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REVIEW ARTICLE

Grape phylloxera (*Daktulosphaira vitifoliae*) – a review of potential detection and alternative management options

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Abstract

The management options for grape phylloxera, *Daktulosphaira vitifoliae*, a monophagous insect pest of *Vitis* species are reviewed. Although in a worldwide context, grape phylloxera is managed predominantly by the use of resistant rootstocks developed through conventional breeding of hybrid crosses of American *Vitis* species, this management aspect is largely excluded from the review so that emerging technologies in the field of detection, quarantine and alternative management are discussed. In some viticulture regions of the world, where grape phylloxera's geographic distribution is limited (e.g. Australia), the pest is managed through a combination of surveillance, detection and quarantine. Although some alternative management options for grape phylloxera exist they have received relatively limited research attention because of the relative success of resistant rootstocks. The resilience of resistant rootstocks as the primary management option could also be challenged in the future by host-plant interactions with diverse grape phylloxera clonal lineages and by potential impacts of climate change on both grapevine and grape phylloxera distribution. A range of control options exist which could be integrated into an improved management system for grape phylloxera. Priority areas for future evaluation and further development include early detection techniques, investigation into the use of biological control agents and development of an integrated approach to grapevine phylloxera management.

Introduction

Grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), is a small, invasive, sap-sucking insect (Family *Phylloxeridae*) that causes substantial physical and economic effects on commercial grapevine, *Vitis vinifera* L., production. Grape phylloxera is native to the Northeastern United States (Wapshere & Helm, 1987) and was unintentionally imported to major viticultural centres in mainland Europe on American rootstocks, originally introduced to manage grapevine powdery mildew (Gale, 2002).

The discovery of grape phylloxera in France in 1868 and its subsequent spread over the next decade are well documented. The pest devastated the French wine industry, destroying over 1 million ha of ungrafted *V. vinifera* vineyards by the turn of the century (Ordish, 1972; Campbell, 2004) and had other socioeconomic impacts on rural communities (Banerjee *et al*., 2007; Bignon *et al*., 2011). Over the past 150 years, grape phylloxera has spread to almost every major viticultural region in the world, including North and South America, Asia, Europe, the Middle East, Africa and Australasia (EPPO, 1990).

Grape phylloxera, depending on genetic lineage (Corrie *et al*., 2002, 2003; Forneck & Huber, 2009), feed on the leaves and/or roots of *Vitis* species, inducing the formation of galls. The frequency, severity and distribution of these infestations vary significantly as a function of the innate resistance mechanisms of host plants and the grape phylloxera genetic lineage.

On suitable indigenous hosts (i.e. American *Vitis* spp.) some grape phylloxera strains feed on the leaves, causing leaf galls with marginal populations found on the root system. The resultant impact on general grafted-vine vigour or yield is minimal (Wapshere & Helm, 1987).

Leaf-galling grape phylloxera (gallicolae) strains are widespread in continental USA and Europe on rootstock foliage. High population numbers are linked to marginal decreased vine productivity on some rootstock cultivars (e.g. reduced shoot growth; Granett & Kocsis, 2000), but rarely cause galling on *V. vinifera* leaves. However, the incidence of leaf galls on *V. vinifera* cultivars has been reported in Europe (Molnár *et al.*, 2009). These leaf-galling strains are generally considered far less significant economically than the more damaging root-galling (radicicolae) grape phylloxera strains (Davidson & Nougaret, 1921; Buchanan, 1990).

In contrast, European *V. vinifera* suffers infestation and damage predominantly to the root system which has significant economic impacts on production (Powell, 2008). When root-galling grape phylloxera infests ungrafted *V. vinifera*, colonies establish on both immature (non-lignified) and mature (lignified) storage roots, resulting in nodosity and tuberosity development, respectively. Both nodosities and tuberosities significantly disrupt nutrient and water transportation and absorption. Extensive root damage leads to gradual vine decline (usually over several seasons) and lowered host-plant resistance, increasing the host plant's susceptibility to secondary fungal infection primarily through wounds caused by stylet insertion points (Omer *et al.*, 1995; Edwards *et al.*, 2007). Feeding on mature *V. vinifera* storage roots is considered the primary factor associated with serious damage and, in some instances, can cause complete root destruction and ultimately vine death (Boubals, 1966; Granett *et al.*, 2001; Herbert, 2005). Root-galling in the form of nodosities can also occur on *Vitis* hybrids, bred for grape phylloxera resistance, but tuberosities are rarely formed on these hybrids and hence only limited root damage occurs.

This review brings together some historical and more recent developments (particularly over the last decade) in grape phylloxera management options, other than just the use of grape-phylloxera resistant rootstocks which has been covered in an earlier review (Granett *et al.*, 2001), based on an interdisciplinary research approach. It is structured into three principal sections: detection, quarantine and alternative grape phylloxera management strategies. Current and historical trends in these three areas are explored concluding with recommendations for future research.

Control options

Rootstocks

Grapevine rootstocks are derived from American *Vitis* spp. lineage, which are widely recognised as having developed intrinsic resistance mechanisms toward grape phylloxera through co-evolution in the insects' native range of North America (Granett *et al.*, 1996). Although many rootstocks are available with distinct adaptations to a wide range of abiotic and biotic stressors (e.g. salinity, lime, nematodes, drought), the recommended use of rootstocks for phylloxera management is not without some limitations. In California in the late 1980's, an estimated loss in production of between US$ 1 and 6 billion (Gale, 2011) resulted from the use of a grape phylloxera-resistant rootstock AXR-1, which included some susceptible *V. vinifera* parentage, and the emergence and spread of grape phylloxera biotype B (Granett *et al.*, 1991, 2001). The use of rootstocks as a long-term phylloxera management option has also been extensively reviewed (Granett *et al.*, 2001).

In some countries, rootstock recommendations are primarily based on screening data on grape phylloxera resistance from overseas. For example, the vast majority of rootstock recommendations in Australia are based on screening conducted in Europe and the USA, with minimal or no consideration of which genetic strains of grape phylloxera predominate in the country. The screening of grape phylloxera genetic clones against locally-bred common and novel rootstocks is essential for the development of accurate and timely recommendations to the viticulture industry. Two such screening programmes have recently commenced in Australia (Korosi *et al.*, 2007) and China (Du *et al.*, 2008). Currently, rootstocks present the only viable long-term solution for grape phylloxera management, yet in some countries it remains an uneconomic option. For example, an estimated cost of replanting and lost production in Australia (AUS) is AUS$ 20 000 ha^{-1} (DAFWA, 2006). With some countries reporting potential breakdown of grape phylloxera resistance in certain rootstocks (Walker *et al.*, 1998; Schmid *et al.*, 2003), the sustainability of the viticulture industry depends on continued rootstock screening against known grape phylloxera clonal lineages and further development of alternative management and detection strategies.

In some countries use of resistant rootstocks for grape phylloxera management remains a relatively low priority in part due to restricted grape phylloxera distribution, geographic isolation of viticulture regions, relative expense of grafted rootstocks and strictly enforced and comprehensive quarantine protocols. Despite this, accidental introductions in these countries have occurred. Since its discovery in Australia in 1877, grape phylloxera has caused significant disruption through quarantine restrictions and replanting costs to some major viticultural areas, particularly central and northeast Victoria (Buchanan, 1987), two isolated zones in southeastern New South Wales (Powell, 2008) and also historically in Queensland (Helm, 1983). Yet in Australia, only 2% of all

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vineyards are known to be infested with grape phylloxera (Nicol *et al.*, 1999). Given that established Australian vineyards are largely planted to highly susceptible own-rooted (ungrafted) *V. vinifera*, effective grape phylloxera management strategies are imperative in order to support and protect the long-term success and economic sustainability of the Australian viticulture industry. Grape phylloxera management in Australia has therefore evolved into an integrative approach consisting of: (a) early detection and surveillance; (b) extensive quarantine regulations which encompass disinfestation procedures for plant material (Deretic *et al.*, 2003; NVHSC, 2009), machinery (Korosi *et al.*, 2009), hand-held equipment and footwear (Dunstone *et al.*, 2003) aimed at preventing the spread of grape phylloxera outside of designated grape phylloxera infested zones (PIZ's); and (c) the use of grape phylloxera resistant rootstocks.

Research into alternative management of grape phylloxera has been relatively *ad hoc* (compared to that of rootstock research); encompassing biological, chemical and cultural control options coupled with quarantine regulations and surveillance strategies. Crucial to the successful implementation of alternative strategies is the development of early detection techniques able to assess the status of suspected grape phylloxera-infested vines prior to the expression of physical symptoms, thereby allowing controls to be implemented rapidly and reducing the economic consequences of replanting onto resistant rootstocks.

Detection

Several factors which can affect the establishment and development of grape phylloxera in vineyard environments need to be considered in order to develop effective detection methods. The first consideration is the insect's life cycle, which is influenced by genetic characteristics of both host plant and pest.

