



Biocontrol options for grape phylloxera in Australia

Agriculture Victoria Research Technical Report

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Project RDC Number: DED 1701 – Integrated management of established grapevine phylloxera

Project CMI Number: 106053

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1 ABSTRACT

Our evaluation of classical biocontrol (importing exotic agents) revealed a lack of information on the natural enemy fauna of phylloxera in its native range (USA). Nevertheless, we found reports of 16 predatory species, of which only two attack root-feeding phylloxera. A predatory syrphid fly is particularly promising and warrants further investigation. We also suggest targeted field surveys in eastern USA to search for root predators and insect pathogens (entomopathogens). Similarly, in Europe most predators were of the leaf-galling form. In Australia, similar native predatory guilds occur in vineyards and could be encouraged through habitat manipulation for conservation biocontrol. There is enormous scope to further investigate the use of entomopathogenic nematodes and fungi as biological pesticides. Combining multiple biocontrol approaches will be more effective than either functional group used in isolation.

2 EXECUTIVE SUMMARY

Grape phylloxera (*Daktulosphaira vitifoliae*) is a tiny insect of North American origin where it feeds exclusively on American grapevines in the genus *Vitis*. For over 100 years, it has been considered one of the world's most damaging plant pest of grapevines after it spread to Europe causing the Great French Wine Blight in the mid-19th century. It has now spread to most parts of the world where highly susceptible European grapevines (*Vitis vinifera*) are grown, causing severe damage to the root system that may result in vine death within six years.

In Australia, millions of dollars have been spent in an on-going surveillance, detection and quarantine program since grape phylloxera was first detected in Victoria in 1877. However, with more than 85% of Australian vineyards planted with highly-susceptible own-rooted European varieties, the potential impact of grape phylloxera to Australian viticulture is alarming should grape phylloxera spread from current Phylloxera Infested Zones (PIZ) of Victoria and New South Wales. Furthermore, there is evidence that the control of grape phylloxera using resistant rootstocks within Victorian PIZ is under threat from the appearance of new virulent strains of the pest. More concerning is that some of these new strains possess both root and leaf-galling stages which significantly increases the risk of grape phylloxera dispersal to un-infested zones.

Clearly, the continued reliance on resistant rootstocks puts Australia's viticulture industry at great risk, prompting an urgent need to investigate additional means of control.

Through a thorough review of the scientific literature and consultation with experts from North America, Europe and Australia, this project sought to assess the feasibility of biological control of grape phylloxera in Australia using different approaches, including: 1) the release of exotic natural enemies from the pest's native range (Classical Biological Control) and ways to support these populations, 2) the release of commercially-reared predatory insects, including mites or parasitoids (Inoculation Biological Control), 3) the application of entomopathogenic organisms such as nematodes and fungi (Inundation Biological Control) and, 4) attracting resident native predators such as canopy and ground-dwelling spiders and insects through the provision of 'insectary plants' to provide shelter, nectar, pollen and alternative prey (Conservation Biological Control).

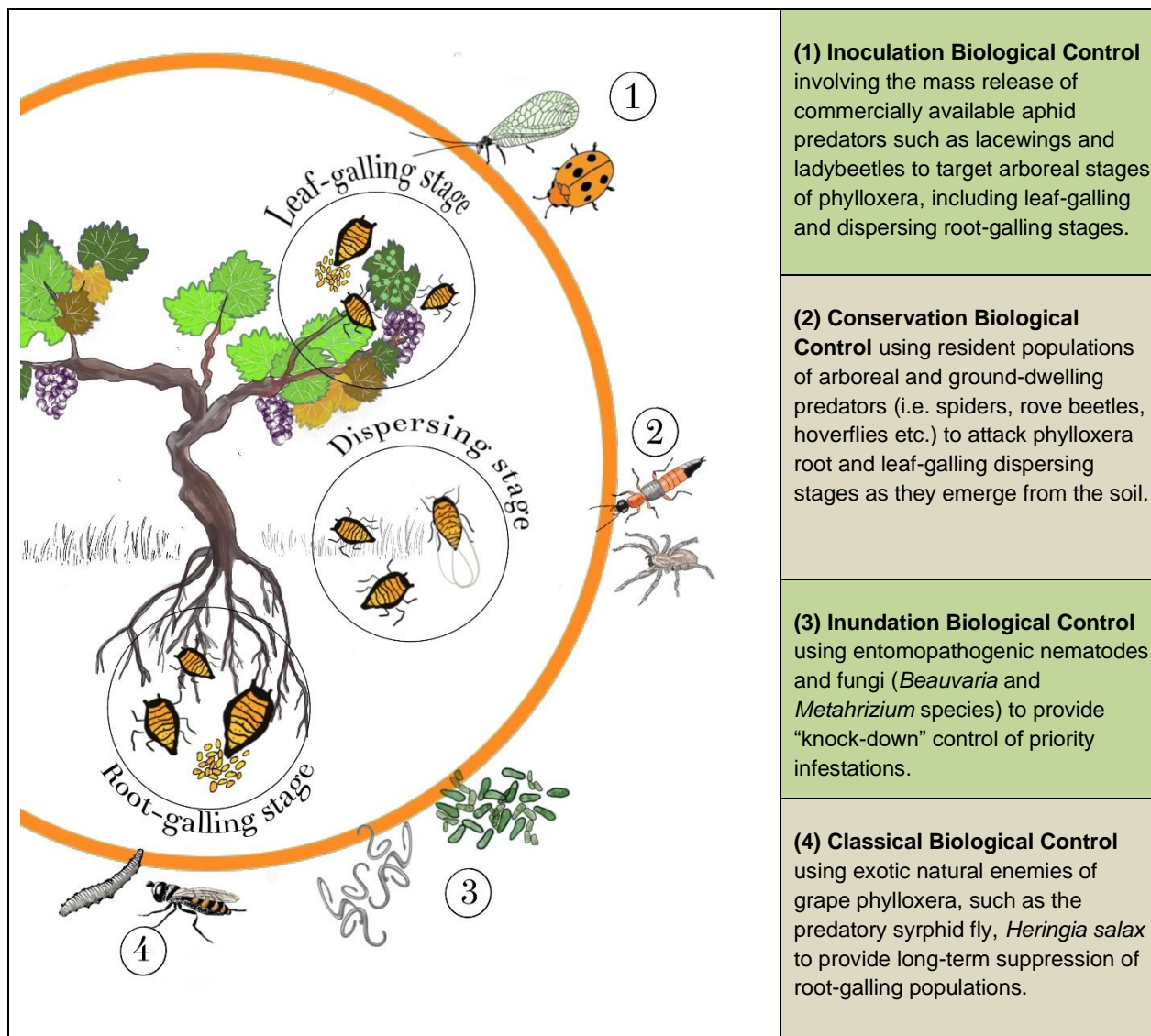
The review identified that enormous potential exists for any one of these approaches to be developed further. For instance, **Classical Biological Control** using a predatory syrphid fly (*Heringia salax*) has considerable potential because it is one of the few documented predators that attacks the adult and juvenile stages of root-galling grape phylloxera in its native range in the USA. In addition to conducting prey-range studies to determine if *H. salax* would be suitable for release into Australia, native range field surveys should be undertaken in North America to identify other predators and pathogens that may make effective biocontrol agents.

Around the world, considerable scientific advances have been made in the development of biopesticides providing **Inundation Biological Control** against a range of soil-dwelling pests using entomopathogenic fungi and nematodes. Surprisingly, relatively little research has been undertaken on the development of biopesticides for grape phylloxera, apart from the studies conducted in Europe that showed that the fungal pathogen, *Metahrizium* sp. provided control of root-galling grape phylloxera for up to three years. Initial research could be conducted to screen the effectiveness of currently available commercial products against Australian strains of grape phylloxera. In addition, research should be conducted to determine if naturally occurring entomopathogenic organisms present in Australian soils could be developed into commercial products, specifically targeting root-galling grape phylloxera.

In other countries, over 30 different predatory insects and mites have been recorded attacking grape phylloxera, yet nothing is known about the predation of grape phylloxera in Australia despite the presence of similar predatory guilds occurring within and around vineyards here (Retallack et al., 2019). Recent research on **Conservation Biological Control** in Australian vineyards has shown that native plant species with specific attributes, such as the provision of shelter and sources of food (pollen, nectar, alternative prey) can attract different types of insect predators. Hence, strategies to promote Conservation Biological Control against grape phylloxera should be initiated through studies that determine which native predators attack different life stages (i.e. sedentary and dispersal) and forms (root and leaf-galling). For instance, the planting of wallaby grasses, *Rytidosperma* spp. between rows could provide habitat for ground-dwelling spiders, earwigs, shield bugs, assassin bugs, lacewings and ground beetles to predate upon dispersing crawlers and winged adults as they emerge from the soil. Similarly, native shrubs that attract aphid-specific predators such as hunter/ambush spiders (Salticidae, Thomisidae), hoverflies, lacewings, earwigs, shield bugs, and ladybeetles could assist in the predation of leaf-galling phylloxera or dispersing stages.

In addition to Conservation Biological Control using resident predators, **Inoculation Biological Control** could also be implemented as an early-season strategy using a range of commercially reared aphid predators such as predatory mites, ladybeetles, lacewings and predatory bugs. Initial research is needed to determine if these predators do feed on phylloxera and if so, what release strategies should be adopted to maximise their impact.

Through an analysis of successful biological control programs against other aphid pests, Australia should adopt a multi-faceted approach that combines different forms of biological control targeting all stages and forms of the pest. This concept is outlined in Box 1.



Box 1. A multi-layered or truly integrated approach to the biological control of grape phylloxera in Australia that combines various approaches to target specific pest stages such as root-galling, leaf-galling and the dispersing stages of both. The different biological control approaches can also be applied depending on the management objective of existing or new infestations. For instance, Inoculation and Inundation methods could be applied where a knock-down approach is required for the rapid suppression of new infestations, while Classical and Conservation Biological Controls could be adopted for the long-term suppression of persistent and widespread infestations.

3 INTRODUCTION

This report is in partial fulfilment of a contractual agreement between Wine Australia and the Victorian Department of Economic Development, Jobs Transport and Resources (DEDJTR) for the project, DED 1701 – Integrated management of established grapevine phylloxera.

3.1 Project outcome

The objective of the project was to conduct a detailed desktop study to evaluate the potential for phylloxera biocontrol through:

- (i) Classical Biological Control using natural predators from the native range,
- (ii) Inoculative and Inundative Biological Control such as the mass application of commercially available predatory insects or entomopathogenic organisms, and
- (iii) Conservation Biological Control that involves the manipulation of the habitat and insecticide practices to enhance the effectiveness of resident natural predators.

3.2 Project background

Grape phylloxera, *Daktulosphaira vitifoliae* (Fitch) (Hemiptera: Phylloxeridae), is a small gall-forming, sap-sucking insect native to eastern North America. Grape phylloxera is monophagous to members of the genus *Vitis*, of which there are 100 accepted species and subspecies found across temperate regions of North America, Asia and Europe (Anon, 2013).

In North America, the frequency, severity and distribution of infestations vary significantly depending on the innate resistance mechanisms between American *Vitis* species. On suitable native hosts, grape phylloxera predominantly feed on the leaves, with marginal populations found in the root system, presenting no noticeably harmful effect on general grapevine vigour or yield (Wapshere and Helm, 1987).

In contrast, European grapevines (*Vitis vinifera*) have little or no tolerance to this insect. Leaf galling is rare and root feeding on both non-lignified (immature) and lignified (mature) roots results in nodosity and tuberosity development respectively, which significantly disrupt the uptake and transportation of nutrients and water. Extensive root damage leads to the gradual decline in vine health, lowering the plant's resistance to invasion by pathogenic fungi at phylloxera-feeding sites and eventually leading to the death of the plant. As such, phylloxera is regarded as the most economically destructive and geographically widespread pest species of commercial grapevines occurring in most grape-growing countries around the world.

In Australia, grape phylloxera was first detected in Geelong, Victoria in 1877 and in New South Wales in 1884 near Camden. Through an on-going surveillance, detection and quarantine program, the spread of grape phylloxera beyond Victoria and New South Wales has been largely successful. However, with the phylloxera-free status of many Australian grape producing regions, the relatively high costs of rootstocks (up to \$60 k per hectare) and the time taken for grapevines to reach full production (up to five years), many growers have been reluctant to plant grape phylloxera tolerant or resistant rootstocks. With more than 85% of Australian

vineyards planted with highly susceptible own-rooted, *V. vinifera* vines, the potential impact of grape phylloxera to Australian viticulture is alarming.

