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.

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 phyto-sanitary 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 phyto-sanitary 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.

How the work was done

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 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.

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.

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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 discolor, 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.
Breathalysing fruit to find FCM

**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-1,3,7-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.

“When the outew, 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 (L2) 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 unit to evaluate the ability of the equipment to detect FCM-infested fruit. Wayne concluded that CT scans could detect all infestations from six days onwards, including all second instar (L2) and larger larvae, as well as larvae that had entered the flesh. However, damage in the rind and albedo could not be detected.

**Gas chromatography-mass spectroscopy (GC-MS)**

The GC-MS trials had four aims.

- Identify volatile emissions associated with FCM-infection 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-MS work will continue once a unit is made available to CRI.