innovate

SUSTAINING AN INNOVATIVE AND GLOBALLY COMPETITIVE FRESH HORTICULTURE INDUSTRY

POST-HARVEST
INNOVATION PROGRAMME

The PHI Programme wishes to thank every person who contributed to this publication with his or her expertise, time, passion and commitment. Thanks to your dedication, INNOVATE is a true re ection of the Programme's commitment to excellence and industry advancement.

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Foreword

RESEARCH AND DEVELOPMENT is the source of innovation. Whether it takes place in a university laboratory or a workshop on a farm, the rigours of trial and error, and of understanding the variables that in uence a solution, are inherently part of the process that births better ways of doing things.

In the context of innovation, the Post-Harvest Innovation (PHI) Programme's mandate is to provide funding access to investigate practices and technologies that can enhance the quality and shelf life of fresh produce after harvesting; and it has to build academic and research capacity while doing so. The ultimate aim is to enhance the competitiveness of South Africa's fresh horticulture produce industry through innovation.

The PHI Programme is a public-private partnership that enjoys support from the Department of Science and Technology (DST). Currently in its third three-year cycle, the total funding the programme has received since 2007 amounts to R60 million. This publication highlights the results of our efforts from 2014 to the end of 2016.

We are extremely proud of the fact that the DST views the PHI Programme, which is one of seven Sector Innovation Fund (SIF) programmes, as the blueprint for how a public-private investment in research and development can and should work.

With its 35 projects, the third cycle of the PHI Programme (PHI-3) supported 44 post-graduate students. Importantly, a recent impact study has shown a signi cant uptake of the PHI Programme-supported students by the industry.

PHI-3 has generated a wealth of valuable knowledge. In some studies the results showed a clear path forward; in others, we learned what did not work. In all cases, science and the cause of innovation were advanced.

In this third phase, the programme's scope was broadened to include fresh horticultural products other than fruit, such as onions, tomatoes and cut owers. Its industry partners - Hortgro Science, Citrus Research International, the South African Table Grape Industry, the Subtropical Growers' Association, the Pomegranate Producers Association of South Africa, Cape Flora SA, the Tomato Producers' Organisation and Korkom Onion Producers – identi ed priority R&D areas and co-funded projects.

One of the most pressing competitiveness challenges faced by the local fresh horticulture industry, is maintaining the phytosanitary integrity of its produce. To a large extent, PHI-3 has been dedicated to solving phytosanitary challenges. Hence, in reading through this book, you will nd exciting work being done with innovative approaches such as irradiation, green chemistry and pest preconditioning treatments; sensory research; and ways to predict physiological disorders and product quality.

We are proud to present this body of work for a variety of reasons, chief among them the fact that the PHI Programme continues to serve competitiveness through innovation.

Anton Kruger
CEO of the Fresh Produce Exporters' Forum and the chairman of the PHI Programme management committee.

Anton Kruger.

Junette Davids, manager of the PHI Programme.

PHI-3 areas of focus and impact

Agricultural engineering

Researchers explored the contribution different engineering elds, such as mechanical, systems, electric, electronic and chemical, can make in the post-harvest sphere.

Container and cold-storage technology

Projects explored a variety of topics, including the validation of time and temperature tolerances (TTTs); alternative cold-storage technologies for minor crops; effective low-technology cooling systems; integrity and ef ciency of reefer trucks and reefer containers; validation of vent settings in containers; defrost management; and air ow management.

Economics

Money talks, hence the necessity to quantify the return on investment delivered by post-harvest research and development and innovation projects and programmes.

Integrated packaging solutions

Research teams worked on alternative packaging materials; package design to optimise payload, cooling ef ciency and the maintenance of product quality; bulk packaging effects on quality; tailor-made packaging for new/minor crops; and modi ed atmosphere packaging.

Logistics

Studies in this eld covered integrated multi-modal transport; management of short shipments; cargo management in ports; air freight cold chain management; traceability systems; and the effect of logistics on product quality.

Post-harvest disease and insect control, including phytosanitary compliance

Phytosanitary matters commanded much time and attention, covering aspects such as monitoring systems; mitigation treatments (inorganic chemicals, GRAS treatments, alternative treatments like irradiation and insecticidal CA, green chemistry, optimising cold sterilisation treatments, alternatives to cold-steri and combined treatments); pest preconditioning treatments; and protocols for effective post-harvest control.

Post-harvest physiology

Efforts in this area were related to the physiology of the product, including determination of harvest maturity through destructive and non-destructive methods; prediction and control of physiological disorders and product quality; conditioning treatments; handling, storage and shipping protocols; sensory research (including aroma and off-taste); product maturation, ripening and senescence; and respiratory and ethylene metabolism.

Resource ef ciency and sustainability

Underlying most of the projects was the evergreen quest to optimise the use of resources like energy and water; increased use of clean energy; and the use of waste products.

Technology transfer

The value of innovation is only fully realised when knowledge is effectively and ef ciently transferred to participants in the value chain.

Temperature and humidity control

Areas of interest in this area were protocols for cold chain management, including optimal temperatures and humidities; alternative storage and shipping temperatures; monitoring equipment; moisture loss control; and technology transfer on equipment and systems.

Contents

Avocado

It is unclear when the first avocados reached South Africa, but it could be as far back as the 1500s. Records exist that prove avocado cultivation by the late 1920s, but the first orchards in South Africa were planted during the 1930s by Dr Merensky at Westfalia, and Lanion Hall at HL Hall & Sons.

By 1970, plantings of around 2 000ha existed; by 2003 a total of 12 000ha were dedicated to avocado production.

The rapid growth of the avocado industry led to the establishment of the South African Avocado Growers' Association (SAAGA) in 1967. A forum to promote communication between producers and other interested parties, its main aim was to regulate the flow of avocados to the European market and stabilise prices.

For a while producers made handsome profits, but by the late 1970s the industry was in trouble. Phytophthora was rampant without any control measures; black spot caused huge losses; post-harvest diseases were common but causes were unknown and there were no control measures. Adding to producers' woes was the increasing incidence of cartons collapsing and rail trucks with avocados disappearing, only to be discovered months later on some obscure rail siding.

The industry acknowledged the need for a coordinated research effort and the SAAGA Research Committee was founded.

Thanks to its work, a greater understanding of the post-harvest handling of avocados emerged. It was discovered, for instance, that fruit quality depended on storage period, and that avocados could be artificially ripened with ethylene gas.

Today the local industry remains mainly export orientated, with black-skin cultivars, such as 'Maluma', 'Hass' and 'Lamb Hass', being the most popular with overseas consumers.

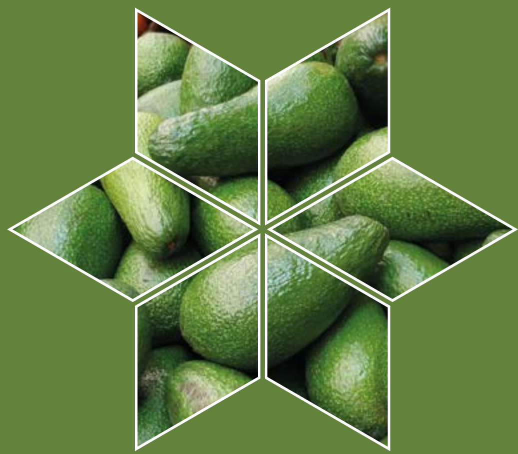
The need for research also remains.

Uneven ripening is an example of a challenge that should be addressed in earnest. Similarly, the incidence of grey pulp remains unacceptably high, despite the causes being well known. In some instances, it would appear that exporters put financial gains ahead of the reputation of the South African industry by exporting high risk fruit.

The use of photo-selective shade nets hold potential, but also risk. The microclimate created by the nets differs significantly from an open orchard and may, eventually, give rise to new diseases and defects, offering unique research opportunities.

FAST FACTS

- In 2014, a record year, South Africa produced almost 140 000 tonnes of avos, and exported just about 15 million 4kg-cartons.
- The local production season runs from February to September.
- Almost 79% of South Africa's export avocados are destined for the EU. The UK, at around 19%, is the second biggest market.
- Avocados originated in an area stretching from central Mexico, through Guatemala and into Central America.
- Avocados are extremely fastidious – different cultivars require very speci c climatic and soil conditions to perform at their best.
- South Africa produces subtropical avocados; the tropical varieties grow in truly hot, lowland climates.
- In 1987, South Africa hosted the rst Avocado World Congress.



Limpopo Province: Levubu, Soutpansberg, Tzaneen, Mooketsi; Mpumalanga: Hazyview, Kiepersol, Nelspruit, Barberton; KwaZulu-Natal: Eshowe, Melmoth, Wartburg, Kranskop, Howick, Richmond; Eastern Cape: Adelaide, Hankey. Western Cape: George.

> Many opportunities exist to improv post-harvest condition of South Afric

Satisfying the out-of-season appetite for avos

South African consumers have developed such a taste for avocados that they want to enjoy them year round. A study funded by industry and the PHI Programme is looking at options to improve local production and storage methods to meet this demand without having to depend on out-of-season imports.

> "The option to market highly productive late-season cultivars instead of imported fruit during the off-season is of extreme importance to the South African avocado industry."
>
> Dr Frans Kruger

GLOBALLY, CONSUMPTION OF avocados is growing at around three percent per annum. Production is struggling to keep up, which is driving a healthy and lucrative export trade.

In South Africa, out-of-season demand has grown to the point where the local avocado industry has started to import fruit from Northern Hemisphere production regions during this period. It is an expensive exercise, and importers regularly claim to make a loss. Making matters worse is the fact that, in many cases, the quality of the imported fruit is poor while the retail price is out of range for most consumers.

In view of the above, it only makes sense for the domestic industry to invest time, effort and resources into extending the local season as much as possible. Various initiatives are already underway. One approach is to establish orchards in late-production areas. These are either at high altitudes or in the more southern regions of the country.

Two cultivars that have proven to consistently produce high yields during the late season are 'Reed' and 'Lamb Hass'. In August 2014, a study funded by the PHI Programme and the avocado industry aimed at measuring the ultra-late-season quality and storage potential of these cultivars, started.

Led by Dr Frans Kruger, a researcher at Lowveld Post-harvest Services, the study set out to achieve three objectives:

1. Determine for what period after the conventional harvest season can 'Lamb Hass' and 'Reed' avocado fruit from high altitude orchards still be harvested, stored and ripened.
2. Launch a trial aimed at establishing what effect the late hanging of the fruit has on tree health and alternate bearing.
3. Develop appropriate harvest and storage protocols.

According to Frans, the option to market highly productive late-season cultivars instead of imported fruit during the off-season is of extreme importance to the South African avocado industry. "It will have an enormous impact on the pro tability of producers with late-season orchards, as well as the ripeners and pre-packers who service the industry."

Frans' two team leaders were Dr Bombiti Nzanza, a horticulturist who manages the R&D

department of one of the world's largest tomato and avocado farms, and Danie Lemmer, a post-harvest specialist with extensive knowledge of avocado storage technology.

Points of departure

Although late-season storage trials in 2006 and 2007 with the 'Hass' and 'Fuerte' cultivars yielded reasonably good results under carefully controlled laboratory conditions, commercial attempts at applying the technology gave mixed results due to challenges in managing the process as carefully as was done under experimental conditions. This emphasised the need to use true late-season cultivars, such as 'Lamb Hass' and 'Reed', for further investigation.

Two approaches can be followed when supplying the very late-season market with locally produced fruit.

The rst is to let the fruit hang until the projected marketing date is reached. This is the best option for fruit quality. However, it can be detrimental to tree health. Certain cultivars, such as 'Hass', bear very poorly the next year. Both 'Reed' and 'Lamb Hass' are more robust when it comes to this aspect and, after fruit set, both cultivars mature at a slow rate, making them suitable candidates for the late-season market.

The second is to let the fruit hang as long as is practical and then to store it for an additional period. The present study aimed to determine whether it is feasible to use 'Reed' and 'Lamb Hass' to supply the late-season market and to develop appropriate harvest and storage protocols for these cultivars.

The study

Starting in August 2014 and covering two seasons, the study planned for the 'Lamb Hass' trials to be performed in the Nelspruit and Mooketsi areas in the Lowveld region of South Africa's Mpumalanga and Limpopo provinces. The 'Reed' trials were planned for the Schagen and Mooketsi areas.

Early on, however, reality forced a change in plans. When project work started in September 2014, the Schagen fruit was already over-mature and therefore not suitable for the trials.

"The rst lesson we learnt was that, although both varieties are described as late cultivars in literature, they must be planted in a cooler area to ensure late-hanging potential," says Frans. As a result, the team then concentrated on high-altitude orchards.

The storage and ripening trails

'Lamb Hass' and 'Reed' fruit were harvested from ZZ2 orchards located on the Olyfberg farm (altitude 1400 mamsl) during, respectively, mid-October 2014, mid-November 2014, mid-December 2014 and mid-January 2015.

The samples collected on the rst three dates were stored for one month; those collected during mid-January were stored for two weeks only. All the fruit were stored at 2°C, 4°C or 6°C after being treated with 0ppb, 200ppb, 300ppb or 500ppb 1-methylcyclopropene (1-MCP).

The results revealed stem-end rot

2

PROJECT TITLE
The development of harvesting and extended storage protocols for late season 'Lamb Hass' and 'Reed' avocado fruit

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Two years

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R207 000 & R197 000

LEAD INSTITUTION
Lowveld Post-harvest Services, Nelspruit

BENEFICIARY
Sub-tropical fruit

FOCUS AREA
Post-harvest physiology and pre-harvest aspects

PUBLICATIONS
Three

PRESENTATIONS
Three

Dr Frans Kruger with Precious Novela, a ZZ2 researcher, in the Boekenhoutsbult packhouse at Mooketsi.

The 'Lamb Hass' avocado fruit is characterised by a blunt ending neck and a rough skin that readily separates from the esh when ripe.

> ‘Lamb Hass’ avos grown at the appropriate altitude have excellent late-hanging and storage potential.
>
> Dr Frans Kruger

infection as the factor that determines when late-season avocados should be picked and for how long they can be stored. There was a direct correlation between the length of the ripening period and the incidence of stem-end rot infections. ‘Reed’ proved to be more susceptible than ‘Lamb Hass’.

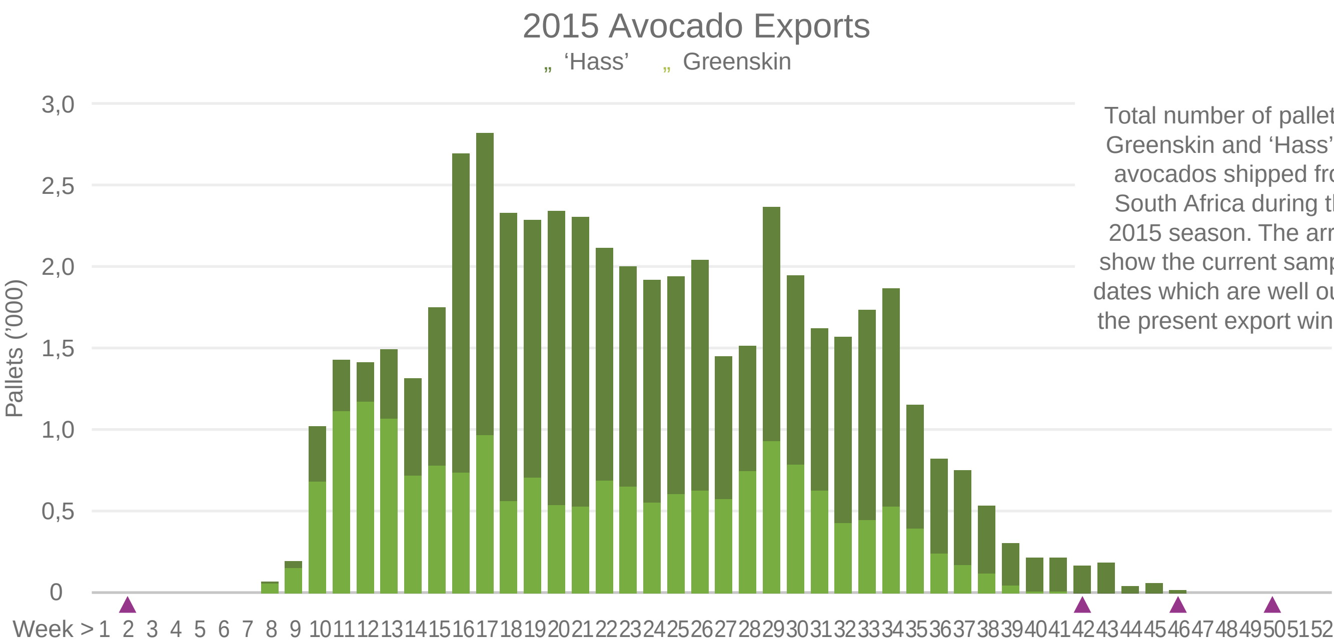
‘Lamb Hass’ fruit stored at 6°C had no stem-end rot when harvested during October, November and December and stored for one month. The fruit harvested during January, however, had around 18% stem-end rot after two weeks of storage.

Stem-end rot aside, various other physiological disorders were present in the fruit harvested during mid-October and mid-November. Nevertheless, it was concluded that internal quality of the fruit should be acceptable after storage for one month at 6°C. The fruit harvested in mid-December and mid-January, stored for respectively one month and two weeks, had a less acceptable appearance. The most important cosmetic aws were bruising, vascular browning, vascular hardening and grey pulp.

“Further trials should aim to reduce the appearance problems by pinpointing harvest dates and maximum storage periods, as well as handling recommendations to reduce bruising,” says Frans.

The results also showed that, because of their ripening retardation effects, neither 1-MCP nor the lowest storage temperature settings, namely 2°C and 4°C, were appropriate for achieving ultra-late-season marketing purposes.

Based on these results, the team provisionally recommended that ‘Lamb Hass’ fruit harvested in December should be stored



Total number of pallets of Greenskin and ‘Hass’-like avocados shipped from South Africa during the 2015 season. The arrows show the current sampling dates which are well outside the present export window.

for two weeks at the most, and that fruit harvested in January be immediately marketed.

The rst season's results con rmed the potential of ultra-late season 'Lamb Hass'. 'Reed', however, proved to be unsuited for the purpose. This outcome informed the decision to proceed with 'Lamb Hass' only in terms of measuring the effect that the late hanging has on tree phenology.

The tree health study

The pilot trial on the effect that late hanging has on 'Lamb Hass' tree health and alternate bearing involved four rows of 'Lamb Hass' trees (132 trees in total) in the Olyfberg orchard.

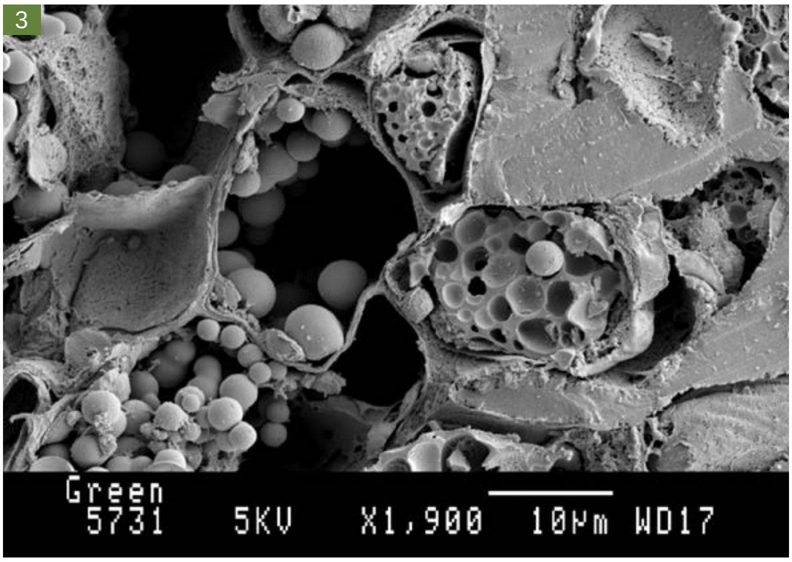
The number of 2014 fruit hanging on each tree was counted and the size of the 2015 set was scored. Fifty 2015 fruit on 20 of the trees were subsequently marked and the rate of fruit drop measured. The rate of fruit size increase was also measured on eight of the trees.

The study found a negative correlation between the numbers of 2014 fruit counted per tree and the 2015 fruit abundance score. However, the effect was not signi cant enough to justify the abandonment of late-hanging trials, neither did the late hanging cause an increase in fruit drop. The preliminary results indicated that trees on which the 2014 season's fruit were retained for a longer period did not seem to shed more of the 2015 season's fruit than those from which the 2014 fruit were stripped at an earlier stage. This observation was con rmed during the 2016 season.

An unexpected spin-off for exporters

Until recently, exporters have struggled to land 'Lamb Hass' fruit in an acceptable condition in Europe. One of the mistakes made was the use of low storage temperature regimes employed for 'Hass' during the late season. During 2016, certain producers exported 'Lamb Hass' at 6°C (which is an unusually high temperature for the time of season) with excellent results. Not only did the fruit land in a good condition, but excellent prices were attained. This was due to the fruit's high oil content and superior taste when compared with the relatively immature Northern Hemisphere fruit marketed at the time.

"We have come to the conclusion that 'Lamb Hass' grown at the appropriate altitude has excellent late-hanging and storage potential," says Frans. "Given the considerable commercial potential of these results, the trials should be expanded during coming seasons."



Harvest-mature 'Lamb Hass' (left) and ripe 'Lamb Hass' (right).

Emè Volschenk, a research assistant at Lowveld Post-harvest Services, taking fruit growth measurements in an avocado orchard in the Tzaneen area.

Letting 'Lamb Hass' fruit remain on the tree for an extended period after the fruit's traditional harvest season resulted in mature fruit with a higher oil content (see oil globules in the cells of 'Lamb Hass') and subsequent superior eating quality for which consumers are willing to pay excellent prices.

Keeping up with tastes that are a-changing

Researchers are putting a new local avocado cultivar through its post-harvest quality paces in order to capitalise on European consumers' changing preferences.

1


THE EUROPEAN AVOCADO market is steadily moving away from green skin cultivars in favour of 'Hass' avocados that change colour as they ripen. Unfortunately, 'Hass', which is the benchmark cultivar, bears quite poorly in certain South African growing regions. Therefore, to protect and grow its export market share, the local industry had to identify and develop alternative, high-bearing 'Hass'-like cultivars.

'Maluma' has emerged as a cultivar that meets these demands. This highly productive 'Hass' selection has been extensively planted over the last few years. As an open cultivar, any grower is free to produce it and all exporters may market it. The only restrictions concern the licensing of nurseries that supply the trees.

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Despite all these advantages, the industry is not yet certain that 'Maluma's' export quality can be guaranteed, mainly because appropriate quality assuring cultivation practices, harvest methods and, importantly, storage/ripening procedures have not yet been developed.

According to Dr Frans Kruger, researcher and consultant at Lowveld Post-harvest Services, the cultivation of a tree crop, such as avocados, is only pro table when export quality can be guaranteed. "Even a high-yielding cultivar such as 'Maluma' is of little value if the post-storage quality of the fruit varies from one season to the next," says Frans.

There is, furthermore, signi cant competition between South Africa, Peru and Chili. To succeed, any new cultivar must have similar storage, ripening and organoleptic qualities to 'Hass' (or qualities better than). "If we can combine increased production with stable quality, the result will most de nitely be increased competitiveness for the industry as a whole," says Frans. "Producers in areas that are marginal in terms of 'Hass' production will be the big winners when it comes to pro tability."

In pursuit of these outcomes, Frans designed a study to upgrade the 'Maluma' cultivar's export protocol. Working with him was Dr Bombiti Nzanza, a horticulturist with considerable expertise in tomato and avocado production, storage and export. The third team member was Danie Lemmer, a PhD student and post-harvest specialist with extensive knowledge of avocado storage technology. The researchers were assisted technically by husband and wife team Otto and Emè Volschenk.

Objectives and methodology

This study, funded by industry and the PHI Programme, ran from January 2015 to December 2016 and had ve objectives:

1. Establish appropriate harvest maturity parameters.
2. Develop appropriate harvest protocols.
3. Establish appropriate storage temperature settings.
4. Develop appropriate ripening protocols
5. Determine how phenological, climatic and horticultural variables in uence the quality of the fruit.

'MALUMA'S' MALADIES

The 'Maluma' cultivar has been shown to be susceptible, to a greater or lesser extent, to these seven disorders.

- Soft landings
- Grey pulp
- Black cold damage
- Vascular staining
- Variable ripening
- Poor external colour
- Post-harvest fungal infections

To achieve these, the researchers set out to answer three questions:

1. How soon after harvest do 'Maluma' fruit need to be put into cool storage?

Six trials were done to answer this question.

- Two at Mooketsi with 'Maluma' fruit only,
- Two at Tzaneen with 'Maluma' fruit only, and
- Two at Nelspruit with both 'Maluma' and 'Hass' avocados.

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PROJECT TITLE
Upgrading the 'Maluma' avocado cultivar's harvest, storage and ripening protocols

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FOCUS AREA
Post-harvest physiology and pre-harvest aspects

PUBLICATIONS
Three

PRESENTATIONS
Three

Dr Frans Kruger
'Maluma' is characterised by a rough skin that is less easily damaged during harvest, export and ripening.
A correctly trained young 'Maluma' avocado orchard near Tzaneen.

1

PhD student, Danie Lemmer is a post-harvest specialist in avocado storage technology.

The shape of the 'Maluma' avocado tree lends itself to high density planting; thus rendering this cultivar ideal for cultivation under shade netting.

The study paid close attention to the development of appropriate commercial ripening practices for 'Maluma' avocados.

'Maluma' avocados have a favourable seed-to- esh ratio.

'Hass' stored at different temperatures resulted in different degrees of ripeness. 'Maluma' generally ripens faster and more synchronised than 'Hass' which makes it an excellent cultivar for ripe-and-ready programmes.

In these trials, the fruit was kept at room temperature for four different time periods before going into cool storage. After storage for 30 days, the fruit was ripened at 20°C and a full set of quality analyses performed.

2. How long can 'Maluma' fruit be stored before arti cial ripening must start?

For this question, 18 trials were performed.

- Two at Mooketsi with 'Maluma' fruit only,
- 14 at Tzaneen with 'Maluma' fruit only, and
- Two at Nelspruit with both 'Maluma' and 'Hass' fruit.

In these trials, the fruit was stored for either 20, 25, 30 or 35 days. After storage, the fruit was ripened at 20°C and a full set of quality analyses performed.

3. How does the post-ripening 'soft life' of arti cially ripened 'Maluma' fruit compare with that of the other commercial cultivars?

To answer this question, six trials were carried out at a commercial ripening facility in Lanseria using the following sets of fruit:

- Four from Mooketsi ('Maluma' and 'Fuerte' avocados), and
- Two from Nelspruit ('Maluma' and 'Hass' avocados).

Before ripening, the fruit was stored for 30 days at four different temperatures. After storage, the avocados were subjected to a full set of quality analyses.

The same quality analyses were done for all three research questions, namely the ripening period (number of days to ripen (DTR)); the incidence and intensity of physiological disorders at the ready-to-eat stage; and the incidence and intensity of fungal infections at the ready-to-eat stage.

Due to the importance of comparative information to the industry, early-season comparisons were made with the 'Fuerte'

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cultivar, while 'Hass' was added to the mid-season trials.

Results

The findings suggest that 'Maluma' avocados must be put into cool storage within eight hours after harvest. Interestingly, the data showed that this recommendation applies to 'Hass' as well.

In terms of how long 'Maluma' fruit can be stored before artificial ripening must commence, export samples from various producers indicated that fruit from older trees with a lower nitrogen status can be stored for 30 to 35 days. Fruit from younger trees with a high nitrogen status should not be stored for longer than 25 days.

One of the most exciting findings was that the post-ripening 'soft life' of artificially ripened 'Maluma' was not inferior to that of 'Hass' and 'Fuerte'. In fact, it even appeared to be superior in certain of the trials.

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'Maluma' fruit fro
older trees can b
stored for up to 3
days before arti c
ripening must
commence, ie. 1
days longer than
from younger tre

Dr Frans Kruger

Shade nets light the way to avocado quality

Nets that manipulate light to the bene t of the plants that grow under them is not science ction. It is a new agro-technological concept that is boosting the post-harvest quality of 'Hass' avocados.

Project leader, Prof. Dharini Sivakumar.

For this study, an avocado orchard of 1,15ha was covered with different colours of photo-selective netting. Depending on the colour used (red, yellow, green, blue, white, grey or pearl), the nets manipulate light quality to induce favourable responses in plants.

TOO MUCH HOT sunshine, wind and hail can in ict damage on avocados that detracts from their marketability long before they arrive at the packhouse. Nets are an obvious solution to these problems and are widely used on farms to physically protect crops against the elements of nature, as well as external pests.

Recent research, however, suggests that nets can do more than just protect. By ltering and manipulating light, they can make a material difference to fruit quality.

Photo-selective coloured netting is a new agro-technological concept. Depending on the colour a producer chooses (red, yellow, green, blue, white, grey or pearl), the nets manipulate light quality to induce favourable responses in plants.