To date, grape phylloxera management around the world has predominantly relied on the use of resistant rootstocks, but there is evidence that this resistance is being overcome by new strains of this pest (Bao et al., 2015; Forneck et al., 2016; Umina et al., 2007). In Australia, 83 strains have so far been identified, of which around 49 are confined to roots only, 23 are confined to leaves only, and 11 strains attack both leaf and root material (Fig. 1) (Umina et al., 2007). The evolution of new strains necessitates both the development of new rootstocks and research on integrated control methods, including biological control. However, research into the latter, such as the use of natural enemies of grape phylloxera from the native range, the manipulation of the vineyard habitat to encourage native predators, and the mass release of insect pathogens such as nematodes, bacteria and fungi, has been limited in comparison to chemical control and rootstock breeding (Benheim et al., 2012).



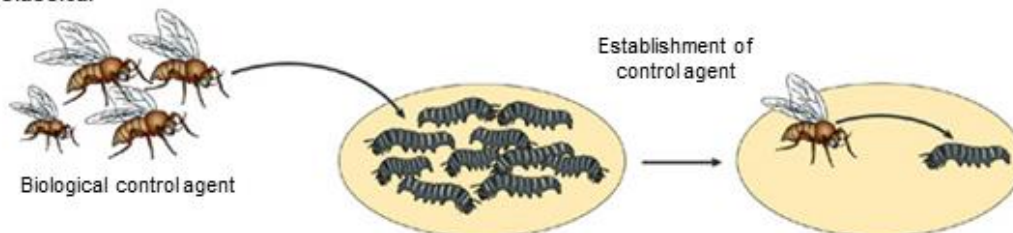
Figure 1. Leaf-galling form of grape phylloxera in north-east Victoria. (Photo: B. Carmody DJPR-Rutherglen).

Biological control, or biocontrol, can simply be explained as the use of natural enemies (biological control agents) to reduce the impacts of a pest. In practical terms, biocontrol consists of four main types: (1) Classical Biological Control, (2) Inoculation Biological Control, (3) Inundation Biological Control, and (4) Conservation Biological Control (Eilenberg et al., 2001), which can be used either alone, or in combination (see Box 2).

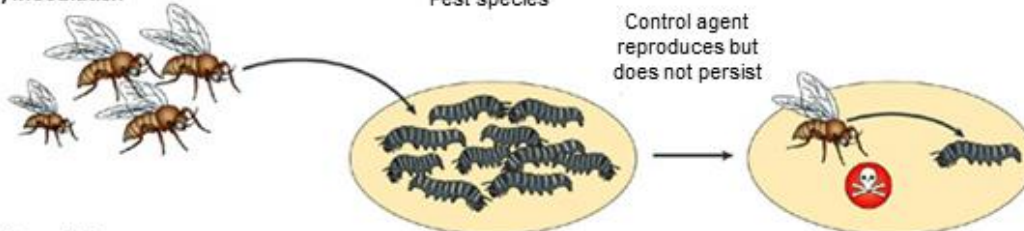
(a) Classical Biological Control involves the intentional introduction of an exotic natural enemy for permanent establishment and long-term pest control. The natural enemy (also known as 'biocontrol agent', or 'agent') can be a parasite, pathogen or predator and is typically collected from the native range of the pest. The agent enemy must be sufficiently specific to the target pest to be approved for release by regulatory authorities.

(b) Inoculation Biological Control involves the intentional release of biocontrol agents with the expectation that they will multiply and control the pest for an extended period, but not permanently. This approach is often used in glasshouses or periodic crops such as the annual release of egg parasitoid wasps (*Trichogramma* spp.) for Lepidopteran pests of corn, cotton and other broadacre crops.

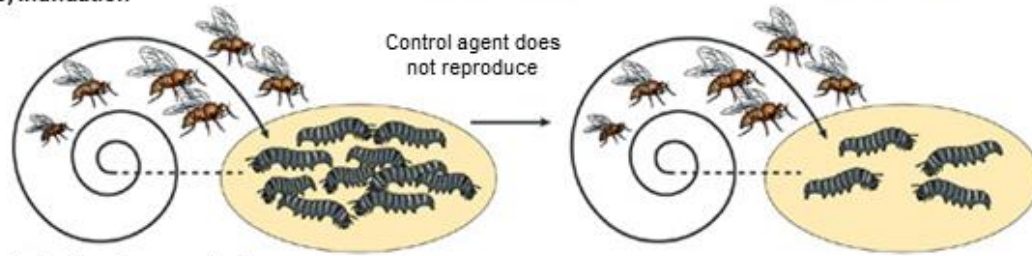
(a) Classical



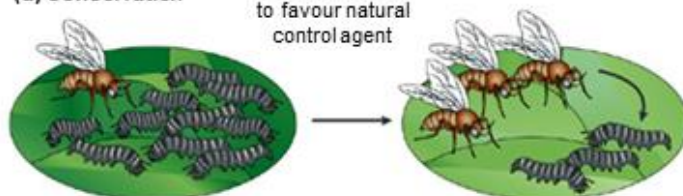
(b) Inoculation



(c) Inundation



(d) Conservation



(c) Inundation Biological Control involves the mass release of natural enemies that are typically produced on a commercial scale. Control is achieved exclusively by the released organisms themselves, hence reproduction of the organism is not expected after release. Certain entomopathogenic fungi, bacteria, viruses and nematodes can be readily cultured *in vitro* and applied using conventional pesticide techniques. In essence, these pathogenic organisms are applied as a microbial insecticide.

(d) Conservation Biological Control involves the alteration of the habitat or cultural practices to increase the numbers or efficiency of resident populations of predators, parasites or pathogens. Such alterations might include providing refuges within or adjacent to crops to provide food and shelter for natural enemies; and changing the timing of cultivation and pesticide applications.

Box 2. Types of biological control used against arthropod pests (adapted from Roderick and Navajas (2003).

In **Classical Biological Control** surveys are conducted in the pest's native range to identify natural enemies that have co-evolved with the pest species. The natural enemies are assessed for their potential as candidate biological control agents based on their impact on pest populations, their degree of host specificity (i.e. the range of hosts that they will attack), and the ability of the natural enemy to maintain persistent populations in the regions targeted for its release (Hajek, 2004). Because classical biological control involves the introduction of organisms to areas outside their natural range, there are quarantine protocols and assessments of the risks to the environment to be satisfied – for example, will the new introduction attack non-target species? In Australia, classical biological control is regulated through a formal risk assessment by the Department of Agriculture and Water Resources (DAWR) under the Biosecurity Act 2015, and by the Department of Environment and Energy (DoEE) under the Environment Protection and Biodiversity Conservation Act 1999 (further information is available at: the <http://www.agriculture.gov.au/biosecurity/risk-analysis/biological-control-agents>). While classical biological control programs can take between 10 to 15 years to complete, the longer-term economic and environmental benefits significantly outweigh the initial research costs due to the on-going nature of classical biological control. For instance, following the introduction of the hymenopterous parasite, *Trioxys complanatus* into Australia in 1977, the spotted alfalfa aphid, *Therioaphis trifolii* ceased to be an economic problem on lucerne crops within six years (Hughes et al., 1987). At the time, the value of the biological control of spotted alfalfa aphid, in terms of reduced insecticide costs and need to replant with aphid-resistant varieties was estimated to be in the order of \$2 million per year in New South Wales alone.

Inoculation and **Inundation Biological Control** are similar in that biocontrol agents are produced *en mass*, often on a commercial scale, and are not expected to persist in the environment, as with classical biological control. Rather, the distinction between inoculation and inundation lies in whether it is the released organisms themselves (Inundation Biological Control) or their progeny (Inoculation Biological Control) that results in suppression of the pest.

With **Inoculation Biological Control**, releases are conducted early in the season to provide adequate time for the released organisms to multiply and reduce the target population. This approach is commonly used for the control of green peach aphid, *Myzus persicae* in glasshouses using the parasitic wasp, *Aphidius colemani*.

With **Inundation Biological Control**, successful control depends solely on the released organisms and not on their progeny and the assumption is that control will decrease significantly over time and there is no expectation of long term pest control (Eilenberg et al., 2001). Agents used for inundative releases are often micro-organisms (entomopathogenic fungi, bacteria, viruses and nematodes) and are commonly called 'biopesticides'. In Australia, most biopesticides are referred to as 'biological agricultural products' and therefore must be registered by the Australian Pesticides and Veterinary Medicines Authority (APVMA, 2019). Macroscopic predators and parasites, including entomopathogenic nematodes, are excluded, but may be covered under other legislation such as the Biosecurity Act 2015 if they are exotic agents.

Conservation Biological Control involves the deliberate modification of the environment or change in existing pesticide practices to attract or improve the fitness of natural enemies (Jonsson et al., 2008). Conservation practices generally adopt a combination of approaches such as the limited and selective use of pesticides and the planting of vegetation adjacent to or within crops that provides habit for shelter (i.e.

overwintering, aestivation), and a source of additional food sources such as pollen, nectar and alternative prey (Gurr et al., 2000). Conservation biological control can be distinguished from other strategies in that natural enemies are not released but rather, are 'conserved' due to actions taken to protect or maintain existing populations (Eilenberg et al., 2001).

In Australia, predatory arthropods such as spiders, lacewings, ladybirds, carabid beetles, parasitic wasps, earwigs, predatory bugs and mites are commonly found in vineyards (Thomson and Hoffmann, 2009). Recently, Retallack et al. (2019) found clear associations between native insectary plants and predatory arthropods, demonstrating that different native species have unique attributes that attract and support different functional types of predators. For instance, the native perennial wallaby grass (*Rytidosperma* spp.) supported more ground-dwelling predators such as wolf spiders, shield bugs, earwigs, ground beetles and rove beetles (Staphylinidae) compared to Christmas bush (*Bursaria spinosa*) and prickly tea-tree (*Leptospermum continentale*). Hence, understanding the types of functional predators associated with different pests, provides considerable potential for Conservation Biological Control to be tailored to specific pest species, such as grape phylloxera. Yet, despite considerable information being available on the predatory arthropods in Australian vineyards, to date, no studies have determined what role these may have on grape phylloxera infestations, particularly in attacking dispersing or leaf-galling arboreal stages.

4 METHODOLOGY

4.1 Literature review of grape phylloxera

A list of predators and pathogens associated with grape phylloxera in the native range (North America) and introduced ranges (predominantly Europe) was built through intensive internet and literature searches. In addition, data were gathered from the grey literature and through direct communication with phylloxera experts via face-to-face meetings or email correspondence (refer to the Acknowledgements section).

References were examined for their relevance to this study and organisms found to be associated with grape phylloxera were further checked for information on their current taxonomy and nomenclature, host-range, mode of action and location and summarised in easy-to-read tables. For each group of natural enemies found to be associated with grape phylloxera, such as predatory Coccinellidae (lady beetles), a brief overview is provided on the importance of the organism in the biological control of aphids in general, and the prospects of the group for the biological control of grape phylloxera through classical, inoculation, inundative and conservation approaches.

5 RESULTS AND DISCUSSION

5.1 Natural enemies of grape phylloxera in the native and invaded ranges

Despite an extensive literature search, there was surprisingly-little published information available on the natural enemies associated with grape phylloxera in the native range of North America. It appears that no studies have ever systematically surveyed and catalogued the natural enemy flora and fauna. Most of the information was gathered from the annual reports provided by the British-born American entomologist Charles Valentine Riley during his term as state entomologist of Missouri during the 1870's (Fig. 2).

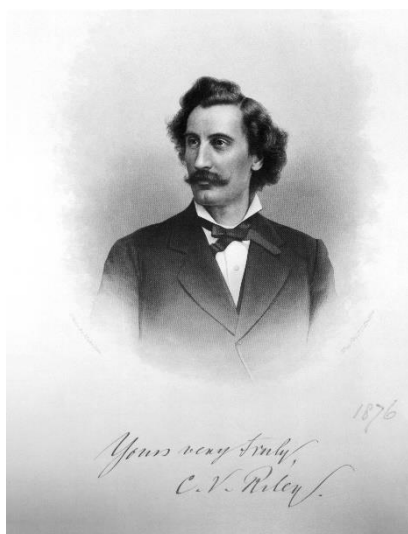


Figure 2. Portrait of Charles Valentine Riley (USDA National Agricultural Library).

5.1.1 Parasitoids

No records of parasitoids associated with grape phylloxera or other closely-related genera were found in the literature, suggesting that members of the family Phylloxeridae have few parasitoids. Riley (1874) fleetingly mentioned “certain smaller Hymenopterous parasites” amongst his list of natural enemies of grape phylloxera in Missouri, however it is uncertain whether he was referring to parasites of phylloxera or hyperparasitoids (i.e. parasites of parasites).

5.1.2 Predators

In the native range, the literature reports that grape phylloxera is attacked by 15 arthropod species, consisting of two coccinellid ladybirds, five species of predatory flies, three lacewings, four predatory bugs, a thrips and one mite. Most species were observed preying upon the leaf-galling phylloxera form (Fig. 3, Table 1). The only natural enemies reported attacking phylloxera in the roots were the syrphid fly, *Heringia salax* and the mite, *Tyroglyphus phylloxerae*.

