

Victorian research the first line of defence for a threatening strawberry pathogen, *Xanthomonas fragariae*

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- *Xanthomonas fragariae* (*X. fragariae*) is a bacterial pathogen that causes a disease known as angular leaf spot (ALS) in strawberries.
- Whilst not found in Australia, it is a high priority for the strawberry industry capable of impacting plant growth, fruit quality, and production, as well as putting Australia's strawberry exports at risk.
- As part of the Boosting Diagnostics for Plant Production Industries project, Agriculture Victoria is working on surge capacity for *X. fragariae*

Signs and symptoms of *X. fragariae* include small water-soaked lesions or spots on the underside of the leaves. The edges of new lesions may look 'angular' in shape. These symptoms can be very similar to other pathogens that affect strawberries, so how do we know if we have found this new pathogen? The answer is, we need experts and specialised diagnostics to find out for us.

As part of the **Boosting Diagnostics for Plant Production Industries** project, Agriculture Victoria is working on surge capacity for *X. fragariae*. They are focusing their efforts on laboratory diagnostics, in-field detection, and updating the National Diagnostic Protocol to prepare for this pathogen.

Boosting Diagnostics Project Officer Maddy Quirk speaks to Dr. Fiona Constable, Dr. Rachel Mann, Elisse Nogarotto, and Jodie Crowder from Agriculture Victoria Research.

Protecting industry

While Australia remains free from *X. fragariae* and therefore ALS, there have been three successful eradication efforts over the last fifty years.

In 1975, ALS was detected in New South Wales, in 1994, ALS was detected in South Australia, and in 2010, ALS was detected in Queensland.

But eradication is time consuming and costly, so it is important to have the most updated tools and technology to identify pathogens rapidly and accurately.

Agriculture Victoria's research is seeking to achieve this, so that if *X. fragariae* is ever detected, Australia can diagnose, eradicate, and manage the problem faster than ever before.

Reviewing the literature

Before any scientific testing can be used in common practice, reviewing internationally available tests is essential. Agriculture Victoria researchers reviewed the literature to understand the types of tests available for detection of *X. fragariae*, including the most sensitive and suitable testing.

Using the literature, the researchers narrowed down their recommendations to two key methods: high throughput PCR testing for laboratory purposes, and loop mediated isothermal amplification (LAMP) testing for in-field detection. All the information from the literature review will assist with updating the National Diagnostic Protocol (NDP) for *X. fragariae* based on the best available information at the time. NDPs are the documents that Australian scientists need to follow to accurately diagnose a specific pest or pathogen.



Xanthomonas fragariae lesions on a strawberry leaf.

Photo credit: Gerald Holmes, Strawberry Center, Cal Poly San Luis Obispo, Bugwood.org

Exploring different sampling methods

To diagnose a pest or disease accurately, you first need a good sample taken from the part of the plant you are most likely to find the pathogen. For laboratory testing of strawberries, DNA is extracted and analysed. But what we may not know is that it can be extremely difficult to extract DNA from strawberries and the tissue type and the time of the year it is sampled can influence the quality of the DNA. For this reason, Agriculture Victoria is testing different extraction procedures to understand what part of the strawberry sample provides the best quality, and which can be applied to high throughput PCR testing in the lab.

‘To evaluate sampling and extraction procedures, we collected wild strawberry samples in summer, December 2020, and material from a commercial strawberry variety in autumn, March 2021,’ the research team explained.

Sampling in both December and March was critical, allowing comparison of a few different extraction methods across different seasons to see which part of the plant and at what time of year would provide the best result.

Dr Rachel Mann explained ‘Knowing what to sample and when to sample is your baseline for testing. If you don’t get decent DNA, the testing can be the best test in the world, but the sensitivity is poor.’

In-field techniques

It is critical to focus efforts on in-field testing techniques that are recognised internationally, and as such Agriculture Victoria has been looking at international research to understand the best in-field techniques for Australia. These techniques are known as LAMP tests, which are used in field and can produce a result in approximately an hour.

Agriculture Victoria are currently assessing the specificity and sensitivity of two LAMP tests and from there, they will be able to recommend which LAMP will be the best fit for Australia. The use of a LAMP tool has proven to be useful for Queensland fruit fly across the Victorian cherry industry to support market access, and this could become the norm for the strawberry industry and *X. fragariae* if it ever became established in Australia.

Dr Fiona Constable said ‘The LAMP would not just be for fruit crops but could also include runner production. Runners get transferred across Australia, and this could be really important for that purpose, to provide evidence that the material isn’t infected before it is sent interstate.’

Biosecurity agencies, which sit between Agriculture Victoria’s diagnostic laboratory and industry, may also be responsible for using this in-field technology.

Validating tests

Agriculture Victoria's role is to make sure that they aren't detecting any false positive results when creating tests specific to *X. fragariae*. In both PCR and LAMP technology, they are working to detect all strains of *X. fragariae*, but also make sure that they're not accidentally detecting anything outside of that target species.

Dr Rachel Mann explained 'We don't always have all the genetic information available to us to design the test. Sometimes the test might pick up something in the environment that wasn't initially accounted for.'

This could include closely related bacteria or another organism altogether, but regardless it is critical to validate these tests across a broad range of material from different regions to make sure they are not going to get false positive results. To assist, Agriculture Victoria has sourced a large collection of positive DNA controls internationally. This has enabled the team to demonstrate that these tests detect *X. fragariae*.

Working collaboratively

Agriculture Victoria will be working with other laboratories across Australia to receive interstate samples, which will assist with the validation process.

However, several interstate laboratories will also be interested in adopting the technology themselves. To facilitate adoption by interstate labs Agriculture Victoria plans to run a workshop to demonstrate the PCR and LAMP technology, which will form an important part of the extension element of this project.

This technology will be included in the updated NDP and will be available for all to access. For industry, this work means that strawberry growers will be guided as to which season and which tissue type will be best to sample for *X. fragariae*.

For industry representatives who may be doing field testing, they will also know how to sample to get an accurate result.

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