According to Prof. Dharini Sivakumar, SA Research Chair in Phytochemical Food Network to Improve Nutritional Quality for Consumers, at the Department of Crop Sciences, Tshwane University of Technology, photo-selective netting can improve the post-harvest physiology of fruit to obtain reduced physical damage and incidence of post-harvest diseases, and improved ripening patterns.

The technology is based on plastic net products into which various chromophores and light dispersive and re ective elements are introduced during manufacturing. The nets are designed to screen various spectral bands of solar radiation, and/or transform direct light into scattered light. The spectral manipulation promotes desired physiological responses that are light regulated, while the scattering improves the penetration of the modi ed light into the inner plant canopy.

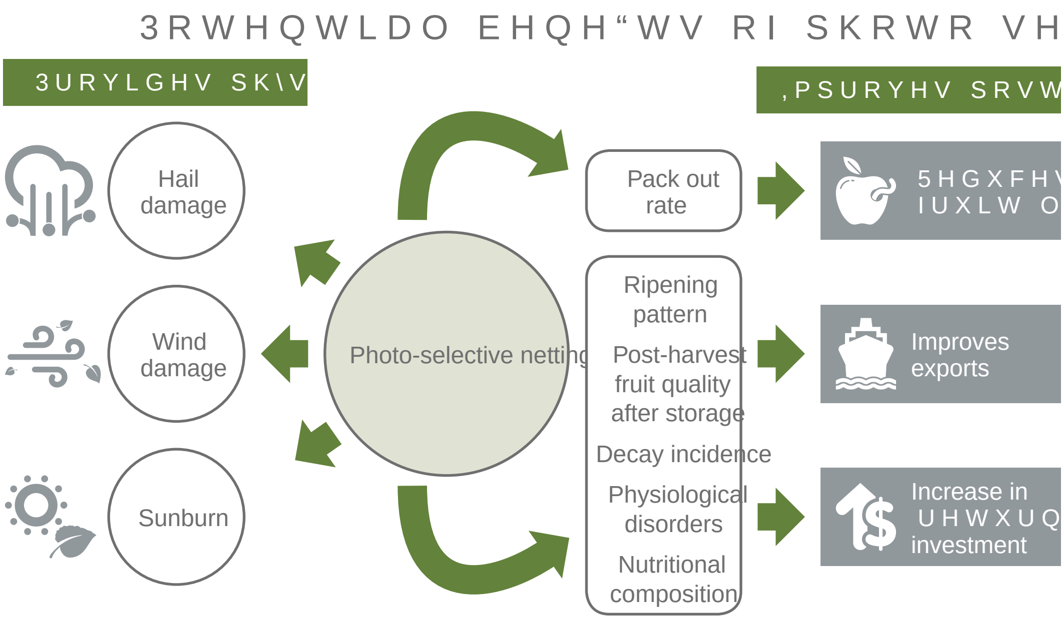
"The available literature on photo-selective nets de nitely points to the fact that the modi cation of light quality and micro-climate conditions caused by these nets can improve the fruit quality both at harvest and during post-harvest storage," says Prof. Sivakumar.

She points out that the word "quality" includes the nutritional value of fruit. Studies Prof. Sivakumar previously carried out on tomatoes and sweet peppers have proven that the manipulation of light quality through the use of photo-selective netting can improve the dietary phytochemical composition of the produce. "Strengthening avocados' growing reputation as a health-promoting food is thus further motivation to conduct in-depth research into the in uence of photo-selective nets on post-harvest storage quality," she says.

Although avocados are not grown under netting in South Africa, growers' interest in this technology is mounting as they seek to reduce the physical damage that hail, wind and sun cause.The interest would increase exponentially if photo-selective nets prove to be an opportunity to increase exports by improving pack-out rates and ripening patterns, while decreasing post-harvest losses due to defects and decay in the packhouse, and improving the nutritional status of the fruit.

The research study

Building on previous research ndings, Prof. Sivakumar led a study jointly funded by the



PHI Programme and industry to investigate the effect of different colours of photo-selective shading nets on the post-harvest quality of 'Hass' avocados. Working with her were Dr Zelda van Rooyen from Westfalia Technology Services, and Peter Tinyane, a PhD student.

The study set out to measure:

- The incidence of sun damage, dry matter and oil content of the fruit, resulting in improved pack-out rates.
- Post-harvest fruit quality parameters, namely fruit rmness, incidence of anthracnose, stem-end rot, number of days to ripen after harvest and after cold-storage, ethylene emission, incidence of physiological disorders (vascular browning, grey pulp), and eating quality.
- Post-harvest fruit quality parameters (same as above) in avocados that are subjected to the ready-to-eat ripening programme (Westfalia fruits).
- The status of dietary phytochemicals and fatty acid composition, cell wall softening enzymes and antifungal compounds after post-harvest storage.

Prof. Sivakumar emphasises that the study outcomes were not only focused on the improvement of pack-out rates. "We wanted to address the ripening quality of 'Hass' avocados, especially in the context of ready-to-eat programmes."

HOW THE SUN INFLUENCES AVOCADOS

Previous studies have shown that pre-harvest exposure to the sun in uences post-harvest responses of 'Hass' avocados. Higher fruit surface temperatures caused by prolonged exposure to sunlight delay ripening, affect the enzymes that soften cell walls, and increase the fruit rmness in avocados. Exposure to higher temperatures can, therefore, negatively affect the ready-to-eat ripening programme.

2

PROJECT TITLE
Photo-selective nettings to improve post-harvest fruit quality of 'Hass' avocados

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DURATION
One year and ten months

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R407 210 & R197 210

LEAD INSTITUTION
Tshwane University of Technology (Department of Crop Sciences)

BENEFICIARY
The subtropical fruit industry

FOCUS AREA
Post-harvest physiology and pre-harvest aspects

HUMAN CAPITAL DEVELOPMENT
One D-Tech student

PRESENTATIONS AND PAPERS DELIVERED
Two

PUBLICATIONS
Pending


1

marketable fruit – according to SAAGA standards – was determined over two years.

To determine the impact of the nets on post-harvest quality, 100 disease-free uniformly shaped avocados, without any injuries or defects, were selected per experimental unit. The fruit was stored separately for all the parameters. Subsequently, a set of 14 fruits was packed in commercial cartons and stored at 5,5°C and 85% relative humidity for 28 days, and thereafter at 25°C to simulate market shelf conditions. At the end of the storage period, the fruit was evaluated for chilling injury, number of days to ripen, fruit rmness after storage and at ripe stage, and the fruit quality parameters (incidence of anthracnose, stem-end rot, esh colour, and eating quality).

For the ready-to-eat ripened avocados, the same methodology was followed, except that the storage period at 5,5°C was 21 days, instead of 28. Following that, the fruit was subjected to commercial ripening. After ripening, the fruit was evaluated for anthracnose and stem-end rot incidence, fruit rmness (softness), and eating quality.

Methodology and results

Photo-selective nets were installed at a Lombard's farm in the Tzaneen area of the Limpopo Province during January 2015. Over the next two years, Prof. Sivakumar and the student who worked with her monitored the micro-climate that was created under the nets, as well as the post-harvest quality of the avocados that were grown there.

Quality and pack-out rates were measured by sorting 60 to 80 avocados per replicate net in the packhouse in order to identify sun-damaged fruits. The total number of

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Analyses of the dietary phytochemicals, fatty acid composition, non-structural C_7 sugars, and antioxidant capacity were done on a set of 16 fruits per replicate net.

The researchers found that shade nets signi cantly reduced sun damage. The different colour nets delivered different results:

- Total yield was lower under the pearl net.
- Pack-out rate was higher under the blue and white nets.
- Fruit size distribution was better under the blue net.
- Post-harvest ripening was delayed in fruits produced under the pearl and red nets, and in the open eld.
- Oleic acid was high under the white net, whereas major phenolic acids, vitamin E, and C_7 sugars were high under the red net and in the open eld.

Based on their results, the research team concluded that blue and white photo-selective nets improved yield and pack-out rates, and can be recommended for growing 'Hass' avocados.

LEARNING FROM OTHER CROPS

Research projects on the effect of photo-selective netting on the post-harvest quality of tomatoes, sweet peppers, lettuce varieties and aromatic herbs have been carried out by the Department of Crop Sciences, Tshwane University of Technology, between 2010 and 2016.

Results obtained over two growing seasons showed that different tomato cultivars responded differently during post-harvest storage. The photo-selective nets improved the marketability of the crops after low-temperature storage more so than the commercially used nets. The incidence of post-harvest decay and physiological disorders were signi cantly reduced, and at least one the cultivars' dietary phytochemicals were improved under the photo-selective nets.

Too much sunlight damages avocado skin, delay ripening and affect the enzymes that soften cell walls.

A set of 16 fruits per replicate net was selected and analysed to determine the composition of dietary phytochemicals, fatty acids, sugars, and antioxidants.

White nets improve yield and pack-out rates, while red nets ( ) delay post-harvest ripening.

For his PhD thesis, Peter Tinyane investigated the in uence of photo-selective shade netting on the quality of avocados at harvest and during post-harvest storage.

4

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Solving the 'Hass' skin colouring riddle

When consumers feel they cannot trust the external appearance of an avocado to tell them when the inside is ready to enjoy, exports come under threat. Fortunately, a recently completed three-year study provided some answers.

Dr Nhlandhla Mathaba, project leader.

From left: Mr Sakhile Mathe (PhD student), Dr Nhlanhla Mathaba (project leader), Mr Justice Mlimi (laboratory assistant) and Mr Jan Ntandane (eld assistant).

Consumers are willing to pay more for the convenience of buying pre-ripened 'Hass' avocados.

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THE SKIN COLOUR of 'Hass' avocados changes from green to purple to almost black when they ripen. This movement through the colour spectrum has become an established ripening indicator for growers and consumers alike.

In recent years, however, this ripening guide seemed to have failed markets importing 'Hass' avocados from South Africa. In some consignments, variable colouring and colouring that did not correspond with the softening of the avocado esh, have resulted in quality challenges.

Consequently, consumers in especially our most lucrative export markets have started questioning the reliability of colour changes as a ripening or softening indicator.

Realising the threat that such doubts pose to the South African avocado industry's credibility and ultimately market share, the South African Avocado Growers' Association (SAAGA) commissioned the Post-harvest Technologies Division of the Agricultural Research Council's Institute for Tropical and Subtropical Crops (ARC-ITSC) and Lowveld Post-harvest Services to determine the pre- and post-harvest factors that could be causing the problem.

Leading the study was Dr Nhlandhla from the ARC-ITSC, assisted by Dr Thieho Mafeo from the University of Limpopo.

"Our local industry cannot afford to compromise its competitive position," says Nhlanhla. "It might not be able to reclaim lost ground, given that countries like Chile, Peru and Australia, whose production seasons are similar to ours and that don't have the 'Hass' colouring problem, will only be too happy to step into the breach."

The industry and PHI Programme-funded study was done over two years, with each season's ndings used to inform the objectives of the next year. The outcome was a cumulative set of results that painted a clear picture of the factors that in uence variable colouring.

2014

In 2014 SAAGA funded the research. Building on previous studies that have indicated that skin colour change is affected by ripening temperatures, Nhlanhla designed a study to investigate the role of orchard block slope, harvest maturity and ripening temperature on variable colour change in 'Hass' avocado fruits.

'Hass' avocados were harvested in May, June and July – to represent early, mid and late season fruit – from two blocks, each with an upper and lower slope, in the Kiepersol area. The fruit was taken to the ARC-ITSC post-harvest laboratory where it was sorted and graded, and then stored at 5,5°C for up to 28 days. After withdrawal from cold-storage, fruit samples were ripened at 16°C, 21°C and 25°C, respectively. During ripening, the avocados were evaluated at 0, 2, 4, 6 and 8 days for rmness, skin eye colour, chromatic skin colour, and external and internal chilling damage.

> The results from this study con r
> variable skin colour development c
> is predominantly an early-season
> regardless of production area, orc
> fruit canopy position, ripening ter
> or post-harvest treatment with 1

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The research team concluded that the incidence of variable colouring in 'Hass' avocados during ripening is prevalent mainly in early harvested fruit, irrespective of ripening temperatures.

Interestingly, fruit harvested from orchard blocks with slopes was found to be most susceptible to variable colouring during ripening. In addition, variable colouring is escalated by external chilling damage, and external chilling damage is associated with fruit harvested from lower slopes.

2015

The 2015 study, which was funded by the PHI Programme, built on the 2014 outcomes, mainly to verify the pre-harvest factors that had been identi ed. In addition, it took inspiration from studies that had found that 1-MCP (1-methylcyclopropene) had an insigni cant effect on the colour development of 'Hass' fruit during ripening.

The aims of the continuing research were therefore to investigate the effects of:

- 1-MCP and harvest time;
- production region, harvest time and orchard slope; and
- production region, harvest time and fruit canopy position, on 'Hass' avocado skin colour development during ripening.

For the 1-MCP and harvest time experiment, avocados were harvested in mid and late season in the Tzaneen area. They were sorted and treated with 1-MCP, and then placed in cold-storage at the ARC-ITSC post-harvest laboratory.

For the production area, harvest time and orchard slope experiment, avocados were harvested in early, mid and late season in the Tzaneen and Hazyview areas. The harvested fruit was sorted, graded and placed in cold-storage at the ARC-ITSC post-harvest laboratory.

For the production area, harvest time and fruit canopy position experiment, avocados were harvested in early, mid and late season in the Tzaneen and Hazyview areas, from both inside and outside the tree canopy. They were transported to the ARC-ITSC post-harvest laboratory in Nelspruit for sorting, grading and storage.

In all cases, the fruit was cold-stored at 5,5°C for 28 days, and then ripened at 21°C. During ripening, the fruit was evaluated for rmness, and subjective and objective colour.

The results from this study con rmed

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PROJECT TITLE
Colour change problem in 'Hass' avocado

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DURATION
Two years

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R627 200 & R247 200

LEAD INSTITUTION
ARC-Institute for Tropical and Subtropical Crops

BENEFICIARY
The subtropical fruit industry

FOCUS AREA
Post-harvest physiology and pre-harvest aspects

HUMAN CAPITAL DEVELOPMENT
One PhD student and one MSc student

PRESENTATIONS AND PAPERS DELIVERED
Four

PUBLICATIONS
Three

The impact of 1-MCP on the colour development of 'Hass' fruit during ripening is illustrated by the poor skin colour development of untreated avocados during early season.

that variable skin colour development during ripening was predominantly an early-season occurrence, regardless of production area, orchard slope, fruit canopy position, ripening temperature or post-harvest treatment with 1-MCP.

The researchers did nd that production site played a role, given that fruit from Tzaneen showed a higher de-synchronised colour change even during mid and late season. Similarly, inside canopy fruit seemed to display skin colour de-synchronisation even during mid and late season, as did fruit harvested from the lower parts of orchard slopes. The latter, however, was production site dependent.

2016

In the 2016 season, Nhlanhla and his team investigated whether girdling, which is a pre-harvest cultivation practice, drives sugars towards the fruit skin and, therefore, enhances anthocyanin synthesis. However, they found that girdling only improved fruit size.

The team concluded that the environment had a minimal impact on how colour developed in 'Hass' avocados, and that colour development could mainly be manipulated at protein and enzyme level.

Consequently, a new research proposal on the impact of proteins and enzymes on anthocyanin synthesis and skin colour change will be drafted and submitted to the industry for consideration.

1

KNOW YOUR AVOCADO OIL

As the worldwide drive to live healthier lifestyles gathers momentum, avocado oil is becoming increasingly popular. It is not surprising, considering that the oil is derived from a fruit known for its health properties.

Avocado oil is packed with mono-unsaturated omega-3 and omega-6 fats, and is naturally cholesterol free.

Its high smoking point of 250°C means avocado oil is safe for high temperature cooking methods, such as frying, baking and roasting, and makes it a good substitute for sun ower and canola cooking oils.

Because of its neutral avour, avocado oil can be used for a virtually unlimited variety of dishes without in uencing the taste of the food.

In South Africa, Westfalia makes avocado oil from locally grown 'Hass' avocados.

For the 1-MCP and harvest time experiment, avocados were harvested in mid and late season. They were sorted and treated with 1-MCP, and placed in cold-stored at 5,5°C for 28 days, and then ripened at 21°C. During a six day shelf life simulation period it became evident that variable skin colour development during ripening was predominantly an early-season occurrence, regardless of treatment with 1-MCP. The team also found that fruit from orchards planted on slopes was more prone to variable colouring during ripening.

Citrus

According to Jan van Riebeeck's diary, the first orange trees arrived in the Cape on the ship "de Tulp" from St. Helena on 11 June 1654. By 1661, more than 1 600 orange and lemon trees had been planted in his private garden.

However, it took almost 250 years for South African citrus to start making its mark internationally. In 1907, the shipping of 3 000 cartons of citrus to the United Kingdom marked the first official exports.

Today South Africa is one of the world's major citrus exporters. This formidable position is no small achievement, given the complexity of the citrus export scene. Producers and exporters have to manage fruit from both winter and summer rainfall areas, meet an enviable array of cultivar requirements, satisfy destination market and regulatory requirements, and cope with the competitiveness of marketers and supermarkets.

The European Union is the biggest consumer of South African citrus, followed by Asia.

The first local citrus producers' organisation, the South African Cooperative Citrus Exchange (SACCE), was founded in August 1926. Its purpose was to create a single channel market structure that would increase bargaining power and to collect levies from members.

Understanding the need for research and development, SACCE invested funds in post-harvest research and, in 1974, opened the Outspan Citrus Centre (OCC) in Nelspruit. As the home of industry-driven research and extension, the OCC set out to improve fruit quality and cultivar availability, and establish Outspan as an international brand name.

Following deregulation in 1998, the industry had to regroup. The Citrus Growers' Association (CGA) was formed and, in 2001, took over the OCC and transformed it into Citrus Research International (CRI), the institution that now manages almost all citrus research in South Africa.

Some of the citrus industry's greatest challenges currently arise from food safety concerns, particularly the lowering of minimum residue levels (MRLs). Too often the requirements imposed by supermarkets have no scientific basis and are used for marketing purposes. The biggest concern, however, is disease management. MRLs that are too low allow spores to spread and could undermine sustainable production in the long run.

The South African citrus industry achieves continuous improvement by focusing on three areas:

- Responsive quality management;
- Research that addresses industry-determined focus areas; and
- Technology transfer involving all tiers of production staff.

FAST FACTS

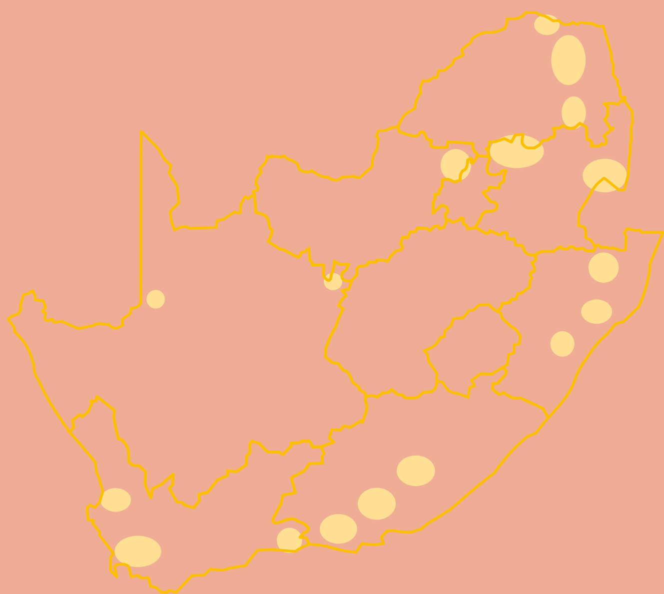
In 2015, South Africa exported a record 118,7 million 15-kg equivalent cartons of citrus.

James Percy Fitzpatrick, the author of Jock of the Bushveld, was a pioneer of commercial citrus farming in South Africa. In 1922, he started marketing citrus grown on his farms in the Sundays River Valley under the Outspan brand.

In 1937, Outspan became the trading name for all South African export citrus.

In the 1970s and 1980s, the Outspan brand was the focus of an anti-apartheid consumer boycott campaign in Europe and Britain.

Citrus is traditionally regarded as winter fruit, but the local season starts in March with easy peelers, mandarins and lemons, and stretches as far into summer as November, when the last oranges are harvested.



Western Cape: Boland, Citrusdal, Ceres; Eastern Cape: Patensie, Eastern Cape Midlands, Sundays River Valley; Kwa-Zulu Natal: Pongola, Nkwalini, KZN Midlands; Northern Cape: Vaalharts, Orange River region; Mpumalanga: Onderberg, Malelane, Nelspruit, Senwes; Limpopo: Hoedspruit, Letsitele, Vhembe.

> Only through industry-wide coopera
> achieving effective, industry speci c pr
>
> Dr Wilma du Plooy, Post-harvest Research and Programme Coordinator (CRI)

Keeping up appearances

A project that set out to validate models that predict the rind quality of citrus fruit has con rmed the role that pre-harvest conditions, notably canopy position, play in maintaining the looks of mandarins, oranges and grapefruit.

Dr Lembe Magwza, project leader (centre), with Dr Samson Tesfay (left) and PhD student, Olaoluwa Olarewaju, who participated in the research.

Rind breakdown disorder only starts developing during storage, about three to ve weeks after harvesting.

CONSUMERS BUY WITH their eyes. Whether they shop for furniture, fashion or, indeed, fresh fruit, appearance counts – and determines prices. Existing fruit grading systems handle fruit with slight external defects in one of two ways. It is either graded and marketed with unblemished fruit, thereby reducing the value of the entire batch, or it is graded and rejected together with seriously damaged fruit, causing unnecessary nancial loses.

Against this backdrop, the University of KwaZulu-Natal, with the support of the citrus industry and the Post-Harvest Innovation Programme, is investing time and resources into nding ways to predict and detect physiological rind disorders that make export citrus less attractive, and limit storage potential.

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One of the main dif culties related to physiological rind conditions, such as rind breakdown disorder (RBD), is their progressive nature. RBD does not manifest during harvest or fruit grading in the packhouse; symptoms only start developing during storage, about three to ve weeks after harvesting. In the case of export fruit, this window matches the commercial shipping period and the fruit's arrival at the point of sale. The result is customer complaints at best, and huge nancial losses at worst.

Physiological rind disorders have long been the eld of study of Dr Lembe Magwaza of the Agricultural Plant Sciences Department at the University of KwaZulu-Natal. Building on studies that have shown that different microclimatic conditions during the growing season and within a tree canopy in uence the physico-chemical properties of citrus rinds and play a signi cant role in fruit susceptibility to physiological rind disorders in 'Nules Clementine' mandarins, Lembe's own research con rmed that 'Nules Clementine' mandarins harvested from inside the canopy had lower rind dry matter content (DMC) and were more susceptible to RBD.

These ndings suggested that rind DMC at harvest could be used as a pre-symptomatic indicator of fruit susceptibility to RBD. As a possible benchmark to reduce RBD, Lembe recommended that rind DMC should not be below 30g/100g fresh weight (FW) at harvest.

The limitations of DMC limits

Valuable as it was, Lembe's DMC benchmark had its limitations, chief among them the measurement of DMC. Current practice entails conventional destructive techniques on representative samples of a batch of fruit. Although widely used, these techniques are time-consuming and the results only re ect the DMC properties of the speci c fruit sample evaluated.

"Given how widely rind attributes vary

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between fruit batches, we had to nd innovative and non-destructive tools for predicting rind quality parameters and fruit susceptibility to physiological rind disorders," says Lembe.

This quest led him to considering near infrared spectroscopy (Vis/NIRS). Among non-destructive quality assessment techniques, Vis/NIRS is the most advanced with regard to instrumentation, applications, accessories and chemometric software packages. As such, it is widely used to carry out non-destructive, post-harvest quality assessments of fruit and vegetables.

Studies that Lembe conducted in 2011 and 2012 demonstrated the feasibility of Vis/NIRS to predict the rind physico-chemical pro le of 'Nules Clementine' mandarins. Partial least squares (PLS) models to predict rind physico-chemical properties were developed using fruit in the Stellenbosch, Citrusdal, Paarl and Porterville areas, and very high prediction accuracy was obtained. The good correlation between spectral information and sugar concentrations and DMC demonstrated the potential of Vis/NIRS as a non-destructive tool to predict mandarins' susceptibility to physiological rind disorder. Principal component analysis (PCA) and PLS-discriminant analysis (PLS-DA) models were developed to sort fruit based on canopy position and susceptibility to RBD.

All these models, however, were validated using fruit from the same cultivar ('Nules Clementine') and the same four citrus-growing regions in the Western Cape.

The next phase

To be useful to the South African citrus industry as a whole, the models that Lembe had developed needed to be validated for the citrus-growing regions in KwaZulu-Natal, the Eastern Cape and Mpumalanga, and for other citrus fruit, such grapefruit and oranges. In addition, the potential of Vis/NIRS-based hyperspectral imaging to differentiate between structures with different densities – and thus its use to visualise rind microstructures associated with rind disorders – had to be explored.

This workload was formalised into a study that started in March 2015 with the overall aim of developing non-destructive methods to predict the quality of citrus fruit.

To achieve its aim, the study has three objectives:

- Validate and test the robustness of the Vis/NIRS PCA and PLS models developed for 'Nules Clementine' mandarin fruit to predict the rind quality of 'Benny' Valencia oranges and 'Marsh' grapefruit.
- Investigate the robustness of Vis/NIRS PCA and PLS models developed for the Western Cape to predict the rind quality of fruit harvested in other citrus-growing regions in South Africa.
- Investigate the feasibility of hyperspectral

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PROJECT TITLE
Non-destructive prediction and monitoring of post-harvest rind quality of citrus fruit using Vis/NIR spectroscopy

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DURATION
Two years

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R595 219 & R145 219

LEAD INSTITUTION
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BENEFICIARY
The citrus fruit industry

FOCUS AREA
Post-harvest physiology and pre-harvest aspects

HUMAN CAPITAL DEVELOPMENT
One PhD student and two MSc students

PUBLICATIONS AND PAPERS
Five

PRESENTATIONS
Three

3

1 2 3 4

Different levels of stem-end rind breakdown on 'Marsh' grapefruit after nine weeks of post-harvest storage. Left to right shows increasing intensity from no (1) to little (2), moderate (3) and severe (4).

1

Dr Magwaza scans intact 'Marsh' grapefruit with a laboratory benchtop re ectance near infrared spectrophotometer.

Different levels of rind pitting on 'Marsh' grapefruit after nine weeks of post-harvest storage. Left to right shows increasing intensity from none (1) to little (2), moderate (3) and severe rind pitting (4).

HARVEST AREAS

- 'Nules Clementine' mandarins – Eastern and Western Cape.
- 'Benny' Valencia oranges – Crocodile Valley Estates (Nelspruit), Mpumalanga, and Limpopo.
- 'Marsh' grapefruit – KwaZulu-Natal, Limpopo and Mpumalanga.

spectroscopy to characterise rind quality and structural changes associated with chilling injury, rind breakdown and rind pitting disorders.

According to Lembe, this non-destructive technology development research has an important long-term aim. "Our eventual goal is the commercial implementation of non-destructive technology in packhouses. Armed with the robust, stable and accurate models for predicting rind quality that will come out of this study, we will approach the National Research Foundation (NRF) for funds to build a prototype for the practical and commercial in-line application of Vis/NIR spectroscopy."

Methodology

During May 2015, mandarins, oranges and grapefruit were harvested from orchards in the Eastern and Western Cape, Mpumalanga, Limpopo and KwaZulu-Natal.

In all orchards, 30 uniform trees were identi ed and marked for sampling. On each tree, 30 fruit of uniform size from the sun-exposed (outside) and six from shaded canopy positions (inside) were randomly selected and harvested at commercial maturity. The fruit was transported to the Post-harvest Research Laboratory at the University of KwaZulu-Natal and to Stellenbosch University where it was sorted and weighed, before being treated and stored according to post-harvest industry practices.

A further 100 blemish-free fruit, representing all orchards and cultivars, was selected for non-destructive evaluation with Vis/NIRS. Following the Vis/NIRS scanning, the fruit was stored for 12 weeks between -0,6 and 0,5°C, a temperature that is known to cause the highest degree of RBD, rind pitting or chilling injury incidence. During the 12 weeks, the fruit was scored biweekly for incidences of rind pitting and rind breakdown disorder. Disorders were scored by visual inspection on a subjective scale from none (0) to little (1), moderate (2), and severe (3) rind breakdown.

DISORDERS DISFIGURING C

The appearance of citrus fruit is marred by different types of post-harvest rind disorders, including:

- chilling injury,
- rind pitting in oranges and grapefruit,
- rind breakdown disorder (RBD) in 'Nules Clementine' mandarins, and
- peteca spots on lemons.

Results

The results showed that the physico-chemical properties of fruit are affected by position in the canopy. Fruit inside the canopy were greener and less mature, and weighed less than fruit on

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1 2 3 4

the outside.

Similarly, the evaluation of rind pitting and chilling injury showed that the outside fruit was less susceptible to physiological rind disorders.