In the invaded range, predators of invasive populations of grape phylloxera have been noted in Italy, Germany, France, Austria and the Ukraine, from similar predatory guilds to those present in the native

range. A total of 35 species were represented, with the most common predators being coccinellid ladybirds (12 species), predatory mites (8 species) and lacewings (5 species) (Fig. 3, Table 2). The higher number of predators (over double) recorded from the invaded range is most likely due to sampling effort, especially from the Ukraine where Gorkavenko (1976) purposefully documented the predators of grape phylloxera. In Australia, there have been no studies of the predators of phylloxera although unidentified nematodes (Powell, 2012) and mites (Catherine Clarke personal communication January 2019) have occasionally been observed in glasshouse phylloxera cultures.

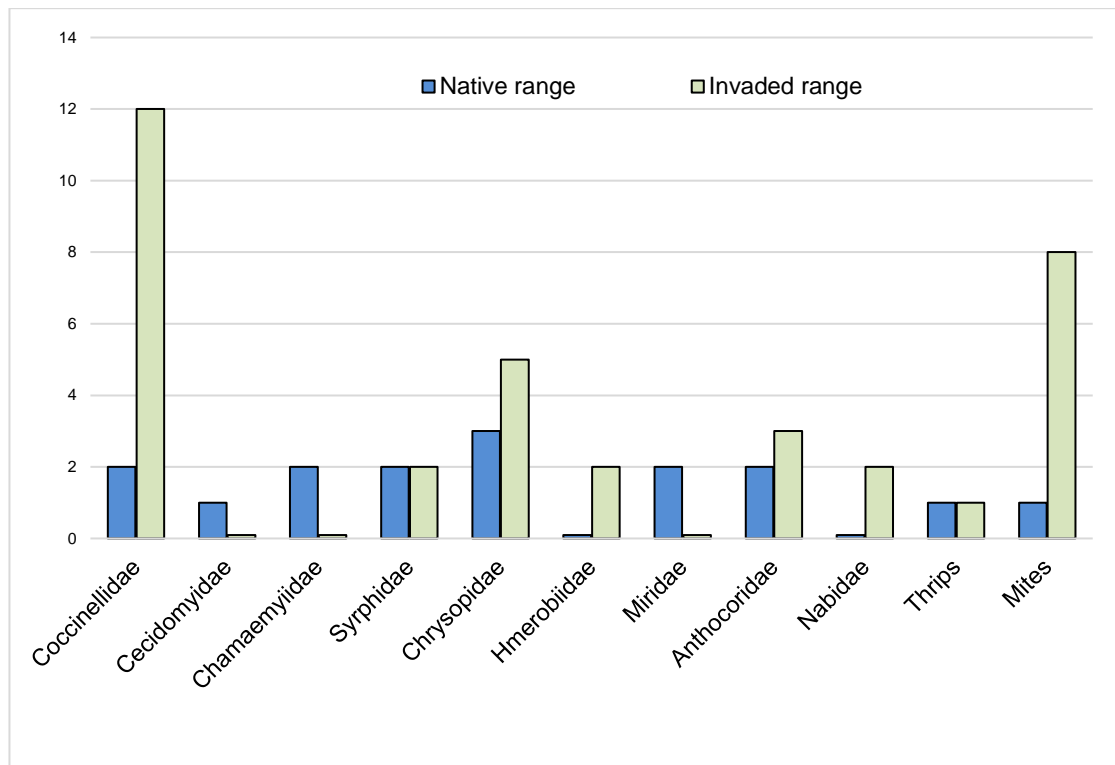


Figure 3. Taxonomic position of predator groups associated with grape phylloxera in the native range and invaded ranges.

Table 1. Natural enemies associated with phylloxera in the native range (USA).

Order: Family	Species	Mode of attack	Feeding guild	Host range	Origin	Reference
COLEOPTERA						
Coccinellidae	<i>Scymnus</i> (ladybirds)	Predacious larvae	Leaf galls		USA	Riley 1874
	<i>Scymnus cervicalis</i> Mulsant	Predacious larvae	Leaf galls	Phylloxera on <i>Vitis riparia</i>	Canada, USA	(Stevenson, 1967); Wheeler and Jubb (1979)
	<i>Diomus terminatus</i> (Say)	Predacious larvae	?	Various aphid species	USA	(Wheeler and Jubb, 1979)
DIPTERA						
Cecidomyiidae	<i>Lestodiplosis grassator</i> (Fyles, 1883) (syn <i>Diplosis grassator</i>)	Predacious larvae pale orange/salmon-colored	Leaf galls on wild grapes (<i>Vitis riparia</i>).	Other Phylloxerians and Pemphigians	Canada, USA	(Fyles, 1883; Riley, 1883; Stevenson, 1967)
Chamaemyiidae	<i>Leucopis phylloxera</i> (as unofficially named by Riley, but is probably synonymous with <i>L. simplex</i>)	Predacious larvae orange colored	Leaf galls	Phylloxera	USA	Riley (1883); (Stevenson, 1967),
	<i>Leucopis simplex</i> Loew	Predacious larvae	Leaf galls on wild (<i>V. riparia</i>) and cultivated (<i>Vitis</i> spp.) grapes	Phylloxera	Canada	(Stevenson, 1967)
Syrphyidae	<i>Syrphus</i> spp	Predacious larvae	Leaf galls		USA	Riley 1874
	<i>Heringia salax</i> (Loew) (syn. <i>Pipiza pistica</i> Williston, 1887, <i>Pipiza radicum</i> Walsh and Riley, 1869)	Predacious larvae	Root galls	Apple tree root louse?	USA	Riley 1874
NEUROPTERA						
Chrysopidae	<i>Chrysoperla carnea</i> Stephens (syn <i>Chrysopa plorabunda</i>) (Fitch) (weeping lace-wing)	Predacious larvae	Leaf galls	Polyphagous	USA	Riley 1875
	<i>Chrysoperla rufilabris</i> (Burmeister) (syn. <i>Chrysopa tabida</i> Fitch)	Predacious larvae	Leaf galls		USA	Riley 1875
	<i>Chrysopa oculata</i> Say	Predacious larvae	Leaf galls		USA	Jubb and Masteller (1977)
HEMIPTERA						
Miridae	<i>Ceratocapsus modestus</i> (Uhler)	Predatory bug	Leaf galls	<i>Quercus</i> spp	USA	Wheeler and Henry (1978)
	<i>Hyaliodes vitripennis</i> (Say)	Predatory bug	Leaf galls	leafhoppers	USA	Jubb et al. (1979b)
Anthocoridae	<i>Anthocoris insidiosus</i> Say (Flower-bug)	Predatory bug	Leaf galls		USA	(Riley, 1874)
	<i>Orius insidiosus</i> (Say)		Leaf galls on wild grape, <i>Vitis riparia</i>		USA	Jubb et al. (1979b)
THYSANOPTERA						
Thripidae	<i>Thrips phylloxera</i>		Leaf galls			Riley 1874
ACARI						
Acaridae	<i>Tyroglyphus</i> (=Acarus) <i>phylloxerae</i> Planchon & Riley	Adults prey on phylloxera	Root galls	Phylloxera	USA, Canada	Riley 1874, Stevenson 1967
	<i>Hoplophora arctata</i> Riley	Believed to be an overwintering form of <i>T. phylloxera</i>	Root galls	Phylloxera	USA	Riley 1874

Table 2. Natural enemies associated with phylloxera in invaded ranges.

Order: Family	Species	Mode of attack	Feeding guild	Host range	Origin	Reference
COLEOPTERA						
Coccinellidae	<i>Scymnus haemorrhoidalis</i> Herbst	Predatory larvae	Leaf galls	Aphids	Italy	Del Bene (1987)
	<i>Scymnus capitatus</i> Fabricius	Predatory larvae	Leaf galls	Aphids	Italy	Memorie della Società entomologica italiana, Volumes 20-29 Fratelli pagano - Tipografi editori, 1941
	<i>Scymnus rubromaculatus</i> (Goeze)	Predatory larvae	Leaf galls	Aphids	Ukraine	Gorkavenko (1976)
	<i>Scymnus frontalis</i> (Fabricius)	Predatory larvae	Leaf galls	Aphids	Ukraine	Gorkavenko (1976)
	<i>Scymnus suffrianioides j.sahlberg</i> (syn: <i>Scymnus apetzi</i> (Muls))	Predatory larvae	Leaf galls	Aphids	Ukraine	Gorkavenko (1976)
	<i>Scymnus (Pullus) ferrugatus</i> (Moll)	Predatory larvae	Leaf galls	Aphids	Ukraine	Gorkavenko (1976)
	<i>Coccinella septempunctata</i> Linnaeus	Predatory larvae	Leaf galls	Aphids	Europe	Gorkavenko (1976); (Kögel et al., 2011)
	<i>Oenopia conglobata</i> (Linnaeus, 1758) (Syn: <i>Synharmonia conglobata</i> L.)	Predatory larvae	Leaf galls	Aphids	Ukraine	Gorkavenko (1976)
	<i>Adalia (Coccinella) decempunctata</i> L.	Predatory larvae	Leaf galls	Aphids	Italy	(Del Bene, 1987)
	<i>Adalia bipunctata</i> (Linnaeus)	Predatory larvae	Leaf galls	Aphids	Ukraine)	Gorkavenko (1976)
	<i>Nephus bipunctatus</i> (Kugelann)	Predatory larvae	Leaf galls	Aphids	Ukraine	Gorkavenko (1976)
	<i>Harmonia axyridis</i> (Pallas)	Predatory larvae	Leaf galls	Aphids	Biocontrol agent from Asia	(Kogel et al., 2013)
DIPTERA						
Syrphidae	<i>Syrphus ribesii</i> (Linnaeus)	Predatory larvae	Leaf galls	Aphids	Ukraine	Gorkavenko (1976)
	<i>Episyrphus</i> (Syrphus) <i>balteatus</i> (De Geer)	Predatory larvae	Leaf galls	Aphids	Ukraine	Gorkavenko (1976)
NEUROPTERA						
Chrysopidae	<i>Chrysopa</i>	Predatory larvae	Root galls		Austria, Italy	Del Bene (1987), Riley (1874)
	<i>Chrysoperla carnea</i> Schneider (syn: <i>Chrysopa vulgaris</i>)	Predatory larvae	Leaf galls	Aphids	Europe, Ukraine	Gorkavenko (1976); (Mondal, 1972)
	<i>Chrysopa perla</i> Linnaeus	Predatory larvae	Leaf galls	Aphids	Europe, Ukraine	Gorkavenko (1976); (Mondal, 1972)
	<i>Chrysopa formosa</i> Brauer	Predatory larvae	Leaf galls	Aphids	Ukraine	Gorkavenko (1976)
	<i>Chrysopa pallens</i> (Rambu) (syn. <i>Chrysopa septempunctata</i> Wesmael)	Predatory larvae	Leaf galls	Ukraine	Ukraine	Gorkavenko (1976)
	Hemeroybiidae	<i>Hemerobius nitidulus</i> Fabricius	Predatory larvae	Leaf galls	Aphids	Ukraine
<i>Hemerobius marginatus</i> Stephens		Predatory larvae	Leaf galls	Aphids	Ukraine)	Gorkavenko (1976)

Table 2. Natural enemies associated with phylloxera in invaded ranges (continued)

Order: Family	Species	Mode of attack	Feeding guild	Host range	Origin	Reference
HEMIPTERA						
Anthocoridae	<i>Anthocoris pilosus</i> (Jakovlev)	Predatory bug	Leaf galls	?	Ukraine	Gorkavenko (1976)
	<i>Anthocoris nemorum</i> L.	Predatory bug	Leaf galls	Small insects and mites	Germany	(Herrmann and Forneck, 2001)
	<i>Orius niger</i> (Wolff)	Predatory bug	Leaf galls	Thrips, mites, aphids	Ukraine	Gorkavenko (1976)
Nabidae	<i>Nabis punctatus</i> A.Costa (Syn: <i>Nabis feroides</i> Rm)	Predatory bug	Leaf galls	?	Ukraine	Gorkavenko (1976)
THYSANOPTERA						
Aeolothripidae	<i>Aeolothrips intermedius</i> Bagnall	Predatory adults	Leaf galls	Thrips, aphids, mites	Ukraine	Gorkavenko (1976)
ACARI						
Trombidiidae	<i>Allothrombium</i> sp	Predatory adults	Leaf galls	?	Italy	(Del Bene, 1987)
	<i>Allothrombium pulvinum</i> Ewing	Predatory	Leaf galls	Aphids	Ukraine	Gorkavenko (1976)
	<i>Allothrombium fuliginosum</i> (Hermann)	Predatory	Leaf galls	Aphids	Ukraine	Gorkavenko (1976)
	<i>Trombidium holosericeum</i> (Linnaeus)	Predatory adults	Leaf and root galls	Polyphagous	Italy, Germany	(Del Bene, 1987; Herrmann and Forneck, 2001)
Acaridae	<i>Rhizoglyphus echinopus</i> Fumouze & Robin (syn. <i>Tyroglyphus echinopus</i>)	Predatory adults	Roots	Predominantly phytophagous	France, Austria	Riley (1874)
Tarsonemidae	<i>Tarsonemus</i> sp.	Predatory adults	Leaf galls	unknown	Germany	(Forneck et al., 1998)
Phytoseiidae	<i>Euseius finlandicus</i> (Oudemans)	Predatory	Leaf galls	Mite, scale insects	Ukraine	Gorkavenko (1976)
MESOSTIGMATA						
Laelapidae	<i>Gaeolaelaps aculeifer</i> (G.Canestrini) (Syn: <i>Hypoaspis aculeifer</i> (Canestrini))	Predatory	Root galls	Generalist predator of soil-dwelling arthropods	Ukraine	Gorkavenko (1976)

