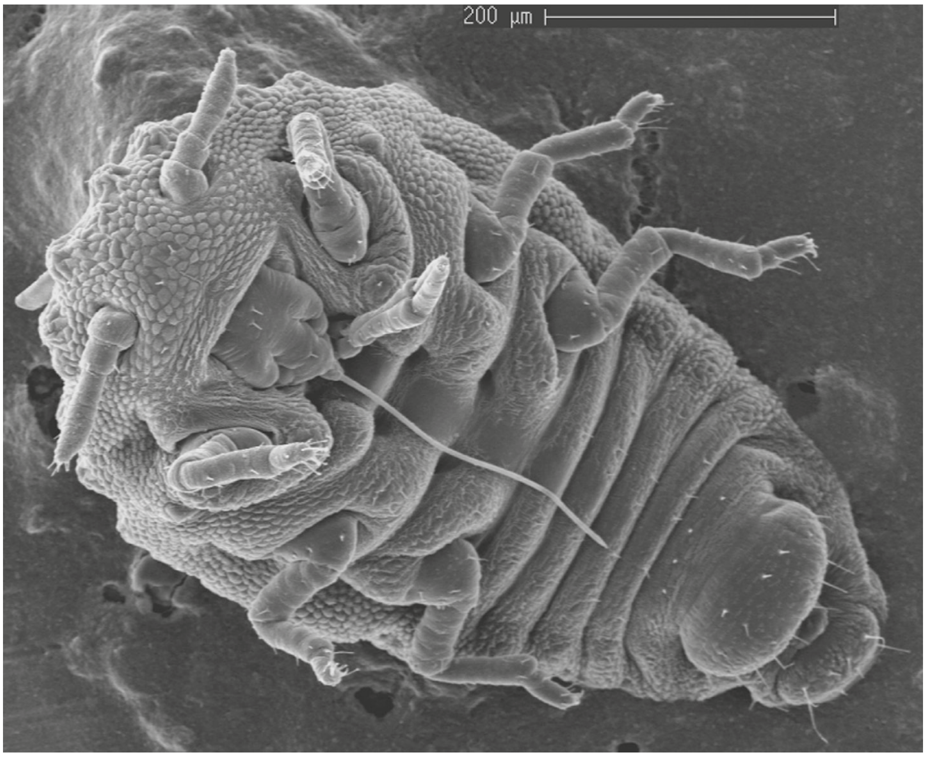
New phylloxera research: actions for industry

A three-year phylloxera project completed by Agriculture Victoria Research (AVR) has uncovered important new knowledge about phylloxera survival, reproduction and genetic strains that exist in Australia. Vinehealth Australia has summarised this research, highlighting key findings for industry. AVR provide insights into a new tool for identifying phylloxera and supply details of the next phase of phylloxera research.

The Agriculture Victoria Research (AVR) phylloxera project has reached an important milestone, with a report released in September, entitled 'Integrated management of established grapevine phylloxera'.

Vinehealth Australia has summarised key research outcomes from the project highlighting actions for industry. There are four key findings that are important for industry to note and incorporate into standard operations and farm-gate hygiene practices.



A phylloxera bug. Photo courtesy of Agriculture Victoria (Rutherglen)

1. Phylloxera lives longer

Intensive survival studies of five of Australia's key endemic phylloxera strains (G1, G4, G19, G20, G30), each representing a different group of genetic lineage, were completed. Survival was measured under a range of ambient temperatures, both in the presence and absence of food, and under wet and dry conditions.

It was found that in the absence of food (root material) but in the presence of water (approximating soil moisture), the G20 phylloxera strain survived for up to 29 days at 18˚C.

The length of survival for this strain is eight days longer than that found for any strain tested in previous strain-survival studies. This has implications as farm-gate hygiene practices until now, were based on a 21-day survival without food.

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This new finding means that the window of opportunity to spread phylloxera to another vineyard has increased. Therefore, to mitigate this risk, changes to farm-gate practices must be introduced immediately to reflect the longest survival time known for an endemic phylloxera strain.