Grapefruit from the same orchard differed in their susceptibility to chilling injury and rind pitting disorders. The difference correlated with parameters that indicated the fruit's physiological properties, which differed based on where the fruit was positioned on the tree.

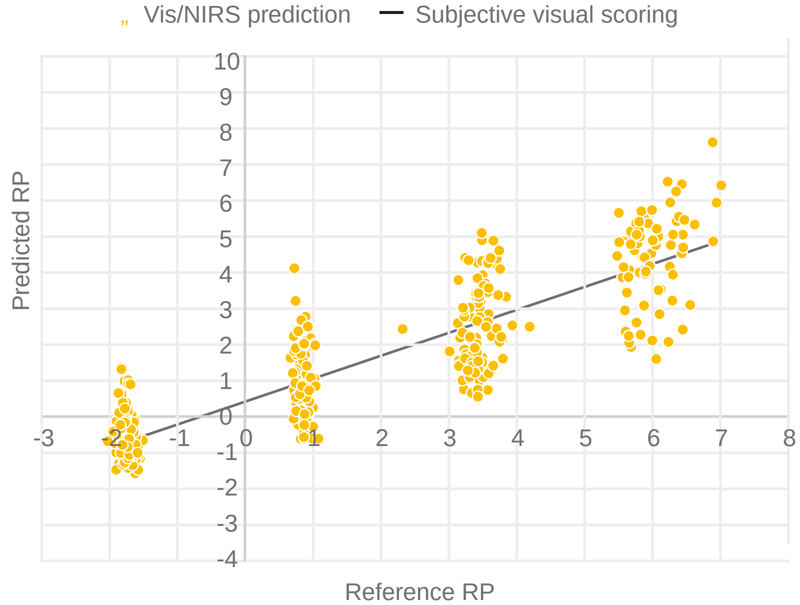
Fruit that grew inside the canopy in the two orchards in Limpopo and KwaZulu-Natal had higher chilling injury incidence (27% and 24%) compared to their counterparts outside the canopy (12% and 0%). In both sites, rind pitting was lower on inside (13% and 2% respectively) than on outside canopy fruit (19% and 9%). Fruit from inside the canopy had higher concentrations of sucrose, fructose and antioxidant activity, and lower dry matter, compared with outside canopy fruit.

The relationship of parameters to rind disorders was also examined as a way to predict the possibility that fruit could develop rind disorders. Parameters with positive correlations to rind pitting did not show a positive correlation to chilling injury, which prompted the idea of using parameters to predict possible disorder incidences.

Lembe's team successfully developed PLS models to predict internal quality parameters based on total antioxidant capacity, carotene, total carotenoids, chlorophyll a, chlorophyll b, dry matter, sucrose, glucose and fructose. Rind pitting, for example, was predicted with an accuracy of 78% (Fig. 1).

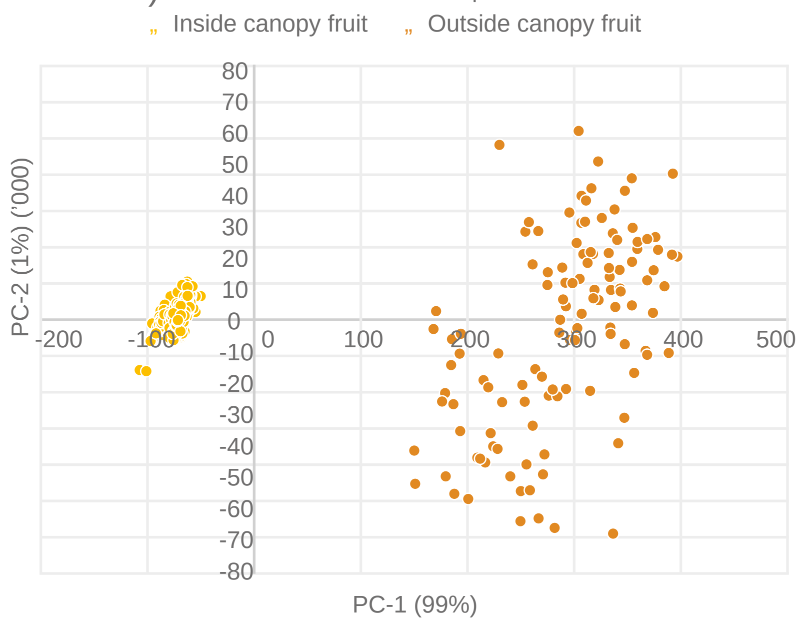
The spectral information from fruit harvested from different canopy positions indicated enough differences to con rm the potential of Vis/NIRS spectra to segregate fruit based on canopy position (Fig. 2). The ability of Vis/NIR spectroscopy, coupled with chemometric analysis, to cluster fruit based on original canopy position during sorting and packaging is recommended as a secondary approach to identify fruit with a high chance of developing rind pitting.

)LJXUH 3/6 SORWV RI JUDS



The correlation of rind pitting values predicted using Vis/NIRS compared to subjective visual scoring.

)LJXUH 3&$ SORWV RI JUDS



The principal components analysis shows the difference that canopy position makes to fruit quality.

Big Brother is watching you, FCM

Two projects, both supported by the PHI Programme, are developing cutting-edge technol detect false codling moth (FCM) infestations as part of a post-harvest systems approach t management. FCM is nally running out of places to hide.

> Manual grading on packhouse lines detects only around 20% of FCM-infested fruit.

FCM IS ONE of the most important pests on citrus, causing notable nancial losses to the Southern African industry. These losses occur because of dropped fruit in the orchard, waste in cartons due to decay, and access issues in sensitive markets.

The EU, which is by far the largest market for South African citrus, is becoming increasingly strict on the phytosanitary risks of FCM. A pest risk assessment (PRA) recently completed by the European Plant Protection Organisation (EPPO) concluded that FCM in citrus from Africa poses a meaningful risk to certain countries within the EPPO region. The implication is stricter control measures. However, cold sterilisation to these markets would not be economically viable for local producers.

Several effective pre-harvest control measures exist for FCM, but there is no silver bullet that can ensure no infested fruit reach the packhouse. Given that there is zero tolerance for FCM, the industry has to urgently investigate post-harvest detection methods to avoid phytosanitary interceptions and prevent enforced cold sterilisation.

Spearheaded by Citrus Research International (CRI), two studies that were jointly funded by the CRI and the Post-Harvest Innovation Programme, were done to address this need. The one project, led by Wayne Kirkman, a technician and researcher at CRI, focused on automatic sorting equipment. The other project was led by Dr Sean Moore, Portfolio Manager: IPM at CRI, and sought to use volatile emissions to detect FCM infestation.

FCM detection goes online

Wayne Kirkman, project leader.

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COLLABORATIVE RESEARCH WITH sorting equipment manufacturers to develop systems to detect FCM-infested fruit in an online grading system has been conducted over the past few years. Although there has been progress, no system can yet detect FCM at all stages of infestation.

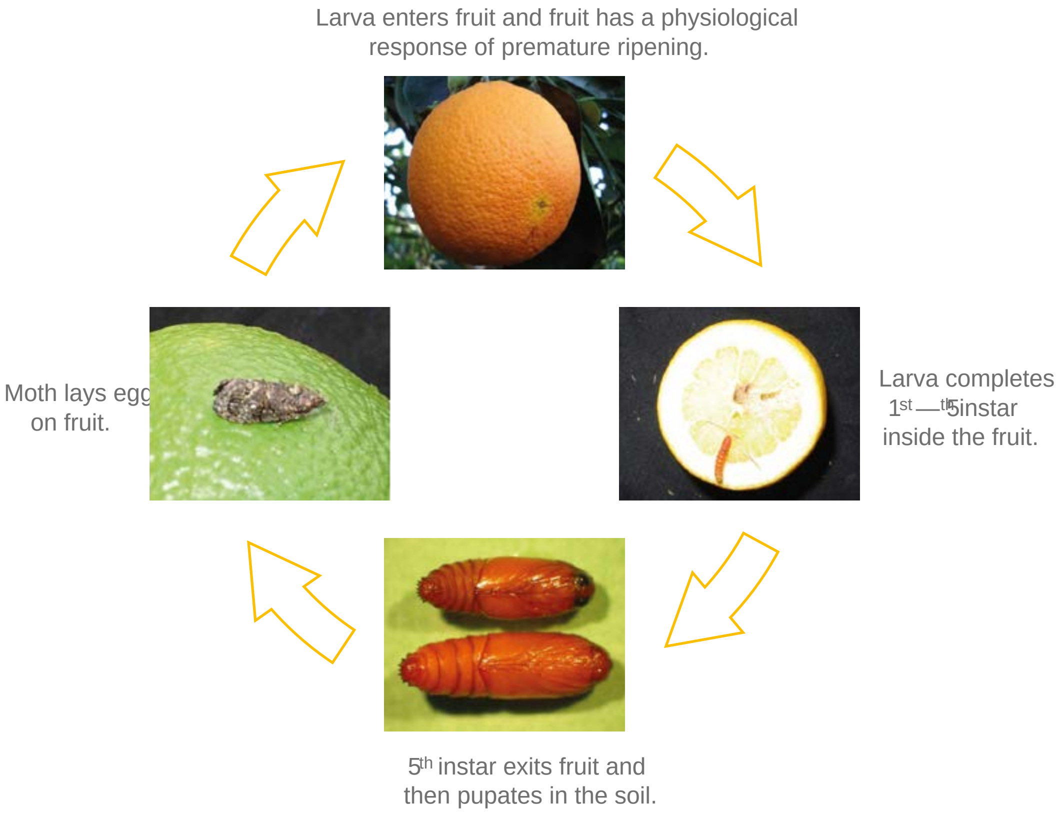
The matter is complicated by the fact that most of the leading manufacturers are based in Europe and, due to FCM's phytosanitary status, research cannot be conducted there. The solution was for CRI – with experts on the pest and its behaviour – to do the latest round of research in South Africa, using equipment provided by the manufacturers and working with their experts to interpret results and ne-tune the systems.

Wayne Kirkman, the principal investigator, signed up two manufacturers (Company A and Company B) in a project that stands to bene t all involved. CRI will provide local citrus producers with a solution to one of their most serious business risks, while the manufacturers can gain a competitive edge by improving the ability of their automatic, online grading systems.

In January 2015 the project started, with these two objectives in mind:

1. Assist the two manufacturers to improve the ability of their automatic online grading systems to detect FCM in citrus fruit. The main aim was to detect all fruit infested with 3rd, 4th and 5th instar larvae. (Previous trials have shown that abbreviated cold treatment

)LJXUH)&0 OLIH F\FOH



– 2 C for 18 days – kills all the smaller larvae.)

2. Evaluate the sorting equipment after improvements have been implemented.

The goal is for CRI to be able to make informed decisions about the role that automatic grading units can play in a systems approach towards mitigating the risk of FCM in export fruit.

How the work was done

Company A

Company A has a sorting unit that employs four different visual detection technologies that collectively take 80 images of each fruit at full packhouse line speed.

Technicians and researchers from the company were present on two occasions when several hundred oranges (naturally infested with FCM) were run through their unit. Where visible, penetrations were marked with a permanent marker, so that the programmers could see exactly where infestation took place, and could use these images to improve algorithms to detect FCM.

Several other batches of fruit (laboratory and naturally infested) were run through the system at two packhouses in the Sundays River Valley, and images were downloaded in Europe. All the oranges were dissected after scanning to verify infestation, and this information was sent to the programmers.

Company B

Company B has a demonstration unit and

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PROJECT TITLE
Detection of FCM with automatic sorting units

PRINCIPAL INVESTIGATOR
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DURATION
Two years

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R87 058 & R77 058

LEAD INSTITUTION
Citrus Research International (Pty) Ltd

BENEFICIARY
The South African citrus industry

FOCUS AREA
Post-harvest disease and insect control, including phytosanitary compliance

PUBLICATIONS
Pending

PRESENTATIONS AND PAPERS
One

Mature false codling moth (Courtesy: Pest and Diseases Image Library, Bugwood. org).

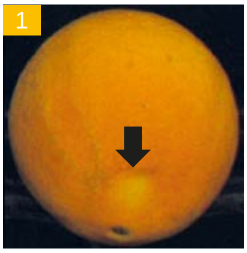
line at its of ces. Wayne visited the company on three occasions, where several hundred oranges, laboratory and naturally infested, were run through the demonstration unit. On the third visit, two new technologies, which are not currently part of the automated system, were tested in isolation on each fruit.

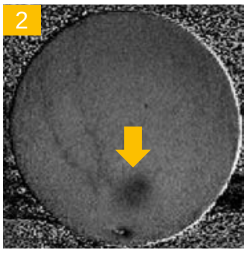
Two batches of laboratory infested fruit were couriered to Company B for further evaluation. Images were taken and sent to the head of ce in Europe for evaluation and algorithm development. All the oranges were dissected after scanning to verify infestation, and the information was sent to the programmers.

Results

Company A

Company A's technology is advanced for visual aspects, as shown in Figure 1, and 2 where the infestation point of a naturally infested Navel orange is highlighted by image saturation. The system was able to detect a high percentage of naturally infested fruit.





However, when laboratory-infested oranges were run through the unit, only a low percentage of FCM penetrations was detected. This was due to the fact that when oranges are arti cially infested they do not discolour, or form a blush, around the infestation point.

The nding that the unit was highly reliant on visual aspects to detect infestation, and that it missed early signs of decay, was relayed to the company. Algorithms are being updated.

Company B

The demonstration unit could detect FCM penetration points on naturally and laboratory-infested fruit (Fig. 3 to 5). The two new technologies, which were tested individually, both showed much promise. These technologies have been incorporated into Company B's demonstration unit.

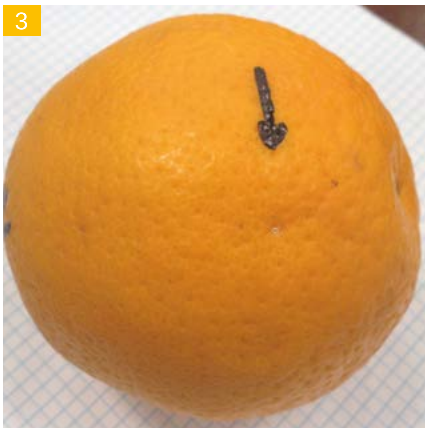
Collaboration with the two companies has led to improvements in their ability to detect FCM.

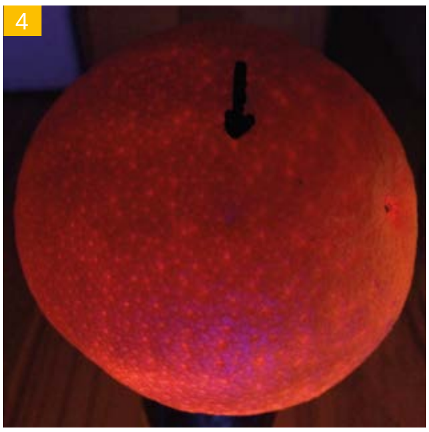
Company A is heavily reliant on its visual technologies, but is working on improving the ability to detect early signs of decay. Company B has added two new technologies to its demonstration unit, which should enhance its capabilities.

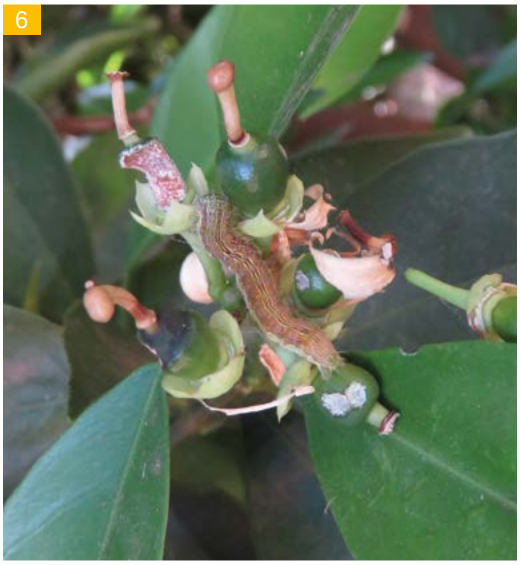
The project was terminated at the end of 2016, to give the companies time to improve their equipment and programming. The project could be resumed after a few years.

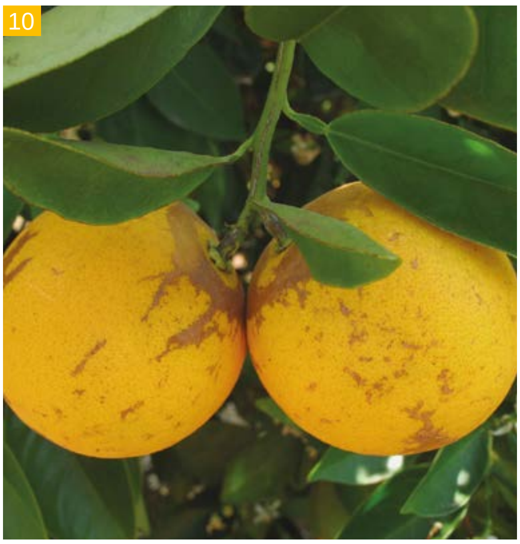
Images show how technologies were used to highlight FCM infestations. Algorithms were updated by company programmers, and this led to improved FCM detection. A naturally infested Navel orange ( ), with the FCM penetration point highlighted by image saturation ( ).

New technologies tested individually, and added to the unit of Company B, improve FCM detection.









Some of the most common pests in citrus orchards. These pests can cause cosmetic damage (reduced exportable fruit), crop loss, stunted growth of trees, and even tree deaths. (Images courtesy Peter Stephen (CRI).)
Bollworm larva and damage.
Fruit y laying eggs.
Mealybug.
Psylla.
Thrips damage.
Red scale on unripe citrus fruit.

Breathalysing fruit to nd FCM

HEALTHY AND INFESTED fruit have different volatile emission pro les, suggesting that volatiles analysis has great potential as a post-harvest screening method.

Using a solid phase micro-extraction (SPME) probe, which has been shown to effectively trap and concentrate headspace volatile compounds surrounding intact fruit, a previous study has found that infested oranges release ve major volatile compounds. These are D-limonene, 3,7-dimethyl-1,3,6-octatriene, (E)-4,8-dimethyl-1,3,7-nonatriene, caryophyllene and naphthalene.

Sean's study, on which he was assisted by Wayne Kirkman, wanted to build on this previous work by verifying results and re ning processes to avoid unwanted variables. "We also wanted to identify compounds unique to infested fruit, which could be detected instantaneously by other technologies, such as near infrared spectroscopy," says Sean. "The ultimate aim was to develop a detection system that could expose infested citrus fruit on a packhouse line."

Packhouse lines run at speeds of up to 10 fruit per second, which might be too fast for a gas chromatograph-mass spectrometer (GC-MS) system to detect infested fruit. However, once the volatile compounds associated with FCM-infested citrus have been determined, other faster technologies could be employed to instantaneously recognise these compounds.

"From the outset, we knew this work could have great scienti c value," says Sean. "If successful, these techniques could be used to identify citrus fruit infested by other pests; the technology could also be applied to different fruit types."

How the work was done

Late mandarins and Navel and Valencia oranges were inoculated with FCM on several different dates before they were ready to be harvested.

In the laboratory, the fruit was subjected to four different scanning technologies.

X-ray technology

Microfocus radiography produced a single 2-dimensional image, on which the damage was not clearly visible.

Microfocus tomography (CT scan) produced perfect images, but at 25 minutes per scan, it was too slow for practical use. The trial's focus changed to speeding up the scans, resulting in an eventual scan speed of 86 seconds.

Wayne concluded that CT scans could detect all infestations from six days onwards, including all second instar (L_2) and larger larvae, as well as larvae that had entered the esh. However, damage in the rind and albedo could not be detected.

Electronic nose

Collaborative research was conducted with the University of Leeds to explore immuno-sensor technology in the form of the "Bloodhound" electronic nose (e-nose). Wayne visited RoboScienti c in Leeds to be trained on the e-nose, and brought back an experimental unit to evaluate the ability of the equipment to detect FCM-infested fruit.

The e-nose uses an array of sensors (electrodes coated with semi-conducting polymers) to recognise bouquets of volatile emissions. The manner in which volatiles bind to the polymers affects the electric current running through the electrodes, enabling the e-nose to distinguish volatile bouquets, as opposed to individual compounds.

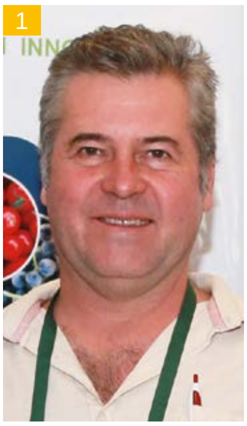
The aims of the e-nose research were to:

- Evaluate the ability of the unit to detect FCM infested fruit.
- Examine the reaction of each individual sensor to the volatiles.
- Construct a new array of sensors consisting of the best sensors.

During the trials, fruit was put into sealed bags or jars and left for 15 minutes to allow the headspace to equalise. An inlet needle was

Dr Sean Moore, project leader.

The gas chromatography-differential mobility spectrometry (GC-DMS) device, also known as the "suitcase", can detect Phytophthora-diseased rhododendron plants from leaf volatiles by essentially breathalysing the foliage. One of its great advantages is its portability.



inserted and the e-nose activated. The software then allocated the fruit to either the infested or the clean group.

Gas chromatography-mass spectroscopy (GC-MS)

The GC-MS trials had four aims.

- Identify volatile emissions associated with FCM-infestation of citrus fruit.
- Determine differences in levels of volatiles between healthy and infested fruit.
- Find compounds unique to infested fruit.
- Develop a method to rapidly detect these compounds.

The search for compounds unique to infested fruit entailed the analysis of segments of clean and infested fruit, as well as larvae extracted from fruit. The researchers found that oxime and methyl eugenol were present in infested fruit and in larvae, but not in clean fruit.

Differential mobility spectrometry (DMS)

Collaborative research was conducted with University of California, Davis on DMS. This technology was chosen because compensation voltage (CV) and retention time (RT) are unique for each volatile compound.

Although DMS has successfully detected Phytophthora-diseased rhododendron plants from leaf volatiles in trials, it will only be tested for FCM detection once a unit becomes available. In order to work in a commercial environment, the DMS unit will have to be much smaller. Work on miniaturising the unit has started.

Results

By the end of 2016, the X-ray work was completed. The technology can be used to scan consignment samples as part of a systems approach to FCM risk management.

The e-nose trials were successful. A new array of sensors, consisting of the ones that performed best, is set to further improve its detection capability.

The GC-MS study is still work in progress, but it is signi cant that use of this technology has, for the rst time, shown signi cant differences between clean and infested fruit by using volatile ratios.

The GC-DMS work will continue once a unit is made available to CRI.

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PROJECT TITLE
Identifying volatile emissions associated with false codling moth infestation of citrus fruit

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DURATION
Two years

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R381 591 & R271 591

LEAD INSTITUTION
Citrus Research International (Pty) Ltd

BENEFICIARY
The South African Citrus Industry

FOCUS AREA
Post-harvest disease and insect control, including phytosanitary compliance

HUMAN CAPITAL DEVELOPMENT
One PhD student

PUBLICATIONS
Pending

PRESENTATIONS AND PAPERS
One

Fumigation innovation knocks out phytosanitary pests

It is not often that one post-harvest treatment addresses a variety of challenges. Vapormate®, a fumigant that has been around for at least 90 years, could be one of those exceptions.

PHYTOSANITARY, OR QUARANTINE, pests are the bane of exporters' existence. Even one insect can cause whole consignments of export fruit to be rejected.

Trying to control these pests in the eld at levels that are acceptable to some export markets is extremely dif cult and compromises integrated pest management and biological control. Hence exporters are looking to post-harvest treatments for solutions.

For several pests, extended post-harvest cold treatments are required to guarantee "clean" consignments. The cold treatments may be detrimental to the crop and, in the case of grain chinch bug, fail to kill the target pest.

Fumigants offer a relatively quick solution without the risk of chilling injury. Until a decade or two ago, methyl bromide was the post-harvest fumigant of choice. However, due to environmental issues, the use of this product has been phased out in most countries. Phosphine is increasingly being offered as a solution, but with some limitations. It is only safe for fruit in a pure gas form, blended with nitrogen, and used at temperatures below 6°C. High-tech application equipment is also required, hence the treatment can only be done in a large, equipped-for-purpose cold room, as may be found at a port.

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Dr Tim Grout, project leader.

Grain chinch bug take refuge in the navel opening of an orange.

Finding a better way

In an attempt to nd a better way, Dr Tim Grout, Manager: Research and Technical at Citrus Research International (CRI), has focused his research on Vapormate®, a patented combination of ethyl formate and carbon dioxide that is a GRAS (generally recognised as safe) fumigant. It is usually effective after shorter exposure periods than phosphine, and is more effective at ambient than cold temperatures. In other countries, Vapormate® is already being used on a variety of fruit, flowers and vegetables, and Tim's earlier research showed that it is also safe on citrus. His objective with further research was to make GRAS fumigants available to growers who can use them in their packhouses without worrying about worker safety, the requirement of high-tech equipment, or needing to pay an expert to conduct the fumigation in a cold room.

Most other uses of Vapormate® are for pests that are found on the outside of the commodity, hence research on the impact on internal phytosanitary pests also needed to be conducted, along with the possible combination of fumigation with shorter cold treatments. If successful, this would result in a reduced likelihood of the kind of chilling injury that currently prevents lemons from being exported to cold-treatment markets like the USA, China and Thailand.

"Most citrus packhouses have degreening chambers that could be used to apply a GRAS fumigant to crates of fruit on arrival, before the fruit move over the packline and are waxed," says Tim.

Project scope

With the increasing rejections for the grain chinch bug and indigenous mealybugs, and the need for an alternative to cold disinfestation for false codling moth, Tim believes that GRAS fumigants could play a role in the future, either alone or followed by a short cold treatment. With PHI Programme and industry support, he started work on a two-year project at the CRI premises in Nelspruit in January 2015. It had two objectives:

- Develop specific Vapormate ® treatments that will guarantee citrus fruit to be free from external phytosanitary pests, such as the grain chinch bug, mealybugs, scale insects and Fullers rose beetle.
- Develop the most effective combination of fumigation with Vapormate ® or carbon dioxide and a short cold treatment at 2,0°C for the control of fruit fly and false codling moth, both internal phytosanitary pests.

PROJECT IMPACT ON THE CITRUS INDUSTRY

It can provide a post-harvest treatment alternative to sprays in the orchard for some external pests, leading to improved integrated pest management practices.

If external insects such as mites, mealybug, grain chinch bug, or armoured scale insects are found on fruit overseas, Vapormate® could be applied to prevent the fruit from being destroyed or diverted.

Fumigation with high concentrations of CO_2 followed by a short cold treatment, allows for a shorter cold treatment time than cold alone. This will allow access to closer markets and reduce chilling injury that occurs during the current 22-day cold treatment for false codling moth.

To meet the first objective, the research team built on preliminary research and international Vapormate® registrations that have indicated approximate dosages and treatment times for the control of external pests. They bracketed the "best guess" treatments with more severe and less severe treatments and evaluated them against relatively large numbers of individuals.

Regarding the second objective, preliminary research had shown variable levels of

PROJECT TITLE
GRAS fumigants for phytosanitary pests

PRINCIPAL INVESTIGATOR
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DURATION
Two years

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R307 218 & R297 218

LEAD INSTITUTION
Citrus Research International (Pty) Ltd

BENEFICIARY
The citrus, table grape and pome fruit industries

FOCUS AREA
Post-harvest disease and insect control including phytosanitary compliance

PUBLICATIONS
One

PRESENTATIONS
Two

After 14 days in storage, the untreated orange on the left displays young, white mussel scale (Lepidosaphes beckii) around adult scale. The orange on the right was fumigated with Vapormate® for four hours and then stored for 14 days. The scale on it is all dead and there is no sign of young scale developing.

Grain chinch bug in the cavity of a cut pear.

'Forelle' pears infested with grain chinch bug before fumigation.

Grain chinch bugs on the bark of a blue gum tree.

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penetration into different citrus cultivars with both Vapormate® and carbon dioxide alone. The new study wanted to determine whether differences in permeability between cultivar types were consistent, or whether fruit condition and time after harvest played a role.

Results and conclusions

In total, 38 861 grain chinch bug adults were killed, with no survivors, using 250g/m^3 Vapormate® for four hours at 15°C. The treatment did not damage Valencia oranges, stone fruit or 'Packham's Triumph' pears, but did increase waste in 'Abate Fetel' pears that had been badly handled and kept in cold-storage.

Vapormate® at 250g/m^3 for four hours at 25°C killed 100% of Siculobata sicula, a beetle mite that was under the calyx of Valencia's in KwaZulu-Natal and responsible for export rejections. It was also very effective in controlling two different armoured scale insects on citrus.

Vapormate® was effective at low dosages against motile mealybug life stages. However,

MORE ABOUT VAPORMATE®

The use of ethyl formate as a fumigant on dried fruit dates back to 1927. However, the compound can be explosive at higher concentrations. As a result, its popularity and use were limited until the CSIRO in Australia thought to eliminate the explosion risk by adding carbon dioxide to ethyl formate. The result is Vapormate®, a patented fumigant that is generally recognised as a safe (GRAS), eco-friendly, non-residual fumigant.

