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

D. Benheim[1,2,3], S. Rochfort[1,4], E. Robertson[1], I. Potter[1], & K.S. Powell[3*]

[1]Deparment of Chemistry. La Trobe Institute for Molecular Science, La Trobe University, Bundoora, Victoria 3083, Australia.

[2]Department of Primary Industries, Biosciences Division, Bundoora, 1 Park Drive, Bundoora, Victoria 3083. Australia.

[3]Department of Primary Industries, Biosciences Division, RMB 1145, Chiltern Valley Road, Rutherglen, Victoria 3685, Australia.

[4]La Trobe University, Bundoora, Victoria 3083, Australia

Correspondence

Department of Primary Industries

Biosciences Division, Rutherglen Centre

RMB 1145 Chiltern Valley Road

Rutherglen, Victoria, Australia 3685.

Ph: 02 6030 4542

Email: kevin.powell@dpi.vic.gov.au

Keywords

Insecticides, emergence traps, disinfestation, biocontrol, phylloxerid, detection, quarantine, *Vitis*

Abstract

Grapevine phylloxera, *Daktulosphaira vitifoliae*, is a monophagous insect pest of *Vitis* species. In a worldwide context, it is managed predominantly by the use of resistant rootstocks developed through conventional breeding of hybrid crosses of American *Vitis* species. In some viticulture regions of the

world, such as Australia, where phylloxera's geographic distribution is relatively limited it is also managed through a combination of surveillance, detection and quarantine. Although some alternative management options for grapevine phylloxera exist they have received relatively limited attention because of the relative success of resistant rootstocks to-date. However the resilience of resistant rootstocks as the primary management option could be challenged in the future by host-plant interactions with diverse phylloxera clonal lineages and by potential impacts of climatic change on both grapevine and phylloxera distribution. A range of control options exist which could be integrated into an improved management system for phylloxera. These are reviewed and recommendations for future research are provided.

Introduction

Grape phylloxera, *Daktulosphaira vitifoliae* (Fitch) is a small, invasive sap-sucking insect (Family *Phylloxeridae*) which causes substantial physical and economic effects on commercial grapevine, *Vitis vinifera* L., production. Phylloxera is native to the North Eastern 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 phylloxera in France in 1868 and subsequent spread over the proceeding decade which devastated the French wine industry is well documented, with over one million hectares of ungrafted *V. vinifera* French vineyards destroyed by the turn of the century (Ordish, 1987; Campbell, 2004). Over the past 150 years, 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).

Phylloxera, depending on genetic lineage (Corrie *et al.*, 2002; Corrie *et al.*, 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 phylloxera genetic lineage. On suitable indigenous hosts (i.e. American *Vitis* spp.) phylloxera feed on the leaves causing leaf galls with marginal populations

found on the root system. The resultant impact on general vine vigour or yield is minimal (Wapshere & Helm, 1987).

Leaf galling (gallicolae) phylloxera strains are widespread in continental United States and Europe on rootstock foliage and high population numbers can on some cultivars decrease vine productivity, in the form of reduced shoot growth (Granett & Kocsis, 2000) but rarely cause galling on *V. vinifera* leaves. However, recently their incidence on *V. vinifera* cultivars has been reported in Europe (Molnár *et al.*, 2009). These strains are generally considered far less significant economically than the more damaging radicicolae phylloxera strains (Davidson & Nougaret, 1921; Buchanan, 1990).

In contrast, European *V. vinifera* L. suffers infestation and damage predominantly in the root system which has significant economic impacts on production (Powell, 2008). Infestation, by root-galling (radicicolae) phylloxera strains, on ungrafted *V. vinifera* results in the development of phylloxera colonies on both immature and mature storage roots, causing nodosities and tuberosities respectively, which 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 plants' susceptibility to secondary fungal infection primarily through wounds caused by stylet insertion points (Omer *et al.*, 1995; Edwards *et al.*, 2007). Feeding on *V. vinifera* mature storage roots is considered the prime factor associated with serious damage and, in some instances, can cause complete root 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 phylloxera resistance, but tuberosities are rarely formed on these hybrids.

Control options

Rootstocks

Grapevine rootstocks are derived from American *Vitis* spp. lineage, which are widely recognised as having developed intrinsic resistance mechanisms toward phylloxera through co-evolution in its 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 is not without some limitations. In California in the late 1980's extensive losses in production resulted from the use of a phylloxera-resistant rootstock AXR-1 with the emergence and spread of phylloxera biotype B (Granett *et al.*, 1991; Granett *et al.*, 2001).

In some countries rootstock recommendations are primarily based on overseas phylloxera resistance screening data. 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 phylloxera predominate in the country. Screening of phylloxera genetic clones against commonly used and novel rootstocks bred for local conditions is essential for the development of accurate and timely recommendations to the viticulture industry, and has recently commenced in Australia (Korosi *et al.*, 2007) and China (Du *et al.*, 2008). Currently rootstocks present the only viable long term solution for phylloxera management, yet in some countries it remains an uneconomic option due to an estimated cost of $20,000AU/ha in loss of production and replanting (DAFWA, 2006). With some European sources reporting potential breakdown of phylloxera resistance in certain rootstocks (Walker *et al.*, 1998; Schmid *et al.*, 2003), continued screening and further development of alternative management strategies is imperative for the sustainability of the viticulture industry.

