallets. Ontwikkeling van plastiese palette	Tilania Fourie Dawid Groenewald
30 Aug	Nelspruit	CBS bespuitings beplanning vir 2021/22	MC Pretorius Chris Kellerman James Warrington Providence Moyo
31 Aug	Stellenbosch: Vaughan Hattingh	Een-op-een vergadering	Vaughan Hattingh Hannes Bester
	Croc Valley	Psylla net proef op nuwe aanplantings – beplan en uitleg	MC Pretorius Aruna Manrakhan Croc Valley personeel
1 Sep	Nelspruit	Jan Landman – voorgestel aan personeel, kantoorspasie uitsorteer en skryfbehoeftes ens	MC Pretorius Jan Landman

2 Sep	Karino	J Landman voorstel aan A Muller, besoek pakhuis en cultivar boord	MC Pretorius J Landman A Muller Hannes Breedt
3 Sep	Nelspruit	FRAC meeting – Croplife insake Mancozeb beskikbaarheid vir komende seisoen se CBS bespuitings	MC Pretorius
6 – 9 Sep	KZN – Nkwaleni, Carrisbrooke	Scout kursus, pakhuis en boord + produsent besoeke	MC Pretorius Wayne Mommsen Jan Landman
7 Sept 2021	CFB	CFB oudit	Hannes Bester Michael Nell Jacolene Meyer
8 Sept 2021	Farmable Teams meeting: André Kruger & Kaye Hope	Farmable app	Hannes Bester
9 Sept 2021	FBT: John Perold	Na-oes chemie en wakse	Hannes Bester Natasha Jackson
9 Sept 2021	Sappi Technology Centre	Beplanning vir 2022 akkreditasie toetse en Verpakkingswerkgroep vergadering.	STC personeel Dawid Groenewald
13 Sept 2021	Vivo: Spitskop Bdy: Louis vd Walt, Jan-Carel Kalkoven Agri: Rikus en Steven Fick Limpopo Sitrus: Pietman Pieterse Sweetspot Citrus: Mynhardt Botha	Produsente-besoeke met Wayne Mommsen en Rudolph Kruger (Inteligro)	Hannes Bester Wayne Mommsen
13 Sep	Alkmaar/ Schagen	Boordbesoeke – koueskade bespreking	MC Pretorius Johan Joubert James Warrington
14 Sept 2021	Vivo Boeredag	Scout kursus Snoei-opleiding	Wayne Mommsen Hannes Bester
14 & 15 Sep	Nelspruit	CBS bespuitingsbeplanning en Proefperseel reelings vir blaarverwyderings proef	MC Pretorius Chris Kellerman James Warrington Providence Moyo Tian Schutte
15 Sept 2021	Letsitele: Groep 91: Jaco Lindeque & Albie Kotze	Boordbesoeke	Hannes Bester Wayne Mommsen
17 Sept 2021	Nelspruit: Henri en Gert	Laptopprobleme	Hannes Bester
17 Sep	Nelspruit	CBS – CRI bespuitings beplanning en prof bespreking	MC Pretorius Vaughan Hattingh Jan v Niekerk Providence Moyo
18 – 23 Sept	Jan-Louis Pretorius & Henk van Rooyen	4Y Plan terugvoer	Hannes Bester Wayne Mommsen

18 – 23 Sep	Tulbach Wes Kaap	NSSA simposium	MC Pretorius
29 Sep	Nelspruit	Friedenheim; CBS proef evaluasie en implimentering	MC Pretorius Chris Kellerman James Warrington Providence Moyo
	Stellenbosch: Paul Cronje	Citriculture navorsing	Hannes Bester Paul Cronje
	Coenraad Fraenkel	Posbeskrywing en verantwoordelikhede	Hannes Bester Coenraad Fraenkel
	Stellenbosch: Pieter Raath, Hein Gerber, Coenraad Fraenkel	Bemestingkursus beplanning	Hannes Bester Pieter Raath Coenraad Fraenkel
7 Sep	CFB	CFB oudit	Hannes Bester Michael Nell Jacolene Meyer
8 Sep	Farmable Teams meeting: André Kruger & Kaye Hope	Farmable app	Hannes Bester
9 Sep	FBT: John Perold	Na-oes chemie en wakse	Hannes Bester Natasha Jackson
9 Sep	Sappi Technology Centre	Beplanning vir 2022 akkreditasie toetse en Verpakkingswerkgroep vergadering.	STC personeel Dawid Groenewald
13 Sep	Vivo: Spitskop Bdy: Louis vd Walt, Jan-Carel Kalkoven Agri: Rikus en Steven Fick Limpopo Sitrus: Pietman Pieterse Sweetspot Citrus: Mynhardt Botha	Produsente-besoeke met Wayne Mommsen en Rudolph Kruger (Inteligro)	Hannes Bester Wayne Mommsen
14 Sep	Vivo Boeredag	Scout kursus Snoei-opleiding	Wayne Mommsen Hannes Bester
15 Sep	Letsitele: Groep 91: Jaco Lindeque & Albie Kotze	Boordbesoeke	Hannes Bester Wayne Mommsen
	Jan-Louis Pretorius & Henk van Rooyen	4Y Plan terugvoer	Hannes Bester Wayne Mommsen
16 Sep	Letsitele: Laeveld Sitrus: Ben Vorster	4Y Plan terugvoer	Hannes Bester Wayne Mommsen
	Mahela Bdy: Clive Lacock & Eddie Vorster	Boordbesoeke	Hannes Bester Wayne Mommsen
	Gubitz Bdy en RB meeting: Sean Thackeray, Richard Schulze (Wenkem), Ken Gamble, Luan Botha	FCM-beheer	Hannes Bester Wayne Mommsen
	Mahela Bdy: Berend Vorster	4Y Plan terugvoer	Hannes Bester Wayne Mommsen
	Mahela Bdy en RB meeting: Sean Thackeray, Ken Gamble, Carl Fourie, Clive Lacock, Richard Schulze	FCM-beheer	Hannes Bester Wayne Mommsen

	Houers: Wimpie Mostert	Verpakkings-aangeleenthede	Hannes Bester
17 Sep	Nelspruit: Henri en Gert	Laptopprobleme	Hannes Bester
20 Sep	Nelspruit: Jan Landman	Posbeskrywing en verantwoordelikhede	Hannes Bester Jan Landman
	Johan Joubert	Laeveld Sitrus cultivars	Hannes Bester Johan Joubert
	Jan Landman en Catherine Savage	Na-oes voorligting beplanning	Hannes Bester Jan Landman Catherine Savage
21 Sep	Jan Landman en Catherine Savage	Kwaliteitsbeheer boord to pakhuis	Hannes Bester Jan Landman Catherine Savage
	Liezl vd Linde	Beplanning en een-op-een bespreking	Hannes Bester Liezl vd Linde
	Catherine Savage	Een-op-een bespreking	Hannes Bester Catherine Savage
22 Sep	Coruseal Karton-aanleg en koelkamers, Springs	Besigting van hulle nuwe karton-aanleg en koelkamers	Johan Nel Bentley Daniel André Cuturi - Corruseal Dawid Groenewald
28-29 Sep	Addo	Bemestingskursus	Hannes Bester Pieter Raath Vivian Whyte Hein Gerber Coenraad Fraenkel
30 Sep – 1 Oct	Simondium	Bemestingskursus	Hannes Bester Pieter Raath Vivian Whyte Hein Gerber Coenraad Fraenkel

**Opsomming van aktiwiteite vir periode Oktober – Desember 2021**

Datum	Studiegroep/Aktiwiteit	Onderwerp/Aksies	Betrokkeses/ Sprekers
1 Okt	Simondium	Bemestingskursus	Pieter Raath Vivian Whyte Hein Gerber Coenraad Fraenkel Hannes Bester
1 Okt	Burgersfort - IPM	IPM Witluse eierlegging in grond	Wayne Mommsen
5 Okt	Tzaneen Vergadering	Hygrotech: CBS Bestuur	Wayne Mommsen Charl Kotze
5 Okt	Kavango Sitrus vergadering: Paul Rossouw en Annemarie McDonald	Sitrusprojek in Namibia	Hannes Bester
4 Okt	Robertson/Ashton Packhouses	Packhouse visits	Natasha Jackson
	Nelspruit	FarmAg bekendstelling en vergadering	MC Pretorius Tim Grout Paul du Randt