However, it was methyl bromide's fall from grace that rekindled interest in ethyl formate, leading to research into its potential. In 1987, Vapormate® was proven effective to kill California red scale on citrus. A 2013 study con rmed that it was effective against all stages of the scale insect and that fumigation periods could be quite short.

It has also been evaluated for the control of cereal pests in silos where it proved effective against beetles.

Vapormate® does not penetrate plant tissue readily, which restricts its ef cacy against internal pests like fruit y larvae and false codling moth. Commercial use will therefore largely be for external pests.

at 250g/m^3 for four hours at 25°C, 2% of citrus mealybug eggs still hatched. Extending fumigation time to 24 hours still resulted in 0,1% egg hatch. The team concluded that Probit 9 assurance for the control of citrus mealybug using Vapormate® would not be possible within an economical time frame. Similarly, egg hatching in vine mealybug was not completely prevented with Vapormate ® at 250g/m^3 for 24 hours at 25°C; 3,4% of eggs still hatched.

Waxing fruit before fumigation with Vapormate® had no effect on treatment ef cacy against internal pests. However, variability in ef cacy between citrus cultivars, and within the same cultivar between seasons, was too extreme for further research to be justi ed.

Carbon dioxide fumigation alone gave more consistent results against internal pests than Vapormate®. The sequential combination of carbon dioxide fumigation with a short cold treatment was synergistic and could be developed into a commercial treatment.

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Plotting the causes of pitting

Rind pitting is a citrus fruit disorder that develops post-harvest during shipping. If its causes can be identi ed, citrus growers would feel the difference in their pockets.

Dr Paul Cronjé, project leader.

POST-HARVEST PHYSIOLOGICAL RIND disorders, such as staining and pitting, affect most citrus cultivars and have a signi cantly negative impact on return on investment for producers.

Despite this, many questions are still being asked about the causes of these physiological disorders.

Over the last decade, several publications have indicated that citrus fruit quality, and speci cally susceptibility to rind disorders, depends on pre-harvest conditions, including ambient temperature, speci c temperature and relative humidity (RH) changes.

However, data published in Spain and Florida has highlighted the importance of rind water content. These studies revealed that the dehydration of the rind during low RH conditions, followed by rehydration at high RH conditions, can result in turgor stress in the transition cellular zone between the avedo and albedo. This stress is thought to cause cellular collapse, which manifests in visible staining or pitting lesions on the fruit rind.

In addition, mineral nutrient levels in the rind have been found to in uence susceptibility to post-harvest rind breakdown.

In a preliminary study during 2012 it was found that pitting and staining in 'Nadorcott' mandarin were in uenced by the type of rootstock (known to in uence water supply to fruit), as well as post-harvest handling practices (low vs. high RH). The results indicated a signi cantly higher susceptibility of fruit from rough lemon rootstocks compared to fruit from the Carrizo citrange rootstock.

Furthermore, fruit that was waxed within 24 hours after harvest, and thus suffered relatively little dehydration, showed signi cantly lower levels of rind disorders compared to fruit that was waxed later on.

The data concurred with ndings on different citrus rind disorders, where a dramatic water loss due to high vapour pressure de cit (VPD) resulted in an inadequate adjustment of the water status of the rind, leading to cellular collapse and tissue damage.

Taking the existing body of work into account, Dr Paul Cronjé, a researcher at Citrus Research International (CRI), developed a couple of hypotheses to be tested:

1. Post-harvest handling practices could aggravate the incidence of rind disorders. Therefore, known and implementable post-harvest practices, such as removal of eld heat and reduction of fruit VPD, could decrease post-harvest citrus rind disorders.
2. Incorrect nitrogen (N) fertilisation could result in an increased sensitivity of mandarin fruit to post-harvest physiological rind disorders due to its impact on rind colour.

In addition, Dr Cronjé wanted to shine the light of enquiry on the in uence of pre-harvest water stress on the citrus fruit rind as it undergoes post-harvest stresses, as well as the potential of the pre-harvest application of synthetic plant hormones to improve the rind condition.

Given that the post-harvest application of thiabendazole (TBZ) is known to reduce chilling injury in citrus, it was decided to include TBZ in the study as well.

At the beginning of 2015, Dr Cronjé received funding from the PHI Programme and industry for an 18-month study with ve objectives:

1. Gather information on the impact of post-harvest water stress on pitting of citrus fruit.
2. Determine whether the same cultivars produced on farms in the same area show a variation in sensitivity.
3. Determine the impact of pre-harvest water stress on rind sensitivity of mandarin.

4. Determine the impact of N application on rind quality.
5. Determine the ef cacy of TBZ application on pitting of Valencia oranges.

Mandarin methodology

'Nules Clementine' and 'Nadorcott' mandarins, harvested in the Citrusdal and Riebeeck Kasteel areas, were used for the study.

Soil applications of N at 20kg·ha^{-1} and 40kg·ha^{-1} were done on 21 January and 26 March in 2015 and 2016. This was in addition to the standard 250kg·ha^{-1} N provided by the producer. During 2015 a 1% urea foliar application was sprayed.

After harvesting, the fruit was dehydrated at 25°C and 60% to 80% RH for two days, followed by rehydration at 100% RH for one day. Subsequently, the fruit was stored at either -0,6°C or 4°C for a 30-day period.

To determine the impact of pre-harvest water stress, the soil below the trees was covered with plastic sheets three weeks prior to harvest to exclude rainfall and irrigation.

The effect of post-harvest stress was established by dehydrating and rehydrating fruit at 0,7kPa to 1,1kPa VPD for different periods after harvest. Wax was applied on day 5; thereafter, fruit was stored at 4°C for 30 days.

Valencia methodology

Various plant growth regulators, as well as the post-harvest application of TBZ, were tested to determine which, if any, could reduce susceptibility to pitting.

The oranges were all subjected to post-harvest hydration–rehydration stress to induce pitting.

Mandarin results

In general, no signi cant differences in fruit colour or size were recorded across the areas and seasons studied.

Furthermore, no consistent differences were seen among the N treatments after the fruit had been stored at either -0,6°C or 4°C. Mineral nutrient analysis recorded that N applications did not increase the N levels in the leaves, indicating that the N concentrations could be increased in future projects to determine a threshold level. The study, therefore, did not con rm the expected decrease in rind colour due to increased N application.

Although incidences of pitting were recorded, they were generally at levels too low for all the treatments to warrant being included in the data analysis and therefore could not be correlated with N treatments. However, the lack of disorders did indicate that N treatment and storage temperature did not lead to higher pitting susceptibility.

It was also not possible to link pre-harvest rind mineral content with additional N applications with the eventual incidence of rind pitting during storage. The interaction between N application and irrigation indicates that available soil moisture plays a role in N uptake.

Studies on post-harvest disorders are

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PROJECT TITLE
Reducing the post-harvest rind pitting of Valencia and Mandarin citrus fruit

PRINCIPAL INVESTIGATOR
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DURATION
21 months

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R708 957 & R533 957

LEAD INSTITUTION
Citrus Research International (Pty) Ltd

BENEFICIARY
The citrus fruit industry

FOCUS AREA
Post-harvest physiology

HUMAN CAPITAL DEVELOPMENT
Three MSc Agric students

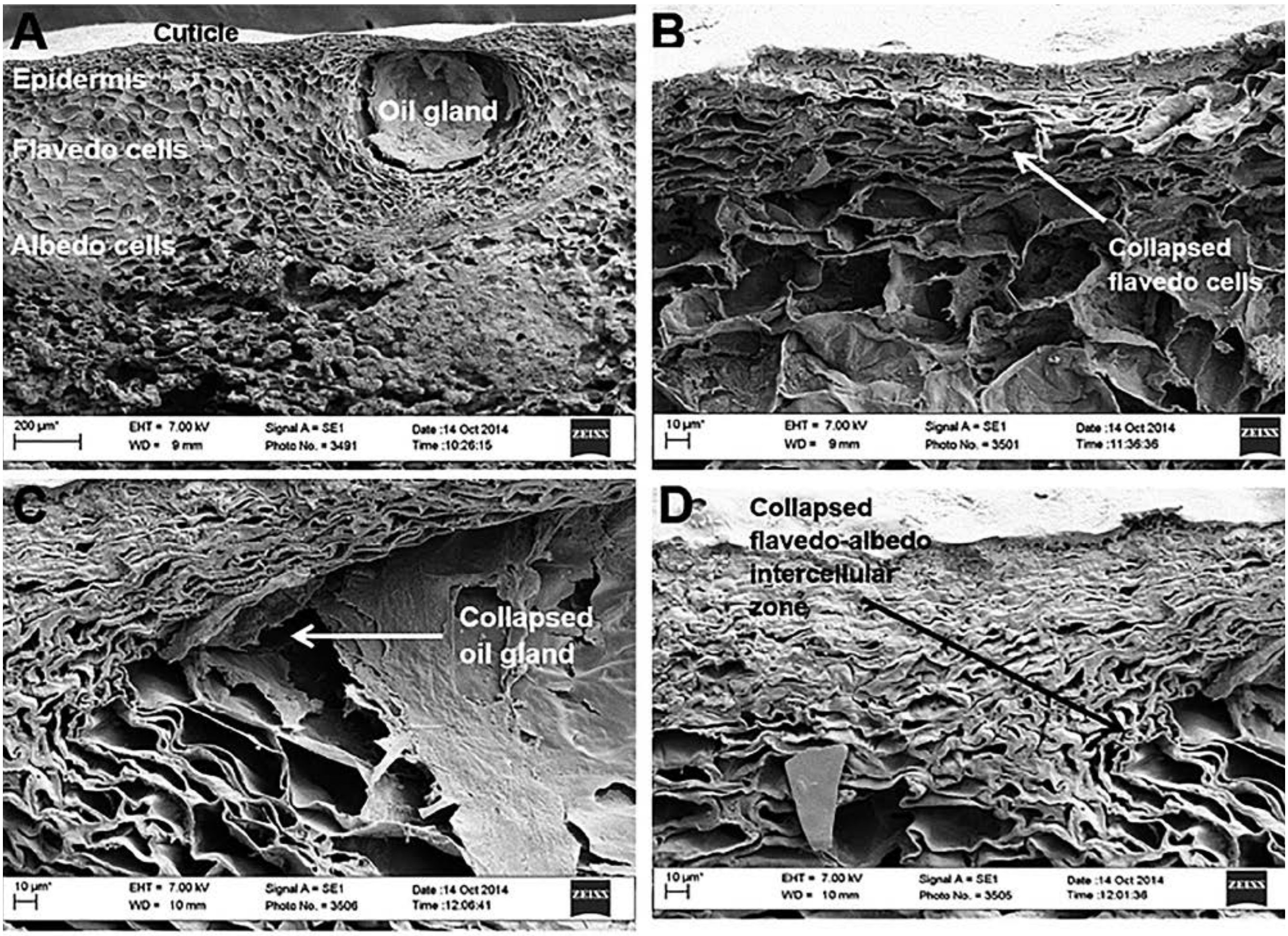
PUBLICATIONS
Two

PRESENTATIONS AND PAPERS
Nine

Rind pitting on Valencia oranges ( ) and Mandarin oranges ( ).

)LJXUH $ '

Scanning electron microscopy (SEM) comparative cross-sections of different cellular sections of ‘Nadorcott’ mandarin fruit rind at storage at -0,6°C for 30 days, showing no rind disorder lesions (A) and collapse of the rind due to rind disorders (B–D).



extremely dif cult due to their erratic (and low) incidence between seasons. Although this study could establish certain correlations between factors in uencing rind quality (climatic in uence, moisture loss and colour) and rind disorder incidence, no single factor emerged as the main underlying cause of rind disorders.

However, the “induction” of rind disorders in some instances by stressing the fruit pre-harvest, and the lack of expected decreased rind colour due to increased N application, can be regarded as positive results. This information can help unravel the factors that predispose citrus fruit rind to progressive post-harvest disorders.

Valencia results

The study con rmed that cultivar plays a signi cant role in pitting susceptibility, with ‘Benny’ Valencia being more susceptible than ‘Turkey’ Valencia.

More mature fruit on the outside of the canopy seems to be more affected, possibly because of greater exposure to changes in environmental conditions, resulting in increased water stress pre-harvest. This aspect should receive more focused research attention.

The synthetic auxins 2,4-D and 3,5,6-TPA signi cantly reduced rind pitting when applied at 10mg·L^{-1}, possibly due to increased sink strength, increased transport of water and nutrients, and increased cell growth in the rind.

An application of 10mg·L^{-1} 2,4-D or 3,5,6-TPA after physiological fruit drop is recommended to reduce pitting susceptibility. In addition, fruit should be packed and placed within the cold chain as soon as possible to minimise post-harvest environmental variation and water stress that result in dehydration.

Post-harvest application of the systemic fungicide TBZ reduced post-harvest pitting in ‘Benny’ Valencia oranges and a dip treatment at 2000µg·mL^{-1} for one minute directly after harvest is recommended.

However, TBZ applied in wax after stress treatment did not have a similar effect.

Conclusion

Even though the symptom development or lesions of rind pitting look similar, the mechanism responsible for the cellular collapse is probably unrelated in the different cultivars, even within cultivar groups such as mandarin and Valencia oranges.

In general, high moisture loss during the time from pick to pack is problematic and in uences pitting incidence. From these results the indications are that TBZ does have a clear mitigating impact on pitting and should be used as advised.

To reduce potential nancial losses due to post-harvest pitting, producers should determine the likelihood of fruit to develop the disorder. This should be done for each orchard by factoring in cultivar knowledge, orchard history and environmental conditions. With this information, producers can then minimise the risk through management practices such as reducing the time between harvest, application of TBZ and optimising the cold chain.

Once the risk of fruit to develop pitting has been determined, the producer and exporter should formulate a strategy to manage the supply of fruit to high-risk markets. The UK, for instance, has little or no tolerance for rind pitting, but is also the market that yields the highest rate of return.

Fruit from orchards with a history of developing pitting should rather be exported to the Middle East or EU markets where lower penalties are imposed on fruit that arrives with pitted rind.

In extreme cases, it would make best nancial sense to sell fruit from highly susceptible orchards at the packhouse.



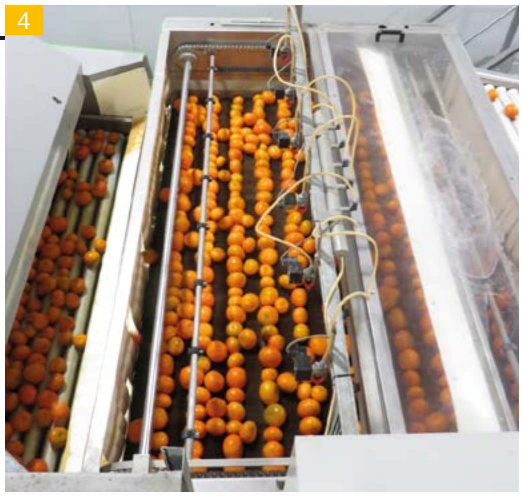
The three MSc Agric students taking part in this PHI Programme-funded project, are Jacques Ehlers ( ), Helen Marais ( ), and Jéanine Joubert ( ).

Waxing citrus fruit within 24 hours after harvest yields fruit that suffers relatively little dehydration and shows signi cantly lower levels of rind disorders compared to fruit that is waxed later on.

WATER LOSS IN FRUIT

Water loss occurs when moisture moves out of a fruit towards the drier atmosphere. The temperature of the fruit and ambient environmental conditions are the factors that primarily in uence the VPD value and the eventual water loss.

Citrus fruit, being a hesperidium berry with a leathery rind, primarily loses water from the rind instead of the pulp. The water content of the rind is therefore directly related to any water loss from the fruit.



A combination that makes all the difference

Cold protocol treatments and irradiation are recognised phytosanitary treatments in their own right. Combining them, however, can revolutionise South Africa's citrus export industry.

SMALL AS THEY are, insect larvae are a massive hurdle in the fresh fruit export industry. Because larvae present the possibility of a quarantine pest establishing a population in the importing country, infested fruit consignments are rejected. Efforts to minimise and control infestations are therefore ongoing.

The cold protocol treatment is the post-harvest treatment most widely used to prevent the export of South African citrus fruit with live false codling moth (FCM) larvae. However, the current protocol of 22 to 24 days at -0,6°C is detrimental to fruit quality and often causes chilling injury.

Ionising irradiation has long been known to be effective in sterilising and killing pests in fruit. As far back as October 2002, the United States Animal and Plant Health Inspection Service (APHIS) approved irradiation as a quarantine treatment for fruit and vegetables at a generic dose of 150Gy for fruit y (Tephritidae) and 400Gy for all other pests, including FCM.

Studies by Citrus Research International (CRI), however, have shown that, at these doses, irradiation is not an option as a stand-alone treatment as it causes prohibitive external quality losses. South African researchers have therefore joined the ongoing international effort to develop additional phytosanitary irradiation treatments and reduce the generic dose of 400Gy for FCM.

In recent times, new developments in phytosanitary regulations have opened up alternative options for the use of irradiation, namely combination treatments and sterile insect technology.

The former combines irradiation and cold treatments, both at reduced doses, and has been proven to suppress larvae and their successive development stages more effectively than the individual treatments alone. Probit-9 level ef cacy was achieved with 60Gy of ionising

FAST FACTS

- Food irradiation is permitted by more than 60 countries.
- About 500 000 metric tonnes of food is irradiated annually worldwide.
- NASA astronauts eat meat that has been sterilised by irradiation to avoid getting food-borne illnesses in space.

1

Paul Cronjé. Irradiation damage on oranges and lemons.

radiation followed by cold exposure for 16 days at 2,5°C.

Sterile insect technology, or low-dose irradiation, affects the fecundity and fertility of pests, also resulting in Probit-9 mortality.

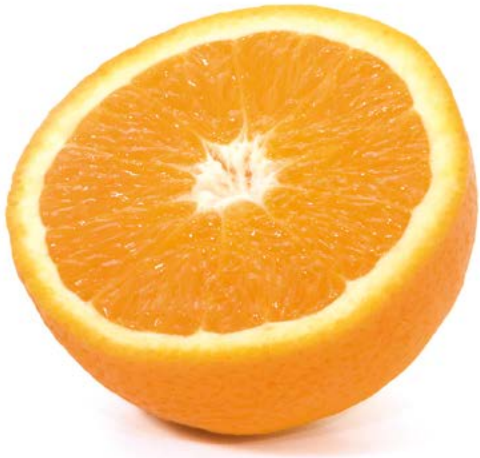
Towards a new protocol

In 2014, Dr Paul Cronjé, a researcher at CRI, initiated a project funded by industry and the PHI Programme to establish a new disinfestation protocol for South African citrus, based on the potential of combined treatments. The rst two steps towards this goal had already been completed by CRI entomological researchers, namely determining insect response to the lowest ef cacy dose and to the combination of irradiation and cold treatment.

What remained to be determined was the maximum irradiation doses that could be tolerated by the major citrus fruit types exported by South African producers. Given that irradiation is not distributed evenly through a pallet of fruit, some of the fruit is exposed to doses twice or three times the minimum required dosage.

"We need the maximum and minimum values to determine if irradiating a speci c cultivar group at a dosage that will be determined by the importing country is actually viable and will not lead to fruit quality losses," explains Paul. "In addition, we wanted to determine the effect on fruit quality of a combination treatment."

With this rationale in mind, Paul designed a study with two objectives:



1. Determine the threshold for fruit quality when irradiating various citrus types, namely, 'Clementine' mandarin, lemon, grapefruit, and Navel and Valencia oranges.
2. Determine variation in dose within each carton box (dosimetry study).

Working at the HEPRO facility in Cape Town, Paul and Jade North subjected eight pallets of different citrus fruit cultivars to gamma irradiation at 200, 300, 400 and 500Gy. Afterwards, the fruit was cold stored at 2°C or 7°C for 40 or 60 days, and then evaluated for internal and external quality.

The rst group of fruit, harvested from May to early June, consisted of 'Nules Clementine' mandarin, 'Nova' mandarin, 'Washington' Navel, 'Eureka' lemon and 'Star Ruby' grapefruit. The second group, harvested in August, included 'Eureka' lemon, 'Navelate' Navel, 'Nadorcott' mandarin, and 'Turkey' and 'Midnight' Valencia orange.

For the dosimetry studies, and to con rm treatment levels of irradiation, a dosimeter was placed outside and inside the short wall, inside the long wall, centrally at the top, and at the middle and bottom of each carton.

Results and recommendations

In terms of external quality, the researchers found that 'Nova' mandarin and 'Turkey'

PROJECT TITLE
Effect of irradiation levels on internal and external citrus fruit quality

PRINCIPAL INVESTIGATOR
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DURATION
Two years

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R590 352 & R560 352

LEAD INSTITUTION
Citrus Research International (Pty) Ltd

BENEFICIARY
The citrus fruit industry

FOCUS AREA
Post-harvest physiology and post-harvest disease and insect control, including phyto-sanitary compliance

PUBLICATIONS
Pending

PRESENTATIONS AND PAPERS
One

2

3

1

FRUIT IRRADIATION ...

- is a technology to extend the shelf life of fruit and eliminate insects,
- does not leave residue and does not make fruit radioactive,
- does not compromise the fruit's nutritional quality, and
- can change the texture or appearance of fruit by impacting rind quality.

WHAT IS PROBIT-9?

Probit-9 mortality is a standard for treatment effectiveness that has its origin in fruit y research, and has been adopted by the United States Department of Agriculture for fruit ies and several other pests.

HOW DOES IRRADIATION W

Irradiation disrupts the DNA molecules in the cell of an organism, making it impossible for the cell to replicate. As a result, the organism becomes sterile and/or dies.

Valencia oranges were highly sensitive, only tolerating doses of up to 200Gy. 'Nadorcott' mandarins and the lemons could tolerate doses below 300Gy. 'Nules Clementine' mandarin and the Navel oranges remained unaffected up to 400Gy, while 'Star Ruby' grapefruit and 'Midnight' Valencia oranges were the least sensitive, tolerating doses of up to 500Gy.

As far as internal quality is concerned, the expert taste panel could not identify the fruit exposed to 500Gy in any of the cultivars. "We could therefore con dently conclude that taste is not a commercial problem," says Paul.

The only internal quality parameter that showed any impact was a reduction in citric acid in the highly sensitive 'Turkey' Valencia and the 'Eureka' lemon, a high acidic cultivar.

The researchers concluded that there was a low incidence of disorders in the 200–300Gy range, combined with cold-storage at 2°C.

"We now know that compliance with combination treatments is indeed possible," says Paul. "Additional analysis will indicate if our data can be used to motivate a new combined protocol."

Laboratory technician, Jade North, measures citric acid levels of citrus juice to determine the effects of treatments on juice quality.

Increase in severity of irradiation damage in lemon fruit.

Rind damage seen in Navel orange fruit due to irradiation.

Rind collapse of 'Turkey' Valencia orange.

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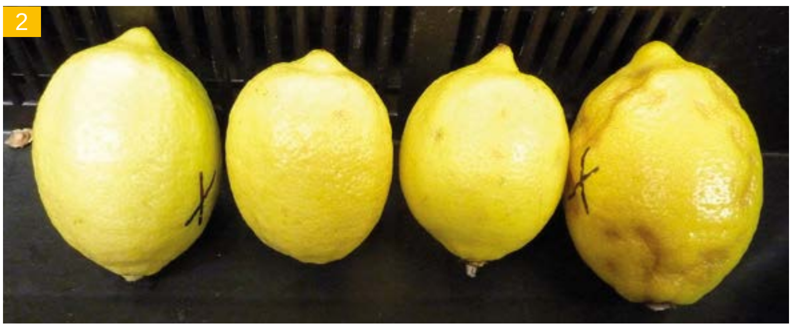

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THE SCIENCE THAT IS FOOD IRRADIATION

Irradiation is a technology that improves the safety and extends the shelf life of food by reducing or eliminating micro-organisms and insects. Like pasteurising milk and canning fruits and vegetables, irradiation can make food safer for the consumer.

Under the blanket statement of making food safer, irradiation can serve many purposes:

- Prevent food-borne illness by effectively eliminating organisms such as Salmonella and Escherichia coli (E.coli).
- Preserve food by destroying or inactivating organisms that cause spoilage and decomposition, and thereby extending the shelf life of foods.
- Offer phytosanitary applications by sterilising insects in or on fresh produce that is exported globally.
- Increase longevity by delaying sprouting (eg. in garlic) and ripening of fruit.
- Sterilise food intended for hospital patients with severely impaired immune systems, such as people with Aids or those undergoing chemotherapy. Foods that are sterilised are exposed to substantially higher levels of irradiation than levels approved for general use.

Food that has been irradiated carries the Radura logo and/or it is stated on the packaging “Treated with radiation” or “Treated by irradiation” or “Radurised”.

In South Africa, irradiation technology has been used for the control of food-borne disease since the 1960s.

Bulk foods, such as fruit and vegetables, must be individually labelled or a label has to be shown next to the container in which they are displayed.

It is important to remember that irradiation is not a replacement for proper food handling practices by producers, processors and consumers. Irradiated foods need to be stored, handled and cooked in the same way as non-irradiated foods, as they could still become contaminated with disease-causing organisms if the rules of basic food safety are not followed.

The safety of food that has been irradiated is undeniable. The technology has been endorsed by eminent global bodies, including the World Health Organization (WHO), the Food and Drug Administration (FDA), the Centre for Disease Control and Prevention (CDC), and the US Department of Agriculture (USDA).

Scientists agree that irradiation does not make food radioactive, compromise nutritional quality, or noticeably change the taste, texture, or appearance of food. Studies have furthermore shown that there is no signi cant loss of nutrients after food has been irradiated. Only small amounts of some vitamins are lost, similar to the amounts lost during other food processing methods such as refrigeration, canning and drying.

New mandarins cope with the cold

South Africa’s newest export-quality citrus cultivars have shown that they can withstand sub-zero sterilisation without losing quality. The industry has every reason to be pleased.

Dr Nhlanhla Mathaba, project leader.

1

CONSUMERS’ TASTE IN fresh fruit – as in fashion – changes. Therefore, in order to remain competitive, exporting countries like South Africa have to continuously update their cultivar catalogue.

The citrus industry, through Citrus Research International (CRI), the Agricultural Research Council, CitroGold and other partners, has responded to this challenge by developing a new selection of soft mandarin cultivars, all with promising internal and external qualities.

Spoiling the industry’s fun, however, is growing populations of Bactrocera invadens, a recently identified fruit fly species. Given its status as a phytosanitary pest, exporters already have to take strictly regulated precautions to prevent fruit fly infestation in consignments. As expected, the arrival of a new variety only made matters worse: sub-zero cold sterilisation has been passed as a mandatory treatment against eggs and larvae in fruit.

While cold sterilisation eliminates the pests, it also has the potential to cause cold damage, or chilling injury, in most tropical and subtropical fruit. Chilling injury is physiological rind damage, which manifests as dark spots or lesions and reduces the marketability of fruit after storage.

According to Dr Nhlanhla Mathaba, from the Post-harvest Technologies Division of the Agricultural Research Council’s Institute for Tropical and Subtropical Crops (ARC-ITSC), cultivars that cannot withstand the required phytosanitary treatments have no place in the export portfolio. “Therefore, before these new mandarin cultivars could be fully registered, sub-zero cold sterilisation experiments had to be performed. Sterilisation at -0,6°C is a phytosanitary requirement for lucrative markets such as Japan and USA. The new cultivars have potentially superior characteristics; the missing piece of the puzzle is their physical and physiological response to sub-zero temperatures.”

To fill the gap in knowledge, Nhlanhla embarked on a two-year study (funded by industry and the PHI Programme), working with Professor Tieho Paulus Mafeo from the University of Limpopo, to evaluate the effect of sub-zero cold sterilisation and ethylene degreening on the internal and external quality of the new mandarin cultivars.

Three objectives were formulated to achieve this aim; these were the following:

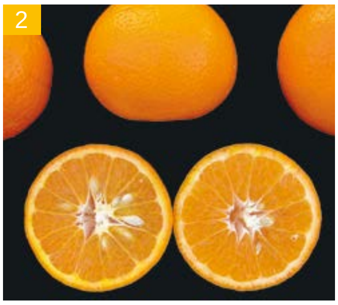
1. To establish the response of the newly bred mandarin selections to sub-zero sterilisation under export simulation conditions;
2. To establish if ethylene degreening has an effect on the development of rind disorders in the newly bred mandarin selections after withdrawal from cold sterilisation; and
3. To establish the effect of sub-zero cold sterilisation on the internal quality of the newly bred mandarin selections after withdrawal from cold-storage.

The tested cultivars were ‘Sonet ARC’, ‘B24’, ‘I22’, ‘M37’, ‘Nova’ and ‘Nova ARC’.

Research methodology

De-greened and non-degreened ‘Sonet ARC’, ‘Nova’ and ‘Nova ARC’, ‘B17’, ‘I22’ and ‘M37’ mandarins were sourced from an Addo farm in the Eastern Cape. The fruit was waxed with polyethylene citrus wax, packed and then transported to the ARC-ITSC laboratory in Nelspruit.