Grape phylloxera exhibits cyclic parthenogenesis including both asexual and sexual components (Coombe, 1963) and the life cycle has been extensively reviewed (Forneck & Huber, 2009). For the purposes of this review, our focus will be primarily on the anholocyclic (asexual) root-galling forms which are economically the most important, and to a lesser extent, leaf-galling forms. Genetic diversity exists between different geographical regions for the two forms. For example, in Australia 83 distinct genotypes have been characterised using six common microsatellite markers (Umina *et al.*, 2007). Of these the majority are root-galling, with leaf-galling forms being sporadic and limited in distribution (Corrie *et al.*, 2003). In China, 13 haplotypes have so far been characterised, which are predominantly root-galling (Sun *et al.*, 2009).

The detection of leaf-galling grape phylloxera strains is evident by visual inspection of rootstock foliage (either as suckers on grafted vines or within rootstock nursery plantings) for gall symptoms, which occur in spring and summer. Currently, visual inspection of foliage and digging to examine root symptoms (as some leaf-galling genetic strains also establish on the root system) are the sole methods of assessing grafted vineyard areas for the presence of leaf-galling grape phylloxera. Although feasible it is unlikely that an improved detection method, such as leaf-imaging technology, will be developed to detect leaf-galling grape phylloxera because this form causes relatively minimal economic damage, compared to root-galling phylloxera.

Detection of root-galling grape phylloxera is far more important economically, particularly in grape-growing regions where commercial plantings of ungrafted *V. vinifera* or rootstocks with some *V. vinifera* parentage predominate, for example in Armenia, China and Australia. Because of its predominantly subterranean habitat and relatively high economic damage once an incursion occurs, several different approaches for improved detection of root-galling grape phylloxera strains have been explored and are currently under development (Bruce *et al.*, 2011*a*).

Conventional detection

The early visual above-ground indications of grape phylloxera infestation on ungrafted *V. vinifera* are typically isolated to only a few vines, principally expressed as a gradual decline in canopy vigour followed by premature yellowing of foliage and an incremental reduction in grape yield. These symptoms alone are not strong indications of grape phylloxera infestation as some grapevine phytoplasma diseases such as *flavescence dorée* and *bois noir*, and field conditions such as dehydration and sustained high temperatures, cause similar symptoms (Hardie & Considine, 1976; Dry & Loveys, 1999). However, when grape phylloxera is the causative agent, these symptoms progressively become more widespread over a period of either 2–3 years or several decades, depending on the virulence of the grape phylloxera genetic strain present (Herbert *et al.*, 2010). Infestation eventually leads to reduced functional root mass, canopy decline, reduced crop yield and occurrence of satellite spots throughout the infested vineyard as a result of spread by machinery, wind or human traffic (Powell *et al.*, 2009). Left untreated, an infestation can eventually result in vine death. However, this is more likely to occur with highly virulent grape phylloxera strains when optimal conditions for survival and development prevail.

The time-frame from initial infestation to eventual death of ungrafted *V. vinifera* has been estimated as 3–6 years (Buchanan, 1990). However, rates of vine decline have also been correlated to grape phylloxera genotype (Corrie, 2003) and, in some instances where low virulent genetic strains are present, visual symptoms may not be evident even after 40 years (K. Powell, DPI Victoria, personal observation). Conventional detection of grape phylloxera infestation can use manual ground surveys either alone or in combination with some form of remote aerial imaging to assess canopy decline and rate of spread (Wildman *et al.*, 1983; Johnson *et al.*, 1996; Renzullo *et al.*, 2004; Bruce *et al.*, 2009).

Detection of grape phylloxera infestation differs on ungrafted compared to grafted *V. vinifera*. Infestation on *V. vinifera* grafted onto American *Vitis* spp. rootstocks can be characterized by leaf-galling on rootstock suckers and development of nodosities on the young non-lignified expanding root tips. However, there is no associated reduction in vine vigour, premature yellowing or tuberosity development on lignified roots (Buchanan & Hardie, 1978; Granett *et al.*, 2001; Granett *et al.*, 2007). In contrast to field observations, rootstock screening under glasshouse conditions has recently confirmed the development of tuberosities on some rootstocks (Korosi *et al.*, 2007), further highlighting the complexity of grape phylloxera–host interactions and the potential for rootstock breakdown depending on environmental conditions and genotypic characteristics of both host and pest.

Manual ground surveys

Conventional detection of grape phylloxera involves manual excavation and visual inspection of the grapevine root system for the presence of grape phylloxera and associated galls (Buchanan, 1987). Optimal ground survey timing typically coincides with peak grape phylloxera activity during summer months (Powell *et al.*, 2000). This detection approach, although widely used, has a number of disadvantages: (a) reliance on successful visual recognition of symptoms relating to grape phylloxera infestation in both foliage and root systems, which is prone to human error and requires an effective training program (Mee *et al.*, 2011); (b) it is usually economically unviable to sample every vine in single or multiple vineyards as part of an area-wide surveillance scheme and consequently repeated annual surveys may be required; (c) surveys are climate-dependent; i.e. rain and high temperatures make soil excavation and identification of grape phylloxera on roots difficult, and in the case of extreme heat and drought, will also influence the degree of stress expressed in the canopy and the abundance of grape phylloxera on the root system, (d) surveys are often conducted late as visual signs of decline typically do not manifest until at least 2–3 years after initial infestation and (e) low virulence genotypes, or high virulence genotypes with low abundance may elicit no visual signs of host plant stress symptoms. The associated difficulties with manual ground surveying have in the last few decades led to the development and validation of other detection and surveillance strategies.

Novel detection and surveillance

Trapping

A range of conventional insect trapping methods have been used to monitor population dynamics of different grape phylloxera strains (Fig. 1) (Powell *et al.*, 2000; Herbert *et al.*, 2006). More recently the emergence trap technique has been modified as a tool to detect and quantify the risk of spread of grape phylloxera populations aboveground in ungrafted *V. vinifera* vineyards (Powell *et al.*, 2009). The technique has recently been validated in conjunction with, and shows similar efficacy to, a phylloxera-specific probe which assesses phylloxera DNA presence in soil samples (Herbert *et al.*, 2008*b*). Emergence traps have also been shown to be effective in grafted *V. vinifera* vineyards (Trethowan & Powell,

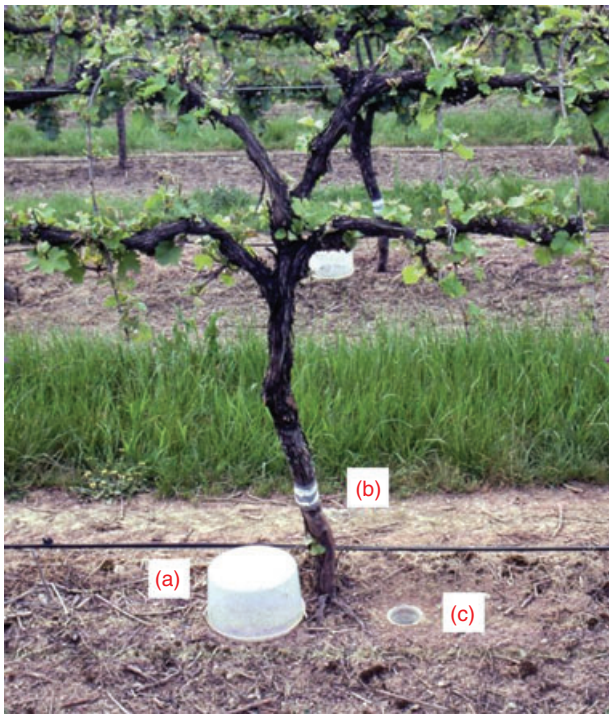

Figure 1 Insect traps used in field surveillance for detection and population monitoring of radicicolae grape phylloxera. Three primary trap types are shown: (a) emergence, (b) trunk and (c) pitfall trap.

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2007), where lack of visible canopy symptoms make the use of manual ground surveying less effective. Although not yet fully evaluated emergence traps do offer a relatively cheap potential method, at AUS$ 2 per trap, for both area-wide and localised surveillance (Powell *et al.*, 2009) when combined with soil and/or vegetation mapping as part of a targeted surveillance strategy (Bruce *et al.*, 2009). Trap placement and sample collection is a relatively simple procedure which can be conducted by vineyard staff with minimal training (Powell *et al.*, 2009). However, identification of phylloxera would require taxonomical skills to differentiate between grape phylloxera and other morphologically similar Hemiptera. Emergence traps are only effective, in detecting dispersive stages of grapevine phylloxera moving aboveground, when soil temperatures favour dispersal (Herbert *et al.*, 2006).

Spectral fingerprinting

Remote aerial and ground-based photography and spectral imaging has been used to detect the extent of invertebrate-induced damage or stress caused by aphids (Pope, 1957; Yang *et al.*, 2004; Smith *et al.*, 2008) and mites (Fitzgerald *et al.*, 2004) in agricultural and forestry production systems. Aerial imaging has some distinct advantages over labour- and time-consuming manual ground surveys. It can provide area-wide coverage over short time periods and identify 'weak spots' for targeted ground surveys in instances when canopy decline occurs. It can also offer temporal surveillance of known pest infestations to determine rates of spread over consecutive seasons under different climatic and soil conditions.