5 Okt	Swellendam Packhouses	Packhouse Visits	Natasha Jackson Jan Landman Coenraad Fraenkel
	Nelspruit	Friedenheim – CBS blaarverwyderings proefperseel besoek	MC Pretorius Chris Kellerman Providence Moyo
6 Okt	CSF meeting	Agenda	Natasha Jackson Jan Landman
6 Okt	CSF meeting	Agenda	Hannes Bester Catherine Savage
6 Okt	Nelspruit	Teams: Soilborne Disease Interest Group meeting CGA – sustainable agriculture	MC Pretorius
7 Okt	Simondium/Franschoek Packhouses	Packhouse Visits	Natasha Jackson Jan Landman Coenraad Fraenkel
8 Okt	Citrusdal/Clanwilliam Packhouses	Packhouse Visits	Natasha Jackson Coenraad Fraenkel
8 Okt	Witrivier	Ezigrow – kwekery media verskaffer besoek – patogeen strategie bespreek	MC Pretorius Nico van Wyk Tammy Marsberg
11 Okt	Kavango Sitrus: Paul Rossouw	Sitrusprojek	Hannes Bester
11 – 14 Oct	Packhouse visits	Weipe/Tshipise	Catherine Savage Jan Landman
12 Okt	Letsitele Junction	Studiegroep	Wayne Mommsen Sean Thackeray
12 Okt	NUST: Solomon Mbai	Cooperation between NUST and CRI	Hannes Bester
	CRI, NAB en NCA meeting	Vestiging en uitvoer van sitrus in Namibia	Hannes Bester Wayne Kirkman Coenraad Fraenkel
13 Okt	Sappi Technology Centre	Akkreditasie toetse vergadering/BCT waardes	Laboratorium personeel Dawid Groenewald
	Nelspruit	Disease Management Navorsings kommitee	MC Pretorius Tim Grout Jan v Niekerk
13 Okt	Mariental Produsente- vergadering	Biosekuriteit CIS Ekonomie Kultivars	Hannes Bester Wayne Kirkman Coenraad Fraenkel
13 Okt	Komati - Croc Valley	CBS bespuitings vergadering en boord besoeke	MC Pretorius Karlien Grobler Henry Cawood
13 Okt	Tzaneen Kantoor Online	IPM Research Comittee Meeting	Wayne Mommsen Sean Moore Tim Grout
13 Okt	Citrii introduction meeting	Stem-end mould, current postharvest research	Catherine Savage Natasha Jackson Jan Landman
14 Okt	Outjo Produsente- vergadering	Biosekuriteit CIS Ekonomie	Hannes Bester Wayne Kirkman Coenraad Fraenkel

		Kultivars	
	Tsumeb vergadering	Produsente- Biosekuriteit CIS Ekonomie Kultivars	Hannes Bester Wayne Kirkman Coenraad Fraenkel
14 Okt	Visit XSIT in PE	Introduction and visit	Natasha Jackson Andre Combrink
	Patensie	Meeting with Phillip Dempsey	Natasha Jackson André Combrink
15 Okt	Groblersdal	Produsent besoeke	MC Pretorius P Nel P Engelbrecht J Gouws
15 Oct	Northern Packhouse Forum	Region Agenda	Catherine Savage Wayne Mommsen Jan Landman
15 Okt	Outjo: Roelie van Wyk Gerhard Burger	Boordbesoeke	Hannes Bester
18-20 Okt	Kat Rivier Vallei	Produsent Besoeke	André Combrink Jannie de Villiers Dassie de Villiers Barry Mildenhall Jock Danckwerts Chris de Wit Shaun Brown Errol Hewson
20 Okt	Kakamas: Karsten Bdy	Boordbesoeke	Hannes Bester
20 Okt	Tzaneen Kantoor Online SUPPLANT	Teams meeting Water monitoring in Orchards	Wayne Mommsen Jan Van Der Merwe
21 Okt	Kakamas: Zwartbooisberg	Boordbesoeke	Hannes Bester Coenraad Fraenkel
	Nelspruit	PALS – vergadering op uitnodiging bygewoon	MC Pretorius Milan Talwitzer Marinus Neetling
22 Okt	Nelspruit	Vergadering CBS en PALS terugvoer Karino – Viroied besmetting op Nadorcot ondersoek	MC Pretorius Vaughan Hattingh Glynnis Cook Kobus Breytenbach Chanel Steyn
25 Okt	CRI Nelspruit	Witrivier Viroloog besoek CRI – organiese sitrusaanplanting beplanning vir medisinale waarde; bespreking	MC Pretorius Dr Ahmad Haeri Mazanderani
25 – 27 Okt	Swadini	CRI Bemestings Kursus	Wayne Mommsen Pieter Raath Vivian Wright Coenraad Fraenkel
26 Okt	FMS Working Group	Changes to the FMS for 2022	Vaughan Hattingh Elma Carsten Sean Moore Hannes Bester Paul Cronje Tarl Berry

	FNB Teams meeting	Bedryfs-inligting	Hannes Bester
	Nelspruit	Produsente besoek: Friedenheim, Rayton	MC Pretorius Flip Walters W Blom
27 – 29 Okt	Groblersdal	CRI Bemestings Kursus	MC Pretorius Pieter Raath Vivian White Coenraad Fraenkel
27 Okt	XSIT/RB	Navorsingsproef	Hannes Bester André Combrink
28 Oct	Nelspruit Packhouse Forum	Agenda	Catherine Savage Jan Landman Wilma du Plooy
1 -3 Nov	KZN packhouses	Packhouse visits and one-on-one time with Dole	Catherine Savage Jan Landman
2 Nov	Nelspruit	Teams: CIS soilborne vergadering	MC Pretorius Paul Fourie Elaine Basson Jacolene Meyer Michael Nel
2 Nov	Tshipise Region	Bied Scout Opleiding	Wayne Mommsen
3 Nov	PE	IPM Planning Meeting	Wayne Mommsen Sean Moore
3 Nov	Vaughan Hattingh	Een-op-een sessie	Hannes Bester Vaughan Hattingh
	Packaging Working Group meeting	Nuwe A15C S2 karton	Dawid Groenewald Hannes Bester Tarl Berry Paul Cronje
	Paul Cronje	Citriculture navorsing	Hannes Bester Paul Cronje
	CGA-GDC Peddie Inligtingsdag	Aanbieding: Blaaspootjie, Witluis, Rooi Dopluis	André Combrink Tamryn Marsberg
	Tarl Berry	Nuwe A15C karton	Hannes Bester Tarl Berry
4 Nov	ETP meeting	Agenda	Hannes Bester Catherine Savage Jan Landman Natasha Jackson Vaughan Hattingh Tarl Berry Wilma du Plooy Paul Cronje
	Steve Turner	Tegniese aangeleenthede	Hannes Bester
	Kat Rivier Vallei	Produsent Besoeke	André Combrink Isabel Sparks
	George Hall: Unipack	Bespreek nuwe A15C karton	Hannes Bester
5 Nov	Houers: Wimpie Mostert	Tegniese- en pakhuisprobleme met nuwe A15C karton	Hannes Bester
5 Nov	Swellendam Packhouse Forum	First meeting of the forum	Natasha Jackson
9 Nov	CRI Na-oes werksinkels Beplanningsvergadering	Agenda	Hannes Bester Dawid Groenewald

