Development of an additional phylloxera detection method

A new DNA based method of phylloxera detection, suitable for grower use, has been developed during a Vinehealth Australia-led project.

Root galls casued by grape phylloxera. Image courtesy Agriculture Victoria (Rutherglen)

Background

Of the pests and diseases already in Australia, our greatest biosecurity threat to grapevines is grape phylloxera. This tiny insect destroys vines by feeding on their roots, and once infested, vines will die within 5-6 years and must be replanted with vines on resistant or tolerant rootstocks. There is no treatment for phylloxera.

Currently, phylloxera is confined to regions in Victoria and New South Wales, while South Australia, Western Australia, Northern Territory and Tasmania are recognised to be free of phylloxera.

The Australian wine industry contributes $40.2 billion in gross output to the Australian economy each year, and we have some of the oldest vines in the world at 175 years old. With estimates that at least 70% of Australia's winegrape vineyards are planted on own roots, and are thus susceptible to phylloxera attack, continued work on improving phylloxera detection methods is vital.

Active surveillance for grape phylloxera must be prioritised, and that requires practical and effective tools for growers and regulators alike. Traditionally, surveillance for phylloxera is undertaken by a third party, often regulators and not the growers themselves. But growers are in their vineyards every week. They are our eyes and ears.

The current endorsed phylloxera detection method (known as visual root inspection, or the 'dig method'), requires a small portion of roots to be dug up and inspected for insects and characteristic galling by trained personnel. This method is time consuming, expensive and relies heavily on the capabilities of the inspector. Another detection method known as the 'emergence trap method' is often employed by researchers and involves placement and then collection

of field traps to collect the insects. This method is not yet nationally endorsed.

A simple, rapid, sensitive and accurate method for the detection of phylloxera is therefore needed to enhance grower uptake of surveying their own properties and to simplify surveillance activities for regulators. This will improve the chance of detecting where phylloxera is and is not, and thus enable protection of vineyards from phylloxera. Additionally, the development of a test that enables rapid collection and analysis of samples will greatly assist both general surveillance activities, and management in the event of an incursion.

Vinehealth Australia was the lead agency in a collaborative phylloxera research project which concluded in mid-2018 to develop an additional phylloxera detection system using DNA extracted from soil samples. Outcomes of this project are expected to support identification and verification of area freedom status to facilitate market access for growers, as well as improving proactive management strategies for phylloxera.

The five-year project was funded by Wine Australia and the Plant Biosecurity Cooperative Research Centre. Partners in the project included the South Australian Research and Development Institute, Rho Environmetrics, the University of Adelaide, the Victorian Department of Economic Development, Jobs, Transport and Resources, Biosecurity SA (PIRSA) and the NSW Department of Primary Industries.

Project Summary

This research project successfully developed a field sampling protocol for collecting soil cores and validated a diagnostic protocol using qPCR (quantitative polymerase chain reaction) for the detection and quantification of phylloxera DNA, known as the 'DNA method'. It is recommended that this DNA method be offered as a commercial service to industry and regulators once endorsed, and that the DNA method is integrated into national and state phylloxera protocols and regulations.

Components of the soil sampling kit for collecting soil cores for the DNA method. Image courtesy Vinehealth Australia

Project recommendations include that:

1. The draft protocol for soil sampling to detect phylloxera DNA using the qPCR be adopted, which includes the following recommendations:
   a. To limit DNA degradation, soil samples for DNA extraction should be:
      i. stored at no more than 20°C during transport and reach the laboratory within 48 hours of collection; and
      ii. dried within approximately 24-48 hours of receival at the laboratory and stored unfrozen at approximately 10-20°C pre-drying.
   b. Soil cores to be collected from the top 10cm of soil and within 10cm of the vine trunk.
   c. The current field sampling density outlined in the National Phylloxera Management Protocol (NPMP) of sampling

A soil core collected as part of the DNA phylloxera detection method. Image courtesy Vinehealth Australia

one vine in every 3rd row in every 5th panel, has been shown to be suitable for the DNA method. However, higher sampling density above this standard recommendation may be warranted for any blocks suspected to have a low or isolated phylloxera infestation.

d. A sampling protocol based on composite samples of five soil cores is appropriate for phylloxera detection by qPCR. However, in instances of suspected low incidence and severity of phylloxera in a block, either less compositing of individual cores or higher numbers of cores per block (rather than per vine) may be considered in the surveillance plan to avoid phylloxera being diluted below the point of detection with the compositing process.

e. Samples can be collected successfully throughout the year for analysis given no significant difference in the frequency of detection across all seasons and sites sampled. However, with a trend of higher quantities of phylloxera DNA in early autumn (March), it is preferable to sample at this time if possible.

