

Victorian diagnostics project leads the way in exotic spotted wing drosophila preparedness

Maddy Quirk, AUSVEG Project Officer

You may already be familiar with the spotted wing drosophila (SWD; *Drosophila suzukii*), a high priority exotic pest for Australia's berries, soft fruit, and viticulture industries; but did you know that Agriculture Victoria are currently investigating ways that we can prepare in case of its arrival?



Spotted wing drosophila (*Drosophila suzukii*) adults on raspberry. Photo credit: Hannah Burrack, North Carolina State University, Bugwood.org

SWD is a temperate pest that has made a major impact overseas. If it made its way into Australia, it could cause significant damage and economic losses to horticultural commodities.

At just 2-3mm long, the tiny fly could hitchhike its way into Australia through movement of infested produce and goods, or in the luggage of travellers.

Recognising the risk that this pest poses to Australia, Agriculture Victoria's project focuses on new molecular surveillance tools for early detection of SWD using high-throughput metabarcoding techniques and rapid in-field assays. Recently, AUSVEG Project Officer Maddy Quirk interviewed Dr Mark Blacket to find out more.

Surveillance methods achieving a common goal

This project aims to improve SWD surveillance methods across Australia, as part of a larger "Boosting Diagnostics" project through the Plant Biosecurity Research Initiative.

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Currently, surveillance for SWD in Australia is based on microscopy. Picking out SWD from our native *Drosophila* takes a lot of time and expertise.

While adult males usually have a distinctive spot on the wing (hence the common name Spotted Wing *Drosophila*), this feature is absent on larvae and female flies, which currently require examination of microscopic features by skilled entomologists. This becomes a bottleneck for diagnosticians because current trapping methods for SWD also catch hundreds of harmless native *Drosophila*, which must be sorted through.



In our last season, we had over 13,000 *Drosophila* flies caught in the traps. To sort through each trap by eye is very time consuming and will significantly delay any possible management response if SWD is detected.

Molecular methods improve the accuracy of SWD identification and vastly increase the number of specimens that can be identified at once, meaning that surveillance results can be provided rapidly to industry and government. The molecular methods that Agriculture Victoria have developed can provide a rapid first-screen of traps and suspect flies, with the aim of supporting, not replacing, current identification methods.

Metabarcoding explained

“Metabarcoding is a very broad test where you sequence a short diagnostic piece of DNA (i.e., a DNA barcode) from everything in a trap sample simultaneously. When you compare these DNA sequences to a reference database you can identify all the species present in a bulk trap sample, without having to sort through the flies individually,” Dr Blacket explained.

Traditionally a diagnostics lab would identify each insect in a trap morphologically or via PCR to determine if it was the target species. Metabarcoding instead uses high throughput sequencing, which is a complex process involving new laboratory technology and computational analysis. It can take about a week to run through the whole process, but many trap samples each containing thousands of insects can be analysed at once.

As well as providing a way of rapidly screening many traps for SWD, metabarcoding can identify all the other insects that are present in a trap, providing a complete picture of what species are present in the area.

“In the SWD project so far, we’ve picked up ten species of native *Drosophila* in our surveillance traps.

We also receive information on all other species that are in the sample, including beneficial wasps that parasitise *Drosophila*, which might prove important for biocontrol of SWD if it ends up in Australia.”

SWD pilot surveillance program

As a part of the National Plant Health Surveillance Program, Agriculture Victoria is conducting trapping across high-risk sites around Melbourne.

The first season, which was undertaken in 2020/21, surveyed community gardens around points of entry in Melbourne as well as multiple U-pick berry farms in the Yarra Valley, which pose a high risk of establishment should SWD enter Australia.

The surveillance program is continuing this season, with increased trapping in community gardens, and additional U-pick berry farms being included.



We are using commercially available state-of-the-art traps which are specifically designed for attracting SWD. The traps include a liquid preservative to make sure the DNA doesn’t degrade in our hot summer weather.

Agriculture Victoria are also coordinating this project nationally, with interstate collaborators from New South Wales Department of Primary Industries (NSW DPI). The samples from NSW DPI are being sent to Agriculture Victoria’s laboratory for analysis.

In the future, Agriculture Victoria may ask other interstate collaborators to provide samples, but with COVID-19 restrictions, the Victorian scientists haven’t been able to do any interstate trapping themselves.

Confirming the sensitivity of the test

Although Agriculture Victoria set up an extensive SWD trapping network, the species still (thankfully) hasn't been found in Australia. So how do we confirm our metabarcoding test will detect something that isn't yet here?

"It's actually quite simple, we deliberately spiked a number of samples with dead SWD that we sourced from an overseas stock centre. Because SWD isn't currently present in Australia, if our test works, SWD should only be found in the samples that were spiked."

The research has demonstrated that the metabarcoding test can successfully detect individual SWD flies mixed in with hundreds of native *Drosophila*. DNA sequences of SWD were only found in samples that were spiked, which shows the test is working and there were no false negatives.

This is an extremely promising result and proves that the metabarcoding assay is highly sensitive and effective.

In-field techniques

Agriculture Victoria have also validated a new rapid, portable, in field technology to identify pests. This technology, called a Loop-mediated Isothermal Amplification (LAMP) assay, can play a complementary role to metabarcoding in early detection of SWD—like the way rapid antigen tests (RAT) are now being used as a component of Australia's COVID-19 response.

"Unlike metabarcoding which is a lab-based test which can identify all species in a trap, LAMP is a very specific test for a single target species. The advantage of this test is that it can be used with a portable machine in the laboratory or field. You run this test and within an hour you have your result."

Previously, Agriculture Victoria developed LAMP assays for other pest insects, including *tephritid* fruit flies (e.g., Queensland fruit fly), *phylloxera*, Khapra beetle and fall armyworm.

LAMP assays for SWD have been published by international groups, but up until now they have not been validated for use in Australia.

Our role is to make sure these existing tests work in an Australian context, then incorporate them into the National Diagnostic Protocol, which is a standard process that everyone across Australia will use.

This ensures Australia has a consistent process and diagnostic methods for laboratories nationally, meaning all labs are using the most up-to-date methods to confirm that a species is, or is not, SWD.

A significant challenge is that SWD larvae (maggots) look like any other common *Drosophila* larvae; it is not possible to tell SWD larvae apart from *Drosophila melanogaster*, the common vinegar fly.

"This makes in-field techniques such as LAMP tests very useful for people finding unusual or suspect pests in their orchards," Dr Blackett continued.

If produce is being sent interstate or overseas, for instance through the market, and they're finding *Drosophila* larvae, LAMP could be used to test these. While this isn't currently set up in the markets, LAMP tests could be used to ensure produce isn't held for too long while waiting for an identification.

Where to from here?

From here, Agriculture Victoria will be training biosecurity personnel in LAMP diagnostics methods so that they can start using them at places such as the markets.

In the future, SWD traps from surveillance programs or individual growers may be processed through Agriculture Victoria's commercial diagnostic service Crop Health Services.

Having both Metabarcoding and LAMP methods in place in this laboratory will make the diagnostics more accurate as well as allow entomologists to cope with larger numbers of samples, so that new pest introductions can be rapidly detected and responded to.

The berry industry, Agriculture Victoria, and AUSVEG will also be looking to collaborate on a field day or workshop throughout 2022.

In the meantime, growers are encouraged to send in suspect SWD samples to their state diagnostic service. In Victoria, the state diagnostic service is Agriculture Victoria's Crop Health Services.

**For more information, please contact
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