			MC Pretorius Catherine Savage Natasha Jackson Jan Landman Liezl vd Linde Paul Cronje Jan van Niekerk Wayne Mommsen Tarl Berry Wilma du Plooy
10 Nov	Voorligting Beplannings- vergadering	Agenda	Hannes Bester MC Pretorius Wayne Mommsen Coenraad Fraenkel André Combrink Liezl vd Linde Natasha Jackson Catherine Savage Jan Landman
11 Nov	Glenfair	Vergadering oor die nuwe A15C-S2 karton	Tarl Berry Dawid Groenewald
11 Nov	Katrivier	CBS ondersoek en besoek aan Jock Dankwarts	MC Pretorius Andre Combrink Jock Danckwerts
11-12 Nov	Letsitele Du Roi Nursery Hoedspruit Kwekery	Nursery Audits	Wayne Mommsen Michael Nel Paul Fourie
12 Nov	Technical Working Group meeting	New A15C carton die- drawing	Tarl Berry Dawid Groenewald Hannes Bester
	Agrigate One: Greg Whitaker	Supply chain issues	Hannes Bester
15 Nov	Technical Working Group meeting	New A15C carton die- drawing	Tarl Berry Dawid Groenewald Hannes Bester
15 Nov	Citrii meeting	Stem-end mould	Catherine Savage Natasha Jackson Wilma du Plooy Steve Turner
16 Nov	CRI Board meeting	Agenda	Vaughan Hattingh Hannes Bester Jon Pinker Tim Grout
16 Nov	CGA-GDC Patensie	Aanbieding: Plaagbeheer	André Combrink Zamazima Njili
16 Nov	Weipe	Bied Scout Opleiding	Wayne Mommsen
	Malelane	CIS Kwekery oudit ; Ngwenja en Komati kwekerye	MC Pretorius Paul Fourie Michael Nel
16 – 18 Nov	Global Citrus Congress	Online - agenda	Catherine Savage Natasha Jackson Coenraad Fraenkel
17 Nov	Nelspruit	CIS Kwekery oudit Montana Kwekery	MC Pretorius Paul Fourie

			Michael Nel
18 Nov	Technical Working Group meeting	New A15C carton die-drawing	Tarl Berry Dawid Groenewald Hannes Bester
18 Nov	CGA Board meeting	Agenda	Vaughan Hattingh Hannes Bester Tim Grout
18 Nov	Patensie, Aerobotics informasie dag	Aanbieding: belangrikheid van esproeiings en boord eenvormigheid	André Combrink
18 Nov	DALRRD Citrus Coordinating meeting	Agenda	MC Pretorius Catherine Savage Natasha Jackson
	Karino	Jaareind terugvoer	MC Pretorius
19 Nov	Nelspruit	CBS bespreking en beplanning	MC Pretorius Tian Schutte
22 Nov	Sondagsrivier Packhouse Forum Meeting	Agenda	Natasha Jackson
	Dalrus Packhouse visit	New packouse Boertjie Nel	Natasha Jackson
22-26 Nov	SRV, Gamtoos Vallei	CIS Kwekery Audits	André Combrink Paul Fourie Michael Nel
23 Nov	DALRRD Roadshow	Agenda	Hannes Bester
23 Nov	Innovation Hub	Vergadering met Palkor 'n paletvervaardiger	Janus Roets Dawid Groenewald
	Sudwala	Boordbesoek – nuwe boord aanplanting	MC Pretorius Justin Swart
24 Nov	Vaughan Hattingh	One-on-one meeting	Hannes Bester
24 Nov	MooiNooi CGA –GDC Farmers Day	FCM Control	Wayne Mommsen Jacomien De Klerk Andrew Mbedzi Melton Mulaudzi
24 Nov	Patensie Citrus	Agenda	Natasha Jackson
	Patensie	First packhouse forum meeting	Natasha Jackson André Combrink
25 Nov 2021	DALRRD Inland Regional Roadshow Karino	Agenda Valley View – boord besoek	MC Pretorius Catherine Savage
25 Nov	Wouter Schreuder Jnr	Meeting Port Elizabeth	Natasha Jackson
26 Nov	Karino	Studiegroepvergadering bespreking	MC Pretorius Hannes Breedt
26 Nov	Hishtil Nursery Mooketsi	CIS Nursery requirements	Wayne Mommsen
29 Nov	Nelspruit	Corteva en PVR Landboudiense – nematode middel en proef bespreking	MC Pretorius Jean de Waal Pieter J v Rensburg
	Namibië-ontwikkeling Kombat	Sitrus Ontwikkeling bespreking Kombat- Namibië	Coenraad Fraenkel
30 Nov	Kwekery Oudit	Clanwilliam / Citrusdal kwekerye oudit	Paul Fourie Michael Nel Coenraad Fraenkel
1 Des	Hennie Ehlers	Bedryfs-aangeleenthere	Hannes Bester
3 Des	Nelspruit	CBS bespreking vir boord oudits; CRI en DALRRD	MC Pretorius Elma Carstens Tankiso Mpholo

			Vaughan Hatting
6 Dec	Sitrus Konsultante Forum	Vergadering met verskeie onafhanklike sitrus konsultante in Wes Kaap	Pieter Raath Coenraad Fraenkel Hein Gerber Jacques Crous Ockert Botha
6 Des	SRV	Produsent Besoeke	André Combrink Willem Bouwer Wium Bouwer
6 Des	CRI Nelspruit Croc Valley Citrus	Bied Scout opleiding	Wayne Mommsen MC Pretorius
7 Des	BAC meeting	Agenda	BAC members
8 Des	Hennie Ehlers	Substandaard kartonne	Dawid Groenewald Hannes Bester Natasha Jackson
8 Des	Katrivier	Riverside boomsteftes en uitteruitgang ondersoek	André Combrink Errol Hewson DC Botha
8 – 9 Dec	eSwatini Packhouse	Packhouse visit and discussions	Catherine Savage Wilma du Plooy
8 Des	Groblersdal	IPM kursus	MC Pretorius Wayne Momsen Sean Moore
9 Des	Sappi Technology Centre	Vergadering om hulle in te lig oor die nuwe A15C-S2 karton	Donald Nonyane, Brian Percival Dawid Groenewald
9 Des	Nelspruit	Studiegroepvergadering IPM kursus	MC Pretorius Sean Moore Wayne Mommsen Aruna Manrakhan Johan Joubert
9 Dec	Citrii Meeting	Stem end mould	Natasha Jackson
10 Des	SRV	Produsent Besoek	André Combrink Hennie Ehlers
10 Dec	Stellenbosch	Besoek van Namibië delegasie rakend sitrus ontwikkeling in Katima, Namibië	Joshua de Gouveia Ivo de Gouveia Coenraad Fraenkel Earle vd Watt
13 Des	Phillip Dempsey	Pakhuisklagtes oor nuwe A15C karton	Hannes Bester
14 Dec	Extension meeting	Agenda	Hannes Bester MC Pretorius Wayne Mommsen Coenraad Fraenkel André Combrink Liezl vd Linde Natasha Jackson Catherine Savage Jan Landman
17 Dec	Working group citrus peduncles	Meeting with European regarding long stems	Natasha Jackson Elma Carstens Glynnis Cook
20 Des	Sappi Technology Centre	Toets van die 1ste A15C -S2 kartonne	Ronnie Bothma Dawid Groenewald

21 Des	Aanlyn vergadering	Vergadering met Farmable oor wat die platform behels vir produsente	Kaye Hope Coenraad Fraenkel
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**Opsomming van aktiwiteite vir periode Januarie - Maart 2022  
(Hannes Bester)**