2. Weak vines must continue to be evaluated and sampled as part of any phylloxera surveillance strategy.
3. Recommendations 1 and 2 are incorporated into a submission for the Subcommittee on Plant Health Diagnostics to update the National Diagnostic Protocol for grape phylloxera.
4. Diagnostic laboratories that commercially undertake the DNA method, also incorporate a *Vitis* qPCR assay as an internal quality control check on all samples analysed for phylloxera DNA.
5. Endorsement of the DNA and emergence trap methods is actively facilitated by the Plant Health Committee, and both methods are integrated into the NPMP as alternate primary phylloxera detection methods.
6. There is a need to develop a national phylloxera surveillance plan which appropriately utilises the three primary detection methods (DNA, emergence trap and visual root inspection) as well as secondary surveillance methods for area freedom maintenance, delimiting of new incursions and zone status upgrades. This surveillance plan must ensure repetitive surveillance over time, particularly with respect to upgrading phylloxera management zone status. This research showed that the three detection methods, detected phylloxera at different rates, and each method failed in some cases to detect phylloxera even though phylloxera was present. It is therefore impractical to give a guarantee that an area is phylloxera-free based on a single detection method conducted at one point in time only.
7. If the DNA method is utilised as part of a surveillance plan, an additional detection method will need to be employed thereafter if positives are detected, to facilitate collection of insects for formal identification and strain typing.
8. A value-add for testing of other soil-borne pathogens, e.g. root-knot nematodes, be considered by the diagnostic laboratories for soil samples collected for the DNA method.

Industry and regulators will be advised once the DNA method has been officially endorsed by the Plant Health Committee and is commercially available.

Future Research

Through the implementation of this project, the project team has identified various items for future research focused on areas of improving the understanding of phylloxera biology as well as improving surveillance strategies:

1. Life table studies on seven key endemic strains under different environmental conditions with and without food.
2. Greater understanding on how phylloxera moves and survives.
3. Rapid laboratory identification of phylloxera in emergence traps, perhaps using scanning and shape recognition systems to confirm phylloxera identification and trap counts.
4. Strain identification of phylloxera:
   a. Interaction of strains with environmental conditions.
   b. Competition between strains of phylloxera – is it possible to inoculate with a benign strain?
   c. Rootstock-strain interactions.

5. Translation of block-level surveillance to landscape-scale surveillance with protocols for situations of varying incidence and severity levels, as indicated by secondary surveillance methods. Options evaluated side by side, over years and in different locations.
6. Use of existing and emerging secondary detection methods to identify potentially phylloxera-infested vines, to help target areas as part of a surveillance plan.
7. Development of a unique 'spectral fingerprint' for phylloxera to assist in facilitating the identification of weak vines associated with phylloxera infestation prior to visual cues becoming evident.
8. Development of a model using a range of risk factors to predict where to sample in a proposed landscape-scale situation, for example:
    a. Vineyards adjacent to cellar doors attracting tourist visitation;
    b. Blocks that share machinery, equipment and contractor movement;
    c. Own-rooted versus rootstock blocks;
    d. Frequency near gates, wineries etc;
    e. Blocks not planted with propagation material from certified sources; and
    f. Vineyards that have changed ownership frequently.
9. Development of strain typing capability for the DNA method.
10. Improving ground detection methods:
    a. Evaluate the effect of sampling density on detection rates across methods (DNA, emergence trap, visual root inspection).
    b. Is there a nutrient or level of a nutrient that phylloxera must have from vine roots to survive?
    c. What is the frequency of false positives associated with the visual root inspection detection method, arising from scoring root damage in the absence of insects as a positive for phylloxera, when in fact the root damage is due to an alternate cause:
        i. Especially in soils where nematodes are present;
        ii. Could a stain be used in-field to distinguish nematode from phylloxera galls?
    d. Is there potential for an in-field molecular test?

Collecting a soil sample for the DNA method using a soil corer. Image courtesy Vinehealth Australia

Reports

For further information on the findings of the research project, refer to the following:

A final report (http://vinehealth.com.au/wp-content/uploads/2018/04/PBCRC2061-FINAL-REPORT.pdf) and appendices (http://vinehealth.com.au/wp-content/uploads/2018/04/APPENDICES-FOR-PBCRC2061-FINAL-REPORT.pdf) submitted to the PBCRC, titled 'Sampling strategies for sensitive, accurate cost effective detection of grape phylloxera for quantifying area freedom status' completed mid-2018.

A final report (https://www.wineaustralia.com/getmedia/5041e2c2-2b23-4574-9e67-5aa71e9de718/PGI1201-Phylloxera-Final-report-AGWA) to Wine Australia , titled 'Sampling strategies for sensitive, accurate and cost effective detections for quantifying area freedom status', completed mid-2017.

Giblot-Ducray, D., Correll, R., Collins, C., Nankivell, A., Downs, A., Pearce, I., McKay, A.C. and Ophel-Keller, K.M. (2016). Detection of grape phylloxera (*Daktulosphaira vitifoliae* Fitch) by real-time quantitative PCR: development of a soil sampling protocol. *Australian Journal of Grape and Wine Research* 22 469-477.