Two alternative digital imaging systems, currently used in precision viticulture, have also been adapted for grape phylloxera detection. Multispectral systems predominantly use four imaging sensors to detect reflected light from the grapevine canopy in the blue, green, red and near infrared (NIR) wavelengths (Proffitt *et al.*, 2006). Hyperspectral systems record reflectance in up to 256 separate spectral wavebands. Multispectral fingerprinting has been examined as a tool in grape phylloxera detection by evaluating canopy vigour and mapping patterns of leaf area (Wildman *et al.*, 1983). Multispectral colour-infrared (IR) aerial photography was applied to observe weak spots and predict grape phylloxera spread in infested *V. vinifera* vineyards (Wildman *et al.*, 1983). The imagery obtained allowed discrimination between grape phylloxera-damaged vines from that of oak-root fungus, *Armillaria mellea*, and Pierce's disease-affected vines – both of which gave differing spectral signatures. Johnson *et al.* (1996) utilised multispectral NIR aerial reflectance imagery in the Napa Valley, California to monitor a grape phylloxera-infested *V. vinifera* vineyard, which provided a measure of canopy density. Variations in canopy, measured as a function of vegetative cover, were generally associated with either grape phylloxera infestation or soil water-holding capacity (Johnson *et al.*, 1996). Later studies, using high-resolution colour IR photography, conducted in Australia (Buchanan *et al.*, 1996; Powell *et al.*, 2000; Frazier *et al.*, 2004) showed grape phylloxera-infested vines as areas of reduced NIR reflectance correlating to a reduction in vine vigour.

The use of multispectral sensors as the sole method in diagnosis of grape phylloxera infestation is unlikely to be effective due to numerous factors influencing vine vigour including water and nutrient stress, soil variability, diseases and competing flora and fauna (Herbert *et al.*, 2003; Frazier *et al.*, 2004). However, this technique does allow for the identification of weak spots which can be followed up by targeted manual ground surveys and would also be useful for temporal surveillance of known grape phylloxera-infested vineyards. The cost of AUS $ 66 ha^{-1} is not prohibitive and data collected can also be used to improve general management of low vigour vines.

In contrast, hyperspectral imaging employs narrower and significantly more wavelength bands over a contiguous spectral range, with notably enhanced sensitivity when compared to multispectral analysis (Powell, 2008). Hyperspectral leaf-level reflectance imaging has been examined to determine if a unique spectral signature directly associated with grape phylloxera infestation could be detected (Renzullo *et al.*, 2004, 2006). The study concluded that grape phylloxera-infested vines generate similar spectral characteristics to vines experiencing dehydration or nitrogen deficiency, a trend that is also found in other early detection methods (Tucker *et al.*, 2007). Hyperspectral imagery warrants further investigation as it may ultimately prove more effective than a multispectral approach. If phylloxera-specific spectral fingerprints are characterised in the future, this may offer the opportunity for area-wide targeted detection and surveillance using ground, aerial or satellite remote sensors.

Photosynthetic pigment fingerprinting

Symptoms of grape phylloxera presence on infested grapevines include reduced chlorophyll and increased photo-protective pigment concentration in leaves (Baldy *et al.*, 1996; Blanchfield *et al.*, 2006). Photosynthetic pigments play a role in both light harvesting and energy dissipation, and changes occur in response to the significant stresses imposed by the disruption of nutrient

and water transport from the grape phylloxera-damaged root system (Blanchfield *et al.*, 2006; Bruce *et al.*, 2011*b*). These changes in pigment composition are detectable prior to the emergence of visible symptoms in vine foliage and as such, provide the basis for potential further development as an early detection method which could be combined with hyperspectral imagery, as particular wavelength bands may correspond with changes in leaf colour.

Chemical fingerprinting

Metabolomic methods are being increasingly applied to the understanding of plant-pathogen and plant–insect interactions. Metabolic profiling of Esca disease, a complex fungal infection of grapevines, has been investigated using nuclear magnetic resonance (NMR) techniques (Lima *et al.*, 2010). Metabolite profiling of diseased and healthy leaf material found diseased leaves accumulated phenolic compounds and had decreased levels of carbohydrates when compared to healthy leaf material.

Induced metabolic changes in grapevines correlated to grape phylloxera infestation have been observed in feeding sites of both leaf-galling (Warick & Hildebrant, 1966; Schaefer, 1972) and root-galling grape phylloxera (Schaefer, 1985; Kellow *et al.*, 2004; Lawo *et al.*, 2011). Leaf gall tissue *in vitro* revealed a significant decrease in free amino acid content and an increase in total peptides compared to single cell grape stem clones (Warick & Hildebrant, 1966). However, *in vivo* studies of grape phylloxera-induced leaf galls are required to confirm these findings. The role of induced defence responses in grapevines, such as the production of secondary metabolites, could potentially allow for the development of a grape phylloxera-specific chemical fingerprint for detection purposes. In susceptible *V. vinifera* roots, starch and amino acid levels change in the presence of grape phylloxera feeding, but no evidence of specific chemical defence responses have been observed (Kellow *et al.*, 2004; Du *et al.*, 2008). This is in contrast to the response in grape phylloxera-resistant vine roots, where increased lignin, polyphenolics, cellulose and pectin and reduced starch accumulation occur, potentially indicating a defence response (Kellow, 2000; van Heeswijck *et al.*, 2003; Du *et al.*, 2011). Upregulation of polyphenols in infested root material was further confirmed through analysis of the volatile metabolome of a grape phylloxera-resistant rootstock (a hybrid of *V. berlandieri* Planch. × *V. riparia* Fitch) by headspace solid phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS) to (Lawo *et al.*, 2011). The study identified 14 differentially expressed compounds from grape phylloxera-infested vines, with preliminary data suggesting the involvement of the mevalonate and/or alternative isopentenyl pyrophosphate, the phenylpropanoid and lipoxygenase plant defence-related pathways.

Preliminary glasshouse and field studies examining metabolic profile shifts in areas remote from the point of root-feeding (e.g. foliage of *V. vinifera* when grape phylloxera feeds on the root system) have been conducted using NMR methods (Tucker *et al.*, 2007). Principal component analysis of both mature and immature leaves sampled through selected vine growth stages showed chemical separation between infested and non-infested vines (Tucker *et al.*, 2007). An elevation in the linoleic to linolenic acid ratio in the triglyceride component of the leaf extract was observed with grape phylloxera infestation (Tucker *et al.*, 2007). However, this appears to be a general defence response, as elevation in this ratio was also identified in *V. vinifera* infected with the fungal pathogen *Eutypa lata* (Koussa *et al.*, 2002). The NMR spectra from grape phylloxera infested vines were similar to that of vines displaying nitrogen deficiency but not water stress, suggesting possible leaching of nitrogen from the leaves of infested vines (Tucker *et al.*, 2007).

In a recent field-based study, liquid chromatography-mass spectrometry (LC-MS) data collected from leaves of grape phylloxera-infested *V. vinifera* indicated an upregulation of the flavonoid compounds isorhamnetin glycoside, rutin, kaempferol glycoside and quercetin glycoside (Fig. 2) (Benheim *et al.*, 2011). These compounds are involved in both passive and induced defence mechanisms in plants, commonly associated with insect or pathogen attack (Treutter, 2006) and can affect insect development and feeding behaviour (Ghumare *et al.*, 1989; Larsson *et al.*, 1992; Onyilagha *et al.*, 2004). Identification of these compounds as putative biomarkers of grape phylloxera infestation requires validation against environmental, pathogen, water and nutrient stressors. The emerging field of metabolomics and other branches of systems biology present strong platforms for the discovery and validation of these biomarkers against these stressors.

Molecular fingerprinting

DNA probes are designed for recognition of explicit DNA sequences for a given target organism and are used extensively in the detection of soil-borne pathogens such as fungi (Ophel-Keller *et al.*, 1995; Corredor *et al.*, 2000), nematodes (Atkins *et al.*, 2005; Madani *et al.*, 2005), and bacteria (Sayler & Layton, 1990; Rasmussen & Reeves, 1992). Molecular methods for grape phylloxera detection have been explored (Herbert *et al.*, 2008*b*) resulting in the development of a commercially available

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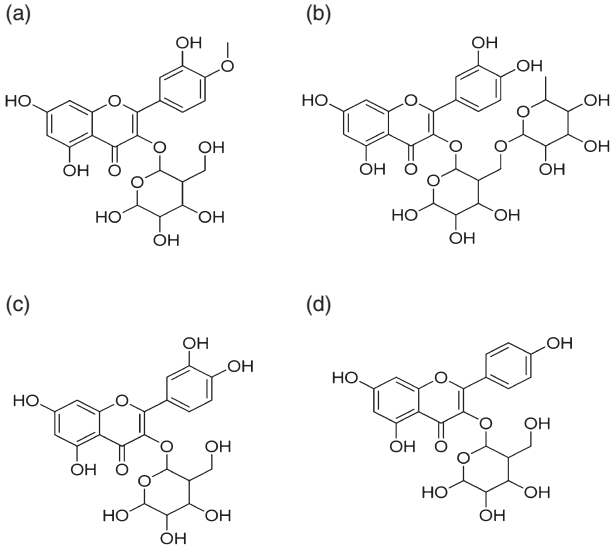

Figure 2 Structures of previously identified flavonoid compounds from leaf extracts taken from phylloxera infested *Vitis vinifera* grapevines: (a) isorhamnetin glycoside, (b) rutin, (c) quercetin glycoside and (d) kaempferol glycoside (Source: Benheim *et al*., 2011).

grape phylloxera-specific DNA soil probe. Validation of the probe, and its utilisation in targetted detection approaches, has been performed under both field and laboratory conditions.