<b>Datum</b>	<b>Studiegroep/Aktiwiteit</b>	<b>Onderwerp/Aksies</b>	<b>Betrokkenes/ Sprekers</b>
12 Jan	Winfield United: Marius Boshoff	Vergadering	Hannes Bester
17 Jan	Voorligting vergadering	Beplanning	Extension Div
25-26 Jan	Limpopo 1 CRI Na-oes werkwinkel	Agenda	Hannes Bester Elma Carsten Tarl Berry Dawid Groenewald Paul Cronje Natasha Jackson Wilma du Plooy Lindo Mamba Meagan van Dyk Jan Landman Liezl vd Linde
27-28 Jan	Limpopo 2 CRI Na-oes werkwinkel	Agenda	MC Pretorius Hannes Bester Elma Carsten Tarl Berry Dawid Groenewald Paul Cronje Natasha Jackson Wilma du Plooy Lindo Mamba Meagan van Dyk Jan Landman Liezl vd Linde
1 Feb	James Warrington	Tegniese bespreking	Hannes Bester
2 Feb	Onderberg: Radley Landgoed Greyvan Bdy	Boordbesoeke met Chris Kellerman	Hannes Bester
3-4 Feb	Mpumalanga CRI Na-oes werkwinkel	Agenda	MC Pretorius Hannes Bester Elma Carsten Tarl Berry Dawid Groenewald Paul Cronje Natasha Jackson Wilma du Plooy Lindo Mamba Meagan van Dyk Jan Landman Liezl vd Linde Catherine Savage
7 Feb	Voorligting beplannings-vergadering	Agenda	Voorligting Divisie
8-9 Feb	Manco meeting	Agenda	Manco

15-16 Feb	Oos-Kaap werkswinkel	CRI	Na-oes	Agenda	André Combrink Hannes Bester Elma Carsten Tarl Berry Dawid Groenewald Paul Cronje Natasha Jackson Wilma du Plooy Lindo Mamba Meagan van Dyk Jan Landman Liezl vd Linde Catherine Savage
17-18 Feb	Wes-Kaap werkswinkel	CRI	Na-oes		Hannes Bester Elma Carsten Tarl Berry Dawid Groenewald Paul Cronje Natasha Jackson Wilma du Plooy Lindo Mamba Meagan van Dyk Jan Landman Liezl vd Linde Catherine Savage Coenraad Fraenkel
21 Feb	Humansdorp: Hoërskool Vergadering: Phillip Dempsey Christo Joubert Henko Smit	Nico	Malan	Landbou kursusse	Hannes Bester
22 Feb	Patensie: Phillip Dempsey			Beplan studiegroep vergadering	Hannes Bester André Combrink
	Patensie: CGA Roadshow			Agenda	Hannes Bester André Combrink Vaughan Hattingh
28 Feb	Bayer Teams meeting			Simposium	Hannes Bester Liezl vd Linde
	Teams meetings			Performance appraisals	Wayne Mommsen Liezl vd Linde Catherine Savage Hannes Bester
1 Mar	PE			Performance appraisals	Jan Landman Natasha Jackson André Combrink Hannes Bester
2 Mar	Teams meeting			Performance appraisals	Dawid Groenewald Hannes Bester
	Een-op-een vergadering			Performance appraisals	Coenraad Fraenkel Hannes Bester
4 Mar	Teams meeting			Performance appraisals	MC Pretorius Hannes Bester
10 Mar	CSR meeting			Simposium beplanning	Hannes Bester MC Pretorius

			Liezl vd Linde
14 Mar	Plaasbesoek	CFB: ondersoek vir grondaankope	Hannes Bester Paul Fourie
	Teams meeting: APL	Nuwe A15C karton	Hannes Bester Dawid Groenewald Tarl Berry
15 Mar	Teams meeting: KingPrice	Simposium borgskap	Hannes Bester Liesl vd Linde
22 Mar	Teams meeting: CGA	Nuwe A15C karton	Vaughan Hattingh Dawid Groenewald Elma Carsten Tarl Berry Paul Cronje Hannes Bester
23 Mar	CMF meeting: Zoom	Agenda	Hannes Bester
23-25 Mar	Grahamstad	RBX strategiese sessie	Hannes Bester Sean Moore André Combrink Wayne Kirkman

**Opsomming van aktiwiteite vir periode Januarie tot Maart 2022.  
(Dawid Groenewald)**

<b>Datum</b>	<b>Studiegroep/Aktiwiteit</b>	<b>Onderwerp/Aksies</b>	<b>Betrokkeses/Sprekers</b>
19 Jan	Sappi Technology Centre (STC)	Toets van kartonne en finalisering van 2022 skedule	STC personeel Dawid Groenewald
25 & 26 Jan	CRI Na-oes werkswinkel Limpopo 1	CRI Na-oes werkswinkel	Produsente, & personeel CRI personeel. Doen voorlegging oor Verpakking
27 & 28 Jan	CRI Na-oes werkswinkel Limpopo 2	Soos bo	Soos bo
1 & 2 Feb	CRI Na-oes werkswinkel Swaziland/KZN	Soos bo	Soos bo
3 & 4 Feb	CRI Na-oes werkswinkel Mpumalanga	Soos bo	Soos bo
15 & 16 Feb	CRI Na-oes werkswinkel Oos Kaap	Soos bo	Soos bo
17 & 18 Feb	CRI Na-oes werkswinkel Wes Kaap	Soos bo	Soos bo
3 Mar	Glenfair	AgriEnterprises Gebruik van herwinbare plastiek in die sitrusbedryf	Craig Murrell Dawid Groenewald
7 Mar	Glenfair	Meeting with CEO of Corroseal to discuss their Triple Wall Board cartons	Rajiv Mehta Dawid Groenewald
8 Mar	Sappi Technology Centre	Pre-season meeting and feedback from Sappi	Sappi Management Dawid Groenewald

25 Mar	Laeveld Agrochem Pretoria	1ste CRI Simposium Gholfdag beplannings vergadering	Chris Thompson MC Pretorius Wayne Mommsen Dawid Groenewald
30 Mar	Lynnwood Bridge	Vergadering met Everest Packaging. Vervaardiging van nuwe A15C kartonne en toets van kartonne	Stuart Esterhuysen Deon Zwanepoel Dawid Groenewald

**Opsomming van aktiwiteite vir periode Januarie – Maart 2022  
(Wayne Mommsen)**

Datum	Studiegroep/Aktiwiteit	Onderwerp/Aksies	Betrokkenes/ Sprekers
3 - 4 Jan	Plaasbesoeke: Komati Sitrus en Gubitz	CRI Navorsing Witluis Spesies ID	Wayne Mommsen
13 Jan	Produsente Besoeke Hoedspruit	Evaluasie van Impak van FMS verandering	Wayne Mommsen Tom van der Meulen
17 Jan	Produsente Besoeke Tshipise: Alicedale	Evaluasie van Impak van FMS verandering	Wayne Mommsen
31 Jan	Plaasbesoeke: Nkwaleni	Voorligting: Witluis monitering vir Korea Mark	Wayne Mommsen
1 Feb	CRI werksinkels Natal	Umhlanga, Na oes werksinkel	CRI Voorligting Span
3 Feb	Constantia Studiegroep	Vrugtevlieg aanbieding	Wayne Mommsen Aruna Manrakhan
7 Feb	Nelspruit Vergadering	Voorligting beplanning	Wayne Mommsen Hannes Bester Andre Combrink Natasha Jackson Jan Landman
8 – 9 Feb	Nelspruit Vergadering	MANCO meeting	Andre Combrink Christine Stoppel Grove Coenraad Fraenkel Hannes Bester Jan Van Niekerk Paul Cronje Paul Fourie Sean Moore Solomon Gebeyehu Tim Grout Vaughan Hattingh Jon Pinker Wayne Mommsen
10 Feb	Nelspruit: Web online	Citrus sustainability Forum Meeting	Wayne Mommsen
16 Feb	Groblersdal Studiegroep	Ondersteuning van NTE data op Anagyrus	Wayne Mommsen
17 Feb	Marble Hall: Opleiding	Spuut operateurs opleiding en Monitering van Phyto plae FCM en VV.	Wayne Mommsen Charl Kotze
1-4 Mar	CGA Roadshow	CRI en CGA aanbiedings	Wayne Mommsen Sean Moore