At the laboratory, the fruit was repacked into smaller cartons, each containing 50 mandarins. Cartons were stored at three different temperatures (-0,5°C, 2,0°C and 0,4°C) – three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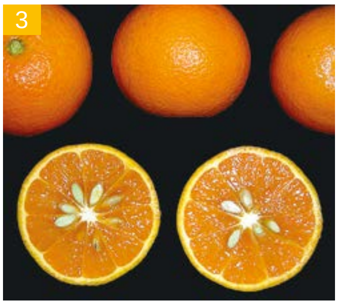
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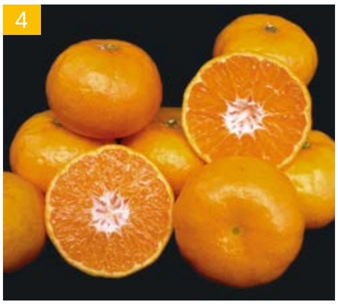
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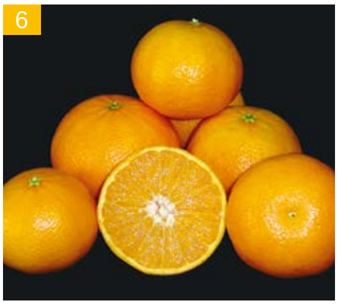
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cartons per temperature – for up to 28 days.

After withdrawal from cold-storage, the fruit was kept at ambient temperature for seven days to allow for the development of rind disorders, mainly chilling injury.

Rind and juice were evaluated for the following physicochemical parameters: chilling injury, fruit weight loss, electrolyte leakage, membrane damage, juice total soluble solids, titratable acidity and rind colour.

Results

The study found that sub-zero cold sterilisation only affected cultivar 'M37'; no effect on the physical and juice quality of the other cultivars was noted.

'M37' showed a signi cant decrease in fruit rmness and weight loss, and an increase in rind electrolyte leakage after cold-storage, irrespective of degreening treatment and cold-storage temperature. The impacts on the quality of 'M37' were associated with severe rind chilling injury. Interestingly, the Brix index of 'M37' improved after cold sterilisation, which might be due to the signi cant water loss.

None of the chilling resistant cultivars showed any change in juice quality after withdrawal from cold sterilisation, irrespective of degreening treatment.

The external colour of the non-degreened fruit seemed to improve during cold-storage, although not signi cantly.

"Our results are good news for the industry," says Nhlanhla. "Given that sub-zero cold sterilisation had a detrimental effect on only one of the new cultivars, South Africa now has the option to register ve new cultivars for export to America and Japan."

This export diversity increases the local industry's competitiveness, while increased plantings of the new selections will lead to increased employment on farms, in packhouses and along the logistics value chain.


7


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The new Mandarin varieties that were tested: 'I22 ARC' ( ), 'M37' ( ), 'Sonet ARC' ( ), 'B24' ( ) and 'Nova ARC' ( ).

A recently identi ed fruit y species, Bactrocera invadens, is yet another phytosanitary pest that producers have to control. (Courtesy: Peter Stephen from Citrus Research International)

Prof. Tieho Paulus Mafeo, from the University of Limpopo, evaluated the effect of sub-zero cold sterilisation and ethylene degreening on the internal and external quality of the new mandarin cultivars.

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PROJECT TITLE
Investigating cold-storage potential of new mandarin citrus selections/cultivars and the effect of ethylene degreening on rind disorders

PRINCIPAL INVESTIGATOR
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DURATION
Two years

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R154 500 & R74 500

LEAD INSTITUTION
Post-harvest Technologies Division of the Agricultural Research Council's Institute for Tropical and Subtropical Crops (ARC-ITSC)

BENEFICIARY
The South African citrus industry

FOCUS AREA
Post-harvest physiology and phytosanitary compliance

HUMAN CAPITAL DEVELOPMENT
One MSc Agric student

PRESENTATIONS AND PAPERS DELIVERED
Four

PUBLICATIONS
Pending

Technology transfer yields excellent results

The transfer of knowledge and skills – known as technology transfer in industry terms – is proving to be a powerful and effective weapon in the citrus industry's competitiveness arsenal.

Hannes Bester, project leader.
Various sponsors also take part in the workshops.

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AS MUCH AS deregulation introduced competition into the fresh fruit export industry, it was not all good news. In terms of citrus, the post-deregulation period was characterised by deterioration in fruit quality and packaging material, handling in the logistical chain and cooling protocols. In addition, ignorance of phytosanitary protocols and a lack of information and knowledge resulted in huge losses to the industry.

Responding to this situation, in 2007, the Citrus Marketing Forum requested that these issues be addressed to minimise losses and improve discipline in the industry. The Forum's plea resulted in the formation of the Citrus Research International Post-harvest Technical Forum (CRI-PTF), whose functions and responsibilities include post-harvest technology transfer. Falling under the CRI Extension Department, CRI-PTF is managed by Hannes Bester, the national extension manager.

CRI-PTF carries out its mandate through, among other initiatives, a series of workshops that helps role players to fully understand phytosanitary issues, export regulations and the post-harvest management practices needed to land their perishable product in the best possible condition in export markets. "Our aim is to minimise losses every season, while enabling long-term sustainable, competitive marketing, ensuring job security and creating wealth," says Hannes.

Enlisting PHI Programme support

Given the increasing importance of technology transfer to the long-term well-being and pro tability of the citrus export industry, Hannes and CRI wanted to extend the reach of the CRI-PTF workshops. To this end, an application was made for support from the PHI Programme.

"The objective of this project was to ensure that, before picking and packing start, all individuals involved in the post-harvest value chain are fully informed of post-harvest phytosanitary requirements and protocols, the latest research ndings and recommendations on pathological and physiological fruit quality issues, packaging research, packaging material speci cations, palletisation protocols, time and temperature protocols, logistics, and handling and cold chain management operating procedures," says Hannes.

With support from industry and the PHI Programme, six two-day workshops were held at the end of January and beginning of February 2015, and a further six workshops in the same months in 2016. The main production regions of the country were covered with two workshops in Limpopo, and one each in Mpumalanga, KwaZulu-Natal and Swaziland, and the Eastern and Western Cape. Funding from the PHI Programme and industry allowed an additional workshop to be held in Nelspruit, making it easier for both commercial and emerging producers to attend.

In 2015, some 764 people attended, and in 2016 the total was 920. They represented the diversity of the industry in terms of economic activity, gender, race, size of enterprises and position in the citrus value chain. Each attendee received a CD with copies of all the presentations, as well as one-page critical

2

PROJECT TITLE
CRI post-harvest technical forum extension

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DURATION
Two years

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R1 356 875 & R1 346 875

LEAD INSTITUTION
Citrus Research International (Pty) Ltd

BENEFICIARY
The South African citrus industry

FOCUS AREA
Technology transfer

PUBLICATIONS
Pending

PRESENTATIONS AND PAPERS
23

THE WORKSHOP CONTENT

- Production region overview
- The citrus value chain
- PPECB feedback:
  - Previous season rejections and quality
  - Export standards for the coming season
- Exchange traded products market feedback
- Export regulations: The Department of Forestry and Fisheries
- Phytosanitary regulations (all markets) and industry's citrus black spot risk management system
- Marking requirements
- Citrus marketing dynamics in the USA
- Packhouse requirements with regards to audits
- Get more miles out of the pre packhouse drench application
- The rst application step in the packhouse: what do we sanitise?
- Update on ooder research
- Resistance development vs. packhouse sanitation practices
- Perspective: imazalil sulphate and pH
- Packaging issues
- Ineffective critical control points
- Practical guidelines for managing physiological disorders
- The effect of packhouse practices on fruit physiology
- Impact of packaging and ambient loading on cooling of fruit in containers
- Recommendations regarding packhouse treatments
- Update on food safety and residues
- Costs and potential losses in the logistical chain
- Packhouse management of FCM for a phytosanitary market
- Post-harvest research projects and priorities

Two-day workshops are powerful technology transfer tools, taking the latest post-harvest research ndings and technology developments from theory to practical application, resulting in a direct and immediate return in the form of increased competitiveness.

control point recommendation hand-outs on the topics of drench application, chlorine application, fungicide bath and wax application.

"The excellent attendance and feedback con rmed the successes of these workshops," says Hannes.

The best indicator of the value added by this initiative, however, is export results. Positive feedback from exporters on the improvement in fruit quality and shelf life, reduced rind disorders, quality of packing material and adherence to phytosanitary requirements is a direct outcome of the fact that producers and packhouse managers apply the research results and recommendations presented during the workshops.

"We nd that packhouse managers who attend the workshops use the presentations for in-house training," says Hannes. "Our post-harvest extension of cer sees vast improvements in packhouse practices, especially the management of critical control points. It is no surprise, therefore, that the Exporters Technical Panel report that the quality and presentation of South African citrus is improving."

These two-day workshops have become one of the most powerful technology transfer tools in the South African citrus industry. They are a vehicle for taking the latest post-harvest research ndings and state-of-the-art technology developments from theory to practical application, resulting in a direct and immediate return in the form of increased competitiveness.

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Hannes Bester

:RUNVKRSV

Tzaneen, Limpopo
26 & 27 January

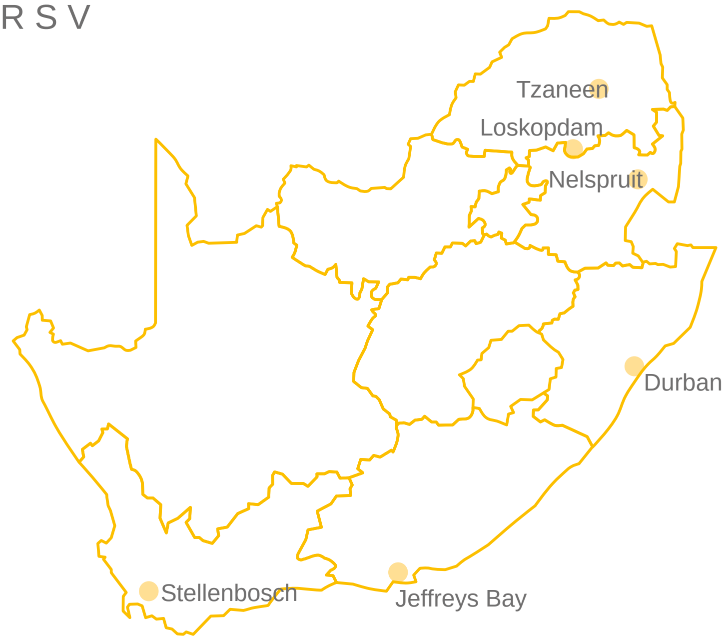
Loskopdam, Mpumalanga
28 & 29 January

Nelspruit, Mpumalanga
09 & 10 February

Durban, KwaZulu-Natal
11 & 12 February

Jeffreys Bay, Eastern Cape
16 & 17 February

Stellenbosch, Western Cape
18 & 19 February



> The workshops successfully and rapidly convert research ndings and technology developments into industry-wide application and value realisation for the industry ar country as a who
>
> Hannes Bester

Nature and nanotechnology join forces

A de ning characteristic of nature is its lack of uniformity and predictability. Nanotechnology offers a way to smooth these rough edges in order to harness the power of essential oils to control post-harvest diseases in citrus.

Prof. Sandra Combrinck, project leader.

Scanning electron microscope (SEM) images of lemongrass-encapsulated silver nanoparticles. The light areas on the left indicate the presence of the nanoparticles.

THE USE OF fungicides to combat pathogens has contributed greatly to sustaining food quality and food security. However, this contribution is threatened by increased consumer awareness of the dangers of synthetically prepared chemicals, global restrictions imposed on fungicide application, and an increase in micro-organism resistance towards fungicides.

As a result, the last decade has witnessed a growing interest in natural mycobiocides, with essential oils (EOs) taking centre stage in the search for alternatives to chemical fungicides.

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The complex nature of EOs impedes the development of resistance by the pathogen. The application of EOs also leaves a smaller carbon footprint, because they are biodegradable without being highly unstable. "The application of essential oils as fungicides is also far more acceptable to consumers and is suitable for the organic market," adds Professor Sandra Combrinck, a researcher in the Department of Pharmaceutical Sciences at the Tshwane University of Technology.

The volatile nature of EOs is both a boon and a bane in their fumigant application. The advantage of their volatility and bioactivity in the vapour phase is that it makes EOs suitable for the protection of stored products. EOs as fumigants have good penetrability, for example into wounds on the rind; low concentrations can be applied, which reduces costs; and the sensory properties of the fruit are not affected.

However, EOs are very volatile and this could reduce the ef cacy of applications, given that the volatile active components have to be released slowly to ensure consistent protection of the fruit over time.

Encapsulating particles is known to have the potential to slow down the evaporation of volatile substances and thus extend their period of release. In the case of EOs, research has included EO-encapsulating zeolites, nanoparticles, and micelles.

In the fruit industry, disease control involving the use of EOs has been focused on modi ed atmosphere packaging (MAP) and coatings. In these applications, the successful introduction of EOs depends on the stability and bioavailability of the oils. "In all cases, the inherent volatility of the oils is a complicating factor," says Sandra.

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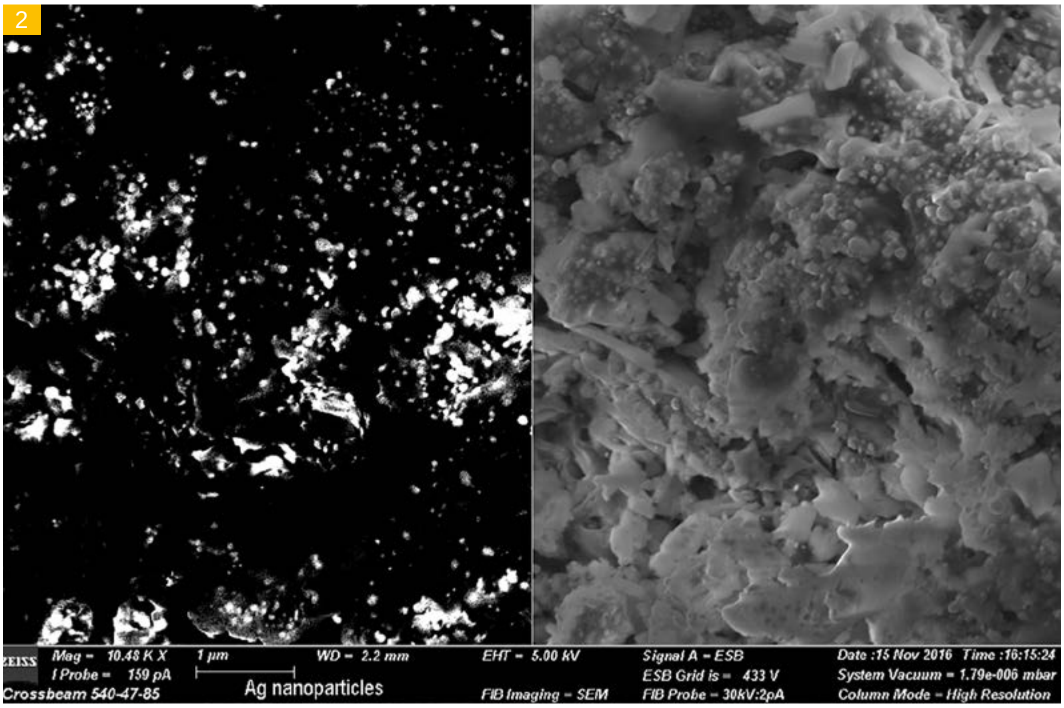

In an industry and PHI Programme-funded study that started in 2015, scientists are now working on combining encapsulation technology and EOs into an innovative approach to control post-harvest decay of citrus.

Project aim and objectives

The aim of the study was to investigate the potential of EOs as post-harvest protection agents, when applied in various encapsulated forms, to inhibit Penicillium digitatum (green mould) and Galactomyces citri-auranti (sour rot) of citrus.

The speci c objectives were to:

- screen a large number of EOs to identify those with high multi-target ef cacy;
- establish various in vitro antifungal assays and compare the results;
- encapsulate EOs with silver nanoparticles and chitosan, and test them as fumigants and in dips and coatings; and
- design and construct a mobile system for accurate headspace analysis of EOs.

Methodology

In terms of methodology, the following steps were proposed and implemented:

- Identify the most active EOs against both sour rot and green mould.
- Identify the components of the EOs

PROJECT TITLE
Improved essential oil application through nanotechnology

PRINCIPAL INVESTIGATOR
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DURATION
Two years and nine months

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R491 275 & R141 275

LEAD INSTITUTION
Tshwane University of Technology (Department of Pharmaceutical Sciences)

BENEFICIARY
The South African citrus and subtropical fruit industries

FOCUS AREA
Post-harvest disease and insect control, including phytosanitary compliance; and post-harvest physiology

HUMAN CAPITAL DEVELOPMENT
One DTech student and one MTech student

PRESENTATIONS AND PAPERS
Six

PUBLICATIONS
Pending

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Prof. Sandra
Combrinck

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by gas chromatography- ame ionisation detection/mass spectrometry (GC-FID/MS) analysis.

- Construct chemomteric models from the GC-FID/MS data to ush out the most active constituents of the EOs.
- Prepare EO-encapsulated nanoparticles and chitosan-EO solutions using the identi ed EOs and their pure compounds, and determine their in vitro antifungal activities.
- Determine the concentrations of the volatile components of the EOs in the headspace using direct sampling of static headspace and Tenax tubes. An appropriate gas chromatograph (GC) sampling system was developed for accurate quanti cation.
- Conduct headspace analysis of the volatile components using the developed sampling system.

Results

The researchers’ analysis identi ed two oils that were highly active against both sour rot and green mould. In subsequent trials, the one was found to have a substantially lower minimum inhibitory concentration (MIC) values than the other oils tested against the two pathogens. This oil will be further tested by Sainclair Bopima, as part of his M Tech study, in in vivo applications; it has potential to be developed into a commercial product.

Three EOs were selected for the modi cation trials, namely lemongrass, spearmint, and thyme since they are easily available, moderately priced, and active against the targeted post-harvest diseases. Lemongrass alone was the most active against both pathogens.

The MICs determined from the microtiter plate serial dilution assay were higher than those from the toxic medium assay. However, the relative trend in activity remained the same. It is necessary to establish the microtiter assay to investigate synergistic or additive effects between EOs.

The results indicated that adding silver ions to EOs considerably enhanced their activity against the pathogens.

NANOTECHNOLOGY CAN EXTEND SHELF LIFE OF FRESH FR

Nanotechnology is an innovative science involving the design and application of small-sized particles measuring one hundred nanometers or less. The miniscule size and large surface area of nanoparticles help to enhance their chemical, mechanical, electrical, optical and catalytic features. Thus, nanotechnology is incorporated into a large variety of consumer and health goods, such as food, food packaging, sunblock, sports clothing, chemical fertilizers and animal feed. Nanoparticles have a tremendous ability to penetrate cells and DNA structures. As chemical reactions occur between particles that are on the surface, a given mass of nanomaterial will be much more reactive than the same mass of material made up of large particles. This means that materials that are inert in their bulk form are reactive when produced in their nanoparticle form.

Source: Food Safety News

Currently, methods of applying these EO-containing particles to pathogen-inoculated fruit are being investigated. It was found that EOs coated with chitosan were more active against the fungi than chitosan or the EO alone.

An understanding of the thermal characteristics of the EOs is important to predict how various active constituents will behave under different temperature conditions, for example in a cold room. The thermogravimetric analysis (TGA) data revealed that the EO-encapsulated silver nanoparticles had a completely different thermal pro le to that of the EOs and their active constituents, indicating release of the volatiles at a higher temperature.

Determination of the evaporation rate of the EOs, terpenoids, and the EO-silver nanoparticles is ongoing. "This analysis will enable us to determine the feasibility of using encapsulated EOs for controlled-release purposes," says Sandra.

A headspace sampling system coupled to a GC was developed to determine the concentrations of active EO components used as fumigants to control decay pathogens. The system, which can be converted to a mobile format for use in cold rooms and containers, can monitor the vapour pressure/concentration of EOs/components at different temperatures and volumetric ow rates.

Once the system has been fully validated for analytical ruggedness, it will be used to determine the rate of release of EOs from the encapsulated structures. This research forms part of the doctoral study of Katlego Phala.

> A nanometre is o billionth of a metr (0,000 000 001m An average huma hair measures 80 000 nanomete in diameter.

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Sinclair Bopima tests the ability of encapsulated essential oils to inhibit fungal growth on citrus.

Dr Wilma Augustyn (left) and Prof. Sandra Combrinck are the supervisors of Katlego Phala and Sinclair Bopima, respectively. Wilma specialises in thermal desorption gas chromatography.

Pennisillium digitatum, also known as green rot or green mould, is a major source of post-harvest decay in citrus fruit.

Katlego Phala uses a gas chromatograph to analyse essential oils.

Spot the post-harvest difference

Pre-harvest prevention remains better than post-harvest cure when it comes to citrus black spot, b this study con rms that packhouse treatments do provide a safety net.

CITRUS BLACK SPOT (CBS) is a major threat to South African citrus fruits' continued access to Europe – their main export market. Despite the disease being purely cosmetic, it has been given A1 quarantine status. As a result, EU market regulators constantly threaten to ban South African imports.

CBS is best managed in the orchard and during fruit set, and much is done to ensure pre-harvest control. However, latent infections can escape these measures, pass unnoticed during local inspection, and only develop into lesions while the fruit is in transit.

Post-harvest treatments may therefore hold the key to inhibiting the development of latent infections – except that not much is known about CBS and its post-harvest behaviour.

In terms of normal packhouse treatments for the control or inhibition of CBS, a 1998 study showed in vitro that the post-harvest fungicides imazalil (IMZ), thiabendazole (TBZ), prochloraz, guazatine (GZT) and sodium ortho-phenylphenate (SOPP) signi cantly reduced conidial germination and appressorium formation of CBS.

A later study showed a three- to sevenfold reduction in CBS lesion viability as a result of post-harvest treatments. However, neither the effects of treatments on the reproductive ability of CBS lesions (latent or visible), nor modern packhouse treatments have ever been evaluated.

A two-year study led by Dr Wilma du Plooy, Post-harvest Research and Programme Coordinator at Citrus Research International, has gone a long way to ll this knowledge gap.

This industry and PHI Programme-funded

Project leader, Dr Wilma du Plooy.

Much is done to ensure pre-harvest control of citrus black spot. However, latent infections present in perfectly healthy looking oranges ( ) can pass unnoticed during local inspections and only develop into lesions while the fruit is in transit.

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WHAT IS CITRUS BLACK SP

Citrus black spot is a fungal disease caused by Guignardia citricarpa. This Ascomycete fungus affects citrus plants in subtropical climates, causing a reduction in both fruit quantity and quality.

Source: Wikipedia

3

research project, which started early in 2015, has three objectives:

1. Compile a thorough literature review of published research conducted in this eld.
2. Investigate the singular and combined effects of standard fungicide post-harvest treatments and cold-storage regimes on the viability and reproductive ability of CBS lesions.
3. Investigate the singular and combined effects of new and alternative fungicides, as well as non-fungicide compounds in heated or ambient aqueous applications on the viability and reproductive ability of CBS lesions.

In addition to updating treatment protocols, the study will identify areas for future research.

"Our project contributes directly to market access and hence competitiveness," says Dr Du Plooy. "Improved post-harvest CBS control will reduce the risk of interceptions, making our fruit more marketable."

Methodology

A signi cant challenge at the outset was nding trees with potential CBS infection, given how well most producers control the disease in their orchards. Initially, two lemon orchards were sourced, one in Brits, in the North West Province, and the other in Kirkwood, in the Eastern Cape.

Two more lemon orchards were later on found closer to Nelspruit, in Mpumalanga.

STANDARD TREATMENTS THAT WERE EVALUATED

Packhouse fungicide applications:

- Drench (TBZ, PYR, GZT and 2,4-D)
- Wash (Chlorine)
- IMZ (Dip 60 seconds at 25°C, 35°C, 45°C and 55°C)
- Wax (TBZ, IMZ and 2,4-D)

Cold-storage regimes:

- Shipping protocol: ve weeks at 7°C for 'Eureka' lemons and 4°C for Valencia oranges
- Cold sterilisation trials: 24 days at -0,5°C for Valencia oranges
- Ambient protocol: ve weeks at 22,0°C

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PROJECT TITLE
Singular and combined effects of post-harvest treatments on viability and reproductive ability of CBS infections on citrus fruit

PRINCIPAL INVESTIGATOR
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DURATION
Two years

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R419 606 & R269 606

LEAD INSTITUTION
Citrus Research International (Pty) Ltd

BENEFICIARY
Citrus industry

FOCUS AREA
Post-harvest disease and insect control, including phytosanitary compliance, and post-harvest physiology

HUMAN CAPITAL DEVELOPMENT
One MSc Plant Pathology student

PUBLICATIONS
Two

PRESENTATIONS AND PAPERS
Eight

Wouter Schreuder Jnr (left) and Dr Wilma du Plooy pose with Jon Pinker, CRI process manager and key contact person between CRI and the PHI Progtramme research project.

Standard packhouse treatment protocols, combined with a brief warm water dip and cold-storage at 7°C, offered signi cant control of new lesion and pycnidia development, with very low viability of pycnidiospores on 'Eureka' lemons.

Two orange orchards were sourced in the area of Nelspruit, and a further two near Brits.

The Valencia oranges and 'Eureka' lemons were subjected to the standard packhouse treatment protocol. In addition, alternative remedies with maximum residue levels (MRLs) accepted in export markets, the European Union in particular, were included in the study.

Applications were rst done in 2015 and repeated in 2016. However, in 2016, changes to the MRL for the pesticide guazatine resulted in it being replaced with propiconazole as a post-harvest chemical.

The fruit was stored under export conditions, removed after ve weeks and then placed in ambient storage for another three weeks.

During this time, lesion development was monitored on a weekly basis, per fruit, per treatment. Lesions were also induced to ooze pycnidiospores during the storage period, and these were single-spored and plated out for viability testing.

Results and conclusions

The existing packhouse protocol was shown to have a suppressive effect on the development of new lesions. In addition, a brief warm water dip at 55°C, as well the cold-storage combination (Valencia oranges at 4°C and 'Eureka' lemons at 7°C) treatments, offered signi cant control of both new lesion and pycnidia development, with very low viability of pycnidiospores.

Three alternative single treatments showed potential to control latent infections: udioxonil, potassium sorbate, and Propirly 270 EC (Propiconazole + Pyrimethanil).

A particularly important nding was that lesions that developed on packhouse-treated fruit did not have reproductive capability and, as such, were not a viable pathway to transmit the disease to unaffected areas abroad.

Dr Du Plooy emphasises that post-harvest treatments can never replace the meticulous pre-harvest management of orchards in areas at risk of CBS infection. However, the study showed that post-harvest measures can provide a valuable safety net.

NEW AND ALTERNATIVE TREATMENTS THAT WERE EVALUATED

Fungicides and alternatives:

- Fludioxonil
- Propiconazole
- Azoxystrobin
- Imazalil + Fludioxonil
- Pyrimethanil + Fludioxonil
- Fludioxonil + Azoxystrobin
- Sodium bicarbonate
- Phosphite salts
- Potassium sorbate

Temperature: 25°C, 35°C and 45°C
Exposure time: 15, 45, 90 and 180 seconds

"Lesions that develop on fruit treated in the packhouse do not have reproductive capabilities."

Dr Wilma du Plooy

THE SOUTH AFRICAN CITRUS INDUSTRY TAKES A PROACTIV APPROACH TO CITRUS BLACKSPOT RISK MANAGEMENT

Despite substantial efforts by the South African citrus industry, an agreement has yet to be reached with the European Union (EU) on the risk of Citrus Blackspot (CBS) transmission by fruit to orchards in the EU. Since 1992 until present day an immeasurable number of actions have been taken by the Citrus Growers' Association (CGA), Department of Agriculture, Forestry and Fisheries (DAFF), and stakeholders within the industry, and procedures put in place to ensure a robust CBS Risk Management System (RMS) can adequately tackle the issue of CBS faced by growers, and the South African export industry at large.

The CGA has engaged grower members at every step of the process, disseminating vital information that has seen the industry stay ahead of the curve, and manage and maintain its position as a leading force in the citrus export market. Communication has taken many forms—weekly newsletters, CBS advisory's and bulletins, research publications, information sessions and brie ngs, websites (www.cri-phytrisk.co.za), CGA roadshows, and the CGA Citrus Summit—and feedback from industry experts, as well as updates on outcomes of meetings held with various international bodies, continues to be presented to grower members on a regular basis.

Compliance measures to further reduce risk of exporting infected fruit in response to the EU's decision to ban citrus imports from South Africa at the end of the 2013 season, saw growers incurring additional and estimated costs of about R1 billion. Furthermore, the submission of its pest risk assessments, by the European Food Safety Authority, who concluded there is a risk of CBS establishing in the EU, are being actively challenged, and have been over the past three years.

Deon Joubert, employed by the CGA as the industry's EU representative in 2014, has been active in addressing the CBS challenge in 2014 and beyond.