Use of the grape phylloxera DNA soil probe has also been examined extensively under field conditions to determine the optimal targeted sampling strategy and to compare its efficacy with other detection techniques (Bruce *et al*., 2011*a*; Powell, 2012). Herbert *et al*. (2008*b*) compared and contrasted the DNA probe technique with that of emergence traps and manual ground surveys in a grape phylloxera-infested vineyard in the Yarra Valley, Victoria, Australia. A strong correlation was found between emergence traps and DNA probes, with manual ground surveys being the least sensitive technique. However, although robust and highly sensitive, the DNA probe technique, at an approximate cost of AUS$ 33 per vine, was relatively expensive. In a more recent study the cost of sampling was reduced to be on a par with ground surveys (Bruce *et al*., 2011*a*). One advantage the DNA probe has over other detection approaches such as emergence traps, is that it can be used year round potentially to detect all grape phylloxera life-stages belowground, rather than just aboveground dispersive stages. However, probe efficiency is reduced markedly in winter months when population levels decline (Powell, 2012). The technique would benefit from further validation against a range of grape phylloxera genotypes under varying edaphic conditions.

Soil sensing

Being predominantly subterranean root-feeding grape phylloxera is influenced by edaphic factors. Like many other soil-dwelling pests, the biophysical and chemical characteristics of soil (e.g. electrical conductivity, moisture, pH and ionic concentration) are principal factors affecting establishment, development, reproductive potential, and spatiotemporal distribution of grape phylloxera (Nougaret & Lapham, 1928; de Klerk, 1972; King & Buchanan, 1986; Bruce *et al*., 2009) and its' host-plant susceptibility. Grape phylloxera development and population dynamics are also influenced by soil temperatures, with grape phylloxera unable to initiate feeding sites or develop beyond hatching at soil temperatures lower than 15–18°C (Turley *et al*., 1996).

Elevated soil electrical conductivity is associated with areas of higher grape phylloxera abundance (Powell *et al*., 2003; Bruce *et al*., 2009). The apparent electrical conductivity (ECa) is influenced by soil moisture, ionic content and salinity (Rodriguez-Perez *et al*., 2011). Grape phylloxera establishment can also be affected by other soil physical and chemical factors including pore size, clay content, pH, nutrient availability, and mineralogy (Reisenzein *et al*., 2007; Rodriguez-Perez *et al*., 2011). Elevated aluminium exchange capacity is associated with inhibiting grapevine root growth (Delhaize & Ryan, 1995). Soils with toxic aluminium levels have been found to be amenable to grape phylloxera establishment (Powell *et al*., 2003), indicating a relationship between root phenology and grape phylloxera.

The study of soil interactions and how they may influence grape phylloxera establishment, development and dispersal affords great potential for the development of an *a priori* risk assessment matrix. This matrix would include the use of novel monitoring techniques, such as emergence traps and/or DNA probes, to identify regions of vineyards with the greatest risk of infestation.

Quarantine

One of the first examples of international quarantine intervention to protect against an insect pest was introduced following the outbreak of grape phylloxera across Europe (Anon, 1975). Although grape phylloxera was largely successfully managed using resistant rootstocks in Europe, the European and Mediterranean Plant Protection Organisation (EPPO) lists grape phylloxera as locally present in the EPPO region and it is therefore classified as an A2 pest (EPPO, 2011). Although the EPPO still recommends member countries to regulate it through the use of quarantine (EPPO, 1990), because of its relatively

widespread distribution, the EPPO also questions whether maintenance of quarantine measures for the benefit of so few uninfested regions is applicable. In contrast, in countries where grape phylloxera is not widespread, such as Australia and China, quarantine protocols are more widely advocated.

The effectiveness of quarantine measures in restricting exotic grape phylloxera incursions and the spread of endemic populations depends on many factors (e.g. regulatory, environmental and biological). Quarantine requires both the analysis of baseline information to determine the risks of spread and scientifically validated grape phylloxera-specific protocols to restrict or reduce the insects' rate of spread.

Risk vectors

Grape phylloxera, due to their compartmentalised gut structure and limited ability to excrete waste (Andrews *et al.*, 2012), can survive without food under suitable ambient conditions and even when immersed in water for several days (Korosi *et al.*, 2009). This survival capacity is an important consideration when developing effective quarantine protocols and assessing the risk of grape phylloxera transfer.

First instar grape phylloxera present the most abundant and mobile life-stage of grape phylloxera with high population levels found on the soil surface, foliage, and fruit through late spring and summer (King & Buchanan, 1986; Powell *et al.*, 2000; Omer *et al.*, 2002; Porten & Huber, 2003) as a result of more favourable dispersal conditions relating to increasing temperatures (Herbert *et al.*, 2006) and changes in grapevine phenology.

First instar grape phylloxera have limited natural dispersal ability (King & Buchanan, 1986) and are spread predominantly through human activity by transfer on viticultural machinery and equipment, footwear and clothing, as well as planting material, soil and some grape products (Deretic *et al.*, 2003; Powell, 2008). Population dynamics studies have confirmed the presence of first instars on both vine foliage and fruit throughout the growing season (Powell *et al.*, 2000). This illustrates an additional risk of unintentional grape phylloxera transfer during the harvest period (Korosi *et al.*, 2009), through transfer on mechanical harvesters (King & Buchanan, 1986), clothing of vineyard personnel involved in hand harvesting and on post-harvest material such as grapes and unfermented pomace or marc (a mixture of grape seeds, skins and stalks; Powell, 2008).

Grape phylloxera is unable to survive composting of green waste and winery waste when conducted using commercial standards (Anon, 1999). Bishop *et al.* (2002) demonstrated 100% mortality of all grape phylloxera life-stages within 3 weeks in a commercial green waste composting process with temperature being the predominant factor influencing mortality (Bishop *et al.*, 2002; Keen *et al.*, 2002). Based on mortality of first instar life-stages, a period of at least 4 days composting of white grape marc is recommended to remove any further risk of grape phylloxera transfer (Korosi *et al.*, 2009). Furthermore, post-harvest red must fermentation (Deretic *et al.*, 2003) and fumigation of table grapes with sulphur dioxide (Buchanan, 1990) have also achieved 100% grape phylloxera mortality.

Quarantine boundaries

Grape phylloxera-specific quarantine within countries is widely practiced by relatively few grape-growing countries including China, Russia, the Netherlands, Armenia, and Australia. One of the most detailed set of legislation and quarantine protocols has been developed in Australia, where distribution of grape phylloxera has been limited to a few grape-growing regions (representing only 2% of production areas) despite the original detection of grape phylloxera dating back to 1877 (Buchanan, 1990). Initial quarantine legislation included the introduction of the Vine Disease Eradication Bill (1878) and the Vine Disease Act (1890). However, grape phylloxera remains a major threat to the Australian viticulture industry and detections outside quarantine zones have increased since 2000, with the latest detection occurring in Victoria in 2010. In response, National Grape Phylloxera Management Protocols (NVHSC, 2009) have been developed for a range of risk vectors. Upon detection of a grape phylloxera infestation outside existing national quarantine boundaries, Australian state legislation requires the declaration of a new grape phylloxera infested zone (PIZ) with a minimum 5 km boundary from the initial detection point (Buchanan, 1990). National grape phylloxera quarantine boundaries are not present in many major grape-producing countries including USA and South Africa. This contrasts with the EPPO member countries and the Russian Federation which focuses predominantly on phytosanitary measures to prevent entry of phylloxera across country borders on plant material (EPPO, 1990; Vasyutin, 2004). Interceptions of grape phylloxera on plant material have recently been made in the UK using this approach (FERA, 2011).

Disinfestation techniques

Disinfestation of grape phylloxera from viticultural machinery, planting material, diagnostic materials, equipment and footwear can employ either heat-based or

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chemical-based treatments. Australia has as a more holistic approach to disinfestation than most countries, as it considers a broader range of risk vectors. Heat treatment of viticultural machinery, coupled with chemical disinfestation procedures for footwear, is recommended to restrict the potential human-assisted transfer of grape phylloxera. The disinfestation of viticultural machinery, such as grape harvesters, requires low humidity heating at 45°C for a minimum of 75 min or 40°C for 2 h (Korosi *et al*., 2009, 2012; NVHSC, 2009). This method has recently been validated against at least two root-galling grape phylloxera strains (Korosi *et al*., 2012). Heat treatment of soil samples for diagnostic purposes is also recommended where samples are dispatched from a PIZ to a processing laboratory for general diagnostic testing (NVHSC, 2009).