			Justin Chadwick Mitchell Brooke Jacomien De Klerk S Sean Thackeray Lukhanyo Nkombisa
8 Mar	Constantia Studiegroep Letsitele	FCM en FMS aanbieding	Wayne Mommsen
9 Mar	Hoedspruit plaasbesoek	Moriah Citrus estate Vrugval ondersoek	Wayne Mommsen
16-17 Mar	Plaasbesoeke Vaalharts	IPM en Bemesting Voorligting	Wayne Mommsen Coenraad Fraenkel
24-25 Mar	Plaasbesoeke Letsitele	IPM en siektebeheer voorligting	Wayne Mommsen MC Pretorius
25 Mar	Meeting Online	Symposium Golfdag Beplanning	Dawid Groenewald Chris Thompson Wayne Mommsen MC Pretorius
30-31 Mar	Letsitele Produsente Vergadering/Online Hybrid	Mahela Seisoen Nabetragting	Wayne Mommsen MC Pretorius Eddie Vorster

**Opsomming van aktiwiteite vir periode Januarie – Maart 2022  
(MC Pretorius)**

<b>Datum</b>	<b>Studiegroep/Aktiwiteit</b>	<b>Onderwerp/Aksies</b>	<b>Betrokkes/ Sprekers</b>
11 Jan	Nelspruit	CBS bespreking en bespuitingsbeplanning weens baie reën	MC Pretorius Chris Kellerman James Warrington
12 Jan	Schoemanskloof	Alternaria uitbraak – Joubert en seuns	MC Pretorius Joubert en Seuns personeel
13 Jan	Karino	Alternaria opname op Karino Koop plaas	MC Pretorius Hannes Breedt
14 Jan	Nelspruit	Alternaria opname – Karino + Halls	Mc Pretorius Hannes Breedt Indigo; Adrian Serfontein Tiaan Snyman
19 Jan	Nelspruit	Studiegroep beplanning – Nelspruit	MC Pretorius Tiaan snyman
20 -21 Jan	Nelspruit	Aankope en laai van werkswinkel benodighede in voertuie	MC Pretorius Liezl v d Linde Jan Landman
24 – 26 Jan	Letsitele	Na Oes werkswinkel	MC Pretorius Hannes Bester Liezl v d Linde Jan Landman CRI Na Oes personeel
27 – 28 Jan	Groblersdal	Na Oes werkswinkel	MC Pretorius Hannes Bester Liezl v d Linde Jan Landman CRI Na Oes personeel
31 Jan - 1 – 2 Feb	KZN	Na Oes werkswinkel	MC Pretorius Hannes Bester

			Liezl v d Linde Jan Landman CRI Na Oes personeel
3 – 4 Feb	Nelspruit	Na Oes werkswinkel	MC Pretorius Hannes Bester Liezl v d Linde Jan Landman Andre Combrink Catherine Savage CRI Na Oes personeel
10 Feb	Karino	Kultivar vergadering	MC Pretorius Hannes Breedt Paul Cronje
16 – 18 Feb	Wes Kaap	US Prof Adele McLoed besoek Na Oes werkswinkel	MC Pretorius A McLoed Hannes Bester Coenraad Fraenkel Liezl v d Linde Na Oes Personeel
23 – 25 Feb	Letsitele/ Weipe	Besoek nuwe aanplantings 140 ha suurlemoen in Weipe	MC Pretorius Eddie Vorster en Mahela personeel
28 Feb	Groblersdal	CGA Road show	MC Pretorius Vaughan Hattingh CGA personeel
1 Mar	Onderberg/Swaziland	1. CGA Road show  2. Phytrisk meeting	MC Pretorius Vaughan Hattingh CGA personeel  Paul Fourie Jan v Niekerk Jaquie vd Waals Providence Moyo
2 Mar	Nelspruit	CGA Road show	MC Pretorius Vaughan Hatting CGA personeel
8 Mar	Nelspruit	Begrafnis – C Kellerman se vrou	MC Pretorius Chris Kellerman
9 – 11 Mar	Champagne Sports Resort	Simposium beplanning en koördinerings	MC Pretorius Hannes Bester Liezl vd Linde
15 Mar	Nelspruit	Indigo bespreking - alternaria	MC Pretorius Adrian Serfontein Tian Schutte Tiaan Snyman
16 – 18 Mar	KZN	CGA roadshow	MC Pretorius CGA personeel
23 – 25 Mar	Letsitele	Probleem boordbesoeke Letsitele kwekery	MC Pretorius Wayne Mommsen
31 Mar	Nelspruit	Studiegroep: Wortelgesondheid, FCM en Vrugtevlieg beheerstrategie	MC Pretorius Aruna Manrakhan Tiaan Snyman

**Opsomming van aktiwiteite vir periode Januarie – Maart 2022 (Coenraad Fraenkel)**

<b>Datum</b>	<b>Studiegroep/Aktiwiteit</b>	<b>Onderwerp/Aksies</b>	<b>Betrokkenes/ Sprekers</b>
11 Jan	Voorligtings materiaal	Vervaardig van Youtube videos (Grond en blaarmonsterneming)	Vivian Whyte Coenraad Fraenkel Hannes Bester
13 Jan	Besproeiings webinaar	Voorligtings kommunikasie	Coenraad Fraenkel
13 Jan	Kwekery en boord ondersoek	Besoek onderpresterende jongboorde – Dasberg (Riviersonderend)	Coenraad Fraenkel Pieter Raath Jan van Niekerk
17 Jan	CRI Symposium vergadering	Eerste beplanning sessie CRI Simposium	MC Pretorius Coenraad Fraenkel Hannes Bester Wayne Mommsen Natasha Jackson Catherine Savage Jan Landman Dawid Groenewald Liezl van der linde
25 Jan	Boord Besoeke	Swartland (ACG, Landau, Loreley, Rooihogte)	Coenraad Fraenkel
26 Jan	Boord Besoeke	Olifantsrivier (Mouton Citrus, Dirkie Mouton, Suiderland)	Coenraad Fraenkel
27 Jan	Boord Besoeke	Overberg (Noordhoek, Suiderland, Dasberg)	Coenraad Fraenkel
7 Feb	Voorligting vergadering	Jaar beplanning	MC Pretorius Coenraad Fraenkel Hannes Bester Wayne Mommsen Natasha Jackson Catherine Savage Jan Landman Dawid Groenewald Liezl van der linde
8 Feb	CRI Bestuur vergadering	Jaar beplanning	CRI bestuur span
9 Feb	CRI Bestuur vergadering	Jaar beplanning	CRI bestuur span
10 Feb	Citrus Sustainability Forum	Aanlyn	Coenraad Fraenkel CGA verteenwoordigers
17 Feb	Na Oes Werkswinkel (Wes Kaap)	Paarl	CRI en CGA verteenwoordigers
18 Feb	Na Oes Werkswinkel (Wes Kaap)	Paarl	CRI en CGA verteenwoordigers
21 Feb	CGA Roadshow - Boland	Ashton	Coenraad Fraenkel Sean Moore CGA verteenwoordigers
22 Feb	CGA Roadshow - Olifantsrivier	Citrusdal	Coenraad Fraenkel Sean Moore CGA verteenwoordigers
24 Feb	M.Sc Verdedigings	Stellenbosch Universiteit - 3 M.Sc (CRI befonds) studies terugvoer	Pieter Raath Paul Cronje Coenraad Fraenkel Vivian White

