Big Brother is watching you, FCM

Two projects, both supported by the PHI Programme, are developing cutting-edge technology to detect false codling moth (FCM) infestations as part of a post-harvest systems approach to risk management. FCM is finally 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 financial 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

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 fine-tune the systems.

Wayne Kirkman, the principal investigator, signed up two manufacturers (Company A and Company B) in a project that stands to benefit 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 – 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.

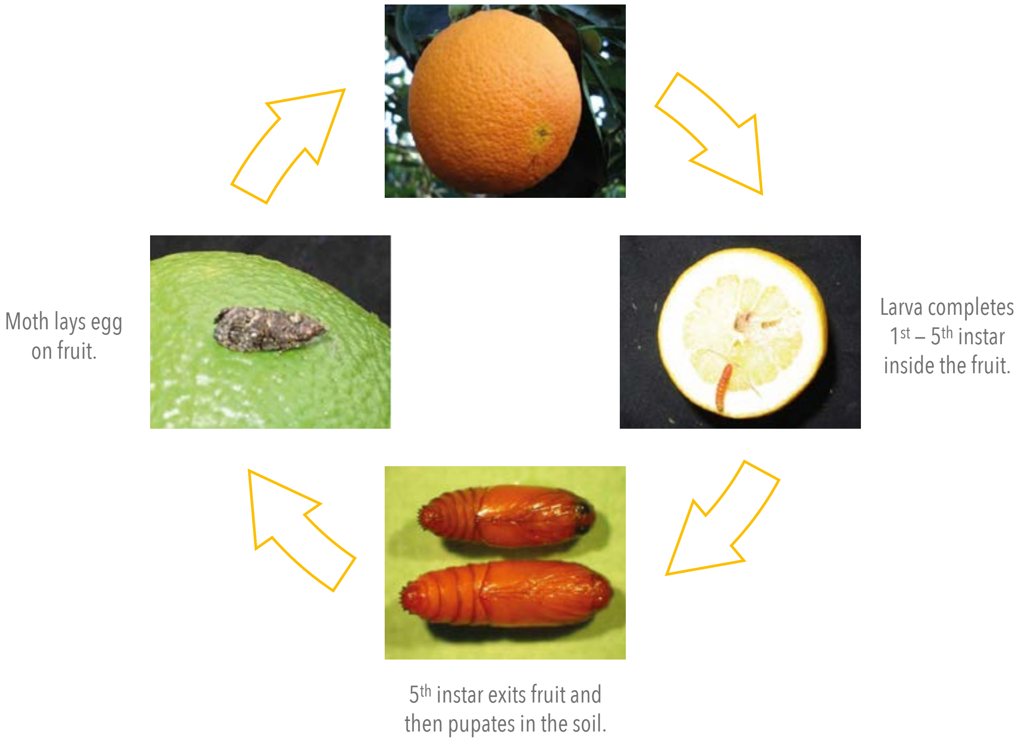
Several other batches of fruit (laboratory and naturally infested) were run through the system at two packhouses in the Sundays River Valley, and images were downloaded in Europe. All the oranges were dissected after scanning to verify infestation, and this information was sent to the programmers.

Company B

Company B has a demonstration unit and ➘

1 Wayne Kirkman, project leader.

Figure 1: FCM life-cycle



PROJECT TITLE	Detection of FCM with automatic sorting units
PRINCIPAL INVESTIGATOR	Wayne Kirkman
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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

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

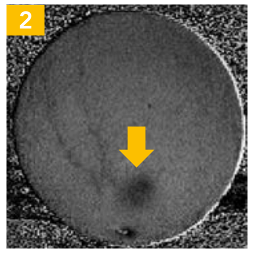
line at its offices. Wayne visited the company on three occasions, where several hundred oranges, laboratory and naturally infested, were run through the demonstration unit. On the third visit, two new technologies, which are not currently part of the automated system, were tested in isolation on each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

Two batches of laboratory infested fruit were couriered to Company B for further evaluation. Images were taken and sent to the head office 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 artificially infested they do not discolour, or form a blush, around the infestation point.

The finding 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.

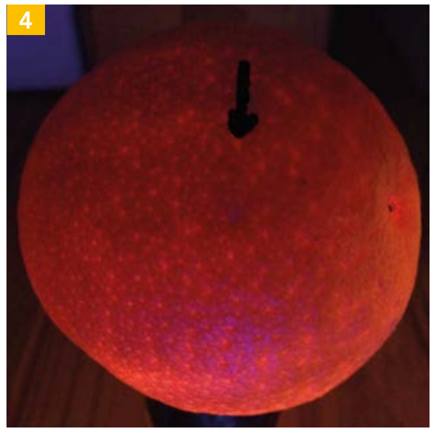
Company B

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.

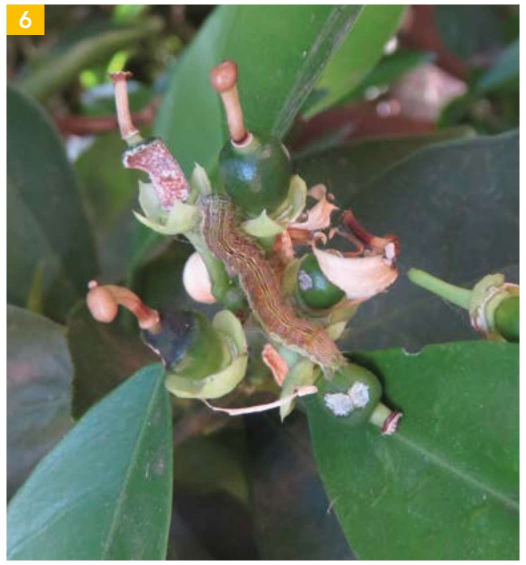
Collaboration with the two companies has led to improvements in their ability to detect FCM.