This survival study highlights that diverse endemic phylloxera strains have different tolerances to temperature, food availability and moisture conditions, and the continued importance of including a range of strains in future such studies to build knowledge on phylloxera survival in Australia.

Actions for industry

- Update your farm-gate hygiene practices now. Ask and check where machinery, equipment and visitors have been for at least the 29 days prior, before assessing the risk to your property and potentially granting controlled access into your vineyards. Refer to the flowcharts in Vinehealth Australia's Hosting Visitors fact sheet (https://vinehealth.com.au/2020/11/hosting-visitors-fact-sheet) for further information.
- Make sure you adjust the wording in your visitor register (https://vinehealth.com.au/tools/visitor-register), site sign-in process, biosecurity training materials and any other documentation you may have in place, to reflect this new time period.

2. Undiluted Dettol is an effective disinfestation alternative

Survival of six key endemic phylloxera strains (G1, G4, G19, G20, G30, G38) was measured after exposure to more than 20 potential disinfectant products

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for durations of 30 or 60 seconds. This study continued the long-term focus on finding an off-the-shelf alternative disinfectant to bleach for footwear and small hand tools.

It was found that immersion of crawlers in undiluted Dettol disinfectant (4.8% chloroxylenol) for 60 seconds with no water rinse thereafter was effective in killing the phylloxera strains tested. Dettol was determined as the most suitable alternative to bleach for its ease in application as a footbath, relative safety to surfaces and grapevines and, availability as an off-the-shelf product from supermarkets and similar outlets.

Immersion for 60 seconds in undiluted Dettol with no water rinse thereafter offers an effective off-the-shelf alternative to bleach for footwear and small hand tool disinfestation.

Although undiluted methylated spirits also proved to be an effective disinfectant against the phylloxera strains tested, its flammability negates its recommendation for broad field use.

Actions for industry

- Consider situations where you may adopt the use of Dettol in preference to bleach for disinfestation of footwear or small hand tools. Cost, pack size and use situation may be contributing factors in this decision. Refer to Vinehealth Australia's Footwear and small hand tool disinfestation protocol.
- Note that if the used hand tool is being moved interstate or between Phylloxera Management Zones, it is important to check the relevant state Plant Quarantine Standard, or equivalent, for approved sterilisation methods. For South Australia's Plant Quarantine Standard, Dettol is not yet included as an approved sterilisation method for used hand tools.

3. Rootstock performance examined

The most recent key endemic phylloxera strain, discovered in 2015 in North East Victoria, is G38. The interaction of G38 with a range of rootstocks was evaluated in the field at a single site over a three-year study, as well as in the glasshouse and laboratory. Prior to this, no formal rootstock screening had been completed against the G38 strain in Australia.

In this study, rootstocks classified as susceptible had the following characteristics:

- Field – nodosities (galls on young fibrous roots) and high populations of phylloxera at all life stages (i.e., eggs, first instars, intermediates, adults) than on own rooted vines.
- Glasshouse (potted vines) – phylloxera that lasted the full eight weeks post inoculation and produced nodosities and necrotic tuberosities on lignified roots and, high populations of phylloxera than on own roots.
- Laboratory (excised roots) – phylloxera survived for 32 days post inoculation and adults produced eggs in higher abundance than on own roots.

It was found that 101-14 and Schwarzmann rootstocks, both of *Vitis riparia* x *Vitis rupestris* parentage, support high numbers of G38 phylloxera in the field, which were above that of own rooted vines. Both rootstocks were therefore classified as 'susceptible' to the G38 phylloxera strain. This is the same resistance rating as for own roots. Potted vine and excised root testing also supported the field susceptibility of these rootstocks to G38.

Other rootstocks in the in-field screening against G38 showed varying levels of resistance, aligned with their parentage. Rootstocks 1103 Paulsen and 99 Richter, both *Vitis berlandieri* x *Vitis rupestris* parentage, were classified as 'resistant' to G38. However, rootstocks with one *Vitis riparia* parent were classified as 'tolerant' to G38. These were 3309C (*Vitis riparia* x *Vitis rupestris*), Sori (*Vitis solonis* x *Vitis riparia*) and 5A Teleki, SO4 and 125AA Kober (*Vitis berlandieri* x *Vitis riparia*). It is important to note, however, that not all of these rootstocks were tested using the three screening methods and where multiple methods were undertaken, some differences in resistance ratings by rootstock resulted.