5.1.3 Coleoptera: Coccinellidae

Lady beetles are known to be voracious predators of plant pests, particularly aphids (Hemiptera: Aphididae). In North America, Riley (1874) reported that “certain small dark-brown species belonging to the genus *Scymnus*, and whose young, thickly covered with white and evenly-shorn tufts of cottony secretion, are frequently found at their good work within the galls”. Two species of scymnine coccinellids, *Scymnus cervicalis* Mulsant and *Diomus terminatus* (Say), have long been known as predators of grape phylloxera in North America (Wheeler and Jubb, 1979). *S. cervicalis* has been reported preying on the leaf form of phylloxera on wild grapes, *Vitis riparia* Michx. in Pennsylvania and Ontario, Canada (Stevenson, 1967; Wheeler and Jubb, 1979). *S. cervicalis* adults lay eggs singly at the entrance of galls. First and 2nd-stage larvae live within the galls feeding exclusively on phylloxera eggs. Wheeler and Jubb (1979) observed an entire phylloxera egg mass of over 100 eggs being devoured by two *S. cervicalis* larvae within two hours. Third and 4th-stage larvae feed on phylloxera eggs and occasionally mature females by inserting their heads into the opening of the gall on the upper leaf surface. Adults also feed on eggs and mature female grape phylloxera. Interestingly, Riley (1874) observed a *Scymnus* larva underground, stating that the larva was “at work six inches below the surface”.

Outside of the native range, six *Scymnus* species have been reported feeding on the leaf galls of grape phylloxera in Europe, from Italy (Del Bene, 1987) and the Ukraine (Gorkavenko, 1976). Little information is available on the biology and host preferences of scymnine coccinellids. *Scymnus* species are thought to be predominantly aphid predators, while *Diomus* are known to attack aphids, mealybugs, scale insects and white flies (Pang and Slipinski, 2009).

In Australia there are eleven species of *Scymnus* and over 100 species of *Diomus* (Pang and Slipinski, 2009; Slipinski, 2013). While none have been recorded feeding on grape phylloxera in Australia, five species of *Diomus* (*D. notescens* (Blackburn), *D. sydneyensis* (Blackburn) and two undescribed species) and an unidentified *Scymnus*, have been collected from vineyards in South Australia (Retallack personal communication December 2018; Thomson and Hoffmann, 2009). However, it is likely that *D. notescens* is a predator of scale insects having been recorded feeding on *Eriococcus* on *Eucalyptus* and *Acacia* (Slipinski 2007), and in vineyards its likely host is the grapevine scale, *Parthenolecanium persicae* (Fabricius).

A further six coccinellid species from five genera (*Coccinella*, *Oenopia*, *Adalia*, *Nephus* and *Harmonia*) have been recorded feeding on grape phylloxera within leaf galls in Europe (Table 2) (Del Bene, 1987; Gorkavenko, 1976; Kogel et al., 2013). While none of these species occur in Australia, congeneric species including *Coccinella transversalis* (Fabricius), *C. undecimunctata*, *C. septempunctata* L. and *Harmonia conformis* (Boisduval) have been recorded in Australian vineyards (Retallack, 2011; Retallack et al., 2019; Thomson and Hoffmann, 2009), where they are most likely feeding on Hemipteran pests such as long-tailed mealybugs, *Pseudococcus longispinus* (Targioni Tozzetti) and grapevine scale, *Parthenolecanium persicae* (Fabricius).

It has often been assumed that aphidophagous lady beetles are highly polyphagous, however there is evidence that not every aphid species is equally suitable for every coccinellid species (Finlayson et al., 2010). For instance, Kogel et al. (2013) investigated the consumption and development parameters of two ladybird species fed on grape phylloxera to assess their potential for biological control of grape phylloxera in Europe. The multicoloured Asian ladybird, *Harmonia axyridis* (Pallas), which has been used for biological control of aphids in several countries, was able to complete its development using grape phylloxera as a food source. In contrast, adults of the seven-spot ladybird *Coccinella septempunctata* (Linné), a native species in Europe, consumed fewer eggs and the larvae did not survive past the 2nd instar stage. Kogel et al. (2003) concluded that “*H. axyridis*, unlike *C. septempunctata* is a predator of *D. vitifoliae* and could potentially reduce grape phylloxera numbers in vineyards”.

5.1.3.1.1 Prospects for biological control

In Australian vineyards, endemic coccinellids already play a vital role in the control of Hemipteran pests, and it is possible that they may opportunistically predate upon arboreal stages of grape phylloxera. As such, strategies that enhance the impact of native coccinellids (i.e. Conservation Biological Control) should be considered over the importation and release of exotic coccinellids (i.e. Classical Biological Control). Surveys for natural enemies in the native range may provide further insights as to the observations of Riley of *Scymnus* larvae attacking phylloxera below-ground.

5.1.4 **Diptera**

5.1.4.1 **Cecidomyiidae**

The larvae of two genera of Cecidomyiidae, namely *Aphidoletes* Kieffer (=Phaenobremia Kieffer) and *Monobremia* Kieffer, feed exclusively as predators on aphids and are the only Cecidomyiidae known to do so (Harris, 1973). Two other cecidomyiid genera, *Endaphis* Kieffer and *Pseudendaphis* Barnes, include species whose larvae live endoparasitically in aphids (Harris, 1973). Unlike hymenopteran aphid parasitoids, these dipterans do not oviposit inside the aphid, but rather lay their eggs on the leaves near the aphid colony or occasionally directly on the aphid body (Muratori et al., 2009). The newly hatched larva actively searches for aphids, crawls onto the aphid abdomen, and penetrates the aphid exoskeleton to develop as a koinobiont endoparasitoid. Once mature, the parasitoid larva emerges from the anus of the aphid, which dies a few seconds after parasitoid emergence. Despite their voracious nature, aphidophagous cecidomyiids have been underutilised for biological control purposes. An exception is the predator *Aphidoletes aphidimyza* Rondani that is currently mass-reared commercially in many countries for the control of aphids in greenhouses, however its polyphagous habits make it less suitable than monophagous parasitoids for classical biological control programs.

Only one cecidomyiid species has been recorded as a predator of grape phylloxera. Described as *Diplois grassator* by Fyles (1883) and (Riley, 1883) (Fig. 4), *Lestodiplois grassator* (Fyles) larvae were found within the leaf galls of grape phylloxera in Ontario, Canada. According to Stevenson (1967), *L. grassator* occurred almost exclusively on wild (*Vitis riparia* Michx) rather than cultivated

(*Vitis* spp.) grapes, feeding on leaf-galling grape phylloxera eggs throughout the season. Interestingly, despite three authors reporting *L. grassator* as a predator of grape phylloxera, Harris (1973) was more doubtful of the association, stating, “Larvae of this genus usually prey on mites and on Cecidomyiid larvae and the original observations (by Fyles 1883) should be confirmed before this species is accepted as primarily aphidophagous”.

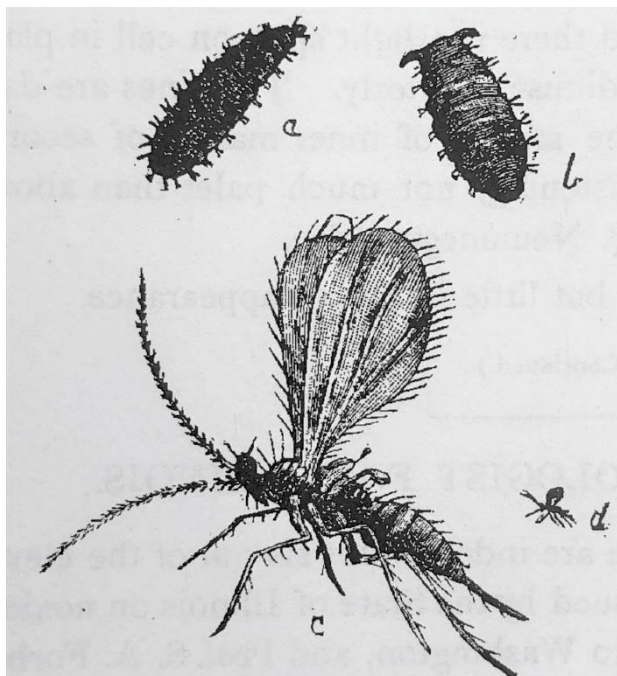


Figure 4. The cecidomyiid fly described as *Diplosis grassator* by Riley (1883).

5.1.4.1.1 Prospects for biological control

As *L. grassator* occurred almost exclusively on wild grapes, its practical importance in relation to cultivated grapes is remote (Stevenson 1967). Further study is required to understand this relationship and its implications for biological control. Its use for Australia is limited given the low incidence of leaf-galling grape phylloxera.

5.1.5 **Chamaemyiidae**

Commonly known as silver flies, Chamaemyiidae represent a group of larval predators attacking sternorrhynchus Hemiptera such as aphids, adelgids, scales and mealybugs (Fig. 5). While some chamaemyiid genera are quite general in their feeding habits, many are restricted to a particular host taxon (Gaimari and N. Tanasijtshuk, 2001). Several chamaemyiids have been utilised for the biological control of aphidine and adelgidine aphids, including *Leucopis glyphinivora* Tanas for the black bean aphid, *Aphis fabae* Scop and *Neoleucopis obscura* against the hemlock woolly adelgid, *Adelges tsugae* Annand (Montgomery and Lyon, 1995; Rad et al., 2003). Gaimari and Turner (1996) developed techniques for the mass rearing of several *Leucopis* species for amentative release programs against cereal aphid pests in the USA.

In North America, one chamaemyiid species, *Leucopis simplex* Loew was recorded predated on leaf-galling grape phylloxera in Ontario. Stevenson (1967) reported that *L. simplex* was the most common predator observed on both wild and cultivated grapes in Ontario over a two-year period with up to 40% of leaf galls infested.



Figure 5 . A *Leucopis* larva predated upon aphids (<https://bugguide.net/node/view/373728> Copyright © 2010 Claude Pilon).

5.1.5.1.1 Prospects for classical biological control

Leucopis simplex appears to have a limited host range and may have promise for the classical biological control in countries where leaf-galling phylloxera occur but would be of limited use in Australia.

5.1.6 **Syrphidae**

Commonly known as hover flies, flower flies or syrphid flies, the family Syrphidae is one of the most species-rich families of the order Diptera, with about 6000 described species belonging to 180 genera from three subfamilies (Syrphinae, Milesiinae, and Microdontinae) (Omkar, 2016). Syrphids are among the most beneficial insects because of their enormous importance as pollinators and because of the major role of predaceous species in natural and biological control of pest aphids and other Sternorrhyncha (whiteflies, scale insects) (Miranda et al., 2013). Adults feed on nectar and pollen, while the larvae of members of the subfamily Syrphinae are voracious predators on aphids, with a single larva consuming the body fluids of hundreds of aphids before entering the pupa stage (Joshi and Ballal, 2013).

Larvae of aphidophagous syrphids can be important biological control agents in agroecosystems (Short 2003 and references therein). Even when few in number, syrphid larvae are capable of effectively reducing or eliminating aphid populations (Michaud and Belliure, 2001). Aphidophagous Syrphidae are either generalists or specialists, with many members of the Tribe Pipizini oligo- or monophagous (Mizuno et al., 1997), and could be suitable for use in classical biological control. However, despite their importance as predators of aphids, they have generally been underutilised in classical and augmentative biological control compared to ladybirds (coccinellids) and lacewings (chrysopids) (Omkar, 2016). This may be due to difficulties associated with the mass rearing and transportation of syrphids for augmentative releases, although new techniques using ovipositional stimulants have been developed for the mass release of eggs of *Episyrphus balteatus* (De Geer) eggs for use against a wide range vegetable aphid pests (Leroy et al., 2010).