Implementation and cost of disinfestation infrastructure, such as heated industrial sheds, is specific to the size of viticultural machinery in use. A heat shed for machinery, including grape harvesters, would cost between AUS $ 50 000 and 170 000 (Hathaway, 2010; PGIBSA, 2011) to build and running costs are estimated to add AUS$ 10 per tonne of grapes. Other infra-structure costs may include a hot water washdown facility installed at an estimated cost of around AUS$ 10 000. Wineries within a PIZ are also required to have hot water dipping facilities, for grape bin disinfestation, which cost around AUS$ 80 000 to install plus running costs (Hathaway, 2010).

Handheld viticultural equipment and footwear can be disinfested with a 2% NaOCl solution for a minimum of 30 s and this is recommended standard practice prior to entering and or leaving vineyards in Australia (Dunstone *et al*., 2003). However, this practice is not conducted in any other grape-growing country, specifically for grape phylloxera. Disinfestation of planting material (vine cuttings) is more widely adopted and necessitates hot water treatment (EPPO, 2009; NVHSC, 2009; Powell *et al*., 2009) or methyl bromide fumigation (Sakai *et al*., 1985). However, methyl bromide can have phytotoxic effects on grapevine planting material (Mordkovich & Chernej, 1994). Its use as a pesticide was phased out as part of the Montreal Protocol (UNEP, 2000) in 2005 because of its impact on the ozone layer, except for allowable exemptions including the Quarantine and Preshipment Exemption, to eliminate quarantine pests. It is therefore unlikely that methyl bromide will be used extensively in the future for grape phylloxera disinfestation, as recommendations have been made for either replacement or reduction in methyl bromide use for phytosanitary purposes (UNEP, 2008).

Gamma irradiation is another option for potential application for disinfestation of both grapes and grapevine planting material, and has been investigated for its effect on the storability and preservation of grape material. It has been shown to reduce survival and fecundity of grape phylloxera (Al-Bachir, 1999; Makee *et al*., 2008). Gamma irradiation of grapevine plant material as a disinfestation procedure is reportedly an effective but relatively slow process, with treated organisms taking weeks to reach 100% mortality (Witt & Van de Vrie, 1985). However, use of this treatment on a commercial basis for grape phylloxera disinfestation has yet to be fully exploited.

Disinfestation treatments for winery waste have also been developed in Australia including composting of grape pomace (Korosi *et al*., 2009) and fermentation of must (Buchanan *et al*., 1996). Other factors have been shown to influence grape phylloxera mortality and may be used in future disinfestation protocols. For example, white juice has recently been shown to reduce survival of first instar grape phylloxera (Powell, 2012) with mortality found to be independent of pH and Baumé concentration, but dependent of cold storage temperature and sulphur dioxide content.

Although rootstocks present the best long-term management solution, the cost of replanting (at AUS$ 25 000 ha^{-1}), and availability of rootstocks remains a major burden to smaller vineyard operators. Comparatively, while the costs associated with implementation of disinfestation procedures are relatively low, it places limitations on market access of grape products. Secondary benefits associated with adoption of disinfestation procedures include control of other pests such as mites (Szendrey *et al*., 1995), some fungi (Clarke *et al*., 2004), phytoplasma and viruses (Caudwell *et al*., 1997).

Ultimately, however effective such disinfestation treatments may be, they rely on adoption by the viticultural industry as a whole and any circumvention of the process is likely to lead to quarantine breakdown.

Alternative management

Early detection may in some instances offer a number of potential options for grape phylloxera management other than the use of resistant rootstocks. Currently, removal of infested grapevines and replanting with grape phylloxera-resistant rootstocks is the predominant long-term management method employed. However, other management options may potentially be useful in the short-term to suppress grape phylloxera populations, with their efficacy dependent on (a) the level of grape phylloxera infestation, (b) genotypic characteristics of both host plant and grape phylloxera, (c) the extent of damage identified in the initial detection period, and (d) the predicted rate of spread. The following sections review historical and recent significant research in the alternative

management of grape phylloxera, encompassing biological, chemical and cultural management options.

Biological control

Biological control of grape phylloxera has been subjected to limited research in comparison to chemical control and rootstock breeding and selection. In general terms, biological control of insect pests requires careful planning and monitoring and is commonly used in tandem with cultural control strategies to ensure successful application (Bernard *et al*., 2007). Biological control of grape phylloxera has been explored in two phases, the late 19th century and mid-late 20th century.

Arthropods

In 1873, a predatory mite *Tyroglyphus phylloxerae* was identified and introduced to France to control the spread of grape phylloxera (Riley, 1881; Gullan & Cranston, 2010). This attempt was unsuccessful (Kirchmair *et al*., 2009). There have been other historical reports of natural predators of grape phylloxera (Anon, 1881), including the millipede *Polyxenus lagarus* (Haller, 1878) and lacewings *Chrysopa* sp. (Riley, 1875). However, these early reports only describe initial observations of predatory behaviour, with their effectiveness in controlling large populations of grape phylloxera having been questioned (Mayet, 1890).

More recently, Stevenson (1967) recorded two *Chamaemyiidae* showing predation on leaf-galling grape phylloxera in Canadian vineyards. Predatory behaviour of the mirid bug *Ceratocapsus modestus* and the coccinellid *Scymnus cervicalis* Mulsant against leaf-galling grape phylloxera has also been noted in North America (Wheeler & Henry, 1978; Wheeler & Jubb, 1979). Predators have also been described for other phylloxerids (von Fulmek, 1857; Jancke, 1954). No recent research on the efficacy of these aforementioned predators has been published. A broad range of natural enemies are present in vineyard ecosystems that could potentially be manipulated for grape phylloxera suppression (Herrmann & Forneck, 2001), particularly life-stages which predominate (i.e. leaf-galling) or are seasonally active (root-galling) aboveground.

Although published evidence of natural predators of grape phylloxera has been limited over the past 40 years, further research in this area is important in the interests of developing non-chemical methods of control and assessing the impact of chemical insecticides on potentially beneficial predators. Manipulation of natural enemies may offer some potential for reducing the risk of grape phylloxera spread by suppressing populations to lower levels, particularly in the spring and summer periods when dispersive life-stages are more active.

Nematodes

Entomopathogenic nematodes, belonging to the *Heterorhabditidae* family, have undergone continued investigation as potent biological control agents against insect pests since their first use in the 1930s (Glaser, 1932). Entomopathogenic nematodes have been tested in a single study against root-galling grape phylloxera in laboratory-based trials (English-Loeb *et al*., 1999) with limited success. Of the nematodes examined, the Oswego strain of *Heterohabditis bacteriophora* Poinar (Hb Oswego), used in Petri-dish trials, reduced grape phylloxera populations up to 80% when compared to experimental controls. In soil-cup trials, the suppressive action of Hb Oswego was significant, but was dependent on high levels of moisture (>13% wt:wt) and nematode density ($>15\,000\,g^{-1}$ soil). As no evidence supporting the ability of Hb Oswego to reproduce post-infection in the grape phylloxera host has been presented, in conjunction with its application difficulties, the commercial use of Hb Oswego as a grape phylloxera management tool is questionable. However, until systematic surveying of vineyard regions for entomopathogenic nematodes has been conducted their future use as potential control agents is unclear.

Fungi

Entomopathogenic fungi have been widely used in agriculture, forestry and land management stemming from interest of their potential use in conservation biological control. Several entomopathogenic fungi have been developed for the suppression of numerous insect pests (Kirchmair *et al*., 2009)

Two anamorphic entomopathogenic fungi in particular, *Beauveria bassiana* (Balsamo) Guillemin (white muscardine) and *Metarhizium anisopliae* (Metschnikoff) Sorokïn (green muscardine), have been a key focus of research due to their broad distribution and natural pathogenicity toward many insect species (Meyling & Eilenberg, 2007). *Beauveria bassiana* has shown successful grape phylloxera control *in vitro*, but has yet to be validated in the field (Granett *et al*., 2001). Kirchmair *et al*. (2004) demonstrated in pot trials the efficacy of *M. anisopliae* as a grape phylloxera control agent, with 80% of treated samples demonstrating an absence of grape phylloxera infestation compared with untreated controls. In addition, certain strains of *M. anisopliae* have demonstrated greater effectiveness against grape phylloxera than others (Huber & Kirchmair, 2007). The

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quantification of pathogenicity levels by *in situ* examination of infected grape phylloxera is difficult as *M. anisopliae* kills and mummifies the insect (Kirchmair *et al.*, 2004; Kirchmair *et al.*, 2009). Field trials have used commercial formulations of *M. anisopliae* such as Granmet® (Kwizda Agro GmbH, Austria & Agrifutur s.r.l., Italy). This treatment reduced population abundance of grape phylloxera 2 years post-application, but persistence of the treatment was reduced to negligible levels 3 years after application (Kirchmair *et al.*, 2007). These results indicate repeated applications would be required to reduce grape phylloxera to manageable levels. *Paecilomyces farinosus* (Holm & Gray) has also been tested against grape phylloxera with reported success (Goral *et al.*, 1975), but has not been investigated further.