> **Rootstocks currently available for use in Australia have limited genetic parentage. This puts them at risk when a breakdown in phylloxera resistance to one or more of these parentages occurs.**
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> INCA LEE

The G38 strain was found to survive on the tolerant rootstocks, and only sometimes displayed nodosities, but characteristically did not produce necrotic tuberosities which are indicative of the rootstocks succumbing to the pest.

New diagnostic tool

Agriculture Victoria Research (AVR) has developed a DNA-based test that enables rapid phylloxera diagnosis both on-farm and in the laboratory. The new LAMP assay (Loop-Mediated Isothermal Amplification) is accurate, quick to obtain a result (less than one hour) and uses a portable device for direct in-field diagnosis.

The major advantage of LAMP for in-field phylloxera diagnosis is that samples can be collected directly from bucket traps, or suspect root material. LAMP now fast-tracks molecular identification by the insect diagnostics team at AVR, and Agriculture Victoria's biosecurity officers. This enables growers to be informed quickly about a phylloxera infestation and hence hygiene protocols can come into play immediately.

Digging for phylloxera in an infested vineyard in the Yarra Valley, Victoria

"The reported susceptibility of 101-14 and Schwarzmann rootstocks to G38 in North East Victoria is concerning. This finding also aligns with Californian reports of phylloxera strains that appear highly adapted to feeding on rootstocks with *Vitis riparia* parentage," said Inca Lee, Vinehealth Australia CEO.

"The tolerant rootstocks to G38 based on the in-field studies – 3309C, Sori, 5A Teleki, SO4 and 125AA Kober will act as reservoirs, or population sources of this phylloxera strain. This means spread can occur to susceptible rootstocks (such as 101-14, Schwarzmann), and to own roots, potentially leading to vine death.

"Rootstocks currently available for use in Australia have limited genetic parentage. This puts them at risk when a breakdown in phylloxera resistance to one or more of these parentages occurs. This could be one possibility to explain the field observations from this single study for the G38 strain only. However, researchers consider that there are likely other variables apart from parentage at play here, and this highlights the need for continued local research into phylloxera strain-rootstock interactions."

Teleki 5C rootstock was also screened against six key endemic phylloxera strains (G1, G4, G19, G20, G30, G38) both in the glasshouse on potted vines and in the laboratory on excised roots. Prior to this, no formal screening of Teleki 5C had been completed against a range of endemic strains Australia.

In potted vines and in the laboratory, it was found that Teleki 5C demonstrated resistance to the G1 strain of phylloxera. Neither nodosities were recorded on young fibrous roots in potted vines nor life stages of G1 phylloxera survived to adulthood at the end of the study.

Teleki 5C was rated as 'tolerant' to the G19, G20, G30 and G38 phylloxera strains in both potted vines and in the laboratory.

In potted vines, nodosities and phylloxera that developed into reproductive adults were in lower numbers compared to own rooted vines. While these endemic phylloxera strains can inflict some damage on Teleki 5C and host reproductive populations of phylloxera, damage and population size was lower than inflicted on own roots.

Teleki 5C was rated as 'resistant' to the G4 strain in the laboratory, but 'tolerant' in the glasshouse.

LAMP testing Agriculture Victoria Research

Pending field testing of phylloxera strain interactions with Teleki 5C, these results suggest this rootstock is likely to support a base populations of G19, G20, G30, G38 and potentially G4, from which spread can occur to susceptible rootstocks and own rooted vines.

Actions for industry

- Carefully consider your rootstock choice using available information and tools. New information from this study has been incorporated by Vinehealth into the rootstock-phylloxera strain interaction table, and by Wine Australia into the Rootstock Selector tool.
- With new phylloxera strains being detected in Australia, and their interactions with rootstocks starting to be understood, it has never been more important to undertake farm-gate hygiene practices to limit the introduction and spread of phylloxera, and importantly the different strains of phylloxera, between vineyards.
- Advocate for further research on rootstock-strain interactions and endemic phylloxera biotype classifications.
- Advocate for all newly-bred rootstocks in Australia to undergo comprehensive screening against key endemic phylloxera strains prior to release.
- Support rootstock breeding programs in Australia which aim to enhance the genetic base of rootstocks available for use in Australia with broad and stable resistance to phylloxera.