Three species of Syrphidae have been recorded as predators of grape phylloxera. In the native range, *Heringia salax* (Loew) (syn. *Pipiza pistica* Williston, *Pipiza radicum* Walsh and Riley) was observed preying upon the leaf-galling phylloxera in Canada (Graham 1965 in Stevenson 1967) and on root-galling phylloxera in Missouri (Fig. 6) (Riley, 1874).



Figure 6. Syrphid *Heringia salax* (Loew) (image Copyright © 2014 Tom Murray from downloaded from <https://bugguide.net/node/view/973735>, accessed 28 Dec 2018).

Outside of the native range, *Syrphus ribesii* (Linnaeus) and *Episyrphus* (*Syrphus*) *balteatus* (De Geer) were reported preying on leaf-galling phylloxera in the Ukraine (Gorkavenko, 1976). In Australia, there have been no reports of syrphids attacking grape phylloxera, however syrphid species are known to occur abundantly in and around Australian vineyards (Retallack personal communication December 2018).

5.1.6.1.1 Prospects for classical biological control

Prospects for classical biological control using syrphids are promising. The syrphid *Heringia salax*, is of particular interest because it was reported attacking grape phylloxera above and below-ground in the native range. *H. salax* belongs to the tribe Pipizini, members of which show a preference for aphids that have both arboreal (leaf gall-forming) and edaphic (root-feeding) colonies (Rojo et al., 2003; Rojo and Marcos-García, 1997). Pipizines are also known to have a narrow host range, making them suitable candidates for classical biological control. For instance, *Heringia calcarata* (Loew) is a pipizine syrphid that is a highly effective, specialist predator of woolly apple aphid, *Eriosoma lanigerum* (Hausmann) in the USA. Adults oviposit at the soil surface and larvae burrow down through the soil to find, feed and develop on buried woolly apple aphid colonies (Bergh and Short, 2008).

Little is known about the biology and host range of *H. salax*. According to Walsh and Riley (1869) and Riley (1874), *Pipiza radicum* (currently accepted as *H. salax*) is a predator of both woolly apple aphid and grape phylloxera. However, Bergh and Short (2008) dispute this, suggesting that the syrphid observed by the Walsh and Riley was actually *H. calcarta*.

5.1.6.1.2 Recommendations:

Further research of pipizine syrphids in the native range is needed to:

- 1) confirm the identity of the species attacking the root and leaf-galling grape phylloxera using genetic and morphological approaches,
- 2) study the biology, ecology and host range of identified syrphid species to determine their prospects for classical biological control of root-galling phylloxera in Australia.

5.1.7 **Neuroptera: Chrysopidae**

The family Chrysopidae, commonly known as lacewings, contains about 1200 species that belong in 75 genera and 11 sub- genera (Pappas et al., 2011). The larvae of all species, as well as the adults of certain species are predaceous and could be important biological control agents of several soft-bodied arthropods, such as aphids, coccids and mites (Fig. 7). Due to their important role in biological control programs, the species of the family Chrysopidae are the most well studied among Neuroptera (Brooks and Barnard, 1990). Research on all aspects of the biology of chrysopids and their use in biological control has been comprehensively reviewed since 1984 (Canard et al., 1984; Pappas et al., 2011).

In the native range, three chrysopid species have been recorded as predators of grape phylloxera. Riley (1884, 1875) reared the weeping lacewing, *Chrysopa plorabunda* Fitch (a synonym of *Chrysoperla carnea* Stephens) and the consumptive lacewing, *C. tabida* Fitch, (a synonym of *Chrysoperla rufilabris* (Burmeister)) of which he stated that the weeping lacewing was the second most efficient predator of leaf-galling phylloxera. In Pennsylvania, Jubb and Masteller (1977) identified five species of Chrysopidae from within and around commercial vineyards, but only the larvae of *Chrysopa oculata* Say were observed preying on grape phylloxera.

In Europe, at least four Chrysopidae have been recorded as predators of the leaf-galling phylloxera (Table 2 and referenced therein). In south-eastern Australia, green (*Mallada signatus* (Schneider)) and brown (*Micromus tasmaniae* (Walker)) have been recorded as important predators of long tailed mealybugs, light brown apple moth and vine scale in vineyards, and are available from commercial suppliers of beneficials (Retallack, 2011). Their role as predators of grape phylloxera has not been evaluated.



Figure 7. Green lacewing larva feeding on an aphid. (accessed http://www.montana.edu/yellowstoneinsects/neuroptera/chrysoperla_carnea.html © 2007 RKD Peterson)

5.1.7.1.1 Prospects for classical biological control

In general, chrysopids have not been widely used in classical biological control and most work has been focused on their application in Inoculation and Conservation Biological Control in agroecosystems. The large numbers of different chrysopid species occurring worldwide have rendered the importation of an exotic chrysopid species to another country, unnecessary. All chrysopid species known to predate upon grape phylloxera are generalists and therefore unsuitable for importation into Australia as classical biological control agents.

5.1.7.1.2 Recommendations:

Further research should determine if Australian native lacewings, including brown lacewings (Hemerobiida) and mantid lacewings (Mantispidae) feed on leaf-galling and dispersing stages of

grape phylloxera. If so, strategies to attract and enhance populations within vineyards using Conservation Biological Control principles should be investigated. Inoculative releases of commercially-reared lacewings could also be an effective strategy.

5.1.8 Hemiptera

Commonly referred to as true bugs, members of several families of Heteroptera, particularly Anthocoridae (flower bugs) (Fig. 8), Lygaeidae (big-eyed bugs), Miridae (plant bugs), Nabidae (damselfly bugs), Reduviidae (assassin bugs), and Pentatomidae (stink bugs) are important generalist predators of aphids (De Clercq et al., 2014; Joshi et al., 2010). Most of the carnivorous heteropterans used for biological control are polyphagous predators feeding on a wide array of arthropod prey. Details on the biology and biological control potential of the main families and species of predatory Heteroptera can be found in (Schaefer and Panizzi, 2000) Schaefer and Panizzi (2000).

In the native range, two mirid (*Ceratocapsus modestus* (Uhler), *Hyaliodes vitripennis* (Say)) and two anthocorids (*Anthocoris insidiosus* Say, *Orius insidiosus* (Say)) have been reported attacking leaf-galling phylloxera (Table 1 and references therein). Ground-dwelling predatory bugs (*Podisus maculiventris* (Say) *Geocoris bullatus* (Say)), are thought to be important predators of grape pests feeding on the vineyard floor (Jubb et al., 1979a), although it's not known if these bugs feed on below-ground or dispersal stages of grape phylloxera.



Figure 8. Anthocorid adult with aphid prey (photo by Bradley Higbee (Paramount Farmin Co)) (accessed <http://www.omafra.gov.on.ca/IPM/english/apples/beneficials/flower-bugs.html>)



Figure 9. The damselfly nymph, *Nabis kinbergii* (accessed <http://www.goodbugs.org.au/Good%20bugs/damselflybug.html>)

In invaded ranges, leaf-galling grape phylloxera has been recorded as being predated upon by three species of Anthocoridae and one Nabidae (*Nabis punctatus* A. Costa) in Europe (Table 1 and references therein). While none of these species occur in Australia, several species of predatory bugs, including the native damselfly nymph (*Nabis kinbergii* Reuter) (Fig. 9.) are commonly found in vineyards in south-eastern Australia (Retallack, 2011; Retallack et al., 2019), and could be predators of grape phylloxera, particularly of dispersal stages migrating along the ground or lower canopy. *N. kinbergii* is also available commercially for inoculative releases, while the minute pirate bug, *Orius*

armatus Gross is currently under development (Australasian Biological Control Association: <http://www.goodbugs.org.au/>).

5.1.8.1.1 Prospects for biological control

None of the predatory bugs known to feed on phylloxera are sufficiently host-specific to be suitable for classical biological control. Inoculation biological control using commercially-available bugs (*Nabis kinbergii* and *Orius armatus*) and conservation biocontrol using endemic species are the only options suitable for grape phylloxera in Australia.

5.1.8.1.2 Recommendations:

Further research should aim to determine if predatory bugs already present in Australian vineyards predate upon dispersal stages of grape phylloxera and if so, how their effectiveness can be enhanced through Conservation Biological Control approaches.

5.1.9 **Thysanoptera**

Thrips are very small, slender insects with mouthparts specialised for sucking and rasping. Thrips are primarily phytophagous, but some species are predaceous and can therefore be important biological control agents, especially of small soft-bodied insects and mites (Saengyot, 2016; Trdan et al., 2005). While some species of predatory thrips are known to feed on aphids (Lewis, 1973; Saengyot, 2016), they are often overlooked as important predators or as potential biocontrol agents of aphids.

According to Riley (1874), *Thrips phylloxera* (as he called it), was the most efficient predator of leaf-galling grape phylloxera in the native range. He describes the egg as three times larger than that of phylloxera, with an ellipsoidal and faceted surface that is deposited within the gall. The larvae, which are blood-red in colour, were noted to “do more than other species to keep the leaf-inhabiting grape phylloxera within bounds”. Despite Riley’s observation, there have been no other reports of phylloxera-predating thrips in the native range and the identity of “*Thrips phylloxera*” is unknown.

In introduced countries, the only species that has been recorded as a predator of grape phylloxera is *Aeolothrips intermedius* Bagnall (Gorkavenko, 1976), a common species widespread across Europe (Trdan et al., 2005).

5.1.9.1.1 Prospects for biological control

Prospects for classical biological control using predatory thrips is considered low as thrips are largely generalist predators and not suitable as classical biological control agents.

5.1.10 **Acari**

Predatory mites are more commonly used in the biological control of phytophagous mites, thrips and whiteflies (Knapp et al., 2018), however several members of the family Anystidae are important predators of aphids. For instance, *Anystis baccharum* Linnaeus is a predator of the apple-grass aphid (*Rhopalosiphum insertum* (Walker)) and English-grass aphid, *Sitobion avenae* (Fabricius) in Britain (Cuthbertson et al., 2003). For the control of edaphic pests or pests that spend part of their life in the soil, four predatory mites of the family Laelapidae are sold commercially namely *Androlaelaps casalis* (Berlese), *Gaeolaelaps aculeifer* (Canestrini), *Stratiolaelaps miles* (Berlese) and *Stratiolaelaps scimitus* (Womersley).

Predatory mites of native grape phylloxera were first observed by Riley in the mid 19th century, feeding extensively on the root-inhabiting form of grape phylloxera (Riley 1874). Based on his belief that the mite, *Tyroglyphus phylloxerae* Riley and Planchon, was largely responsible for “keeping phylloxera in check” in the USA, Riley sent mites to France in 1973 to combat grape phylloxera there. While Riley believed that the mite had established, he made a puzzling post-script two years later suggesting that a synonymy of *T. phylloxerae* known as *Tyroglyphus echinopus* Fumouze & Robin (currently, *Rhizoglyphus echinopus* (Fumouze & Robin)) was already present in Europe (Riley, 1875). Irrespective of whether Riley’s imported mites established or not, *Tyroglyphus* were observed attacking root-galling phylloxera in France and Austria, however their impact was considered disappointing.

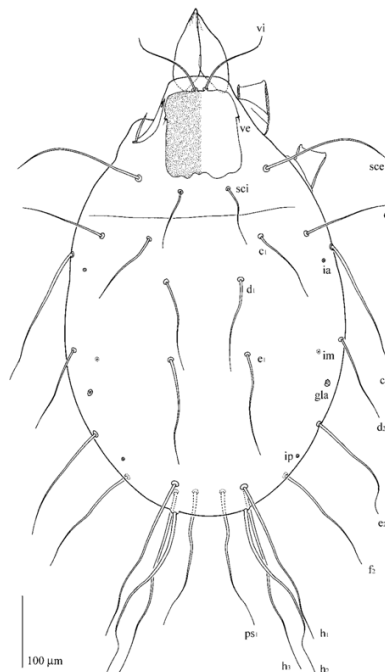


Figure 10. *Rhizoglyphus echinopus* (Fumouze & Robin) (adult homeomorphic male). Dorsal view of idiosoma. (accessed https://www.researchgate.net/publication/268422209_Revision_of_Rhizoglyphus_Claparede_Acari_Acaridae_of_Australasia_and_Oceania/figures?lo=1 Image by Qing-Hai Fan.)

Several mite species have been recorded associated with leaf-galling phylloxera in Europe, including members of the family Trombidiidae, Tarsonemidae, Acaridae and Phytoseiidae (Table 2 and references therein), however their association with phylloxera is uncertain as some of these species, such as *Euseius finlandicus* (Oudemans) are known predators of eriophyid mites (Abou-Elella et al., 2014). Apart from *R. echinopus* the only other mite recorded as a predator of root-galling grape phylloxera was *Gaeolaelaps (Hypoaspis) aculeifer* (G.Canestrini) (Mesostigmata: Laelapidae), observed by Gorkavenko (1976) in the Ukraine.