Effective use of entomopathogenic fungi as a biological control agent relies upon careful selection of specific strains that have overcome host resistance. These are generally rare and host-specific (Jackson & Klein, 2006), such as the B96 strain of *Beauveria brongniartii* which is used for the control of *Hoplochelus marginalis* on sugar cane crops (Jackson, 1999).

The selection of suitable virulent strains of entomopathogenic fungi, or their toxic metabolites, which may be specific to grape phylloxera, is important. Published studies have focussed purely on inoculation with these fungal agents in preference to investigations into their ecology and host-specificity. Upon selection of a suitable strain, application methods targeting root-galling grape phylloxera with entomopathogenic fungi would require a sowing machine in conjunction with a rotary harrow. This was demonstrated by Huber & Kirchmair (2007) with their use of *M. anisopliae*-colonized barley seeds.

Chemical control

An effective chemical control option against both leaf-galling and root-galling grape phylloxera is, despite several studies, still pending further development. A range of chemical options have been examined both historically with a range of insecticide groups and more recently using systemics (Table 1). Although many insecticides have previously reported success in suppressing grape phylloxera populations, their registration as grape phylloxera control agents worldwide is limited.

Soil fumigants, such as carbon bisulphide (*aka* carbon disulphide CS_2), were first used in France in the 1870s and sodium tetrathiocarbonate, which releases CS_2 on breaking down in soil has been used more recently in California (Weber *et al.*, 1996). Both fumigants were relatively ineffective against root-feeding phylloxera most likely due to either minimal impact on winter hibernants and eggs on the roots, or failure of CS_2 to effectively penetrate the full depth of the grapevine root zone. Grape phylloxera has been detected to depths of over 1 m in the soil profile in Australia (Buchanan, 1990) and South Africa (de Klerk, 1974). This ability of grape phylloxera to move down the soil profile is likely to constrain the effectiveness of many insecticide groups.

Carbamates and organophosphates have previously been tested against grape phylloxera. Carbofuran reduced first instar abundance in field trials conducted in the USA (Rammer, 1980) and Australia (Buchanan & Godden, 1989), whereas oxamyl and aldicarb, showed little residual activity against root-galling grape phylloxera (Buchanan & Godden, 1989; Loubser *et al.*, 1992) and failed to suppress grape phylloxera populations. Stevenson (1968) assessed o-isopropoxyphenyl methylcarbamate and phophorothionic acid in field trials and demonstrated successful control of grape phylloxera using drench treatments of both agents but only on grafted-rootstocks.

The organophosphate phenamiphos was ineffective against root-galling grape phylloxera in ungrafted *V. vinifera* vineyards in Australia (Buchanan & Godden, 1989) and South Africa (de Klerk, 1979). Disulfoton had only limited short-term suppression of grape phylloxera in field trials conducted in South Africa (de Klerk, 1979) and Canada (Stevenson, 1968). Endosulfan has shown some suppressive action against leaf-galling grape phylloxera (Stevenson, 1968, 1970); however, evidence indicating suppressive action against root-galling forms of grape phylloxera on *V. vinifera* was lacking.

In the last decade advances in the development of systemic insecticides, such as neonicotinoids and tetronic acid derivatives, has highlighted some potentially more effective control agents for root-feeding grape phylloxera. Thiamethoxam and imidacloprid are both upwardly and downwardly mobile neonicotinoids. Both compounds have been shown to markedly suppress populations of grape phylloxera in laboratory, glasshouse and field trials (Botton *et al.*, 2004; Al-Antary *et al.*, 2008; Herbert *et al.*, 2008*a*; Johnson *et al.*, 2008). Promising suppressive effects on population abundance were observed along with increases in grapevine vigour. However, investigations into the effect of these insecticides on grape quality, potential residue levels in grapes, and residual effects in the xylem of *V. vinifera* have yet to be conducted. Both compounds are relatively toxic towards the honeybee *Apis mellifera* (Iwasa *et al.*, 2004; Tapparo *et al.*, 2012). Spirotetramat, containing tetramic acid and imidacloprid as active ingredients, has been used to control foliar forms of grape phylloxera in the USA on susceptible grapevine cultivars (hybrids of American *Vitis* and *V. vinifera*) (Nauen *et al.*, 2008; van Steenwyk *et al.*, 2009; Johnson *et al.*, 2010;

Table 1 Summary of a range of insecticides used in phylloxera control trials

Compound Class	Active Ingredient (Trade name)	Trial Location	Trial Type	Phylloxera Type	Selected Sources
	Carbon disulphide	France	Field	Radicicolae	Ordish, 1972
	Sulphocarbonates	France	Field	Radicicolae	Ordish, 1972; Campbell, 2004
	Enzone	USA	Field	Radicicolae	R. Loveless (as cited by Herbert, 2005); Weber *et al*., 1996
Carbamates and organophosphates	Carbofuran	USA, Australia	Field and Laboratory	Radicicolae	Rammer, 1980; Granett & Timper, 1987; Buchanan, 1990
	Fenamiphos	Germany, USA and Australia	Field	Radicicolae	Homeyer & Wagner, 1981; Buchanan, 1990; de Klerk, 1979
	Phosphorothioic acid	Canada	Field	Radicicolae	Stevenson, 1968
	Baygono-isopropoxyphenyl methylcarbamate	Canada	Field	Radicicolae	Stevenson, 1968
	Disulfoton	South Africa and Canada	Field	Radicicolae	Stevenson, 1968; de Klerk, 1979
	Oxamyl	Australia	Field	Radicicolae	Buchanan & Godden, 1989; Nazer *et al* ., 2006
	Aldicarb	Australia	Field	Radicicolae	Buchanan & Godden, 1989; Loubser *et al*., 1992
Organochlorines	Hexachlorobutadiene	South Africa	Field	Radicicolae	de Klerk, 1979
	Hexachlorocyclopentadiene	USA	Field and Laboratory	Radicicolae	Cox *et al*., 1960
	Endosulfan	USA and Canada	Field	Gallicolae	Stevenson, 1970; Williams, 1979
Neonicotinoids	Thiamethoxam	Australia and USA	Laboratory	Radicicolae	Granett *et al*., 2001; Nazer *et al*., 2006; Herbert *et al*., 2008*a*,*b*
	Imidacloprid	South Africa, Jordan, USA and Australia	Field and Laboratory	Radicicolae and Gallicolae	C. Coetzee & R. Loveless (as cited by Herbert, 2005); Herbert *et al*., 2008; Nazer *et al*., 2006; Al-Antary *et al*., 2008
	Spirotetramat	USA	Field	Gallicolae	Nauen *et al*., 2008; van Steenwyk *et al*., 2009; Johnson *et al*. 2010

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Sleezer *et al.*, 2011). Spirotetramat has also recently been registered for use against grape phylloxera in Canada (BCS, 2011) but has recently been cancelled for use in the USA due to detrimental effects on honeybees (Erickson, 2010)

Only four insecticides: imidacloprid, acetamiprid, fenopropathrin and spirotetramat are currently registered against the foliar form of grape phylloxera in the USA (Johnson *et al.*, 2009), Europe, and South Africa. In contrast, no insecticide treatments for controlling either root-galling or leaf-galling grape phylloxera have been registered for use in Australia.

Chemical control considerations

Chemical control of grape phylloxera remains an area of continued development. The use of foliar sprays and upwardly mobile insecticide treatments for the control of leaf-galling grape phylloxera are frequently observed to have high efficacy. However, leaf-galling grape phylloxera is less economically important than root-galling forms. In direct contrast to root-galling grape phylloxera, leaf-galling grape phylloxera are completely exposed on the leaf surface and form fibrous galls that offer limited protection from predators and foliar sprays. The chemical management of root-galling grape phylloxera presents a considerably more complex challenge which can be placed into three distinct categories: (a) insect interactions, (b) environmental considerations and (c) host-plant interactions.

Insect interactions

Chemical insecticides for use against both root-galling and leaf-galling grape phylloxera require careful selection. The efficacy and suitability of the insecticide for use is dependent on several characteristics. The active ingredient(s) must ideally (a) have systemic properties, (b) have good interaction with the insect, (c) possess sufficient residual activity in soil and low residual activity in grapes, (d) have effective levels of dispersion and diffusion into the soil, (e) be ambimobile (having both upward and downward mobility) (Granett *et al.*, 2001; Herbert, 2005; Powell, 2008).

The subterranean habitat of root-galling grape phylloxera affords a natural protection from non-systemic foliar sprays and low-dispersive soil drench treatments. Grape phylloxera reproduce rapidly, have a high fecundity, with many overlapping generations, and colonies have been found several metres down the soil profile (de Klerk, 1974; Buchanan, 1990). The spatial distribution and mass of grapevine root systems can vary as a function of grapevine type and soil composition. This tendency leads to a greater potential for grape phylloxera dispersal both in and around the root zone and aboveground (Powell, 2008). An effective chemical insecticide, in addition to having good dispersal and soil diffusion characteristics, must be targeted to specific insect developmental stages. First instar grape phylloxera represent the most active and mobile life-stage, therefore insecticide application timed to coincide with peak periods of first instar abundance would be ideal to suppress population build up, lower the risk of infestation and reduce economic damage.