CGA and DAFF, together with the Perishable Products Export Control Board, and industry stakeholders, continue to actively work and rework the South African CBS RMS to strengthen its effectiveness, maintaining close relations with grower members, to ensure compliance until such time as a resolution regarding the scienti c dispute surrounding the CBS issue is achieved. At incredible cost to the industry, the positive trend of results over the recent years pertaining to CBS risk management clearly demonstrates that it works.

Exotic Cape Flora

The world has six oral kingdoms, the smallest and richest of which is found at the southern tip of Africa. The Cape Floristic Kingdom is the only one to be fully contained within one country, and has one of the highest known concentration of species in the world.

Botanically, the Cape has been on scientists' radars since a Dutch trade group collected the Oleander-leaf Protea (Protea neriifolia) in 1597.

Some 350 years later, in 1953, the Cape's unique owers crossed the divide between science and commercialism when a bouquet of P. cynaroides was sent as a gift to Queen Elizabeth in celebration of her coronation. It was the rst recorded export of fresh Protea from South Africa to Europe.

Today South Africa is the world's leading Protea exporter. The European Union receives around 80% of total exports. The United Kingdom accounts for another 10%, while the remaining owers go to Africa, the Middle East, North America, Eastern Europe and the Far East.

Despite the uniqueness of the Cape ora, the local industry faces signi cant, and growing, competition from other Southern Hemisphere countries such as Australia, New Zealand, Chile and Zimbabwe. They have similar climates but, in many instances, boast advanced transport logistics and post-harvest technologies that increase product quality and reduce transport costs.

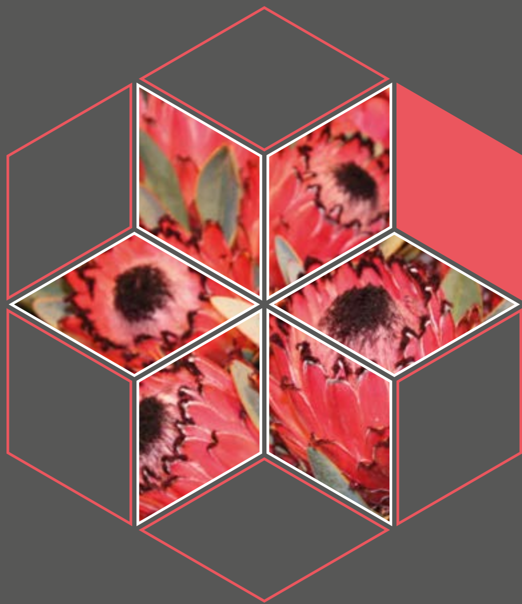
Northern Hemisphere countries, such as Israel, the USA (California and Hawaii), Portugal and Spain (Madeira and Canary Islands), Colombia, Peru, Ecuador and China are also aggressive competitors. Their proximity to European markets, with the associated lower freight costs, as well as a different owering season, gives Northern Hemisphere producers a competitive edge.

Various factors have to be addressed to ensure the ongoing competitiveness of the Cape ora export industry. These include ef cient freighting, cost-effective storage and transport value chains, prolonging post-harvest quality, and the ever-present phytosanitary insect issues.

The issues facing the industry are often interlinked. For example, increased interest in sea freight of oricultural products has allowed for larger volumes to be exported at lower prices and a reduced carbon footprint. However, the extended transport period has serious cold-storage implications. A balance has to be struck between temperatures that are low enough to eliminate pests and manage the effects of water loss, ethylene and leaf yellowing, but high enough to prevent post-harvest disorders such as chilling injury and leaf blackening.

Fortunately, the constant challenges faced by producers and exporters alike promote an industry that invests in research and innovative thinking to secure its place in the international arena.

Source: Chronica Horticulturae, Vol 56, No. 3, 2016



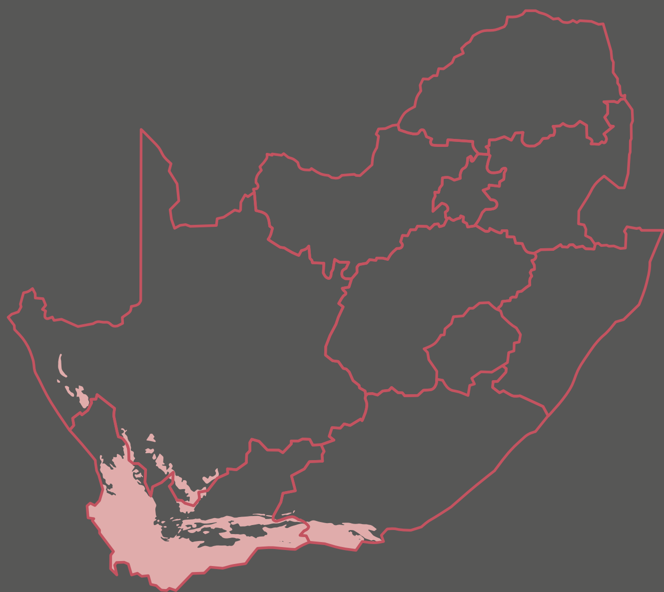
CAPE FLORA FAST FACTS

Cape ora accounts for 90% of South Africa's cut ower exports.

The value of South Africa's cut ower industry is estimated at R1 billion per year, of which exports are close to R500 million.

South African exports earn around R390 million a year on international exotic ower markets.

An estimated 20 076 014 Cape ora cut stems were exported during the 2015/2016 season. (www.cape orasa.co.za)



The Cape Floristic Kingdom is the smallest of the six globally recognised oral kingdoms, but by far the richest. Contained within its 87 892km² is around 9 600 oral species, an estimated 70% of which do not grow anywhere else on earth.

"South Africa's *Protea* species, with their lar
spectacular owers, and the great varie
 ora are high in demand in internatio

New tools to bash bugs

Two novel techniques hold great potential for mitigating the most persistent pests troubling the export of South African Proteaceae to stricter markets.

IN SOUTH AFRICA, Cape ora cut owers are grown and harvested in their natural environment, which includes the insect communities that live there. The insects of the Cape ora biome are diverse, even between growing regions, and are so poorly understood and understudied that many have not been identi ed scienti cally.

The complexity and arrangement of Proteaceae oral structures, not to mention the impressive size of some, provide ample hiding places for a massive array of arthropods. They may inhabit the stem, leaves, receptacle or in orescence, and can occur in staggering numbers.

Regardless, Cape ora is an internationally renowned and highly sought after oricultural product, with more than 20 million stems exported in the 2015/2016 season.

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Dr Shelley Johnson and MSc student Anton Huysamer (right) with the laboratory-scale controlled atmosphere and temperature treatment systems (CATTS) unit at Stellenbosch University.

Core temperatures of 'Barbi's' (Protea magni ca) are recorded throughout the CATTS treatments.

Protea scarlet butter y found in 'Barbi's' receptacle was raised from larval stage to assist in its identi cation.

'Barbi' proteas subjected to various ethyl formate (EF) fumigation treatments inside 14 litre desiccators.

Toxicity with sugar pulsing in Leucospermum leaves was induced under intensive temperature ramping protocols.

The European Union receives around 80% of Cape ora exports, but this does not imply that the owers are not in demand elsewhere. However, access to other markets proves more challenging, due to phytosanitary regulations that are particularly strict, resulting in the danger and harsh reality of consignment rejection.

This is particularly true for countries whose climatic conditions are, in places, similar to the Western Cape's Mediterranean climate, and those that have their own Proteaceae or similar oral families. The risk of South African insects establishing in overseas crops is too great for them to accept our owers without intensive phytosanitary inspections and regulations, and many have a zero-tolerance approach to living and dead insect pests within consignments.

The direct and indirect costs of consignment rejection and subsequent damage to the reputation of the Cape ora cut ower industry are astronomical.

Current mitigation techniques for phytosanitary pests include various pre- and post-harvest protocols. These protocols, although suf cient for owers intended for some markets, have not yet fully met the high standards required for others that have stricter phytosanitary requirements. Effective, reliable and preferably greener post-harvest techniques are required to ensure that consignments arrive pest-free, containing only the breathtaking owers the world has come to expect from the Cape Floral Kingdom.

Looking further a eld for answers, Dr Shelley Johnson, research fellow at the Department of Conservation Ecology and Entomology, Stellenbosch University, realised that two post-harvest techniques that have been approved for and are used in other industries have great potential for use on Cape ora cut owers:

- Controlled atmosphere and temperature treatment systems, known as CATTS, use the stresses created by high temperatures in combination with controlled atmospheres

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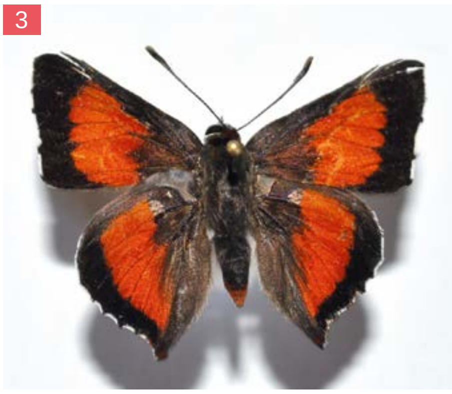

(consisting of 1% oxygen, 15% carbon dioxide in nitrogen) to eliminate pests.
- Ethyl formate (EF) is a naturally occurring compound that, in high enough doses, prevents oxygen reaching the cells of insects, ultimately leading to their demise through asphyxiation.

Both these methods are considered environmentally friendly, as CATTS is essentially chemical free, and EF breaks down into naturally occurring, harmless products.

Shelley and Dr Lynn Hoffman, lecturer at the Department of Horticultural Sciences, Stellenbosch University, designed a study that received funding from industry and the PHI Programme to achieve two objectives:

1. Identify the insect categories that are of highest concern to the Cape ora cut ower industry.
2. Establish the ef cacy of various CATTS and EF fumigation treatments on problematic phytosanitary pests, while maintaining post-treatment ower quality.

Methodology and results

Early in 2016, before the start of the peak export season, numerous pilot trials were performed.

Cut stems from the species Protea magni ca (also known as ‘Queen’ or ‘Barbi’) were subjected to regular atmosphere heat treatments to assess the owers’ response to heat stress. These proved far too harsh. The owers lost an average of 13g in water mass and yielded unsatisfactory results from an aesthetic perspective, particularly in terms of leaf damage.

With these results in mind, amendments were made. The ‘Barbi’s’ were kept at 4°C for no longer than 24 hours after harvesting before treatments commenced. The owers were pulsed using a sucrose solution, and subjected to much faster temperature ramps for shorter durations. The owers then underwent 10-day vase life studies, during which they were graded for both leaf blackening and bract browning of the ower head.

These brief yet intense treatments, in combination with controlled atmospheres,

PROJECT TITLE
Novel technologies for post-harvest treatment of Cape ora owers for control of phytosanitary insect pests

PRINCIPAL INVESTIGATOR
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DURATION
Two years

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R412 951 & R112 693

LEAD INSTITUTION
Cape Flora SA

BENEFICIARY
The Cape ora industry

FOCUS AREA
Post-harvest insect control, including phytosanitary compliance, and post-harvest physiology

HUMAN CAPITAL DEVELOPMENT
One MSc student

PUBLICATIONS
Two

PRESENTATIONS
Three

4

5

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yielded far better results than the standard regimes used on fruit overseas, which were followed in the pilot trials.

The Leucospermum 'Veld re' cultivar was subjected to the amended CATTS regimes, and also yielded more promising results with regard to leaf damage. However, the controlled atmosphere protocols did result in signi cant pin wilting not seen in the same heat treatments performed with regular atmospheres.

Although these treatments improved the quality of both 'Barbi' and 'Veld re' compared to the pilot trials results, they require adjustment to further minimise post-treatment damage and ensure standard vase life quality.

EF fumigation was initially performed on 'Veld re', using the same concentrations that were stipulated in an unpublished pilot study in which Australian wild owers were fumigated. Once again, the treatments were far too extreme, and considerable damage resulted from all concentrations but the lowest one.

When repeated on 'Barbi', the same aesthetic challenges were experienced.

A diverse range of insects, from stem borers and leaf miners to pollen visitors, were subjected to different treatments to determine their overall tolerance to both heat treatments and fumigation concentrations.

Living insects were collected over a period of two months during of cial phytosanitary inspections at the airport in the peak export season. Adult specimens were identi ed, and larval stages were reared to allow for easier and more accurate identi cation.

A total of 10 species were collected, of which four occurred regularly.

The western ower thrip, Frankliniella occidentalis, was of particular concern due to the sheer numbers in which it was present, resulting in multiple rejections. Living specimens were collected from commercial plantations and subjected to the amended CATTS treatments, as well as the lowest concentration of EF fumigation. In both cases 100% mortality was achieved.

This was followed closely by the Protea itch mite, Proctolaelaps vandenbergi, which too occurred in vast quantities. Both regular atmosphere treatments and EF fumigation resulted in 100% mortality.

The banded fruit weevil (Phlyctinus callosus) and Fuller's rose weevil (Naupactus godmanni) were often found, and also in unacceptable numbers. These insects will also be subjected to both CATTS and EF treatments.

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'Barbi' proteas subjected to a 10-day vase life study after EF fumigation treatments.

Severe leaf damage and pin wilting in 'Veld re' after higher EF concentrations.

Typical post-treatment quality in 'Veld re' subjected to regular atmosphere heat treatments.

Unacceptable leaf damage experienced due to higher concentrations of EF.

Vast numbers of monkey beetles, leaf beetles and mites were collected from wild-growing owers and subjected to varying CATTS and EF treatments.

American bollworm caterpillar found boring within the receptacle of a 'Veld re' pincushion ower head.

DID YOU KNOW?

According to Tony Rebelo, author of the book A eld guide to the Proteas of South Africa, there are currently about 1400 species in more than 60 genera in the Proteaceae family. Virtually all the species occur in the Southern Hemisphere, mostly in Australia which harbours 800 species representing 45 genera. About 400 species occur in Africa, of which more than 330 species are in the South-Western Cape. Central and South America host about 90 species, while 80 species occur on the islands east of New Guinea, 45 species in New Caledonia and a few species in Madagascar, Southeast Asia, New Guinea and New Zealand.

Taxonomy
Family: Proteaceae
Genus: Proteaceae (The Sugarbushes), Leucadendron (The Conebushes), Serruria (The Spiderheads), Orothamnus (The Marsch Rose), Vexatorella (The Vexators), Leucospermum (The Pincushions), Hakea (The Needlebushes), Diastella (The Silkypuffs), Brabejum (The Wild Almond), Grevillea (The Silky Oaks), Aulax (the Featherbushes), Mimetes (The Pagodas), Faurea (The Beechwoods), Paranomus (The Sceptres), Spatalla (The Spoons) and Sorocephalus (The Clusterheads)

A ‘protea’ is any member of the Proteaceae family. In order to distinguish between a member of the Proteaceae family and a member of the Protea genus, the latter is sometimes referred to as a ‘sugarbush’.

5

A noteworthy insect was the Protea hister beetle, Platysoma capensis. Although there were never more than two individuals per inspection, it has proven to be incredibly hardy and resistant, with 0% mortality in regular atmosphere treatments and 40% mortality rate achieved using EF fumigation.

Finally, receptacle-boring Lepidopteran caterpillars, such as the Protea scarlet butter y larvae (Capys alpheus) and American bollworm larvae (Helicoverpa armigera) managed to survive all treatments. The CATTS temperature regimes necessary to raise the core of the ower enough to kill them results in severe and unacceptable damage to the owers, and EF fumigation does not penetrate deeply enough to result in their death.

Conclusions and future research

The research results indicate that, in terms of ower quality, fumigation with EF has the potential to be developed as a post-harvest treatment. CATTS has also proven an effective measure with which to control a vast range of insect pests that target Proteaceae.

However, post-treatment ower quality is still not satisfactory. “We have to ne-tune different pre- and post-treatment techniques by, for instance, introducing forced air cooling directly after CATTS treatments, and optimise EF fumigation concentrations and durations,” says Shelley.

She also highlights the need for a greater understanding of the range and complexity of insect pests that trouble the Cape ora industry. “To this end, we will include more Protea and Leucospermum cultivars in our research trials in the last phase of this project. We will continue collecting live problematic insects, particularly during peak season, to identify and then subject them to different CATTS and EF treatments to see which techniques are the most effective while still maintaining ower quality. Only then will rejections of Cape ora be reduced,” Dr Johnson concludes.

6

Longer vase life for Proteas

Leaf blackening is a post-harvest disorder that blots the protea's export copybook. New research suggests that the right sugars at the right time can help these iconic South African owers to turn over a new leaf as far as quality and vase life are concerned.

> The value of a cut ower crop is determined by the post-harvest quality and longevity of the owers.

FOR THE SOUTH African Cape ora industry to be internationally competitive, it has to consistently deliver high quality stems with good storage and vase life characteristics. As air freight costs increase and a growing demand results in larger export volumes, Cape ora is increasingly being shipped to Europe. However, this leaves the industry with a quality management challenge, given that the owers now spend 21 days at sea; air freight used to deliver them to market within three to ve days. While some Protea cultivars can be successfully shipped, there are many that suffer from leaf blackening, resulting that these owers be shipped with their leaves removed.

Currently, the only way to delay leaf blackening in some cultivars is to pulse the cut owers with a glucose solution for a short period, usually less than four hours, as soon as possible after harvest.

Glucose pulsing prevents the owers from using their own carbohydrate reserves once they have been removed from the mother plant. It also maintains osmotic pressure so that the ower can take up water after shipping, and helps to maintain quality and extend vase life.

Glucose pulsing is effective and reliable when administered under controlled laboratory conditions. However, it has proven to be somewhat problematic when applied on commercial scale on the farm, especially when different species, each with their own glucose concentration and exposure time preferences, are harvested at the same time.

Uptake rates are unpredictable, resulting in either ineffective control or glucose toxicity. The latter occurs when stems are exposed to the pulse solution too long, or when they absorb it too quickly.

The stem's water content, which is in uenced by pre- and post-harvest conditions, affects its ability to absorb glucose. Ideally, the uptake of each stem should be measured, but it is impractical and a logistical nightmare.

"The reasons for these inconsistencies in glucose uptake and response are not known," says Dr Lynn Hoffman, lecturer in the Department of Horticultural Science at Stellenbosch University. "Further investigation is needed to nd alternate solutions, such as pulsing with sugars other than glucose. We also need to improve our understanding of how the owers metabolise carbohydrates in order to offer innovative strategies to control leaf blackening."

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Filling the knowledge gap

With all these unanswered questions in mind, Lynn designed and conducted a research project nanced by the Post-Harvest Innovation Programme, Cape Flora SA and Hortgro

Science. Working with her on this project is Werner Truter, a nal year BSc Agric student, and Dr Waafeka Vardien, a post-PhD student.

Project objectives

1. Determine the ability of various Protea species to convert glucose to starch or sucrose, following glucose pulsing, and relate this phenomenon to the ability of glucose pulsing to alleviate leaf blackening.
2. Determine whether glycine betaine and trehalose, a sugar, are possible pulse alternatives to glucose.
3. Determine the potential of sodium nitroprusside (SNP) as a nitric oxide (NO) donor to extend the vase life of Cape ora cut ower products, and develop a method to generate the right amount of NO molecules required for fumigation.
4. Understand nectar biosynthesis inhibition as an alternative mechanism to maintain the carbohydrate status of selected proteas, thereby delaying the onset of leaf blackening.
5. Determine the natural carbohydrate store present in lignotuberous species of Protea and the availability of the carbohydrate pool to the storage ability and vase life associated with lignotubers.

Conducting vase life studies

Pulsing solutions trial – Objectives 1 and 2

The research was done at the laboratories of Stellenbosch University. The owering cut stems were harvested at the 'soft-tip' stage when there is little nectar present in the ower head. The stems of selected, commercially important Protea species and cultivars, prone to leaf blackening, including Protea magni ca, 'Sylvia', 'Pink Ice', 'Brenda', 'Limelight', 'Susara', 'Sharonet' from Helshoogte, Riviersonderend, Napier and Porterville, were brought to the laboratory within hours of being harvested. The stems were stored for 24 hours at 4°C and then cut to a standard length of 45cm. The leaves were reduced to either 10 or 20, depending on the number that remained after commercial grading.

After 24 hours, the owering stems were subjected to pulsing with one of the pulsing solutions (glucose, sucrose, lactulose, trehalose, glycine betaine, ascorbic acid

WHAT IS LEAF BLACKENING?

Leaf blackening is a brown-black discolouration on leaves closest to the in orescence that occurs within three to seven days after harvest. Leaf blackening manifests differently in different species and cultivars.

The exact cause is not clear, but it is likely that leaf blackening occurs because of

- Harsh pre-harvest conditions, such as heat waves;
- The owers draining carbohydrates stored in the green leaves closest to them to open, causing the leaves to turn black; and/or
- Unfavourable storage and transport conditions.

PROJECT TITLE
Developing innovative technologies to obtain a better understanding of the physiological processes critical to maintaining post-harvest quality in Proteaceae cut ower stems

PRINCIPAL INVESTIGATOR
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DURATION
One year and nine months

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R194 810 & R83 492

LEAD INSTITUTIONS
Cape Flora SA and Hortgro Science

BENEFICIARY
The protea and Cape ora industry

FOCUS AREA
Post-harvest physiology

HUMAN CAPITAL DEVELOPMENT
One post-PhD student and one nal year BSc Agric student

PUBLICATIONS
Two

PRESENTATIONS
Two

The Cape ora projects' research team consists of (from left) Dr Waafeka Vardien, Stenford Matsikidze, Dr Lynn Hoffman, Anton Huysamer and Dr Shelley Johnson

2 Vase life evaluation of 'Sylvia'.

)LJXUH ,PDJHV RI Protea ... D WR GHSLFW tR
DQG OHDI EODFNHQLQJ RYHU WKH YD

Water 6% Glucose 2,5% Glycine betaine 2% Lactulose 5% Lactulose

> Understanding *Protea* plants' response to stress and the processing of carbohydrates in and by the lignotuber may provide meaningful insight into nding the most ef cient pulsing solution to prolong the quality and vase life of these beautiful cut owers.

and γ-aminobutyric acid (GABA) for three hours, or until 10ml of the pulse solution accumulated in the stem. Additional stems were placed in either tap water or a 6% glucose solution (industry standard) as controls for the pulsing period.

Thereafter, all stems were transferred to a vase with tap water and evaluated for ower quality and leaf blackening over a period of 10 to 14 days. Additional stems were stored for 21 days at 1°C, before evaluating them for an additional 10 days. Flowers were scored on a ve-point scale, while leaf blackening was determined by the number of leaves that had more than 10% blackening.

Each treatment was replicated six to 10

PULSING SOLUTIONS ON TRIAL

As not all ower types respond in the same way to sugars, putting these solutions to the test (objective 1, 2 and 3) is the only way to see which may be effective to prolong vase life of Cape ora.

Sugars

Glucose ($C_6H_{12}O_6$) – a sugar made during photosynthesis from water and carbon dioxide, using energy from sunlight, and stored as starch for times when the plant needs it. Glucose and other sugars such as sucrose and fructose are soluble. Glucose needs to be linked together in long chains to make starch. Sucrose (table sugar), glucose and fructose (fruit sugar) are often used in pulsing solutions.

Lactulose ($C_{12}H_{22}O_{11}$) – a synthetic disaccharide sugar consisting of glucose and galactose.

Trehalose ($C_{12}H_{22}O_{11}$) – a sugar used by the ower industry to delay symptoms of senescence and associated programmed cell-death, to suppress water loss and provide enhanced vase life.

Glycine betaine ($C_5H_{11}NO_2$) (trading as Greenstim®) – this sugar, found in beetroot, is an organic osmotic compound taken up by cells for protection against osmotic stress, drought, high salinity, extreme temperatures, UV radiation and heavy metals. It plays a role in maintaining cell volume, enzyme and membrane integrity, and uid balance, thus protecting cells from the effects of dehydration.

Senescence inhibitors

Nitric oxide (NO) fumigation – NO is a critical signalling molecule to down-regulate ethylene synthesis; acting as an antioxidant to delay decay of plant tissue and cellular membranes. Fumigation with NO gas reduces respiration rate, stem and ower head wilt (water loss), decreases the incidence of mould growth, and improves the vase life of cut owers.

Sodium nitroprusside (SNP) – a NO donor which acts as a protective signal molecule in plants and is in particular responsible for the regulation of key stress/defence-related antioxidant enzymes to extend the shelf and vase life of fruit, vegetables and cut owers.

times, depending on the amount of material received and the number of treatments per trial. A total of 17 pulsing experiments were conducted.

During vase life studies, the average vase life days, the water content and percentage leaf blackening were scored over the evaluation period.

Results

Leaf blackening develops rapidly in some species and cultivars, for example, at day one or two of vase life, whilst in others the onset of this post-harvest disorder is slower, developing only after day ve. In 'Brenda', leaf blackening develops from day two onward. The quality of untreated (control) owers declined rapidly, whereas those treated with glucose and lactulose maintained quality and had reduced levels of leaf blackening (Fig. 1 and 2).

In 'Sylvia', leaf blackening occurs between day one and four and ower quality can be maintained until day eight, after which it drops and the product is considered non-marketable. Glucose and trehalose signi cantly reduce leaf blackening in 'Sylvia' (Fig. 3 and 4).

For 'Pink Ice', leaf blackening was only visible from day ve, and progressed slowly compared to 'Sylvia' and 'Brenda'. Glucose greatly reduced leaf blackening and good ower quality was maintained throughout the vase life period. For P. magni ca, the onset of leaf blackening was also slower, occurring between days eight and 10. For this cultivar, sucrose had the greatest effect in alleviating leaf blackening. Flower quality was retained throughout the vase life period.

Glycine betaine (trading as Greenstim) in combination with 6% glucose (industry standard) reduced leaf blackening. In cultivars such as 'Sylvia', the effect was better than when using glucose or Greenstim alone. The effect is similar for 'Susara'.

The positive effect of glucose, trehalose, sucrose and lactulose on leaf blackening con rms that the disorder may be largely linked to carbohydrate demand. These sugars not

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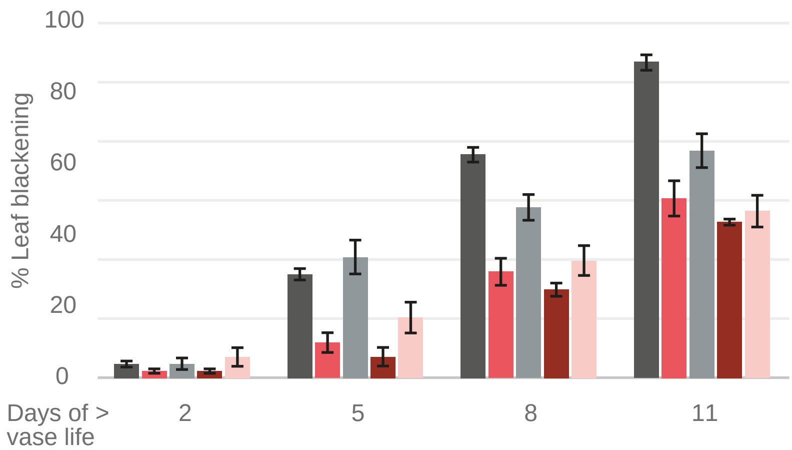

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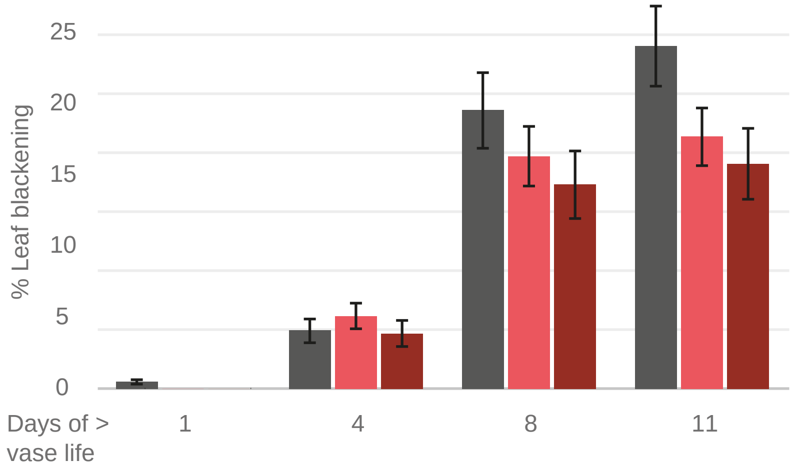

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)LJXUH)ORZHU TXDOLW\ DQG OHDI
Protea FY 6KDURQHW GXULQJ WKH 613 1

> Understanding how Cape ora stems metabol carbohydrates after harvest w enable us to identify which of the new alternatives currently being tested in other major commodity cut owers, such as roses, will be most effective.
>
> Dr Lynn Hoffman

only delay senescence, but may also help to reduce water loss, which ultimately results in better ower quality and longer vase life.

GABA had a positive effect on ower quality in 'Sylvia' stems. Stems treated with 5, 10 and 20mM GABA had increased ower quality scores (averages of 2,25, 2,37 and 2,75, respectively) by day ve5 of the vase life evaluation, which were better than the scores of owers treated with water and glucose. The percentage leaf blackening also decreased with increasing concentrations of GABA and was lower than for water-treated stems. However, glucose-treated stems still exhibited the least amount of blackening.