In Australia, an unidentified species of mite was found on one occasion feeding upon root-galling phylloxera in laboratory rearing cultures (Powell unpublished), however it is not known whether the mite occurs in field populations or is an opportunistic predator under optimum laboratory conditions. Similarly, Forneck et al. (1998) found that a *Tarsonemus* mite was able to destroy colonies of grape phylloxera in the laboratory however its suitability as a biological control agent in the field has not been assessed.

5.1.10.1.1 Prospects for Classical Biological Control

Apart from *R. echinopus* (formerly *Tyroglyphus phylloxerae*) little information is known about predatory mites of grape phylloxera in the native range. There is no value in utilising *R. echinopus* for classical biological control in Australia, as the species is considered an important pest attacking bulbs, corms and tubers of a variety of crops (e.g. onions, garlic and other vegetables) and ornamentals (lily and other flowers) in Australia and other countries (Fan and Zhang, 2003).

5.1.10.1.2 Prospects for Conservation Biological Control

In Australian vineyards, predatory mites provide an essential role in the biological control of pests. A total of 31 species of native predatory mites from the family Phytoseiidae Berlese occur in vineyards providing an essential role in the biological control of eriophyid and tetranychid mites, with *Euseius victoriensis* (Womeley) considered the most important (Bernard et al., 2010; Whitney and James, 1996). It is unknown if any members of the Anystidae also recorded from Australian vineyards (James and Whitney, 1993) are likely to prey upon grape phylloxera.

5.1.10.1.2.1 Recommendations

Further studies are required to identify the key predatory mite species occurring in vineyards within phylloxera infested zones in Australia and to determine what role they play, if any on the predation of leaf and root-galling phylloxera.

5.1.10.1.3 Prospects for Inoculation Biological Control

Gaeolaelaps aculeifer is an oligophagous mite that is used commercially in a number of countries for the biological control of soil-dwelling arthropod pests including fungus gnats (Diptera: Sciaridae) and western flower thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) (Premachandra et al., 2003). *G. aculeifer* is sold by several commercial biocontrol agent suppliers in Australia as *Hypoaspis A (Hypoaspis aculeifer)*, along with another soil-dwelling predatory mite, *Stratiolaelaps scimitus* (Wormersley) (formerly called *Hypoaspis miles*). In the USA, *S. scimitus* is reported by one

supplier to feed on “fungus gnat larvae, pupating thrips, pathogenic nematodes, immature root aphids, and small insects” (<https://www.evergreengrowers.com/stratiolaelaps-scimitus-womersley-hypoaspis.html>).

5.1.10.1.3.1 Recommendations:

Studies should be undertaken to determine if predatory mites can be utilised in augmentation biological control using commercially-available colonies of *G. aculeifer* and *S. scimitus*.

5.2 Entomopathogens

Naturally occurring pathogens such as bacteria, fungi, viruses, protozoa and nematodes are important regulators of insect populations. Many entomopathogenic species, also referred to as microbial control agents, have been selected or developed for use in classical biological control, augmentation and conservation programs. The advantages of entomopathogens over chemical pesticides are many, including a high degree of target specificity, safety for humans and other nontarget organisms, reduction of pesticide residues in food, increased activity of most other natural enemies, and increased biodiversity in managed ecosystems (Lacey et al., 2001). The disadvantages are mostly associated with their persistence, speed of kill, specificity (sometimes too broad or too narrow), and cost relative to conventional chemical insecticides (Lacey et al., 2001).

A large number of pathogens are known to kill aphids, with often dramatic effects in both field and glasshouse environments (Latgé and Papierok, 1998). The most common pathogens of aphids are entomopathogenic fungi, some of which have been developed into biopesticides (Milner, 1997). Bacterial and protozoan infections have not been conclusively demonstrated in aphids, but several baculoviruses and picornaviruses have been found, which can significantly decrease the longevity of infected aphid individuals (D'Arcy et al., 1981; Joshi et al., 2010).

In this section, we review the main entomopathogenic groups that have been previously investigated for use on grape phylloxera and identify prospects for further research.

5.2.1 Nematodes

Nematodes that parasitize insects, known as entomopathogenic nematodes (EPN), have been the focus of a significant amount of research since the early 1980s due to their many favourable attributes as biological control agents of potential pest insects (Kaya and Gaugler, 1993). EPN, mainly from the Steinernematidae and Heterorhabditidae families, have been used in classical, conservational, and augmentative biological control programs (Grewal et al., 2005; Lacey and Georgis, 2012). The free-living, non-feeding infective juveniles of these nematodes, referred to as 'dauer larvae' or infective juveniles (IJ), possess attributes of both insect parasitoids or predators and microbial pathogens. Like parasitoids, they have chemoreceptors and are motile; like pathogens, they are highly virulent, killing their hosts quickly, and can be cultured easily in vitro, have a high reproductive potential, and have a numerical but no functional response (Kaya and Gaugler, 1993). This spectrum of activity is more characteristic of a chemical than biological pesticide, making EPN attractive subjects for commercial development (Gaugler, 1988). However, unlike chemical pesticides, EPN are exempt from registration in many countries as agricultural chemical products, including Australia (<https://apvma.gov.au/node/11196>).

Both steinernematid and heterorhabditid nematodes have mutualistic associations with bacteria in the genera *Xenorhabdus* and *Photorhabdus* Boemare & al. (Enterobacteriaceae), with different nematode species harbouring different species of bacteria. Once a suitable host is found, the IJ enters through the natural openings (mouth, anus, or spiracles) and expels the bacterium from its intestines into the

hemocoel. The bacterium rapidly multiplies, killing the host by septicaemia within 48 hours. The nematodes then feed on the bacteria cells and host tissues, produce several generations and emerge from the host as infective juveniles to search for new hosts (Fig. 11) (Kaya and Gaugler, 1993).

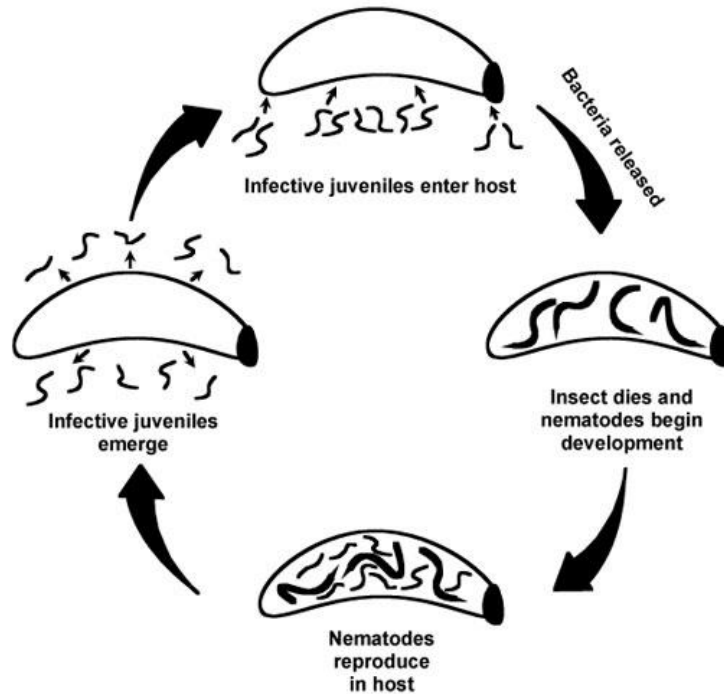


Figure 11. Generalized life cycle of a steinernematid nematode (Shapiro-Ilan and Gaugler).

Extensive research has demonstrated both their successes and failures for control of insect pests of crops, ornamental plants, trees, lawn and turf (Grewal et al., 2005; Stock, 2015). A few studies have also shown that EPN can have direct and/or indirect effects on populations of plant parasitic nematodes and plant pathogens (Grewal et al., 2005; Kaya and Gaugler, 1993; Navarro et al., 2014; Stock, 2015).

While there are many advantages to using EPN in IPM programs, there are also various constraints such as environmental factors (pH, temperature, moisture etc.) that can limit their effectiveness (Table 3). EPN have been most efficacious in habitats that provide protection from environmental extremes, especially in the soil, which is their natural habitat (Gaugler, 1988).

Table 3. Advantages and disadvantages of entomopathogenic nematodes (from Lacey and Georgis 2012).

Advantages	Disadvantages
Broad pest insect host range	Cost of production
Rapid speed of kill	Limited shelf-life and refrigerated storage
Can actively seek or ambush host	Environmental limitations: requirements for adequate moisture (to enable survival and infectivity) and temperatures (above or below that required for optimal infectivity), sensitivity to UV radiation, lethal effect of several pesticides (nematicides, fumigants and others), lethal or restrictive soil chemistries (high salinity, high or low pH, etc.)
Mass in vivo and in vitro production capability	
Possible to use conventional application equipment	
Safety: for all vertebrates, most non-target invertebrates, and the food supply	
Little or no registration required	

Successful examples using EPN against soil pests include the nematodes, *Steinernema kraussei* (Steiner) and *Heterorhabditis megidis* Poinar, Jackson & Klein against the black vine weevil, *Otiorhynchus sulcatus* (Fabricius) (Ansari et al., 2010; Kakouli-Duarte et al., 1997), and soil drenches of *S. feltiae* (Filipjev) to control imported red fire ants *Solenopsis invicta* Buren in Florida (Jouvenaz et al., 1990). In Australia, EPN are commercially available for a range of soil-dwelling pests (see Table 4), produced by Ecogrow Environment Pty Ltd. (<https://www.ecogrow.com.au>).

Table 4. EPN commercially available in Australia for soil-dwelling pests.

Nematode	Soil pest
<i>Heterorhabditis zealandica</i>	Black vine weevil, Argentine scarab African black beetle, Argentine stem weevil, red-headed cockchafer, black-headed cockchafer
<i>Steinernema carpocapsae</i>	Army worm/cut worm, termites, fleas
<i>Heterorhabditis bacteriophora</i>	Black vine weevil
<i>Steinernema feltiae</i>	Fungus gnats
<i>Rhabditis necromena</i>	Portuguese millipedes

Few studies have examined the effectiveness of EPN for soil-dwelling aphids. Brown et al. (1992) investigated the effectiveness of applying *Steinernema carpocapsae* (Weiser) in combination with an experimental systemic aphidicide RH-7988 to control edaphic populations of the woolly apple aphid (WAA), *Eriosoma lanigerum* achieving a mortality rate of 57%. However, Berkvens et al. (2014) evaluated six commercially-available EPN (*Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema carpocapsae*, *S. feltiae*, *S. glaseri* and *S. kraussei*) for WAA with less promising results. Interestingly, they demonstrated that the growth of the EPN-symbiotic bacteria *Xenorhabdus nematophila* and

Photorhabdus luminescens was inhibited by the body fluids of the WAA, and speculated that this antibacterial activity was the cause of the unsuccessful parasitisation of the WAA by EPN. In a recent South African study, all seven nematode isolates screened were able to infect and kill adult WAA, with *S. yirgalamense*, *H. zealandica* and *S. feltiae* giving the best results (Stokwe et al., 2017). However, the WAA mortality rate was low, with the highest rate obtained being 39% with *S. yirgalamense*. Stokwe et al (2017) found that only adult WAA were susceptible to infection and that the small size of WAA crawlers limited their attractiveness to, and their recognition and penetration by EPNs. As with Berkvens et al. (2014), the symbiotic bacteria associated with the EPN failed to reproduce and grow within the host. The bacterial growth assay on insect extracts clearly showed that the WAA haemolymph visually inhibited the growth of the symbiotic bacteria of all 14 EPN strains used in the study. This inability of the symbiotic bacteria to grow and reproduce in the haemolymph of WAA meant that they were unable to convert the inside of the insect effectively into a food source for the nematodes. Although *S. yirgalamense* showed some potential to control adult WAA, its inability to complete its reproductive cycle within WAA limits its effectiveness as a biological control agent.

5.2.1.1 Use of entomopathogenic nematodes for control of grape phylloxera

There has been only one study that has assessed the effectiveness of EPN against grape phylloxera, and this was conducted in the USA 20 years ago by English-Loeb et al. (1999) against the root-galling form. In petri dish trials, the Oswego strain of *Heterorhabditis bacteriophora* Poinar reduced survival of attached grape phylloxera by up to 80% relative to the control treatment, whereas *Steinernema glaseri* Steiner (isolate 326) had no measurable impact and was not used in further experiments. Hb Oswego significantly reduced survival of grape phylloxera on root pieces placed in small cups filled with soil, but only when soil moisture was high (13% water content wt:wt) and when high densities of infective nematodes were used (15,000/g soil). Grape phylloxera exposed to Hb Oswego often turned a brick-red colour, indicating successful infection. However, there was no evidence that Hb Oswego was able to successfully reproduce within the bodies of grape phylloxera hosts. English-Loeb et al. (1999) concluded that despite high mortality being achieved in the laboratory, the use of Hb Oswego in the field in an augmentative release program was likely to be constrained by the need to use high densities, their dependence on moist soils, and their inability to propagate themselves within grape phylloxera hosts. It is uncertain as to why English-Loeb et al. (1999) chose these particular EPN species to trial and not other species, particularly those occurring in vineyards. For instance, a survey of 13 vineyards in Quebec isolated the steinernematid nematode, *Steinernema carpocapsae* from 11 locations (Bélair et al., 2001).