			Hein Gerber
25 Feb	Wes Kaap Sitrus Konsultante forum	Aanlyn – Bespreking oor huidige produksie kwessies in die Wes Kaap	Coenraad Fraenkel Hein Gerber Luan Le Roux Du Toit Prins Ockert Botha Jacques Crous
28 Feb	Outjo Variteit Bespreking	Aanlyn – Bespreking oor variteit opsies vir sitrus ontwikkeling – Dr Burger	Coenraad Fraenkel Johan Joubert
2 Mar	KPA Review	Jeffreysbaai	Coenraad Fraenkel Hannes Bester
3 Mar	Humefert vergadering	Stellenbosch – Bespreking oor humefert en H2i produkte vir sitrus bedryf	Coenraad Fraenkel Jan Du Toit Cornel Broeksma Karen vd Westhuizen
10 Mar	EOS Namibie vergadering	Ondersoek na vestiging van kwekery en lab in Namibie)	Coenraad Fraenkel Charles Cherry Frederico van Wyk
14 Mar	Eureka Climate Smart vergadering	Klimaat projek gefokus op verbetering van weerdata in die Wes Kaap	Ebené Oranje Coenraad Fraenkel
15 Mar	Citrusdal Pakhuis besoek	Aanvang van nuwe pak seisoen besoek	Natasha Jackson Jan Landman Coenraad Fraenkel
16/17 Mar	Vaalharts besoek Danie Mathewson Michael v Niekerk	IPM strategie en produksie riglyne	Coenraad Fraenkel Wayne Mommsen
23/24 Mar	Patensie Studiegroep vergadering	Bestuur van brak grond en water inligting sessie	Coenraad Fraenkel Andre Combrink
23/24 Mar	Besoek aan Citrus Grondvesblok	Leiding rondom grondbestuur en voedingbestuur	Coenraad Fraenkel Jacolene Meyer Mpaballeng Sam
29 Mar	Aerobotics Vergadering	Leiding oor grootste kwessie in sitrus verbouing.	Coenraad Fraenkel Aerobotics verteenwoordigers

**Opsomming van aktiwiteite vir periode Januarie – Maart 2022  
(Andre Combrink)**

<b>Datum</b>	<b>Studiegroep/Aktiwiteit</b>	<b>Onderwerp/Aksies</b>	<b>Betrokkenes/ Sprekers</b>
10 Jan	Vergadering: SRV Nexus en Nulandis	FMS en BD inligtings vergadering	Andre Combrink
11 Jan	CIS Grondvesblok	Ondersoek geskiktheid van grond te koop vir uitbreiding van saadbron	Paul Fourie Andre Combrink
12 Jan	SRCC Agronomie	FMS en BD inligtings vergadering	Andre Combrink
	SRV - Dunbrody SRV – Inteligro		Andre Combrink Andre Combrink

14 Jan	SRV – Sunland Farms / LAC	FMS en BD inligtings vergadering	Andre Combrink
18 Jan	Patensie	Plaasbesoek, Pietlam Ferreira. Onderzoek invloed van soute in gronde	Andre Combrink
	CGA-CRI Crop Protection Needs Zoom meeting	Determine and discuss status of registered pre-harvest fungicides	Andre Combrink
19 Jan	Nutrico SA	Produk bespreking	Andre Combrink
20 Jan	Fafpro/Eden Agri/EASSE, Katrivier BEE Studiegroep	Vergesel studiegroep na Sitrus Rand en Whytes Citrus in die SRV	Andre Combrink
24-26 Jan	Katrivier	Onderzoek boomsterftes op verskeie plase	Andre Combrink Jan van Niekerk
27 Jan	CRI Proef besoek	Besoek Jan van Niekerk se proewe oor Valley Bushveld Decline in die SRV	Andre Combrink Jan van Niekerk
31 Jan	CRI PE Office	Vergader met Sean Moore en Wayne Kirkman oor BD en FMS kwessies	Andre Combrink Sean Moore Wayne Kirkman
2-4 Feb	Nelspruit	Na-oes Werkwinkel	Andre Combrink
7 Feb	Nelspruit	Voorligtings vergadering	Andre Combrink
8-9 Feb	Nelspruit	MANCO vergadering	Andre Combrink
14-16 Feb	Jeffreys Baai	Na-oes Werkwinkel	Andre Combrink
17 Feb	SRCC	Phytrisk vergadering	Andre Combrink Paul Fourie
22 Feb	Loerie Area	Besoek Ashley Ludwig. Onderzoek swak boorde	Andre Combrink
22-24 Feb	Patensie, SRV, Katrivier	CGA Roadshow	Andre Combrink
2 Mar	SRV	A&N Partnership plaas besoek.	Andre Combrink
3 Mar	Patensie	GRV Pakhuis Forum	Andre Combrink Natasha Jackson
	Webinar	Citrosol, Alernative decay control strategies	Andre Combrink
9 Mar	SRCC	Quality manager vegadering	Andre Combrink
14 Mar	CIS Uitenhage	Onderzoek grond te koop vir saadbron	Andre Combrink Paul Fourie Hannes Bester

15 Mar	Patensie	CGA-GDC Plaasbesoeke	Andre Combrink Zama Njili
23 Mar	Patensie	CRI Studiegroep vergadering	Andre Combrink Coenraad Fraenkel
24-25 Mar	Grahamstown	RBX/CRI SIT werkswinkel	Andre Combrink Hannes Bester Wayne Kirkman Srean Moore
29 Mar	Patensie	Onderzoek sprinkaan plaag	Andre Combrink
30 Mar	SRV	Serfontein Boerdery besoek	Andre Combrink
31 Mar	SRV	SRCC Primere Produksie Laslappies Boerdery besoek	Andre Combrink

**Opsomming van aktiwiteite vir periode Januarie – Maart 2022 (Catherine Savage)**

<b>Datum</b>	<b>Studiegroep/Aktiwiteit</b>	<b>Onderwerp/Aksies</b>	<b>Betrokkes/ Sprekers</b>
17 Jan	Extension meeting	General	André Combrink Catherine Savage Coenraad Fraenkel Dawid Groenewald Hannes Bester Jan Landman Liezl vd Linde MC Pretorius Natasha Jackson Wayne Mommsen
1 – 4 Feb	Postharvest workshops	Agenda	Catherine Savage Coenraad Fraenkel Dawid Groenewald Hannes Bester Jan Landman Liezl vd Linde MC Pretorius Natasha Jackson Wayne Mommsen
8 Feb	Citrosol Webinar	Costs, chilling injury, resistance, sour rot control.	Catherine Savage Natasha Jackson Jan Landman
10 Feb	Citrus Sustainability meeting	Agenda	Catherine Savage
14 – 18 Feb	Postharvest workshops	Agenda	Catherine Savage Coenraad Fraenkel Dawid Groenewald Hannes Bester Jan Landman Liezl vd Linde

			MC Pretorius Natasha Jackson
2 Mar	CGA Roadshow Nelspruit	Agenda	Catherine Savage Liezl vd Linde MC Pretorius
3 Mar	Citrosol Webinar	Alternative Decay Control	Catherine Savage Natasha Jackson Jan Landman
23 Mar	CRI-CGA Postharvest actives meeting	Status and concerns over postharvest actives	Catherine Savage Natasha Jackson Wilma du Plooy Meagan van Dyk Jan van Niekerk CGA
28 – 30 Mar	Swaziland Packhouse visits	Packhouse practices	Catherine Savage Jan Landman

**Opsomming van aktiwiteite vir periode Januarie – Maart 2022 (Natasha Jackson)**

<b>Datum</b>	<b>Studiegroep/Aktiwiteit</b>	<b>Onderwerp/Aksies</b>	<b>Betrokkes/ Sprekers</b>
6 Jan	Jeffreys Bay	Preperation for PH workshops	Natasha Jackson Hannes Bester
20 Jan	Teams Call	PH workshop internal presentations	Natasha Jackson Catherine Savage Jan Landman MC Pretorius Meagan van Dyk
25 Jan	Letsitele	PH Workshops	CRI Extension
26 Jan	Letsitele	PH Workshops	CRI Extension
27 Jan	Loskop	PH Workshops	CRI Extension
28 Jan	Loskop	PH Workshops	CRI Extension
1 Feb	KZN Umhlanga	PH Workshops	CRI Extension
2 Feb	KZN Umhlanga	PH Workshops	CRI Extension
3 Feb	Nelspruit	PH Workshops	CRI Extension
4 Feb	Nelspruit	PH Workshops	CRI Extension
7 Feb	Nelspruit	Extension Meeting	Hannes Bester Natasha Jackson Andre Combrink Liezl van der Linde Jan Landman
15 Feb	Jeffreys Bay	PH Workshops	CRI Extension
16 Feb	Jeffreys Bay	PH Workshops	CRI Extension
17 Feb	Paarl	PH Workshops	CRI Extension
18 Feb	Paarl	PH Workshops	CRI Extension
23 Feb	Kirkwood	Zest/Mimosa Packhouse meeting	Johan Bruwer Natasha Jackson Jeanne Smith CJ Meiring
23 Feb	Addo	CGA Roadshow	Andre Combrink Natasha Jackson
3 Mar	Patensie	Technical Forum Meeting	Natasha Jackson Andre Combrink
3 Mar	Webinar	Citrosol Webinar	Natasha Jackson