1

Natural carbohydrate storage in lignotubers – Objective 5

Some species in the Proteaceae family have lignotubers, ie. they have the ability to re-sprout after their above-ground parts have been removed or killed by re. The lignotubers, or underground stems, act as reservoirs of carbohydrates (sugars) and mineral nutrient reserves, which are assumed to play an important role in resprouting after re. After a re, shoots grow from the lignotuber and eventually develop into new trunks.

To determine the natural carbohydrate storage ability of lignotuberous species of the Proteaceae family, Lynn and her team milled lignotuber material from P. cynaroides, also known as 'King Pink', for carbohydrate (sugar) analyses for a preliminary assessment. Baseline samples for carbohydrate analysis (soluble sugars, water soluble polysaccharides and starch) were analysed for both lignotuberous and non-lignotuberous species.

Stems were stored for three weeks at 1 C, whereafter leaf, stem and in orescence material was again sampled for post-storage carbohydrate analysis. In addition, the respiration rate of each representative was measured by placing ve replications of two to three hydrated cut ower products (30cm stem with leaves and ower head) in a sealed airtight container for one hour, where after CO_2, possible C_2H_4 evolution and O_2 consumption were determined by means of gas chromatography. The species' vase life was determined over a 10-day period following long-term storage. Each treatment was replicated 10 times. Carbohydrate analyses for soluble sugars and starch were done.

To determine the effect that girdling might have on the storage ability of lignotubers, potted Protea plants, some girdled above the lignotuber, were obtained from Arnelia farm (Hope eld) in May 2016. The plants were monitored weekly for ower development and the quality of the leaves beneath the developing ower. The plants were harvested and separated into leaves, lignotubers and stems, and carbohydrate analyses were conducted on separated plant organs.

Results

The study showed that the lignotuber of 'King Pink' Protea contains more starch than the stem organs.

Nitric oxide donors – Objective 3

The application of SNP as a NO donor on freshly harvested 'Sylvia', 'Susara', 'Sharonet' (Fig. 5) and 'Brenda' cut stems did not show any positive effect on the reduction or control of leaf blackening. The only trial where SNP showed an ef cacy was in the April-harvested 'Susara' trial. Both concentrations (25µM and 50µM) of SNP that were used in this trial had a positive effect on ower quality. It seems that the better the quality of the freshly harvested owers, the lesser the incidence of leaf blackening. To maintain the quality of the cut stems, they can be stored in a controlled atmosphere with low oxygen and high carbon dioxide levels to reduce the metabolic activity and cellular processes of the cut stems. Applying SNP as a NO donor might be more effective under these conditions.

Slowing down nectar production – Objective 4

The production of nectar after harvest is a major carbohydrate drain, leaving the ower with less energy to look its best for the longest possible time. A better understanding of how nectar production can be held back or

2

Leaf blackening on a Protea magni ca ower from the Porterville area. Hot and dry mid-summer conditions probably subjected the leaves to additional stress.

Leaf blackening, a post-harvest disorder characterised by a dark brown to black discolouration, is found in most commercially important Protea cut ower species and cultivars.

slowed down may be a way to maintain the carbohydrate status of selected Protea cultivars, thereby delaying the onset of leaf blackening and extending vase life.

For this study, stems of 'Sylvia', 'Pink Ice' and 'Susara' were harvested just before the 'soft-tip' stage when only a little nectar is present in the ower head. Solutions of invertase inhibitors, ascorbic acid (Vit. C), Greenstim®, chloramphenicol and cycloheximide were prepared in a range of concentrations. The cut stems were treated with these pulsing solutions and kept at room temperature to allow further ower head development.

Once the ower heads opened, the volume and sugar composition of nectar in the ower head were determined and compared with controls (no inhibitor treatment). During this period, leaves from the stems were periodically sampled to analyse the starch and soluble sugar contents.

A subset of treated and untreated stems were held in water at room temperature to determine the rate of development of leaf blackening.

Results

Neither invertase inhibitor, cycloheximide or chloramphenicol, at different concentrations, delayed the onset of blackening or reduce it. In fact, their application resulted in a higher incidence of blackening. Ascorbic acid did not delay leaf blackening.

> Currently glucos pulsing is seen as effective but cumbersome. Improving this technology, or nd an effective, easy to-apply alternati will ensure our beautiful Cape o stand out in the highly competitiv oricultural world markets.
>
> Dr Lynn Hoffman

Helping Cape ora nd its sea legs

As the preferred mode of transportation for Cape ora shifts from air to sea, the industry is looking for ways to ensure extended ower quality while simultaneously saving costs.

MSc student Stenford Matsikidze and Dr Lynn Hoffman, project leader, observe vase life studies of Protea at the Department of Horticulture at Stellenbosch University.

Conebushes ( ) (Leucadendron) and Pincushions ( ) (Leucospermum) account for about 17% and 14% respectively of all Cape ora products cultivated in South Africa.

Signs of chilling injury on Leucadendron as a result of long-term cold-storage at sub-optimum temperatures.

Kobus van der Merwe, Anel Botes, Prof. Marius Huysamer and Stenford Matsikidze all worked on the Cape ora research project.

IN THE MID-1980s, air transportation of perishable produce became viable for high-value export produce such as owers and exotic fruits and vegetables destined for niche markets.

In recent years, however, high jet fuel prices, concerns about the carbon footprint of the mode of transport, and advances in sea freight technology, notably the automatic ventilation device (AV+), have stimulated a shift from air back to sea freight.

Most shipping lines use the conventional xed open air exchange vents system (AirEx) to introduce fresh air while fresh produce – owers included – is at sea.

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Effective as it is, AirEx is not ideal for Cape ora cut owers as it was developed for fruit. The air exchange rate is likely higher than what owers require, resulting in unnecessarily high cold-storage energy costs and an elevated cold-storage drying factor.

Closed ventilation shipping could hold the key to transport that is not only more cost effective, but also better at preserving ower quality. However, the lack of information on the gas exchange dynamics of Cape ora cut owers stands in the way of the local industry adopting this technology.

According to Dr Lynn Hoffman, lecturer at Stellenbosch University's Department of

THE BENEFITS OF AUTOMA VENTILATION DEVICES (AV+

AV+ intelligently monitors the concentration of O_2 and CO_2 gases inside the container. If concentrations rise beyond the levels that were set for the speci c cargo, a valve automatically opens to let in fresh air in order to maintain the optimum atmosphere.

The technology bene ts the export industry in three ways:

- Power saving – Only the exact amount of air actually needed is allowed to enter the container. The reduction in ambient air exchange results in less air being needed to cool the cargo and less condensation, resulting in a signi cant reduction in energy use.
- Temperature control – Less time spent defrosting translates into more time spent keeping accurate temperature control.
- Faster pull-down – By controlling the entry of ambient air rather than allowing a constant high air exchange, pull-down times can be reduced.


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Horticultural Sciences, new reefer technology cannot be adopted until the ideal rate of fresh air exchange is known. “Suboptimal shipment conditions aggravate the occurrence of leaf blackening and chilling injury,” she says. “Hence the carbon dioxide toxicity level and lower oxygen limit for the respective products are vital pieces of information.”

Acting on a request from the Cape ora cut ower industry, which is investigating the adoption of AV+ and dynamic controlled atmosphere (DCA) technology in order to improve long-term cold-storage, Lynn designed a study that received PHI Programme and industry funding in 2014.

The study had three objectives:

1. Determine the level to which CO_2 accumulates and O_2 depletes in a closed ventilation system with a mixed load of Fynbos products.
2. Determine the effect of closed ventilation shipping on the vase life quality of Protea, Leucospermum and Leucadendron cut owers.
3. Determine the effect of closed ventilation shipping on carbohydrate levels in the leaves of Protea, Leucospermum and Leucadendron cut owers.

Materials and methods

Various Cape ora cut ower products from different genera of the Proteaceae family were used in the study during the 2015/2016 season. The owers were sourced from Floralae in Paarl, Tussenberge in Napier, Berghoff in Poterville, and Fynbloem in Riviersonderend, in the Western Cape.


4

The owers were stored in 610L Janny bins for 21 days at 1°C (±0,5°C) to simulate sea freight conditions. Gas measurements were taken every four days. A hand-held gas analyser was used to monitor the changes in gas levels.

AirEx ventilation rate was the control treatment. Closed ventilation treatment samples were placed in air tight Janny bins with 545–55% free air.


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PROJECT TITLE
De ning sea freight transportation conditions for Cape ora cut ower products to align with new reduced energy consumption shipping technology

PRINCIPAL INVESTIGATOR
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DURATION
Two years and four months

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R365 874 & R92 517

LEAD INSTITUTIONS
Cape Flora SA and Hortgro Science

BENEFICIARY
The protea and Cape ora industry

FOCUS AREA
Container and cold-storage technology

HUMAN CAPITAL DEVELOPMENT
One MSc student

PUBLICATIONS
Two

PRESENTATIONS
Two

Stenford Matsikidze takes gas readings during dynamic controlled atmosphere storage of Cape ora products.

Carefully controlled gas dynamics during cold-storage is the key to extended vase life of Cape ora products.

Evaluations were done on 10 replicates per treatment. Analyses included gas concentration, moisture loss, leaf colour, leaf vase life and carbohydrate content.

Vase life quality was visually scored on a scale where 4 = export grade, 2,5 = minimum acceptable for retail, and 0 = complete senescence.

Results

Gas dynamics

For Leucadendron the O_2 level dropped to 51% and the CO_2 rose to 520% in a closed ventilation system with 545-55% free air (see Fig. 1).

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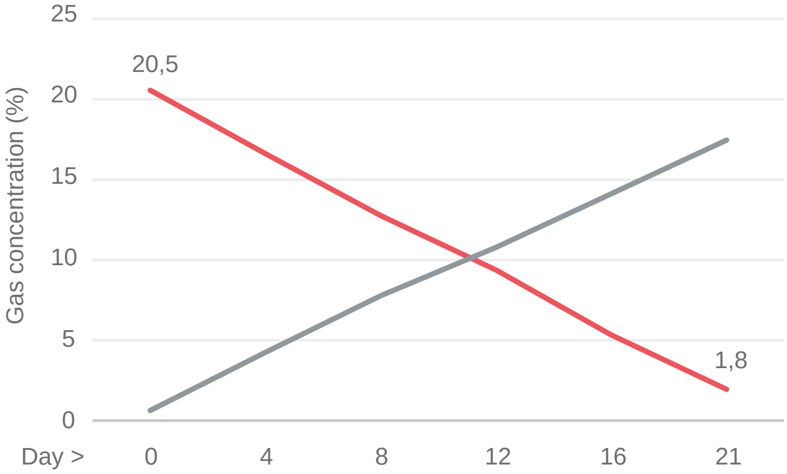

Mass loss

Mass loss was higher under the AirEX system than under closed ventilation for both Leucadendron and Leucospermum products.

Leaf hue angle

- Leucadendron: There was signi cant storage x product x leaf type interaction. Only the hue angle of ‘Safari Sunset’ involucral and mature leaves differed signi cantly with storage. However, differences were not visually noticeable and thus did not affect the visual quality score.
- Leucospermum: There was no signi cant difference in leaf hue angle due to storage or treatment.
- Protea: Differences in ower head hue angle were due to storage effect. Differences in leaf hue angle were due to product variability. The ventilation system had no signi cant effect on hue angle.

Leaf vase life quality

- Leucadendron: Only the position of the leaves on the stem had a signi cant in uence on vase life quality There were no signi cant differences in vase life quality of leaves on day one, but there were signi cant differences in vase life quality on day seven in the 2015 season. However, products had vase life quality that was above the minimum acceptable level for retail. A similar trend was observed in the 2016 season. The Leucadendron cultivar ‘Discolour’, showed the fastest drop in quality after day seven of vase life evaluation.

- Leucospermum: There were no signi cant differences in vase life quality on day one but on day seven the closed ventilation treatment samples had higher quality scores than the control. A similar trend was observed for both 2015 and 2016 season; however, the control treated owers scored lower than the 2,5 threshold level in the 2016 season.
- Protea: There were no signi cant differences in quality on either day one or day seven of vase life evaluation.

Carbohydrate content

- Leucadendron: There were no signi cant differences due to any factor in the 2015 season. 2016 results showed that carbohydrate content was signi cantly in uenced by both product type and carbohydrate type interaction, and also carbohydrate type and treatment interaction. Monosaccharides and oligosaccharides were higher in AirEX treated samples and polysaccharides and starch were higher in closed ventilation treated samples.
- Leucospermum: Monomers and oligosaccharides were higher in control samples compared to those in closed ventilation samples.
- Protea: There was signi cant product x treatment interaction with some products showing higher monosaccharide and oligosaccharide levels under control treatment and vice versa, while other products exhibited no signi cant differences.

Conclusions

Closed ventilation shipping of Proteaceae cut owers resulted in lower moisture loss during storage and better ower head vase life than that after controlled ventilation shipping.

There were no signs of CO_2 toxicity or low O_2 stress in the gas dynamics tests.

Despite having lower monomer and oligosaccharide levels, the closed ventilation samples had higher polysaccharide and starch content. The latter suggests the potential for better vase life quality.

More trials need to be done to con rm this study’s results, investigate seasonal effect, test commercial feasibility and investigate the possible use of controlled atmosphere technology to reduce Botrytis infections. These will be concluded in April 2017 and nal results will be published on the PHI Programme website.

However, the study’s ndings have con rmed that closed ventilation shipping is a promising technology that warrants further research on more Cape ora products. It has the potential to reduce cold-storage energy costs and the carbon footprint associated with the exporting of Cape ora cut owers, while improving their vase life quality.

Cape ora products are graded and packed according to industry standards to ensure only top quality owers are exported.

Immediately after cut ower stems have been packed, they are placed in the pre-cooling unit to remove eld heat. The owers are kept in the cold room until a refrigerated truck transports the cargo to the airport or the Port of Cape Town.

Onion

Onion trading dates back to 2 000BC, and through the centuries it has maintained its status as one of the most consumed crops on the planet.

Onions may have been one of the earliest cultivated crops because they were less perishable than other foods of the time, were transportable, and could be grown in a variety of soils and climates. Since onions grew wild in various regions, they probably entered domestic consumption simultaneously all over the world.

Many documents from very early times describe onions' importance as a food and its use in art, medicine and mummification. During their exodus, the Israelites lamented their desert diet, remembering how they enjoyed abundant fish, cucumbers, melons, leeks, onions and garlic in Egypt.

By the Middle Ages, onions, beans and cabbage were the pillars of European cuisine. Onions were also prescribed as a cure for headaches, snakebites and hair loss, and served as rent payments and wedding gifts.

Today, onions are the third most-eaten vegetable, after tomatoes and potatoes.

Grown in more than 170 countries, it is estimated that over 3,7 million hectares of onions are harvested annually. Approximately 8% of this production is traded internationally. The leading onion producing countries are China, India, Australia, the United States and Pakistan.

In South Africa, onions are produced for the fresh market in all provinces, but mainly in the Western Cape (Ceres), Northern Cape, North West and Limpopo Province.

The Klein Karoo region in the southern Cape, with its dry, warm climate, is specifically favourable for the production of onion seed for clients worldwide.

The onion industry operates in a deregulated environment where the prices are determined by the forces of demand and supply. The industry uses fresh produce markets, informal markets, processors, and direct selling to wholesalers and retailers as marketing channels.

Annually, South Africa exports more than 45 000 tonnes of onions to the European Union, Africa, Indian ocean islands, United Kingdom, Middle East and Asia.

South Africa also imports onions from other countries, especially Namibia.

FAST FACTS

- Onions, garlic, leeks and shallots are all members of the botanical genus Allium.
- The Greeks prepared athletes for the Olympic Games by eating pounds of onions, drinking onion juice, and rubbing onions on their bodies.
- Onions owe their antiasthma, antihistamine, antimicrobial and antiparasitic properties to the organic compound cepaenes, and a variety of thiosul nates that are formed when they are crushed.
- Onions are a good source of dietary bre, folic acid, vitamin C, calcium, iron and quercetin, an antioxidant.
- Onion juice immediately relieves the pain and burning caused by a bee sting.
- The water soluble organic sulphur compound in onions is what stings your eyes and makes you cry.

Western Cape: Worcester, Caledon, Vredendal, Ceres, Kouebokkeveld, Sandveld, Klein Karoo; Northern Cape: Douglas, Prieska, Hopetown, Barkly West; Northwest Province: Christiana, Brits; and Limpopo: Dendron, Vivo, Vaalwater, Polokwane.

"The average annual onion consum
exceeds 6,5kg per person across t

Basal rot makes onion farmers cry

Nature often brings pests or diseases with which farmers have to deal. Such is also the case of onion producers of the Koue Bokkeveld near Ceres, who have to cope with Fusarium basal rot (FBR).

SINCE THE 1980s the Koue Bokkeveld has developed into South Africa's main onion producing region, delivering around 160 000 tonnes per year. It is the only area in the country where the typical brown onion is grown, and the only to export signi cant volumes to countries in Africa and the European Union.

Produce from the Koue Bokkeveld is favoured for their high quality, long shelf life, attractive skin and fair price. Onions from the region also need only a short curing period after being harvest.

In the 1990s, however, a proverbial snake raised its head in this onion-producing paradise: Fusarium basal rot (FBR) – a disease that causes serious economic losses in most of the more than 170 onion producing countries.

1

Prof. Adele McLeod, project leader.

Removing the basal plate of a brown onion.

Symptoms of basal rot in a young red onion.

By 2012, up to 25% of all onions produced in the Koue Bokkeveld were being rejected because of some type of rot. FRB in particular was steadily eating away at producers' pro t.

When Stellenbosch University PhD student Michael Southwood, through a KORKOM-funded study, investigated healthy bulbs from 10 farms stored for three months, he found that FBR caused 30% of all onions to be rejected during the 2013 season, and 18% in the 2014 season.

Producers have tried their best to manage the disease. In heavily infested elds, good irrigation and fertilisation practices are helpful, however, in the presence of environmental conditions that favour the development of FBR, severe losses still occur. Crop rotation is also not effective in highly infested elds, because the pathogen can survive for up to a decade in soil in the absence of host plants.

No known FBR-resistant options are available for the intermediate-day onion cultivars commonly farmed within the Koue Bokkeveld. Fungicides are also not the ideal solution, as most fungicides are not systemic and can therefore not be translocated to roots when applied to the foliage. Furthermore, application of fungicides to soil is generally also ineffective, unless it can be taken up systemically by the plant, which is not a characteristic of most fungicides.

In search of alternatives

Prof. Adéle McLeod, of the Department of Plant Pathology at Stellenbosch University, decided to tackle the FBR problem. Having received funding from the PHI Programme and the onion industry, she tasked Masters student Hanli Kellerman to establish whether phosphonates can be used to ght FBR.

2

Adéle is an expert on fungus-like micro-organisms and soil-borne pathogens. She has been investigating the use of phosphonates to combat, among others, replant disease, or sick soil disease, in apples and avocados.

"Phosphonate is quite a wide term that generally includes the salts and esters of phosphonic acid (H_3PO_3)," Adéle explains. "Phosphites are highly mobile and are translocated upward and downward in plants, which allows for various application methods including foliar applications and soil drenches."

Phosphonates have been used over the years to manage many Phytophthora, and less frequently Fusarium diseases in food crops. Potassium phosphonate, ammonium phosphonate and fosetyl-Al (alkyl phosphonate) are among the registered phosphonate products often used to manage root rot.

WHAT IS *FUSARIUM* BASAL ROT (FBR)?

FBR is caused by a persistent soil-borne pathogen called Fusarium oxypsorum f.sp. cepae (Focep) that infects leek and onion seedlings and mature bulbs. Its spores survive in the soil for years, long after all host plants have been removed, due to its so-called saprophytic ability and ability to produce hardy spores that allows the pathogen to live off dead or decomposed matter.

Bulbs are infected while still growing in the field but symptoms are barely noticeable. Wilting, curving and yellowing of leaves, or tissue damage at the bottom of the bulb itself, is only visible to under highly conducive environmental conditions and in fields containing a lot of the pathogen in the soil.

The disease becomes obvious during storage, in symptoms such as dry rot in an onion's basal plate, and white to pink mycelial growth that further develops into a soft rot.

3

PROJECT TITLE
The potential of phosphonates for managing Fusarium basal rot of onion, and their translocation and persistence in onions

PRINCIPAL INVESTIGATOR
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DURATION
Two years and two months

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R312 051 & R162 051

LEAD INSTITUTION
Stellenbosch University (Department Plant Pathology)

BENEFICIARY
The onion industry

FOCUS AREA
Post-harvest insect control, including phytosanitary compliance, and post-harvest physiology

HUMAN CAPITAL DEVELOPMENT
One MSc student and one BSc student

PUBLICATIONS
Two

PRESENTATIONS
Five

Onion producers have been plagued by Fusarium basal rot, causing rejections up to 30% and resulting in great pro t losses.
Onion seedlings can be infected with Focep in the nursery.
Fusarium basal rot on onion (Fusarium oxysporum f.sp. cepae).*
A micrograph of asexual spores of conidia causing FBR in garden onions. (Courtesy of Bruce Watt, University of Maine, Bugwood.org)
Onion plants showing leaf symptoms of Fusarium wilt in the eld.*
Onion plants showing Fusarium infected basal plates.*
* (Courtesy Howard F. Schwartz, Colorado State University, Bugwood.org)

Importantly, the US Food and Drug Administration (FDA) classi es phosphonates as a GRAS, or generally regarded as safe, product.

This project took the research team into unchartered territory. Only a few studies have been conducted on the use of phosphonates on onions or leeks, but none have yet speci cally investigated its value to suppress FBR.

Adéle's interest in giving it a shot was triggered by a handful of onion farms in the Koue Bokkeveld who were using foliar sprays containing ammonium phosphonate to control downy mildew.

"We heard that these farms had exceptionally high post-harvest quality," she says. "Whether it was in fact ammonium phosphonate that contributed towards improved post-harvest quality had not been quanti ed and determined experimentally on these farms, but warranted further investigation."

The researchers set themselves the following objectives:

- Optimise and develop a method to quantify the development of symptoms caused by Fusarium oxysporum f.sp. cepae (Focep) in arti cially inoculated onion basal plates in onion seedlings and mature bulbs;
- Determine in vitro whether Focep is sensitive to the use of phosphite, as in uenced by phosphate;
- Develop an analytical method to quantify phosphite from onion basal plates and roots;
- Determine whether timing and different dosages and application methods play a role in suppressing FBR with ammonium phoshonate; and

DAY AND NIGHT HOURS

Bulb onions are highly in uenced by the length of days and nights. Different types of onions have different light (and dark) requirements.

Regardless of when an onion is planted, the amount of dark and light that a bulbing onion is exposed to strongly in uences when and if they will bulb, ower and set seed.

The varieties of onions that require a shorter period (11 to 13 hours) of daylight to bulb are termed "short day" onions. Those that require the longest period of daylight (14 hours per day or more) to form bulbs are known as "long day" onions. Those with intermediate requirements (from 13 to 14 hours of light per day to bulb) are called, logically, "intermediate" onions.

Because location (latitude, or distance north or south of the equator) determines day length, some varieties of onions are not as suited for some locations.

- Determine the effect of ammonium phosphonates on mycorrhizal colonisation.

Results and implications

The study standardised various testing methods for South African conditions and onion cultivars.

- DNA extraction and Focep speci c quantitative PCR (qPCR) methods from plant tissue and soil were developed using standardised techniques.
- A sensitive, rapid and speci c liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method for quanti cation of phosphite in onion basal plates and roots.
- A phosphonate agar assay was amended to evaluate in vitro the sensitivity of Focep isolates to phosphonates. It showed that Focep is not sensitive to phosphite based on radial growth.
- The cytotoxicity assay was optimised to test the sensitivity of Focep to phosphonates, using a liquid medium. The assay can also be used to measure whether the addition of phosphite inhibits spore germination.

"qPCR quanti cation of Focep in soil can be valuable for not only selecting elds for trial sites and optimising trial design layout, but also for growers in making management decisions," says Adéle. "From nursery and glasshouse trails we learnt that phosphonate soil sprays work best to attain high phosphite concentrations of phosphite in onion seedlings."

Field trials conducted in 2015 on whether ammonium phosphonate and mycorrhiza can supress FBR did not yield promising results. "It was probably because phosphonate was applied too early in the season, resulting in too low root and basal plate phosphite concentrations," says Hanli.

Come harvest time in 2017, she will know whether her latest series of eld trials have been more successful. "We started spraying phosphonate in the nursery, and continued to do so throughout the season," explains Adéle, while holding thumbs.

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Pome Fruit

Pome fruits are members of the plant family Rosaceae, sub-family pomoideae. They are fruits that have a core of several small seeds, surrounded by a tough membrane. The membrane is, in turn, encased in an edible layer of flesh. Apples and pears are the best known examples of pome fruit; others that are found in South Africa include loquats and quinces.

The history of pome fruit in South Africa dates back to the early 17th century when the world's first multinational company, the VOC (Dutch United East India Company), decided to start a halfway station at the Cape of Good Hope. The sole purpose of this settlement was to supply ships with fresh meat, vegetables and fruit to combat the effects of scurvy that decimated unfortunate sailors on their long voyages.

It was here in the shadow of Table Mountain where Jan van Riebeeck planted the first apple seeds and where the South African history of fruit production began.

By 1916 three million apple trees had been planted in South Africa and by 1966 five million cases of apples were being exported annually. By then, the industry occupied with the export of fresh fruit, mostly to Europe, was well established.

Today South Africa is one of the major players in the international fruit arena and the local industry is buoyant and growing.

South Africa produces approximately 1,3 million tonnes of apples and pears each year. The total value of the pome fruit production amounts to more than R8 billion, with 92% of this generated by fresh fruit sales.

Some 45% of the total pome fruit production is destined for the export market, with the main destinations being the Far East and Asia, Africa and the EU.

The per capita consumption of apples and pears in South Africa is calculated at 4,12kg and 0,96kg, respectively.

According to the 2015 tree census, 36 322ha pome fruit are established in South Africa, with 81% of these plantings situated in the Western Cape and 17% in the Eastern Cape.

The major cultivated varieties per fruit kind are:
- Apples: 'Golden Delicious', 'Granny Smith' and 'Royal Gala'. 'Cripps' Pink' showed the most growth in hectares planted over the last five years.
- Pears: 'Packham's Triumph', 'Forelle' and 'Williams Bon Chretien'.

FAST FACTS

- The word pome is derived from the Latin word p mum, which means fruit.
- The cultivated apple originated in Central Asia, around Kazakhstan, where its wild ancestor (Malus sylvestris) can still be found today.
- China is responsible for around 48% of global apple production; the USA, where apples were introduced by European colonists, is second with 6%.
- There are over 7 500 known apple varieties.
- Pears are thought to have originated in Central Asia, but have been cultivated in Europe for around 10 000 years.
- Even though the South African pear industry is a small international player, about 50% of our pears are exported.
- South African pear producers grow more pears on less hectares than producers in any other country in the world.

Western Cape: Ceres, Groenland, Villiersdorp/Vyeboom, Wolseley/ Tulbagh, Klein Karoo, Southern Cape, Langkloof West, Piketberg, Somerset Wes, Stellenbosch, Worcester, Paarl, Franschhoek; Eastern Cape: Langkloof East; Other provinces producing between 0-3% pome fruit: Limpopo, Northern Province, Northwest Province, Mpumalanga, and Free State.

> "According to an international report,
> tops the rankings for fresh fruit produc

The hunt for Botrytis cinerea

By developing a method to detect Botrytis infection before the rot shows itself, the South African pear export industry hopes to boost its reputation and pro tability.

CONFIDENCE IN THE quality of produce is a cornerstone of international trade in fresh fruit. Post-harvest diseases undermine this con dence, especially when they manifest only after fruit consignments have been delivered to their foreign destinations. The consequent nancial losses can be ruinous to a producer, while the reputational damage can be impossible to repair.

Botrytis rot, caused by Botrytis cinerea, is a major issue in the pear industry. B. cinerea can infect pears at the calyx end, the stem-end, and (or through) puncture wounds. Although infection can occur at any time during the pear's lifecycle, calyx-end infection occurs mainly during the blossom period when pistils and stamens are infected and the inoculum is retained within the oral tube of the fruit. The infection remains latent until visual symptoms nally appear at some point during post-harvest storage.

According to Dr Stephan Ferreira, manager of biotechnology at WestCape Biotech, the unpredictability of especially calyx-end rot is hugely problematic for the local industry. "It would therefore be of great value to have a way to detect and quantify Botrytis cinerea levels within a batch of fruit before visual symptoms appear," he says. "This will allow producers to make informed decisions about corrective action, and help them to plan logistics that will reduce the risk of delivering fruit that is likely to develop Botrytis rot to the market."

At the moment, however, no economically viable method exists to monitor the presence of the Botrytis cinerea inoculum at different stages of the production and supply chain.

Merle Wells-Lee, laboratory assistant (front), and Dr Stephan Ferreira, project leader and biotechnology manager, WestCape Biotech.

The DNA solution

Techniques based on measuring the amount of DNA from a speci c pathogen have become the gold standard in disease diagnostics in all life science elds, including agriculture.