In Australia, nematodes were observed on one occasion attacking root-feeding first instar grape phylloxera on potted *V. vinifera* plants during a glasshouse trial (Fig. 12) (Powell, 2012). As the identification of the nematode was not determined, it is not known if it was an entomopathogenic species or one of the several plant pathogenic nematodes of grapevines.



Figure 12. Unidentified nematodes observed attacking phylloxera (photo Agriculture Victoria).

5.2.1.1.1 Prospects for biological control of grape phylloxera

The limited research conducted to date on EPN of grape phylloxera and other soil-dwelling aphids has not yielded particularly promising results. It appears that only late instar stages and adults are susceptible to attack, and although invading nematodes can themselves cause high rates of mortality of their host, the effects are short-term. In these studies, it was suspected that the aphid haemolymph may have antibacterial properties that prevent symbiotic bacteria from multiplying and killing its host. However, these studies have only tested a limited number of commercially-available EPN species/strains and recent research has shown that a high degree of host specificity exists in some EPN species. To date, no studies have been conducted on EPN within field populations of grape phylloxera in either the native or invaded range, which is a fundamental knowledge gap that must be addressed.

5.2.1.1.2 Classical Biological Control

As a priority, surveys in the native range should seek to determine if EPN occur within root-galling grape phylloxera populations and if so, characterize these species and their symbiotic bacteria. Studies to elucidate the ecology and host-range of grape phylloxera EPN would be required to assess the prospects of utilising EPN for the classical biological control of grape phylloxera in Australia.

5.2.1.1.3 Inundation Biological Control

Firstly, studies are required to determine what defence mechanisms are utilised by root-galling grape phylloxera to protect them from attack by EPN. If the grape phylloxera haemolymph has antibacterial properties as suspected, laboratory bacterial growth assays along the technique used by Stokwe et al., (2017), could be utilised to rapidly assess resistance to EPN commercially-available in Australia. If results are promising, further research into application techniques in vineyards should take priority over classical biological control. This is because this research would be less expensive and risky than importing exotic EPN from the native range.

5.2.1.1.4 Conservation Biological Control

Field surveys should be conducted to determine if endemic EPN are already present in grape phylloxera infestations in Australia. If so, further research should focus on strategies to conserve and encourage resident EPN communities such as habitat manipulation (mulching, cover crops) and irrigation methods and timing.

5.2.2 Entomopathogenic fungi

Aphids can be attacked by entomopathogenic fungi (EPF) of two different classes: the Zygomycetes and Hyphomycetes (but refer to Hibbett et al. (2007) for a recent reclassification of the Zygomycota based on molecular phylogenetic analyses). In nature, many of the fungi considered major pathogens of aphids are from the order Entomophthorales (class Entomophthoromycetes) and are good candidates as prospective biocontrol agents because of their short infection cycle, a high reproductive rate, and ability to cause dramatic epizootics that may occur almost overnight (Barta, 2006). Most of the species have a narrow host range, posing no significant threat to non-target organisms, and some are capable of being cultured or produced in the laboratory or mass-scale (Barta, 2006). However, there are some shortcomings of the Entomophthorales as biocontrol agents, including the fragility of infective conidia, dormancy and an asynchronous germination of resting spores, and the requirement for very specific external conditions to achieve a successful infection cycle, such as very high humidity (Barta, 2006; Milner and Bourne, 1983). Numerous studies have shown that aphids in moist climates, or irrigated crops, are more likely to succumb to fungal disease than aphids on non-irrigated crops or in dry conditions (Milner, 1997). A review of the aphid-pathogenic Entomophthorales, including their host specificity, global distribution and possible prospects for use in biological control, is provided by Barta (2006). Although the Entomophthorales is an important and world-wide distributed group of EPF, only one species (*Conidiobolus thromoides* Drechsler for the control of aphids), has have been developed into a commercial biopesticide (Vega et al., 2012), largely due to difficulties associated with producing infective and stable biopreparations (Ravensberg, 2011).

Entomopathogenic Hyphomycetes includes hundreds of species, but just a few of them are specific to aphids.

5.2.2.1 *Lecanicillium longisporum* (Petch) Zare & Gams (synonym: *Verticillium lecanii* (Zimm.) Viegas

This well-known insect pathogen that has been commercialized as “Vertalec™” and “Mycotal™” for use predominantly against aphids in glasshouses in Europe and North America (Faria and Wraight, 2007; Shah and Pell, 2003). No products containing *L. longisporum* are currently registered in Australia (<https://apvma.gov.au/> accessed January 2019).

5.2.2.2 *Beauveria bassiana* (Balsamo) Vuillemin

This is a filamentous fungus that grows naturally throughout the world infecting many insects including Coleoptera, Diptera, Orthoptera and Lepidoptera. Under natural conditions, it rarely attacks aphids

(Milner, 1997, but see Hatting et al., 1999). On the host, mycelium emerges through the host exoskeleton to form a dense white covering on the surface, giving rise to the common name of white muscardine disease (Sinha et al., 2016). *B. bassiana* can be mass produced by either liquid or solid-substrate fermentation, from which the conidia (spores) can be formulated into a stable and effective mycoinsecticide. More recently, aphid-derived isolates of *B. bassiana* have provided promising results in both laboratory and field trials against Russian wheat aphid (Vandenberg, 2001). For the development of aphid-selective mycopesticides based on *B. bassiana*, further work is needed to develop formulations effective over a range of weather conditions and to assess the likely impact on predators and parasites (Milner, 1997).

5.2.2.3 *Metarhizium anisopliae* (Metschn.) Sorokin

This is a green muscardine fungus that enters insects through spiracles, eventually filling its host with mycelia before breaking through the cuticle to sporulate (Aw and Hue, 2017). Many isolates recognised to be specific were assigned variety status such as *Metarhizium anisopliae* var. *anisopliae*, but they have now been assigned as new *Metarhizium* species (Bischoff et al., 2009). For instance, the commercially important isolate M.a. 43, F52, Met52 and BIPESCO 5 (*M. anisopliae* var. *anisopliae*) has now been assigned to *M. brunneum* Petch (Krell et al., 2018). The potential of *Metarhizium* spp. as a biopesticide for aphid control has not been as well studied compared to that of *B. bassiana* largely due to the fact that a very limited number of isolates have been bioassayed specifically against aphids (Shan and Feng, 2010). According to Faria and Wraight (2007), only three *Metarhizium* products target aphid pests compared to 12 for *Beauveria*.

5.2.2.4 *Isaria farinosa* (Holmsk.) Fr. and *Isaria fumosorosea* Wize

These entomopathogenic fungi, were formerly known as *Paecilomyces farinosus* and *Paecilomyces fumosoroseus*, respectively. Both fungi have a worldwide distribution and a relatively wide host range. While *I. farinosa* currently is of minor importance in research and as biocontrol agent, *I. fumosorosea* is regarded as a species complex, and various strains are successfully used for biocontrol of several pest insects, mainly whiteflies (Zimmermann, 2008). Highly virulent isolates of *I. fumosorosea* from aphids have been discovered and shown significant population reduction of cereal aphids in the USA (Milner, 1997).

5.2.2.5 *Entomopathogenic fungi as endophytes for dual biological control of insect pests and plant pathogens*

In addition to causing diseases in insects, some EPF species (*Metarhizium* sp., *Beauveria* sp) have also been recently shown to act as plant endophytes in a variety of host, as antagonists to plant pathogens, as plant growth promoters and as beneficial rhizosphere colonizers (Jaber and Ownley, 2018). Endophytes are plant associated microorganisms that live part of their life cycle within a plant without causing harm or disease to the host. Fungal endophytes are common in nature and primarily depend on their host plants for nutrition, protection, and development. In return, they confer systemic protection against insect pests and disease usually by inducing a change in the chemical response of

the plant or by the secretion of secondary metabolites (Vidal and Jaber, 2015). The incorporation of such EPF strains in agricultural practices may be incredibly promising, providing multiple simultaneous benefits, ranging from plant root defence to plant growth-promoting properties (Kergunteuil et al., 2016). For situations where the action of the plant pest initiates infection by a plant disease, entomopathogenic endophytes will be highly beneficial. For example, the endophytic fungus *Lecanicillium longisporum* (Petch) Zare & W.Gams provided dual suppression of powdery mildew and aphids in cucumbers (Kim et al., 2007). However, there is still much research to be done before the potential of using entomopathogenic fungi as endophytes against herbivorous pests can be fully capitalized (Vidal and Jaber, 2015).

5.2.2.6 Use of entomopathogenic fungi for control of grape phylloxera

Despite the potential of biopesticides to be used for the biological control of aphids (Milner, 1997) and of insect root herbivores (Kergunteuil et al., 2016), there has been limited research to date on the use of EPF for the biocontrol of grape phylloxera.

The first investigations into the effectiveness of entomopathogenic fungi for grape phylloxera were conducted by Russian scientists in the Ukraine in 1975. Goral et al. (1975) tested the effects of *B. bassiana*, *M. anisopliae* and *P. farinosus* (currently *I. farinosa*), with the root form of phylloxera in the laboratory. All three fungi were able to exert some controlling effect on phylloxera individuals, particularly the nymphal stage but effects on the adults varied. *M. anisopliae* had little effect on the number of females but appeared to stimulate their fecundity. *B. bassiana* affected numbers more but likewise increased fecundity, while *P. farinosus* caused little mortality but strongly inhibited reproduction. Based on these observations, *P. farinosus* was considered to have the greatest effect on population numbers, but as far as we are aware no field trials were conducted.

Granett et al. (2001) mentioned that *B. bassiana* has shown successful phylloxera control *in vitro*, but no follow-up trials were conducted to test efficacy in the field. Recently, Ficiu et al. (2015) evaluated the efficacy and persistence of four different formulations of *B. bassiana* on leaf-galling phylloxera in a greenhouse study. They found that conidia of *B. bassiana* could persist and infest larvae and adult grape phylloxera for at least six days, however concluded that applications would need to be reapplied every 6 days to decrease phylloxera populations to the level achieved by chemical treatments. We are not aware of any subsequent trials being conducted to assess these formulations under field conditions.

In 2003, studies were initiated to evaluate the effectiveness of *M. anisopliae* in bioassays, greenhouse and field experiments. In the first study, *M. anisopliae* var. *anisopliae* isolate Ma 500, colonised on barley kernels, was applied to potted cuttings of the grapevine clone 5BB Klon 13-11 Gm (*Vitis riparia* x *V. berlandieri* Kober) (Kirchmair et al., 2004). After 32 days, no fresh phylloxera infections were observed in 80% of plants and untreated controls showed new nodosity formation and continued population increase. Direct examination of infected phylloxera in the soil is almost impossible to achieve because infected individuals rapidly decompose (Hermann Strasser personal communication, September 2018). However, the laboratory bioassays conducted by Kirchmair et al., 2004 confirmed

that grape phylloxera was susceptible to *M. anisopliae* Ma 500 applications as conidiophores were observed on dead insects in SEM-preparations (Fig. 12).



Figure 12. Conidiophores with conidial chains of *Metarhizium anisopliae* on the abdomen of grape phylloxera (Bar: 10 μ m) (from Kirchmair et al., 2004).

To overcome the difficulty of detecting diseased root-galling grape phylloxera in pot and field experiments, Porten and Huber (2003), devised an assessment method where different classes of root infection by grape phylloxera were categorized, allowing for a correlation between phylloxera control and *Metarhizium* applications to be made.

Based on the successful pot experiments, Kirchmair et al. (2007) conducted field trials in Germany in 2003 and 2004 to assess the efficacy of *Metarhizium* against grape phylloxera and its persistence within the soil. *M. anisopliae*-colonised barley (applied in 2004 as GRANMET, Agrifutur), was applied with a rotary harrow to the tramline (the area between rows). While the authors acknowledge that the application of *Metarhizium* underneath the row where root-infested grape phylloxera occur would have been ideal, it was not manageable with the agricultural machinery routinely in use by winegrowers in Europe at the time. Nevertheless, it appeared that vectors such as springtails (Collembola) carried the *Metarhizium* spores from the tramline to the grapevine roots as *M. anisopliae* was detected up to two years after treatment underneath the row. Further field evaluation indicated that the main effect on root-galling populations occurred three to four years after the application of GRANMET (Lars Huber, personal communication September 2018).