4. More phylloxera strains identified

Extensive phylloxera strain surveys over two seasons were undertaken across eight vineyards in the King Valley in the North East Phylloxera Infested Zone (PIZ) in Victoria, to understand the diversity and distribution of phylloxera strains in the region.

36 phylloxera strains were identified from a population of over 750 insects that were genotyped. Previous surveys in the King Valley had only identified the root-galling strain, G4, as present.

32 of the 36 phylloxera strains found had never been previously identified in Australia. This result is a 39% increase in genetic diversity and takes Australia's endemic phylloxera strain tally to 115 from 83.

The 32 new strains were found to be closely related to each other and to root-galling and leaf-galling strains previously detected in Milewa at the northern edge of the King Valley.

Vines were frequently found to have more than one strain of phylloxera on their roots. The highest genetic diversity found in a vineyard block was 14 phylloxera strains.

One hypothesis put forward by researchers for the occurrence of new strains, is that phylloxera may be reproducing sexually in addition to asexually, the latter being recognised as the predominant form of phylloxera reproduction in Australia.

If this is the case, it would bring with it an increased risk of long-range dispersal of novel strains of the winged form part of the sexual reproduction cycle. Sexual reproduction also tends to enhance adaptability to new or changing environments. It should, however, be noted that the hypothesis phylloxera are sexually reproducing in Australia still remains to be tested scientifically.

Results from this study have facilitated an update to the phylloxera strains distribution map, which is integral to informing decisions on rootstock selection, given a particular rootstock's ability to withstand a particular phylloxera strain.

Action for industry

- Continue to be vigilant with your on-farm biosecurity practices, both within PIZs and outside PIZs, to limit the introduction and spread of phylloxera strains. Let's not become complacent.

Summary

This three-year Agriculture Victoria Research project was conducted from 2017-2020 and was co-funded by Wine Australia and AVR.

The research was undertaken by a team of researchers at AVR: Paul Cunningham, Catherine W. Clarke, Bernadette Carmody, Mark Blacket, Rae Kwong, Isabel Valenzuela, Kevin Farnier, Stephen Tobin, Junji Miyazaki and John Weiss.

Australia's knowledge on the biology of grape phylloxera has advanced as a result of this current research study which builds onto past findings. This knowledge will now be used by the Australian grape and wine sector to enhance management of this destructive vine pest.

This article informs industry of key actions to implement now. To assist, Vinehealth Australia has also updated its posters and fact sheets, freely available on its website: www.vinehelth.com.au.

To read Agriculture Victoria Research's full report visit: https://www.wineaustralia.com/research/integrated-management-of-established-gra

The next phase of the phylloxera project

The biology and ecology of phylloxera underpins the development of policies and management of this vine pest. Recent research at Agriculture Victoria Research (AVR) has explored important knowledge gaps on the interactive effects of temperatures, rootstocks and phylloxera development under laboratory and field studies.

The next phase of AVR research (again co-funded by Wine Australia) will address important ecological knowledge gaps such as life-stage survival through "overwintering" as well as environmental and rootstock-related factors that may account for leaf-galling by phylloxera.

Additionally, research will investigate more effective surveillance strategies to increase confidence in declarations of phylloxera absence. The newly developed rapid diagnostic LAMP will be field validated and evaluated for large scale surveys of phylloxera.

Also, the effectiveness of disinfestation procedures under different hygiene situations will be assessed to support the improvement of industry-aligned protocols. A conservation biocontrol approach for phylloxera that could provide natural pest suppression against phylloxera and other important vineyard pests will continue to be explored.

Resilient rootstocks safeguard vineyards from phylloxera. AVR will continue the screening of commercial and CSIRO newly developed rootstocks to assess their long-term resistance to diverse phylloxera. GW