15 Mar	Citrusdal-Paardekop, Berg en Dal, Abrie van Zyl	Packhouse and Orchard visits	Natasha Jackson John Sinclair Jan Landman Coenraad Fraenkel
16 Mar	Ashton – Unipack, Sonskyn Boerdery	Meeting	Natasha Jackson Jan Landman Joseph Rospel Koos Rabie
17 Mar	Swellendam	PH Forum Meeting	Ettienie Brewis Nicola Kirsten Sarel Neetling CJ Badenhorst Johnny Matthee Ansa Loubser Natasha Jackson Jan Landman
17 Mar	Swellendam	Packhouse Visit Thornlands and Swellenfruit	Jan Landman Natasha Jackson JC Badenhorst Sarel Neethling
22 Mar	Patensie	Patensie Citrus Meeting	Flippie Natasha Jackson
		Mandaryn Packhouse Meeting	Bernard Jansen Natasha Jackson
23 Mar	Teams Call	Postharvest Meeting	Paula Bester Paul Hardman Catherine Savage Wilma du Plooy Jan van Niekerk

**Opsomming van aktiwiteite vir periode Januarie – Maart 2022  
(Jan Landman)**

<b>Datum</b>	<b>Studiegroep/Aktiwiteit</b>	<b>Onderwerp/Aksies</b>	<b>Betrokkenes/Sprekers</b>
17 Jan	Nelspruit	Na-oes werkswinkel	Andre Combrink Catherine Savage Coenraad Fraenkel David Groenewald Hannes Bester Jan Landman Liezl vd Linde MC Pretorius Natasha Jackson Wayne Mommsen
24 – Jan	Letsitele	Na-oes Werkswinkel	Andre Combrink Catherine Savage Coenraad Fraenkel David Groenewald Hannes Bester Jan Landman Liezl vd Linde MC Pretorius Natasha Jackson Wayne Mommsen

27 – 28 Jan	Groblersdal	Na-oes Werkswinkel	Andre Combrink Catherine Savage Coenraad Fraenkel David Groenewald Hannes Bester Jan Landman Liezl vd Linde MC Pretorius Natasha Jackson Wayne Mommsen
31 – 1 Feb	Kwazulu-Natal	Na-oes Werkswinkel	Andre Combrink Catherine Savage Coenraad Fraenkel David Groenewald Hannes Bester Jan Landman Liezl vd Linde MC Pretorius Natasha Jackson Wayne Mommsen
2 – 4 Feb	Nelspruit	Na-oes werkswinkel	Andre Combrink Catherine Savage Jan Landman
7 Feb	Nelspruit	Voorligtings vergadering	Jan Landman Andre Combrink
8 Feb	Citrosol Webinaar	Koste, koue besering, weerstand, suurvrot beheer	Catherine Savage Jan Landman Andre Combrink
14 – 18 Feb	Jeffreysbaai	Na-oes werkswinkel	Andre Combrink Catherine Savage Coenraad Fraenkel David Groenewald Hannes Bester Jan Landman Liezl vd Linde MC Pretorius Natasha Jackson Wayne Mommsen
17 – 18 Feb	Paarl	Na-oes werkswinkel	Andre Combrink Catherine Savage Coenraad Fraenkel David Groenewald Hannes Bester Jan Landman Liezl vd Linde MC Pretorius Natasha Jackson Wayne Mommsen
3 Mrt	Citrosol Webinaar	Alternatiewe verval, beheer strategie	Andre Combrink Catherine Savage Jan Landman Natasha Jackson

14 – 18 Mrt	Citrusdal en Swellendam	Besoek aan pakhuise	Natasha Jackson Jan Landman
17 Mrt	Swellendam	Swellendam Pakhuis forum	Natasha Jackson Jan Landman
28 – 30 Mrt	Swaziland	Pakhuis besoeke	Catherine Savage Jan Landman

### 7.3 OTHER MEANS OF TECHNOLOGY TRANSFER

#### 7.3.1 SA Fruit Journal by Tshidi Ramabu (CRI)

**Table 7.3.1.1** SA Fruit Journal articles in 2021-22 besides Extension Briefs.

Issue		Pages	Title	Author/s
Apr/May	20(2)	72-75	The blue-green citrus nibbler	Evans Mauda and Sean Moore
		76-77	CRI postharvest webinar 2021	Catherine Savage
Aug/Sep	20(4)	95-97	Spinning a new story in citrus: A first record of two spider species	Tamryn Marsberg, Mellissa Peyper and Sean Moore
Oct/Nov	20(5)	70-73	Top-working of citrus	Werner Swiegers, Johan Joubert and Paul Cronje
		76-78	Fungal degradation of wooden pallets in citrus export	Wilma du Plooy
		81-85	Citrus chilling injuries viewed from all angles	Paul Cronje
Dec/Jan	20(6)	59-61	African greening disease management in SA's citrus orchards	MC Pretorius and James Warrington
		62-65	The value of phosphorus fertilisation and microbial inoculants	Pieter Raath, Vivian White and Marli Vermooten
		66-69	Water quality for irrigation of citrus trees	Pieter Raath and Tariena Nel
Feb/Mar	21(1)	30	Sean Moore – visiting professor at Rhodes University	
		31	New handbook on fertilisation of citrus trees in SA	Engela Duvenage
		90-94	Efficacy of fruit fly trapping systems in SA	Aruna Manrakhan, John-Henry Daneel, Rooikie Beck, Claire N Love, Martin J Gilbert, Massimiliano Virgilio and Marc De Meyer
		97-100	A survey of the Australian Bug and the Vedalia beetle in mandarin orchards in South Africa	Leani Serfontein

### 7.3.2 CRI website by Tim G Grout

**Table 7.3.2.1** Visits and page requests on [www.cri.co.za](http://www.cri.co.za) since June 2021.

Month	Users	Page views	Unique page views	Hits
Jun 2021	25	932	381	933
Jul 2021	454	2071	1305	2085
Aug 2021	1500	5380	3622	5430
Sep 2021	1823	7220	4660	7316
Oct 2021	1273	5055	3427	5179
Nov 2021	1328	4976	3394	5023
Dec 2021	978	3108	2206	3143
Jan 2022	2094	8730	5890	8832
Feb 2022	2382	8646	5841	8745
Mar 2022	1976	6174	4374	6211
<b>Total 2021/2</b>	<b>13839</b>	<b>52292</b>	<b>35100</b>	<b>52903</b>

### 7.3.3 Cutting Edge

**Table 7.3.4.1** Cutting Edge issues during 2021-22.

No.	Titles	Month	Author/s
318	Fruit rind symptoms possibly associated with citrus virus A (CiVA)	Apr	Glynnis Cook, Rachelle Bester and Hano Maree
319	Sampling procedure for <i>Phytophthora</i> and citrus nematode analysis and latest price list for services rendered by the Diagnostic Centre	Apr	Elaine Basson, Charmaine Olivier and Jan van Niekerk
320	Ambient / warm loading of citrus – 2021 season	Apr	Paul Cronje, Tarl Berry, and Bernard Henning
321	Consumer assurance update	Apr	Paul Hardman
322	Hydrochloric acid (HCl) shortage in South Africa	May	Wilma du Plooy and Catherine Savage
323	Warning of high counts of Oriental fruit fly in traps in Limpopo	May	Aruna Manrakhan
324	Consumer assurance update	Jun	Paul Hardman
325	Adapting to changed EU MRL for imidacloprid	Aug	Tim Grout and Sean Moore
326	Stem-end saprophyte (Storage mould)	Aug	Catherine Savage and Wilma du Plooy
327	Mealybug control recommendations	Aug	Sean Moore, Wayne Mommsen and Tim Grout
328	Change to Pymetrozine EU MRL	Sep	Paul Hardman