Despite these early promising results, the ability to register *Metarhizium* in Europe was impeded by the lack of clear guidelines for the risk assessment of biological control agents by the European Food Safety Authority (EFSA) (Hermann Strasser and Lars Huber personal communications, September 2018). *Metarhizium brunneum* (BIPESCO 5/F52, previously classified as *M. anisopliae*, var. *anisopliae*) has subsequently been approved as an active substance by EFSA enabling companies such as Novozymes Biologicals and Agrifutur to pursue product registration. In the USA and Canada, the product is registered as Met52 (Novozymes Biological, Franklinton, NC, USA) and its effectiveness against grape phylloxera is currently under evaluation by North Carolina State University (Mark Hoffmann, personal communication December 2018). In Australia, there are five

Metarhizium products currently registered for use against plague locusts, greyback canegrub and red headed cockchafers, but no products containing *M. brunneum* are currently available (APVMA, 2019).

To date, research into the application of entomopathogenic fungi for grape phylloxera has focussed largely on the effectiveness of commercially-available strains that are targeted for other pests, rather than the selection of suitable virulent strains that target root-feeding grape phylloxera.

In Australia, a PhD study conducted through Charles Sturt University is looking at endemic entomophagous fungi to determine what species of *Beauveria* and *Metarhizium* can be isolated from soils within Australian vineyards (<https://www.csu.edu.au/nwgic/about-us/our-people/profiles/postgraduate-students/ginger-korosi>). While the results of the study are not yet available, preliminary results suggest that there are several *Beauveria* and *Metarhizium* isolates that are present in vineyards and could be investigated further for their potential as mycopesticides for grape phylloxera.

6 CONCLUSIONS AND RECOMMENDATIONS

Grape phylloxera is one of the most important insect pests of grapes worldwide. To date, grape phylloxera has been managed predominantly using resistant rootstocks, developed through conventional breeding of hybrid crosses of American *Vitis* species. However, with increasing evidence that this resistance is being overcome by new strains of grape phylloxera, there is a risk of becoming trapped on a “resistant rootstock treadmill” unless new approaches are identified, tested, developed and implemented.

Through our review of published and grey literature and from consultation with relevant experts from North America, Europe and Australia, the prospects for biological control of grape phylloxera through classical, inoculation, inundation and conservation approaches in Australia are encouraging.

6.1 Classical Biological Control using specialised arthropod predators

The exotic origin of grape phylloxera makes it particularly amenable to Classical Biological Control through the importation and establishment of specific natural enemies from its native range in North America. While these natural enemies are poorly documented and even more poorly understood, we know of two insect species that warrant further investigation as candidates for biological control. The siphid fly, *Heringia salax* is of greatest interest because it is known to attack both leaf and root-galling phylloxera and is likely to be host-specific. Other members of this genus have been utilised in classical biological control programs against aphids that have both arboreal (leaf-galling) and edaphic (root-galling) forms, such as *H. calcarata* against woolly apple aphid in the USA (Bergh and Short, 2008). A second fly species, *Leucopis simplex*, which is reportedly the most common predator of grape phylloxera in North America, is considered of lower priority because it is likely to be a predator of only the leaf-galling form. Targeted natural enemy surveys in the eastern USA may reveal the identity of other arthropod predators of root-galling phylloxera with potential as candidate biological control agents for importation and release into Australia. As there are no Australian native species of *Phylloxera*, potential off-target attack of indigenous aphids by grape phylloxera-specific predators is relatively low, making classical biological control a highly feasible option.

6.1.1 Recommendations

- Undertake natural enemy surveys in the native range concentrating on soil-dwelling arthropod predators in collaboration with USA entomologists.
- Through laboratory and field studies in the USA, determine which predators have potential as classical biocontrol agents based on specificity, effectiveness and likelihood of establishment under Australian climate and soil conditions.
- Pending results of native range studies, initiate a classical biological control program against grape phylloxera in Australia.

6.2 Inoculation Biological Control using commercially available biocontrol agents

There are several commercially available biological control agents that are utilised in Australia against soft-bodied pests such as aphids, scale insects and thrips. While it is not known if these species will attack grape phylloxera, similar predatory guilds are key predators of grape phylloxera in other countries and may play a similar role here. Hence the prospects of inoculation biological control of grape phylloxera using commercially available agents warrants further investigation (see Table 5). Of interest are the predatory mites, *Gaeolaelaps aculeifer* and *Stratiolaelaps scimitus*, as these are sold for the control of soil-dwelling soft bodied insects including fungus gnats and western flower thrips. Arboreal and ground-dwelling predators such as ladybirds, lacewings and predatory bugs may be useful in reducing the spread of grape phylloxera within and between vineyards by attacking the vulnerable dispersing juveniles and alate (winged) adults.

6.2.1 Recommendations

- Conduct laboratory bioassays to determine if commercially available biological control agents, particularly soil-dwelling predatory mites predate upon root-galling and dispersal stages of grape phylloxera.
- If so, determine effective agent release strategies and methods to conserve populations for longer-term benefits.

6.3 Inundation biological control using entomopathogenic fungi and nematodes

Entomopathogenic fungi (EPF) and nematodes (EPN) are among the most promising biocontrol agents of root pests because they are naturally soil-dwelling organisms, they are easy to isolate from the field, and they can be readily mass-produced on artificial media and applied using conventional insecticide application techniques. In comparison to chemical pesticides, the multiple mode of action of EPF and EPNs, lessens the possibility of resistance development in insects. Plus, they are often highly host-specific, avoiding unexpected deleterious effects on non-target beneficial organisms (Kergunteuil et al., 2016). However, the use of EPF and EPN against grape phylloxera has to date been surprisingly limited, despite the increasing development of commercial products for other pests from significant advances in the isolation of effective strains, formulation, shelf-life of and application techniques in recent years (van Lenteren et al., 2018).

Entomopathogenic fungi and nematodes have considerable potential for use against grape phylloxera in Australia, and this area of research warrants further investigation. As classical biological control agents, phylloxera-specific species and strains would need to be identified from the native range and subjected to prey-range studies under quarantine conditions to assess their safety for release into the Australian environment. If approved for release, studies would need to be conducted to determine if these entomopathogenic organisms can maintain self-sustaining populations or if they need to be developed into commercial products for inundation biological control. Various commercial species and

strains of EPF and EPN are already available in Australia against other soil-dwelling pests, and these could be easily screened for their effectiveness against grape phylloxera. Finally, further research should seek to identify endemic entomopathogenic organisms present within Australian vineyards and their potential for development as commercial biopesticides.

6.3.1 Recommendations

Native range studies:

- Undertake field surveys to identify the EPF and EPN associated with grape phylloxera in the eastern USA in collaboration with key universities and other research agencies.
- Conduct laboratory and field studies to better understand the role of entomopathogens in grape phylloxera population dynamics.
- For entomopathogenic nematodes, investigate the interactions between these nematodes and their symbiotic bacteria.
- Pending results of native range studies, initiate a classical biological control program against grape phylloxera in Australia.

Australian studies:

- Undertake laboratory bioassays to screen the effectiveness of commercially available strains of EPF (*Beauveria bassiana* and *Metarhizium*) and EPN (*Steinernema* and *Heterorhabditis*)
- Determine if endemic fungi and nematodes present in Australian vineyards have potential for commercial development as biopesticides for grape phylloxera.

6.4 Conservation Biological Control

Australian vineyards are host to a diverse range of predatory arthropods whose populations can be enhanced through the provision of insectary plants that provide ‘SNAP’, an acronym that refers to shelter, nectar, alternative prey and pollen (Retallack et al., 2019). While we do not know if these predators attack grape phylloxera in Australia, it is highly likely that some of them do because similar functional groups are known to attack grape phylloxera in other countries. For instance, two species of *Scymnus* lady beetles in North America (native range) plus a further six species in Europe (invaded range) are known to predate upon grape phylloxera. Therefore, it is likely that the native *Scymnus* identified in Australian vineyards are also predators of grape phylloxera.

6.4.1 Recommendations

- Conduct local ground surveys within Australian phylloxera infested zones (PIZs) to elucidate endemic predators using next generation sequencing of gut contents for the presence of grape phylloxera.
- Identify suitable native plant species that provide ‘SNAP’ resources (i.e. shelter, nectar, alternative prey and pollen) to encourage predators of phylloxera.

Table 5. Prospects for Classical, Inoculation, Inundation and Conservation Biological Control of grape phylloxera (GP) in Australia.

Type	Habitat	Phylloxera target	Potential biological control organisms	Priority for biocontrol
Classical	Soil and canopy	Root-feeding, leaf-galling and dispersal stages	Syrphid fly, <i>Heringia salax</i> (Diptera).	High priority. <i>H. salax</i> is known to attack both root and leaf-galling GP in the native range. Research is required to understand the biology and prey-range of <i>H. salax</i> before its suitability as a biocontrol agent for GP can be assessed.
	Canopy	Leaf -galling	Silver fly, <i>Leucopis simplex</i> (Diptera).	Low priority. A common predator of leaf-galling GP in the native range. Research is required to understand the biology and prey-range of <i>H. salax</i> before its suitability as a biocontrol agent for GP can be assessed.
	Soil	Root-feeding	Entomopathogenic nematodes.	High priority. Nothing is known about the EPN associated with GP in the native range. Native range surveys are required to identify EPNs of root-galling GP and their prospects for classical biological control.
Inoculation	Canopy and ground	Leaf-galling and dispersal stages	Green lacewing, <i>Mallada signatus</i> . Brown lacewing, <i>Micromus tasmaniae</i> . Australian spotted ladybird (<i>Harmonia conformis</i>). Damsel bug, <i>Nabis kinbergii</i> . Orius - minute pirate bug, <i>Orius armatus</i> .	Medium priority. Adult lacewings will persist in the crop if nectar and pollen are present. Practices such as strip intercropping and encouraging flowering plants will give best results. Ladybird beetles are very effective predators of aphids, but they may be harmed by pesticides. The damsel bug is an aphid predator currently under development for commercial use. Minute pirate bug is predominantly a thrips predator but will also feed on aphids.
	Soil	Root-feeding	Predatory mites, <i>Gaeolaelaps aculeifer</i> , and <i>Stratiolaelaps scimitus</i> .	High priority. Commercially sold for soil-dwelling pests such as fungus gnats and western flower thrips, but overseas, similar species attack soil-dwelling aphid pests. Studies are required to determine if species available in Australia will attack GP.
Inundation	Soil	Root-feeding	Entomopathogenic fungi, <i>Beauveria</i> , <i>Metarhizium</i> , <i>Isaria</i> spp..	Medium priority. Identify EPF species and isolates attacking root-galling GP <u>in the native range</u> and assess their potential for commercial development. Medium priority. Identify EPF species and isolates present <u>in Australian vineyards</u> and assess their efficacy against GP and potential for commercial development. High priority. Investigate the potential for off-label use or registration of BIPESCO 5/F52/Met 52 through the APVMA in Australia.
			Entomopathogenic nematodes, <i>Steinernema</i> spp, and <i>Heterorhabditis</i> .	High priority. Conduct screening trials to determine if commercially available EPN are effective against GP.
Conservation	Canopy	Leaf-galling and dispersal stages	Native ladybirds, hoverflies (Syrphids).	Medium priority. Identify which native ladybirds will attack GP and develop strategies to encourage their presence in vineyards.
	Ground	Dispersal stages	Native predatory bugs and rove beetles.	Medium priority Predatory bugs and rove beetles occur in Australian vineyards, many of which predate of ground-dwelling pests. However, their role as predators of GP is unknown.
	Soil	Root-feeding	Native entomopathogenic nematodes, fungi, predatory arthropods.	High priority Little is known of the native soil-dwelling predators or pathogens in Australian vineyards and the role they may play, if any, on phylloxera control.

7 ACKNOWLEDGMENTS

The authors gratefully acknowledge the following for their valuable contributions and advice:

Dr Lars Huber (Senior Manager Regulatory Affairs) and Dr Albrecht Heidemann (Vice President and Head of Agrochemicals and Biopesticides) at the Science Consulting Centre (SCC) in Bad Kreuznach, Germany.

Professor Astrid Forneck, Head Division of Viticulture and Pomology, University of Natural Resources and Life Sciences, Tulln, Austria.

Professor Martin Kirchmair, Dr Sigrid Neuhauser and Dr Hermann Strasser at the University of Innsbruck, Austria.

Dr Mark Hoffman of North Carolina State University.

Dr Mary Retallack, Retallack Viticulture Pty Ltd.

Dr Catherine Clarke, DJPR – Rutherglen.

8 FUNDING

The authors gratefully acknowledge funding from Wine Australia and Agriculture Victoria, Department of Jobs, Precincts and Transport and Regions.

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