Quantitative real-time polymerase chain reaction (qRT-PCR) has been established as a cost-effective, accurate and sensitive method to routinely detect and quantify pathogens in their hosts by detecting and quantifying DNA.

Importantly, several studies have already

> We found that *B. cinerea* DNA was detectable in the blossoms at full bloom and end of fruit harvested at optimal
>
> Dr Stephan Ferreira

confirmed the validity of this technique to determine the levels of Botrytis cinerea in crops. A 2008 study showed that a strong linear correlation existed between the amount of Botrytis cinerea DNA on pear fruit after artificial inoculation and observed stem-end grey mould symptoms. Studies conducted at WestCape Biotech also accurately and sensitively quantified Botrytis cinerea in a variety of crop plants from artificial and natural inoculation experiments.

Building on this existing body of work, WestCape Biotech and its collaboration partners proposed a project to develop a system based on the qRT-PCR technique to accurately, sensitively and rapidly determine the level of Botrytis cinerea present at the calyx end of 'Packham's Truimph' and 'Abate Fetel' flowers and fruit.

"By sampling specific stages of the production and supply chain, we hoped to generate information that would introduce changes in agricultural practices, pesticide application and post-harvest management to address Botrytis cinerea infection levels and financial losses associated with the problem," says Stephan.

He furthermore foresees that the outcome of the team's work could lead to a predictive

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model capable of assessing the likelihood of disease development during the storage and distribution of pears. Such a tool would make a significant contribution in reducing financial and reputational damages to the South African fruit industry.

The proposal received PHI Programme and industry funding and in August 2014 the team, consisting of Dr Stephan Ferreira and Dr Ida Paul, set to work.

Objective, methodology and results

The objective of the project was to develop a method to continuously detect and quantify B. cinerea levels in orchards, packhouses and packed fruit.

To achieve this, pear blossoms were collected from orchards at full bloom, and fruit at optimal harvest maturity. These samples were either artificially inoculated with known amounts of B. cinerea spores or left uninoculated. Genomic DNA was extracted from the samples and used in a qRT-PCR reaction to determine the relative B. cinerea DNA content present

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PROJECT TITLE
Detection and quantification of Botrytis cinerea incidence and severity in harvested pears for the development of a monitoring system to support commercial exports

PRINCIPAL INVESTIGATORS
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DURATION
One year and six months

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R181 000 & R171 000

LEAD INSTITUTIONS
WestCape Biotech (Pty) Ltd and ExperiCo (Pty) Ltd

BENEFICIARY
The South African pome industry

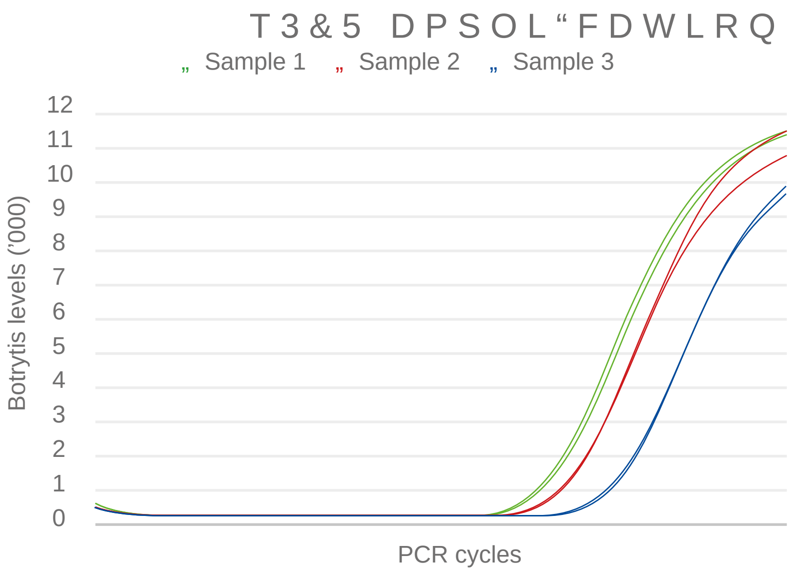
FOCUS AREA
Post-harvest disease control

PUBLICATIONS
Pending

PRESENTATIONS
Two

3

B. cinerea DNA was detectable in the blossoms at full bloom ( ) and in the calyx end of fruit ( ).



in the blossoms and in the calyx end of the mature pears at speci c time points.

The research team found that:

- B. cinerea DNA was detectable in the blossoms at full bloom and in the calyx end of fruit harvested at optimal maturity.
- There was a strong correlation between the B. cinerea DNA content and the number of spores arti cially inoculated in both blossoms and the calyx end of fruit.
- The method that WestCape Biotech had developed previously as a research tool could be used to monitor the increase in B. cinerea DNA in the calyx end of pear fruit in storage.
- There was no signi cant reduction in the B. cinerea DNA content in the calyx end after chlorine ume treatment.
- Infections early in the production period, most probably at bloom, are crucial in determining the inoculum level present throughout the production and storage period.

THE BANE OF *BOTRYTIS*

Botrytis cinerea, the causal agent of Botrytis rot, is a lamentous fungus that infects more than 200 host species, leading to signi cant economic losses worldwide in various commercial crops.

The impact of Botrytis rot in commercial crops is devastating. One study has estimated that around 20% of all crop losses can be attributed to this fungus; another claimed it is responsible for more than 50% of post-harvest rot.

The cost of fungicide used to ght the disease is estimated at around $540 million annually, which represents around 10% of the global fungicide market.

The results showed that WestCape Biotech's method detected and quanti ed inoculum levels sensitively and accurately on pear blossoms and at the calyx end of mature pears. They also con rmed its application as a way to monitor B. cinerea inoculum levels in stored pear fruit.

Consequently, the method can be used to inform packhouse and logistics decisions in the interest of reducing nancial losses incurred due to infected fruit.

Calyx-end decay caused by B. cinerea in an advanced stage. The decayed area appears light brown to dark brown and is spongy.

There was no signi cant reduction in the B. cinerea DNA content in the calyx end after chlorine ume treatment.

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Eliminating astringency's bite

Astringency in pears leaves not only an unpleasant taste in consumers' mouths, it also eats into the viability of a lucrative export industry. Fortunately science is coming to the rescue.

THE TASTE AND texture of a prefect pear is one of life's joys. When consumers bite into a pear expecting that, only to be met with an unpleasant puckering experience, their disenchantment with the fruit can be complete.

Astringency commonly occurs in 'Forelle', a bi-colour pear that has a mandatory 12-week cold-storage requirement to prevent mealiness from developing. This storage requirement delays marketing and causes a gap in bi-colour pear availability between the last volumes of 'Rosemarie' and 'Flamingo', and the onset of 'Forelle'. One of the main causes of astringency is the drive to harvest 'Forelle' as early as possible to start the required 12-week cold-storage period.

In an attempt to bridge the bi-colour pear supply gap, a new cultivar, 'Cheeky'®, was introduced. However, increasing reports of astringency in 'Cheeky'® have been received from overseas supermarkets.

In response, Hortgro Science, on behalf of the South African Apple and Pear Producers' Association (SAAPPA), called on Dr Ian Crouch to investigate possible causes and solutions. Ian is the director of research at ExperiCo (Agri-Research Solutions).

"Unless a solution to astringency is found, there is the risk that supermarkets may refuse to stock 'Cheeky'®," says Ian.

To nd such a solution, Ian designed a two-year study to determine ways in which to predict, and eliminate or control astringency, without compromising the overall quality of the fruit. "Once we know which environmental, pre-harvest and/or post-harvest factors cause astringency in pears, we can put in place protocols to control it," he says.

The study, which started in February 2012 as a Hortgro Science project and continued as a FEMA Programme project from 2015, initially set out to assess the impact of ve factors on the expression of astringency in the 'Cheeky'® and 'Forelle' cultivars. These are harvest maturity, storage duration, SmartFresh™, CO_2 spiking and storage temperature.

In addition, the research team wanted to develop a biochemical method to measure astringency and assess its potential as a means of prediction.

However, following the success of the FEMA programme (see box on page 89), the Hortgro Science Technical Advisory Committee requested that the study's scope and objective be revised. As a result, work on 'Forelle' was stopped. Resources were instead devoted to understanding the physiological pro le of 'Cheeky'® with particular reference to the effects of harvest maturity and storage

> Unless a solution to
> astringency is fou
> there is the risk
> that supermarket
> may refuse to
> stock 'Cheeky'®
>
> Dr Ian Crouch

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Dr Ian Crouch, project leader.

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PROJECT TITLE
Identi cation of factors involved and control of astringency in pears

PRINCIPAL INVESTIGATOR
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DURATION
Two years

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R295 210 & R285 210

LEAD INSTITUTIONS
Hortgro Science and ExperiCo (Pty) Ltd

BENEFICIARY
The pome industry

FOCUS AREA
Post-harvest physiology

HUMAN CAPITAL DEVELOPMENT
None

PUBLICATIONS
One

PRESENTATIONS
Four

1

duration on fruit quality, and the expression of astringency and internal browning. Harvest maturity was deemed particularly important, given that orchards are becoming older and producing higher yields.

Study methods

'Cheeky'® pears from four orchards in two areas – Ceres and Grabouw – were harvested according to speci c maturity parameters during the commercial harvest window, and seven to 10 days on either side of it.

The pears were packed into MO7T telescopic cartons with standard non-perforated pear liners. Bags were closed four weeks before the scheduled examination to simulate packing and shipping.

Each harvest was stored for four, eight, 12 and 16 weeks at -0,5°C regular atmosphere (RA), followed by a shelf-life period of seven days at 20°C.

At harvest and after each cold-storage period, the fruit was examined and results recorded. In all cases, esh rmness was measured using a fruit texture analyser, while skin ground colour was recorded according to the South African industry colour chart for green apples and pears.

Further tests were carried out at harvest to determine the following: percentage of total soluble solids (TSS); titratable acidity, expressed as the percentage of malic acid (MA) equivalents; starch breakdown percentage; and fruit size.

The following were determined post-harvest:

- Internal quality, determined on a random sample of 20 fruit per replicate. The fruit was cut in half and visually assessed for mealiness and internal browning.
- Astringency, by tasting ve fruit per replicate (%).
- Decay (%) on all fruit in a carton.

In addition to internal quality, astringency and decay, the TSS percentage and titratable acidity of the fruit that had been exposed to the seven-day shelf-life period, were also recorded.

Key results

Astringency occurred in later harvests, from six weeks in 2012 and from 12 weeks in 2013. Incidence declined with storage duration and

IT'S (STILL) A MATTER OF TA

The measurement of astringency is not a simple process and currently there is no litmus test that gives an objective astringency value. The most reliable and quickest assessment of astringency remains tasting the fruit, either in the orchard at time of harvest, or in the laboratory after storage.

This method is, however, very subjective, and is the reason why scientists are working on establishing an objective test.

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THE FEMA SOLUTION

An innovative solution to market 'Forelle' pears earlier, and without the 12-week mandatory cold-storage period, has reduced pressure on producers to harvest the fruit as early as possible.

The 'Forelle' Early Market Access (FEMA) programme allows pears to ripen on the tree and then through the use of SmartFresh™, an ethylene blocker, prevents the expression of mealiness by keeping the fruit sweet and crisp. Fruit is sold within four weeks of harvest. As a result, the marketing gap is lled and bi-colour pear continuity is maintained.

was no longer evident after 18 weeks of cold-storage.

No mealiness occurred in 2012, and only low levels after six weeks storage in 2013 and 2014.

No internal browning and very little astringency were evident in the 2015 season. Astringency was only detected in Ceres fruit of optimal maturity that was cold stored for eight weeks.

Depending on seasonal variations, it is important to monitor skin ground colour, as in some years this may be a limiting factor and result in colour break during extended RA storage. In these instances, fruit should be marketed within eight weeks of harvest.

Fruit stored under RA conditions for up to 14 weeks exhibited acceptable esh rmness that almost always ripen normally after a shelf-life period. TSS remained relatively constant over a period of 16 weeks across all harvest maturities, but MA declined. There were possibly more decay, shrivel and internal disorders with extended storage and later harvests.

Mealiness occurred in optimum harvested fruit when cold stored for eight weeks, but not before or after this time.

Recommendations

- Early-optimum fruit should be cold stored for longer than four weeks to ensure normal ripening post shelf life.
- Optimum and post-optimum fruit should not be stored for longer than eight weeks RA to prevent skin ground colour break (*3,0).

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The new red blush pear variety, 'Cheeky'®, is currently being grown on 350 hectares in the Western Cape. (Courtesy of Caldevco (Pty) Ltd)

'Cheeky'®, a new addition to the South African pear cultivar portfolio, was bred and developed by the Agricultural Research Council, and marketed and commercialised by Culdevco (Pty) Limited. (Courtesy of Caldevco (Pty) Ltd)

Can mealiness be nipped in the bud?

Where and how pears grow, and how they are harvested and stored, are all clues to the early detection, and even prevention, of a costly post-harvest disorder.

1 Dr Elke Crouch, project leader.
2 Dr Letitia Schoeman and Dr Walter Fourie, both post-PhD researchers, applied their knowledge of X-ray micro and nano computed tomography to detect mealiness in 'Forelle' pears.

'FORELLE', A SMALLISH bi-colour pear with a distinctively sweet aroma and taste, is the second most produced pear in South Africa by volume. Popular as it is, the cultivar has one aw. It is prone to mealiness, a condition that results in the fruit esh becoming dry, soft and oury.

Previous research has revealed that mealiness is caused by the loss of cell-to-cell adhesion, and fruit that will become mealy has a higher porosity in the neck even before ripening. Researchers also agree that the condition has its roots in pre-harvest conditions, but they don't exactly know which conditions and hence cannot predict susceptibility.

At present, the industry deals with the problem by subjecting 'Forelle' pears to a mandatory 12-week cold-storage period to prevent mealiness from appearing, or by keeping the pears rm with SmartFresh™ (Forelle Early Market Access protocol).

However, some markets do not like pears rm and sweet, but prefer them to be soft and juicy. It is, therefore, not dif cult to understand how valuable it would be to have a way of predicting mealiness susceptibility in the packhouse, and to sort pears accordingly before they even start their journey to the consumer's table.

Dr Elke Crouch, a lecturer and researcher at the Department of Horticultural Sciences at Stellenbosch University, has been investigating the mealiness phenomenon for a number of years.

In a previous project, jointly nanced by the Post-Harvest Innovation Programme and Hortgro Science, her research team established that mealiness was a condition that existed prior to ripening in some 'Forelle' pears. Importantly, they also found that its development was associated with larger-celled fruit and those with large air spaces around their cells, and that in 'Forelle', speci cally, mealiness development seemed to be linked to a high total soluble sugar (TSS) content.

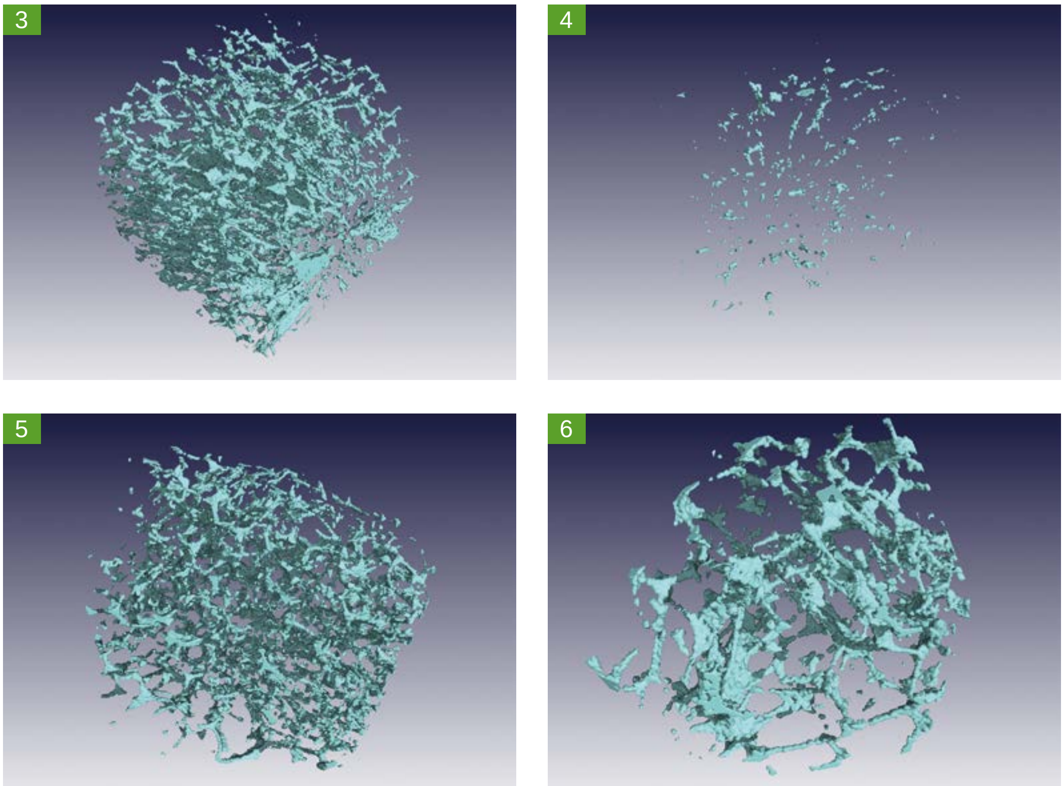
These were important ndings, given that TSS in fruit can be determined accurately with non-destructive NIR spectroscopy methods, while X-ray computed tomography (CT) scanning can detect the air spaces in fruit. Unfortunately, the latter technology is not available to the fruit industry on a commercial basis yet.

Consequently, it is important to nd supplementary ways to determine the post-harvest mealiness risk at harvest, and to reduce it as much as possible.

The project

With this objective in mind, Elke proposed a study that would investigate the link between mealiness and where in the canopy a pear grows and matures.

"We don't know why some fruit on a tree is predisposed to mealiness and others not, and



Above: Three-dimensional images of the porosity distribution (illustrated in aqua blue) in the neck (,) and equator (,) regions of a pollinated (,) and non-pollinated (,) fruit. The fruit was scanned on the GE Nanotom at the Stellenbosch University Central Analytical Facilities.

neither do we know if fruit position in uences ripening rate or tissue density," says Elke. "However, the fact that 'Forelle' seems to have rosier cheeks – in most cases indicating higher TSS concentrations – when they grow on the perimeter of the tree suggests a link between position and mealiness development."

In addition to position, pollination, type of ower in a cluster, number of fruit in a cluster, carbon assimilation due to sink strength, and ripening rate differences are factors that may affect fruit anatomy and physiology, and thus possibly mealiness susceptibility.

The link between TSS, a more intense blush and fruit position made colour an ideal focus for the new study that was designed to test predictors of mealiness. A successful outcome could lead to custom harvesting and storage protocols in order to reduce the risk of mealiness development, while also improving fruit quality after storage and ripening. The accuracy of current pre-sorters have to be tested in order to do this as pear pre-sorters are not commonly available.

Project objectives, methodology and ndings

The project, once more nanced by the Post-Harvest Innovation Programme and Hortgro Science, had four objectives that each required a speci c methodology.

PROJECT TITLE
Post-harvest 'Forelle' mealiness development, detected at harvest by CT-X ray scanning and semi-commercial colour pre-sorting in uenced by canopy position at harvest as well as pollination

PRINCIPAL INVESTIGATOR
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DURATION
One year and nine months

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R852 408 & R262 408

LEAD INSTITUTIONS
Stellenbosch University (Department of Horticultural Sciences) and Hortgro Science

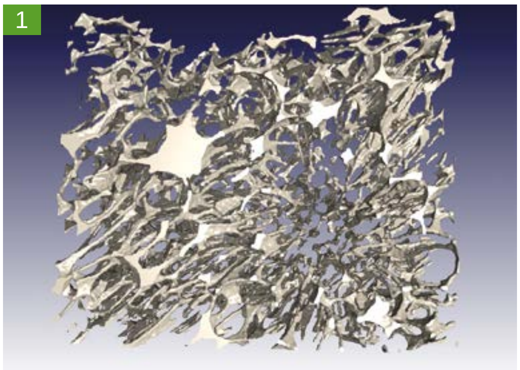
BENEFICIARY
The pome fruit industry

FOCUS AREAS
Post-harvest physiology

HUMAN CAPITAL DEVELOPMENT
One MSc student (Rudolph Cronjé), three post-doc researchers (Young Career Development) (Letitia Schoeman, Walter Fourie, Tavagwisa Muziri)

PUBLICATIONS
Pending

PRESENTATIONS
Three



Visualisation of the porosity in the neck region of a mealy ( ) and non-mealy ( ) 'Forelle' pear. The fruit was scanned on the GE Nanotom at a resolution of three micron. The scanner is located at the Stellenbosch University Central Analytical Facilities.

A mealy 'Forelle' pear does not release juice when squeezed or chewed. All mealiness determinations are done organoleptically by tasting, or visually by squeezing wedges of tissue and measuring juice weight.

OBJECTIVE 1: Map the fruit canopy positions in terms of fruit temperature, light interception, fruit tissue structure and porosity, fruit ripening potential and fruit mealiness development after ripening as well the relation to mealiness.

Trial 1: Fruit was tagged in ve positions of 10 tree canopies with uniform height and shape in one row. Eight pears per tree position were marked and used to measure light irradiation with a quantum sensor attached to a light meter. Fruit surface temperature of the portion of the fruit facing the current position of the sun was measured by using a high performance infrared thermometer.

Light irradiation and fruit surface temperature were measured three times a week, approximately four times a day, on cloudless days between 07:00 and 18:00, from mid-January until early March 2016.

Canopy temperature and relative humidity were measured continuously from mid-January until early March 2016 by means of tiny tags.

A total of 400 tagged pears – 80 fruit from each fruit position – were harvested at optimum harvest maturity on 2 March 2016. Maturity indexing was conducted at harvest and again after eight weeks of cold-storage at -0,5°C, plus seven and 11 days of ripening at 20°C. Standard maturity parameters were measured, such as the fruit background colour, blush percentage, esh rmness and TSS.

Micro-computed tomography was used to visualise fruit tissue density at harvest, after storage and after ripening. Nano-computed tomography was used to assess the quantitative porosity and cellular histology in the neck region of interest for fruit from different canopy positions.

Findings:

- Outer canopy fruit was more prone to mealiness, and was associated with the highest temperature and irradiance levels.
- Mealy fruit had more porous neck tissue compared to non-mealy fruit.
- Euler values indicating connectivity of pore space, cell size per volume and cell size did not differ signi cantly for fruit from different canopy positions.
- However, the consistent differences in Euler values indicating differences in porosity did suggest that inside and outside fruit may indeed be different.

Trial 2: To determine whether mealiness differences within the canopy are related to storage potential and ripening rate differences for fruit from different canopy positions.

Pears from the ve positions were harvested at optimum and post-optimum maturity. They were evaluated for maturity and quality after storage (eight, 12 and 16 weeks at 0,5°C) and ripening (four, seven and 11 days at 20°C).

Findings:

- Canopy position seems to play a larger role in mealiness development than ripening potential of inside versus outside fruit.
- The outside fruit from the rst harvest maturity did seem to exhibit differences in mealiness due to ripening and ethylene levels.
- Inside canopy fruit had higher levels of ethylene and not the highest level of mealiness.

OBJECTIVE 2: Determine whether pollination/ seed count affects mealiness potential.

Two treatments were applied on the western and eastern side of ten randomly selected trees during September 2015.

For treatment one, owers of ve trees were emasculated and hand cross-pollinated with ‘Early Bon Chretien’ pollen. Treatment two consisted of emasculating owers of the remaining ve trees without receiving pollen.

A total of 1 800 owers per treatment were used, and approximately 1 700 fruit were harvested at optimum maturity.

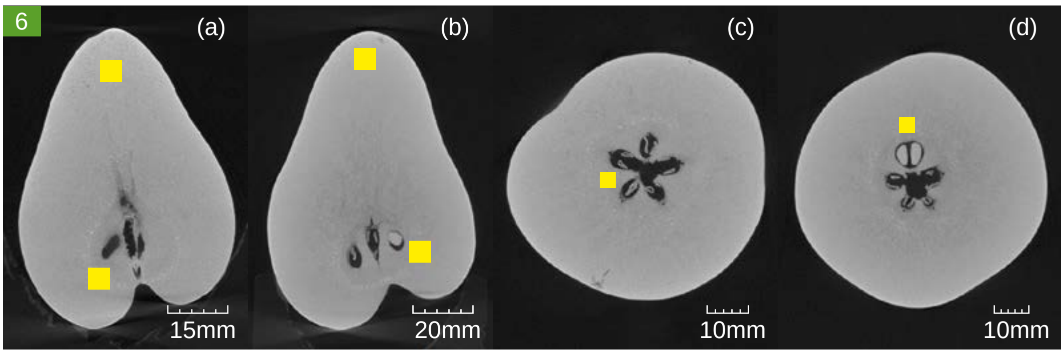
Maturity and quality indices were determined 12 hours after the fruit was taken out of cold-storage and left to reach room temperature, and again after seven days of ripening at 20°C. Maturity indexing at harvest and after eight weeks of storage at -0,5°C each consisted of 180 fruit from both sides of each tree. Double the amount of fruit was used after the seven-day ripening period, as the most mealiness was expected during this period.

X-ray micro-computed tomography (µCT) was used to visualise fruit tissue density. Nano-computed tomography was used for the quantitative porosity and cellular histology.

Findings:

- Pollinated fruit had a lower level of mealiness compared to emasculated fruit.
- There is a difference in tissue density between full viable seeds and parthenocarpic seeds (two types – longer or small type), with the region next to full seeds being denser.
- Pollinated fruit had a lower porosity than non-pollinated fruit in both the neck and equator regions, which may result in a lower incidence of mealiness (see Fig. 1 on page 95).
- The neck region (of both pollinated and non-pollinated fruit) had a higher porosity than the equator, which indicates different levels of mealiness in different regions in the fruit (see 6 below).

OBJECTIVE 3: Test the viability of commercial blush colour pre-sorters to determine mealiness susceptibility.



Rudolph Cronje, MSc student, studies the effect that the canopy position, light interception, fruit and canopy temperature, and pollination has on fruit quality, mealiness and ripening behaviour.

Illustration of the neck and region next to the seeds selected as region of interest (ROI) in a non-pollinated ( ) and a pollinated fruit ( ). The cross-sectional grey scale tomographic slice images also illustrate the ROIs next to the seeds in a non-pollinated ( ) and pollinated pear ( ). Differences in the grey level intensities indicate density variations (light grey = high density; darker grey = lower density), while black areas represent air voids.

1

‘Forelle’ fruit from emasculated owers where anthers were removed and owers not pollinated. The calyx end of the fruit is clearly affected.

‘Forelle’ pears were harvested from four different canopy positions in three orchards and sorted into four colour groups. The colour categories were related to mealiness development in ripened fruit in order to establish if colour can be used as a proxy for mealiness detection and, if so, how accurately a commercial colour pre-sorter can sort for mealiness before the condition develops.

Findings:

- Colour groups were related to mealiness development differences after ripening.
- The grading machine did not accurately detect the four colour groups. However, the machine’s ability can be improved through modi cations to the ramp, rolling action or camera con gurations on packlines speci cally used for pears.

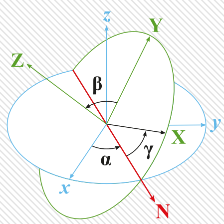
Conclusions

- Direct irradiance and higher temperatures make outside fruit possibly more susceptible to mealiness.
- Mealiness may not be directly linked to ripening. Ethylene levels of inside fruit are in many cases higher, yet they are never as mealy as outside fruit.
- Outside west-facing fruit consistently had the highest Euler number, smallest cell size and highest amount of cells/mm^3, while green inside fruit had the lowest Euler number, largest cells and lowest amount of cells/mm^3. This indicates that inside and outside fruit may indeed be different. However, a larger sample size may be required in order to statistically prove that fruit structure is different for different canopy positions.
- Pollinated fruit had a lower mealiness incidence, perhaps pointing to pollinated fruit having a better source of hormones during fruit set and development, which may in uence porosity.
- CT scanning shows important histological differences between mealy and non-mealy fruit. The mealy fruit shows lower density regions in the neck, attributed to higher porosities.
- Blush colour is related to mealiness development after ripening, making it ideal for pre-sorting. Apple colour pre-sorters are, however, not suitable for bi-colour pear sorting. Pear pre-sorters that overcome this will be tested for classi cation accuracy.

These studies are to be repeated to con rm ndings.

The most important preliminary recommendation is that for fruit harvested on the outside canopy, or fruit that is well blushed, the protocols that reduce mealiness incidence should be followed carefully. These are:

- Harvest at optimum maturity – not over-mature.
- Store fruit for longer than 12 weeks at -0,5°C.
- Orchards that qualify for the ‘Forelle’ Early Market Access (FEMA) protocol should use this for recommended markets as fruit are eaten crisp and sweet.



WHAT IS AN EULER NUMBER?

It is an indicator of connectedness of a 3D complex structure. Higher values indicate poorly connected structures and lower values better connected structures.