329	Citrus Black Spot Spray Programmes 2021 – 2022	Sep	Providence Moyo, Jan van Niekerk, Elma Carstens and Paul Fourie
330	Consumer assurance update	Nov	Paul Hardman
331	Changes to the Citrus FCM Risk Management System (FMS) for the 2022 Export Season	Dec	Elma Carstens, Paul Cronje, Sean Moore, Tarl Berry and Vaughan Hattingh
332	Managing citrus black spot during wet weather periods	Dec	Providence Moyo, Elma Carstens, Tankiso Mpholo and Jan van Niekerk
333	New A15C Supervent cartons for EU exports (2022)	Dec	Tarl Berry, Dawid Groenewald and Paul Cronje
334	Star-grading of certified nurseries in the South African Citrus Improvement Scheme (CIS)	Jan	Paul Fourie, Michael Nell and Jacolene Meyer
335	Management of Alternaria Brown Spot	Feb	Jan van Niekerk, Providence Moyo and Jacque van der Waals
336	Exclusion of lemon from <i>B. dorsalis</i> removal permit requirements	Feb	Aruna Manrakhan, Wayne Kirkman, Elma Carstens and Vaughan Hattingh
337	FCM fruit infestation monitoring in orchards for export to the USA in 2022	Mar	Elma Carstens and Vaughan Hattingh
338	Implications of locust swarms for citrus	Mar	Sean Moore and Hannes Bester

## 8 PUBLICATIONS IN 2021-22

### 8.1 Refereed Publications (or ISI ranked journals)

- Acheampong, M.A., C.A. Coombes, S.D. Moore, M.P. Hill. 2020. Temperature tolerance and humidity requirements of select entomopathogenic fungal isolates for future use in citrus IPM programmes. *Journal of Invertebrate Pathology* 174: 107436.
- Bester, R., Cook, G., and Maree, H.J. 2021. Citrus tristeza virus genotype detection using high-throughput sequencing. *Viruses*. 13: 168.
- Bester, R., Cook, G., Breytenbach, J.H.J., Steyn, C., De Bruyn, R., and Maree, H.J. 2021. Towards the validation of high-throughput sequencing (HTS) for routine plant virus diagnostics: measurement of variation linked to HTS detection of citrus viruses and viroids. *Virology Journal* 18: 61. 19 pp.
- Bester, R., Karaan, M., Cook, G., and Maree, H.J. 2021. First report of citrus virus A in citrus in South Africa. *Journal of Citrus Pathology*. 8 (1).
- Carstens, E., Linde, C.C., Fourie, P.H., Bester-van der Merwe, A.E., Langenhoven, S.D. and McLeod, A. 2021. Spatial and temporal genetic analyses of *Phyllosticta citricarpa* in two lemon orchards in South Africa reveal a role of asexual reproduction within sexually reproducing populations. *Phytopathology* 111: 1238-1251.

- da Graça JV, Cook G, Ajene IJ, Grout TG, Pietersen G, Roberts R, Bester R, Pretorius MC & Maree HJ (2021) A Review of the '*Candidatus Liberibacter africanus*' Citrus Pathosystem in Africa. *Phytopathology* 112: 44-54. doi:10.1094/PHYTO-07-21-0296-FI.
- Dessie, B., D. Shimelash, S. Gebeyehu. 2022. Detection of Huanglongbing, insect vectors and nutritional profile of citrus in Upper Awash, Ethiopia. *J. Plant Pathol.* 104: 17-31.
- Drosopoulou, E., A. Damaskou, A. Markou, S. Ekesi, F. Khamis, A. Manrakhan, A.A. Augustinos, G. Tsiamis and K. Bourtzis. 2021. The complete mitochondrial genomes of *Ceratitis rosa* and *Ceratitis quilicii*, members of the *Ceratitis* FAR species complex (Diptera: Tephritidae). *Mitochondrial DNA Part B* 6(3): 1039–1041.
- Erasmus, A., Lennox, C.L., Korsten, L., du Plooy, W., Kellerman, M., and Fourie, P.H. 2021. Imazalil resistance management for sustainable citrus green mould control: limited options and alternatives. *Acta Horti* 1323:105 – 110.
- Fourie, P.H., Kirkman, W., Cook, G., Steyn, C., De Bruyn, R., Bester, R., Roberts, R., Jose, C., Basimba, D., and Maree, H.J. 2021. First report of '*Candidatus Liberibacter africanus*' from Citrus in Angola. *Plant Disease*. 105 (2): 486.
- Fourie, P.H., Erasmus, A., Lennox, C.L. and du Plooy, W. 2021. Optimisation of postharvest fungicide application in citrus packhouses: low-tech but high impact. *Acta Horti*. 1323:137-142.
- Fuchs, M., Bar-Joseph, M., Candresse, T., Maree, H.J., Martelli, G.P., Melzer, M.J., Menzel, W., Minafra, A., Sabanadzovic, S., and ICTV Report Consortium. 2020. ICTV Virus Taxonomy Profile: *Closteroviridae*. *Journal of General Virology*. 101: 364–365.
- Grout, T.G. and P.R. Stephen. 2021. Are Yeast Autolysate Attractants for *Ceratitis* Species (Diptera: Tephritidae) in South Africa More Attractive and Palatable Than a Currently Used Protein Attractant? *J. Econ. Entomol.* 114(2): 1005-1008.
- Mamba, L.C., Meitz-Hopkins, J.C., Stevens, C., Fourie, P.H., du Plooy, W., Erasmus A. and Lennox C.L. Citrus sour rot management by propiconazole drench application in South Africa. *Acta Horti* 1323:177 -182.
- Manrakhan, A., J.H. Daneel, R. Beck, C.N. Love, M.J. Gilbert, M. Virgilio and M. De Meyer. 2021. Effects of male lure dispensers and trap types for monitoring of *Ceratitis capitata* and *Bactrocera dorsalis* (Diptera: Tephritidae). *Pest Manag. Sci.* 77: 2219–2230.
- Manrakhan, A., Daneel, J.-H., Stephen, P.R., Hattingh, V., 2022. Cold Tolerance of Immature Stages of *Ceratitis capitata* and *Bactrocera dorsalis* (Diptera: Tephritidae). *Journal of Economic Entomology*. <https://doi.org/10.1093/jee/toab263>
- Mwanza, P., M. Jukes, G. Dealtry, M. Lee and S. Moore. 2022. Selection for and Analysis of UV-Resistant *Cryptophlebia leucotreta* Granulovirus-SA as a Biopesticide for *Thaumatotibia leucotreta*. *Viruses* 14, 28.
- Olivier, C., Savage, C., du Plooy, W., Fourie, P.H., Erasmus A. and Lennox C.L. Sanitisation of fungicide drench solution and effects on green mould and sour rot control. *Acta Horti* 1323:183 – 188.
- Serra, Wendy, María B. Lugo Álvarez, Dariel García Rodríguez, Eugenio Alonso-Oliva, Amalia Sanz Llorente, Vladimiro Guarnaccia, Elma Carstens, Jeffrey A. Rollins, Sofe Thijs, Jaco Vangronsveld, Ana M. Manzano León. 2022. Polyphasic identification and MAT1-2 isolates of *Phyllosticta citricarpa* in Cuba. *European Journal of Plant Pathology*. DOI 10.1007/s10658-021-02453-y
- White, VG, AG Hardie & PJ Raath. 2021. Phosphorous Fertilizer Recommendations Considering Single-point P Sorption Capacity and Soil P Test Extraction Efficiency. *Communications in Soil Science and Plant Analysis*. 14 pp. DOI:10.1080/00103624.2021.1993881
- Zeng, Y., Xiong, T., Liu, B., Carstens, E., Chen, X., Xu, J., and Li, H. 2021. Genetic Diversity and Population Structure of *Phyllosticta citrisiana* in China. *Phytopathology* 111: 850-861.