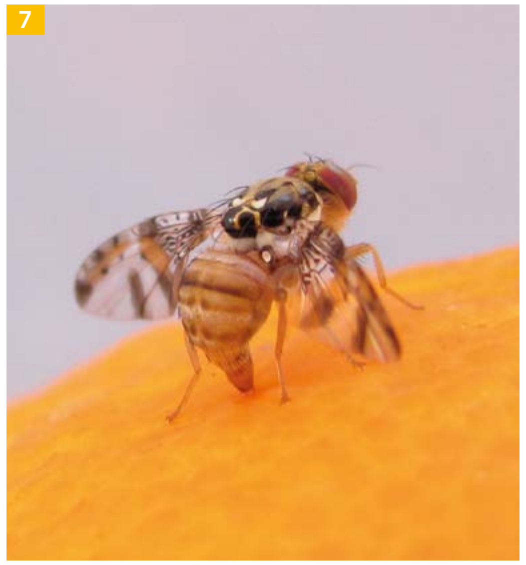
Company A is heavily reliant on its visual technologies, but is working on improving the ability to detect early signs of decay. Company B has added two new technologies to its demonstration unit, which should enhance its capabilities.

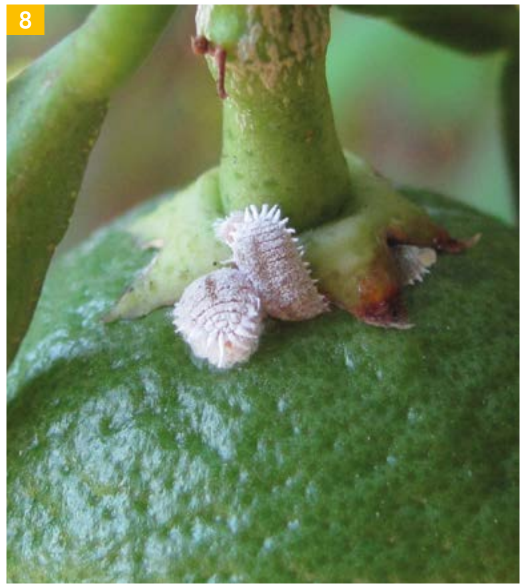
The project was terminated at the end of 2016, to give the companies time to improve their equipment and programming. The project could be resumed after a few years.

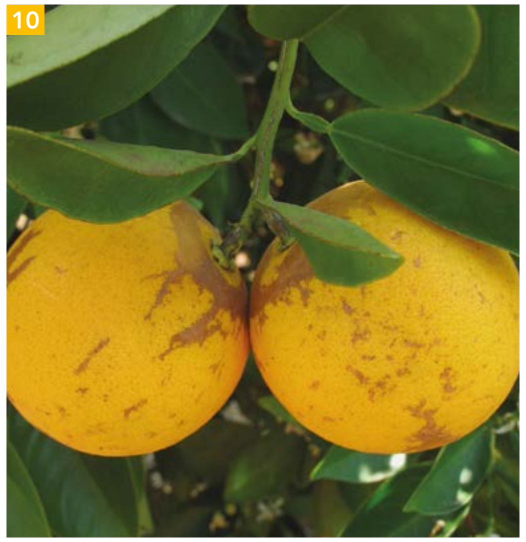


Images **1–5** show how technologies were used to highlight FCM infestations. Algorithms were updated by company programmers, and this led to improved FCM detection. A naturally infested Navel orange (**1&2**), with the FCM penetration point highlighted by image saturation (**2**).
3–5 New technologies 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).*)
6 Bollworm larva and damage.
7 Fruit fly laying eggs.
8 Mealybug.
9 Psylla.
10 Thrips damage.
11 Red scale on unripe citrus fruit.

Breathalysing fruit to find FCM

1 Dr Sean Moore, project leader.
2&3 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.

HEALTHY AND INFESTED fruit have different volatile emission profiles, 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 five 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 refining 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 scientific 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 flesh. However, damage in the rind and albedo could not be detected.

Electronic nose

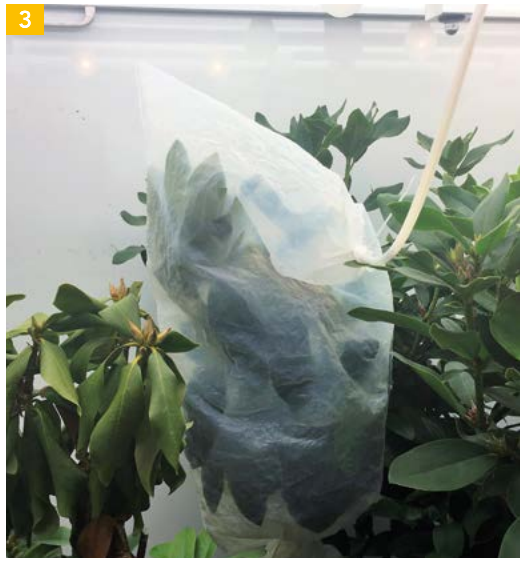
Collaborative research was conducted with the University of Leeds to explore immuno-sensor technology in the form of the "Bloodhound" electronic nose (e-nose). Wayne visited RoboScientific in Leeds to be trained on the e-nose, and brought back an experimental unit to evaluate the ability of the equipment 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 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 significant that use of this technology has, for the first time, shown significant 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
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LEAD INSTITUTION
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HUMAN CAPITAL DEVELOPMENT
One PhD student

PUBLICATIONS
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