Environmental interactions

Environmental factors such as temperature (both soil and air), soil type, rainfall and humidity play fundamental roles in insecticide efficacy. For instance, soil composition (specifically, soil texture; e.g. silt, sand and clay) has been noted to affect the bioactivity of some insecticides (Harris, 1966). Chlorinated hydrocarbons deactivate in dry clay soils, with reactivation occurring only under conditions of high relative humidity (Barlow & Hadaway, 1955; Gerolt, 1961; Harris, 1964). Temperature has been directly correlated with an increase of toxicity of some organophosphates (Satpute *et al.*, 2007). In the case of endosulfan, increased toxicity is achieved through a combination of high relative humidity and temperature.

Some authors suggest that root-galling grape phylloxera populations are more easily established in clay loam soils (Nougaret & Lapham, 1928; Granett & Timper, 1987). As a result, the efficacy of surface-applied agents may be hindered through restrictions in depth penetration, and non-uniform distribution of the insecticide. However, recent studies (Chitikowski & Fisher, 2005) indicated that although some soil compositions may marginally hinder population growth, the establishment of grape phylloxera colonies is independent of soil composition.

The environmental toxicity of some synthetic insecticides has led to persistent organic pollutants (POPs) being banned internationally due to unacceptable environmental effects and residue levels in food. In 2009, carbofuran was banned for use as an insecticide in the USA. In 2010, Australia placed a total ban on the use of endosulfan due to health and environmental concerns (Cubby, 2010) and over 80 countries have either banned or implemented plans for its phase out by mid-2012. In some countries other insecticides previously tested against grape phylloxera are either banned, under restricted use, or under review for potential withdrawal. These include fenamiphos (APVMA, 2003; EPA, 2008), disulfoton (EPA, 2006; APVMA, 2011), aldicarb and HCCPD derivatives.

Many insecticides not only affect the target pest, but also affect biodiversity, air and soil quality and water purity (Miller, 2004). Insecticides also potentially impact beneficial insects in vineyards (Bernard *et al.*, 2007) and could disrupt integrated pest management (IPM) programmes. The application of certain insecticides could potentially disrupt the natural enemies of both grape phylloxera (Gorkavenko, 1976; Wheeler & Henry, 1978) and other pests, thereby possibly causing a secondary pest resurgence.

Host-plant interactions

Phytotoxicity is a secondary effect imparted by numerous insecticides, resulting in damage to foliage and shoots (e.g. leaf burn) and reduced yields (Boutin, 2002). Although there is limited evidence to conclude that commonly used insecticides in viticulture are phytotoxic, carbofuran and aldicarb have been shown to elicit phytotoxic effects on a range of crops (Singh & Maheshwari, 1989). Endosulfan is also phytotoxic to several sulphur-sensitive grapevine cultivars (Johnson *et al.*, 2009). Foliar treatments of oxamyl have shown acute phytotoxicity to *Vitis*, reducing vine vigour and yield, while aldicarb has high residual activity in grapes (Buchanan & Godden, 1989). These phytotoxic and residual effects alone would preclude both insecticides from being used as suitable control agents. Exposure of grapes to chemical treatments has resulted in the establishment of maximum residue limits (MRLs), set through government legislation in numerous countries (AWRI, 2011). Any consideration of a chemical control option for grape phylloxera must also take these limits into consideration.

Overall the potential for using chemical agents for grapevine phylloxera is currently limited, particularly in ungrafted *V. vinifera* vineyards where repeated applications of chemicals would be required. However, there may be some potential for future applications in situations where early detection of grapevine phylloxera may warrant rapid intervention to suppress populations to manageable levels.

Cultural management

Cultural control of grape phylloxera has been explored as an alternative method to the use of rootstocks after reports of potential failure of rootstock resistance (Porten *et al.*, 2000; Granett *et al.*, 2001). One of the earliest examples of cultural management was the attempted eradication of grape phylloxera by flooding of vineyards during the winter months (Riley, 1875; Hilgard, 1876). This submersion method caused a noticeable increase in the vigour of all plants, yet was only applicable to non-permeable soils. Flooding is also uneconomical, particularly where water availability is limited. It requires access to large quantities of fresh water and subsequently fertilizer to replace water-soluble nutrients drawn away during the treatment. Flooding is still used as a means of controlling grape phylloxera in southern France (Campbell, 2004). However, in laboratory trials, grape phylloxera first instars and eggs have been shown to survive for up to 8 days when submersed in water (Korosi *et al.*, 2009), indicating a high resilience to submersion. The efficacy of the submersion treatment is also influenced by temperature and grape phylloxera life-stage, with temperatures of less than 5°C reducing survival of egg and crawler stages (Korosi *et al.*, 2009).

The influence of nutrient fertiliser treatment on grapevine root development and phylloxera damage has received limited research attention, even though growers in some countries apply foliar nitrogen to retain vine vigour under phylloxera-infested conditions (K. Powell, personal observation). In laboratory and field trials, using phylloxera-infested grafted rootstocks, reduced nodosity development was observed (Kopf, 2000) following nitrogen fertiliser application. Although the mechanism of action was unclear, the application of a nitrogen source to grapevines could either directly affect root development or indirectly impact on phylloxera feeding behaviour by influencing the N content in the roots.

Soil type has been shown to affect the establishment and population dynamics of grape phylloxera. Soil characteristics such as low aluminium exchange capacity and acidic pH are associated with high grape phylloxera abundance above and belowground in commercial vineyards in Victoria, Australia (Powell *et al.*, 2003). In Austria, an increase in nodosity formation has been correlated to the level of clay and humus content of soils, with grape phylloxera infestation enhanced by low nutrient availability (i.e. phosphorus, magnesium, copper, zinc and potassium) (Reisenzein *et al.*, 2007).

Soil amendments such as composted winery waste, composted green waste, humus and spruce sawdust mulches (Huber *et al.*, 2003; Powell *et al.*, 2007*b*,*c*) have been investigated as potential grape phylloxera management options in vineyards in Australia and Germany. Annual applications of composted green waste over three consecutive growing seasons increased both the abundance and dispersal of aboveground first instar grape phylloxera. No improvement to vine vigour, grape yield and quality or pruning weight was observed (Powell *et al.*, 2007*c*). Some formulations of composted winery waste consisting of grape marc were shown to considerably reduce grape phylloxera emergence aboveground when compared to untreated vines (Powell *et al.*, 2007*a*). Both studies had limited effect on first

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instar and total life-stage abundance belowground when compared to controls, but have implications for altering the risk of grape phylloxera transfer aboveground on viticultural machinery and vineyard personnel clothing. In contrast, spruce sawdust-based and humus-based compost can reduce phylloxera abundance and improve grapevine health (Porten *et al*., 2000; Huber *et al*., 2003).

In a survey comparing conventionally managed vineyards (CMV) and organically managed vineyards (OMV), CMVs showed a strong correlation between grape phylloxera population density and root necrosis (Granett *et al*., 2001) initiated by secondary fungal pathogens (Omer *et al*., 1995). OMVs reduced root necrosis, with similar grape phylloxera population abundance observed in CMVs. Possible explanations of these results include either differences in microbial ecology; pathogen suppression by discrete soil characteristics, or the induction of systematic acquired resistance (SAR), which if validated, could be of great significance in understanding the intrinsic defence mechanisms of *Vitis* towards grape phylloxera.

Overall the contrasting compost treatment effects, reported by various authors, support our view that selection of appropriate compost formulations is an area that requires further research to elucidate their mechanisms of action.

Genetically modified vines

The development of novel approaches to insect resistance in economically important plants through genetic modification and incorporation of resistance genes into crop species has been successful in some instances against Hemipteran pests (Shi *et al*., 1994; Hilder *et al*., 1995; Gatehouse *et al*., 1996; Rao *et al*., 1998). Although several opportunities exist for the development of genetically modified grapevines for pest resistance (Viss & Driver, 1996), this research area is still largely unexplored.

Although limited research has focussed on the potential modification of grapevine to resist grape phylloxera attack, several studies have investigated the effects of transgenic plants expressing antimetabolic proteins (such as enzyme inhibitors and lectins) on related Aphididae. *Solanum tuberosum* modified with various combinations of the proteins bean chitinase (BCH), snowdrop (*Galanthus nivalis*) lectin (GNA) and wheat α-amylase inhibitor (WAI) reduced fecundity of the peach–potato aphid, *Myzus persicae* (Gatehouse *et al*., 1996). Proteinase inhibitors are also effective against the cereal aphids *Diuraphis noxia*, *Schizaphis graminum* and *Rhopalosiphum padi* (Tran *et al*., 1997), pea aphid *Acyrthosiphon pisum*, cotton aphid *Aphis gossypii* and *M. persicae* in artificial diet bioassays and when expressed in transgenic plants (Rahbe *et al*., 2003; Carrillo *et al*., 2011).

Plant-derived lectins bind to specific carbohydrate moieties in the Hemipteran gut and also have feeding deterrent properties (Kingston *et al*., 2005; Powell, 2008). One particular group of lectins, which are mannose-binding, include garlic lectin (Hossain *et al*., 2006; Saha *et al*., 2006; Sadeghi *et al*., 2007), onion lectin and snowdrop lectin (Rao *et al*., 1998; Miao *et al*., 2011) have been introduced to a range of crops and act as antimetabolites towards Hemipteran pests. The standard practice, prior to conducting *in planta* bioassays is to first screen the lectins using an *in vitro* artificial diet system which is specific for the target pest (Sauvion *et al*., 1996; Powell *et al*., 2003; Kingston *et al*., 2005; Hussain *et al*., 2008; Trebicki *et al*., 2009). Preliminary screening of potential gene products for antimetabolite activity towards grape phylloxera is feasible, following the development of *in vitro* artificial diet systems for both leaf-galling (Forneck & Wöhrle, 2003) and root-galling grape phylloxera (Kingston *et al*., 2007), but no further progress in this area has been reported.

A single *in vitro* study using transgenic *Vitis* species was conducted to assess inducible defence against root-galling grape phylloxera (Franks *et al*., 2006). Three *Sorghum bicolor* genes expressing biosynthesis of cyanogenic glycoside were transferred to *V. vinifera*, producing one hairy root transgenic line capable of releasing cyanide on maceration. Both cyanogenic and acyanogenic lines proved unsuccessful in reducing grape phylloxera development. The successful application of transgenic technologies to grape phylloxera remains uncharted territory and requires significant attention particularly as grape phylloxera is a monophagous pest and potentially more amenable to this type of application than related polyphagous aphids.

Manipulation of plant defence systems

When insects feed on host plants, they can induce defence responses in the host through secondary metabolic pathways. In the case of aphids, jasmonic acid and salicylic acid pathways have been shown to be involved in plant resistance (Moran & Thompson, 2001; de Ilarduya *et al*., 2003; Girling *et al*., 2008). Jasmonic acid has been implicated as a potential resistance mechanism for grape phylloxera (Omer *et al*., 2000).

Plant volatiles can also be released from attacked plants as a defence response to aphid attack, affecting the insect directly through antixenosis or indirectly by enhancing natural enemy predation. Some predators also respond to aphid pheromones. The vast majority of research in this field has focused on aphid species, which attack

aboveground foliage (Powell & Pickett, 2003) rather than belowground herbivores, such as root-galling grape phylloxera. Biochemical pathways which may be induced upon insect attack on the root system have received minimal attention (van der Putten *et al.*, 2001). The exploitation of natural plant defence systems presents an opportunity to enhance resistance to grape phylloxera through both conventional breeding and introduction of foreign genes through genetic manipulation. However, because of the genetic diversity of grape phylloxera, some resistance mechanisms may only be effective against some grape phylloxera genetic strains.

Eradication

There are no reports of any countries where grape phylloxera has been successfully eradicated (Morgan *et al.*, 1973). Following the discovery of grape phylloxera in Australia in 1877 in Geelong, Victoria, attempts were made at eradication by either uprooting and fumigating with carbon disulphide or burning infested grapevines. In 1893, grape phylloxera was found in the Bendigo district, and again an eradication policy was followed. During the late 1890s, however, grape phylloxera was discovered at Heathcote in the Goulburn Valley, in replanted vineyards at Geelong, and in the Rutherglen district (Buchanan *et al.*, 2012). More recently in the Yarra Valley region, Victoria, Australia following a grape phylloxera detection in 2006, a localised eradication attempt occurred in 2007, which included extensive chemical treatment of both vines and insects and removal of 30 ha of grapevines (R. Hamilton, personal communication). The attempt was unsuccessful and cost in excess of AUS$ 75 000 (Hathaway, 2010). The vineyard itself was then sold at lower than market value. An alternative option would have been a gradual replanting of the vineyard onto phylloxera-resistant rootstocks at a cost of $25 000 AUD ha^{-1}. Although it would have taken 4–5 years for these replanted vines to reach maturity, a progressive replanting program would have allowed maintenance of some grape production, and hence minimised economic losses, during the establishment period. However, once established, replanted vines could be potentially productive for at least 25–30 years or longer.

Removal of grapevines from infested regions was also the predominant recommended 'eradication' method in Europe (Börner & Schilder, 1934), yet grape phylloxera remains widespread. In China, grape phylloxera was first described in 1893 and reportedly 'disappeared' during the Cultural Revolution when vineyards were removed and replaced by food crops, but re-emerged in several provinces during 2006–2007 (Du *et al.*, 2011).

Any serious attempt at eradication of grape phylloxera would require a concerted, multidisciplinary approach, ensuring that fundamental interactions between environment, pest, and host-plant are researched and clearly understood, and that quarantine protocols are appropriately implemented to prevent reinfestation. Even if grape phylloxera eradication were to succeed, the risks of accidental reintroduction would remain.

Concluding remarks

Grape phylloxera has, since the late 19th century, been managed in countries where its distribution is widespread, very successfully with the use of resistant *Vitis* rootstocks and phytosanitary protocols. In other countries, where its geographic distribution is relatively limited, it can be primarily managed through an IPM approach combining effective detection, quarantine and alternative management options.

In some countries detailed scientifically validated quarantine protocols specific for grape phylloxera have been developed. However, these protocols are not universally adopted. Future modifications to these protocols could also be expected as our understanding of the insect's physiology is increased and as new disinfestation tools become available.

Several alternative options for grape phylloxera management have been described and could be employed in an integrated management approach, combined with improved early detection, although this may require additional research in some instances. Chemical insecticides are not widely advocated for grape phylloxera control, particularly in ungrafted *V. vinifera* vineyards. Although historically some sulphocarbonates and organophosphates have proven effective against grape phylloxera under field conditions, due to very high toxicity, carcinogenicity and phytotoxicity, their agricultural use is now greatly restricted. More recently systemic insecticides, such as imidacloprid and spirotetramat, have shown suppression of grape phylloxera in laboratory, glasshouse and field based trials (Herbert *et al.*, 2008*a*). However, the use of these compounds under field conditions introduces multiple abiotic and biotic variables which may impact on their efficacy. The subterranean habitat of root galling grape phylloxera in conjunction with consideration of soil type, climatic conditions, vine cultivar, method and rates of application all influence the degree of suppression attained. The final, and arguably, most important factors against their use are the environmental and health risks associated with chemical insecticides. An effective chemical treatment against root-galling grape phylloxera requires a systemic mode of activity, downward mobility, ease of diffusion through the soil, sufficient

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chemical-grape phylloxera interaction and high residual activity, which would not impact on the final commodity product. Currently there is no fully effective chemical-based control for grape phylloxera.

Cultural management strategies are classically used as short-term suppression methods rather than for prevention or eradication. Such strategies aim at creating crop habitats that disrupt the reproductive potential of pests through an understanding of their life-cycle and biological requirements. Investigations into the effects of soil type, composted mulches and flooding on grape phylloxera population abundance have yielded mixed results, with little to suggest that any one method alone would provide a means of sustainable control. The grafting of own-rooted vines on carefully selected grape phylloxera-resistant rootstocks currently remains the most effective means of long-term cultural management following a grape phylloxera outbreak and as a protective measure in case of grape phylloxera incursion. However, reports of collapse in rootstock resistance to grape phylloxera are of concern (Porten *et al*., 2000; Granett *et al*., 2001). The continued development of highly resistant rootstock hybrids, either through conventional breeding or genetic modification, combined with a coordinated international approach to grape phylloxera resistance screening is required. Conventional and novel grapevine breeding for resistance needs to consider the broad genetic diversity of grape phylloxera to ensure 'biotypes' do not arise which could result in future rootstock failures. The breeding of genetically modified rootstocks presents a significant opportunity to develop both 'broad spectrum' and genotype-specific rootstocks possessing genetic traits that render them unfavourable hosts for grape phylloxera. In some grape phylloxera-infested regions a single genetic strain, or a low incidence of recombinant genotypes which lack sexual reproduction among grape phylloxera populations, mitigates the risk of collapse of resistance in either engineered or conventionally bred rootstocks.

Biological control options and the identification of natural predators of grape phylloxera, particularly in its native range, remains a promising, but largely unexploited area. However, due to the accessibility constraints of root-galling grape phylloxera the range of potential biological control agents may be restricted to fungi, other soil-borne pathogens and nematodes. Although some success has been observed using entomopathogenic fungi under controlled and field conditions (Kirchmair *et al*., 2004), the use of biological control for grape phylloxera management remains a promising area which would require substantial further investment.

Significant advances into early detection techniques such as molecular probes, trapping systems, spectral fingerprinting and the use of metabolomics in chemical biomarker discovery remain an important research focus. These techniques should be considered in an integrated approach for early detection and rapid management intervention to reduce the risk of further quarantine breakdown and minimise economic loss.

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