In some countries use of resistant rootstocks for phylloxera management remains a relatively low priority in part due to restricted phylloxera distribution, geographic isolation of viticulture regions, relative expense of grafted rootstocks and strictly enforced and comprehensive quarantine protocls. Despite this, incursions in these countries can cause economic difficulties. Since its discovery in Australia in 1877, although not widespread, phylloxera has caused significant disruption through quarantine restrictions and replanting costs to some major viticultural areas, particularly central and north-east Victoria (Buchanan, 1987), two isolated zones in south-eastern New South Wales (Powell, 2008) and also historically in Queensland (Helm, 1983). Yet in Australia, only two percent of all vineyards are known to be infested with phylloxera (Nicol, 1999). Given that established Australian vineyards are largely planted to highly susceptible own-rooted (ungrafted) *V. vinifera*, effective phylloxera management strategies are imperative in order to support and protect the long-term success and economic sustainability of the Australian viticulture industry. Phylloxera

management in Australia has therefore evolved into an integrative approach consisting of: (i) early detection and surveillance; (ii) effective quarantine regulations which encompass disinfestation procedures for plant material (Deretic *et al.*, 2003; NVHSC, 2009), farming machinery (Korosi *et al.*, 2009), hand held equipment and footwear (Dunstone *et al.*, 2003) aimed at preventing the spread of phylloxera outside of designated phylloxera infested zones (PIZs); and (iii) the use of phylloxera resistant rootstocks (currently in rather limited use).

Historically, phylloxera research in various countries peaks with the incidence of new detections and outbreaks rather than being sustained at a consistent level. Since the successful implementation of rootstocks as the principal phylloxera management technique in the late 19th century, the volume of literature covering the biology, ecology and alternate management options for grape phylloxera has been fairly limited.

Alternative management

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 phylloxera-infested vines early prior to the expression of physical symptoms, thereby allowing controls to be implemented rapidly and reducing the economic consequences of replanting onto resistant rootstocks. This review brings together recent developments in targeted phylloxera management options based on an interdisciplinary research approach and is structured into three principal sections: detection, quarantine and alternative phylloxera management strategies. Current and historical trends in these three emerging areas are explored, concluding with recommendations for future research.

Detection

Detection methods for grape phylloxera need to consider several factors which can affect the establishment and development of phylloxera in the vineyard environment. The first consideration is the insects' life-cycle which is influenced by genetic characteristics of both host plant and pest.

Grape phylloxera exhibits cyclic parthenogenesis and its classical life-cycle includes both asexual and sexual components (Coombe, 1963). There is debate in the literature regarding the relative expression of these two components of the life cycle and variations have been described which have been recently reviewed (Forneck & Huber, 2009). For the purposes of this review our focus will be primarily on the anholocyclic (asexual) root-galling ,which are economically the most importand, and to alesser extent leaf-galling forms.

Genetic diversity exists between different geographical regions for the two life-cycle forms. For example in Australia 83 distinct genotypes have been characterised, using six common microsatellite markers (Umina *et al.*, 2007), the majority of which are root-galling, with leaf-galling forms being less common and sporadic in their occurrence and limited in their 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 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. At the current time other than visual inspection with the naked eye and digging to examine for root symptoms (as some leaf-galling genetic strains also establish on the root system) there are no other methods being used to survey grafted vineyard areas for the presence of gallicolae phylloxera. Whilst the potential for the development of leaf imaging systems exists, it seems unlikely that this type of technology will develop because of the limited economic damage that gallicolae phylloxera strains can impart on grafted grapevines.

Detection of root feeding phylloxera is far more important economically particularly in regions where commercial plantings of ungrafted *V. vinifera* or rootstocks with some *vinifera* parentage predominate, for example in China, Armenia and Australia. Due to its predominantly subterranean habitat and relatively high economic damage several different approaches for detection of root-galling phylloxera strains have been explored.

Conventional detection

Initial above-ground indications of infestation on ungrafted *V. vinifera* are typically isolated to only a few vines principally expressing a decline in canopy vigour, followed by a gradual premature yellowing of foliage and reduced grape yield. These symptoms alone are not necessarily a strong indication of 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 phylloxera is the causative agent, depending on the level of virulence of the phylloxera genetic strain present, these symptoms become more widespread during the next two to three years or over several decades eventually leading to a decline in canopy, 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 when highly virulent phylloxera strains are present and optimal conditions for survival and development prevail.

The time-frame from initial infestation to eventual death of ungrafted *V. vinifera* has been estimated to within three to six years (Buchanan, 1990). However rates of vine decline have also been correlated to phylloxera genotype (Corrie, 2003) and in some instances where low virulent genotypes are present visual symptoms may not be evident even after 40 years (K. Powell, DPI Victoria, personal observation). Detection of phylloxera infestation can use 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 phylloxera infestation differs on ungrafted compared to grafted *V. vinifera*. Phylloxera infestation on American *Vitis* spp. rootstocks can be characterized by potential leaf-galling on rootstock suckers and development of nodosities on the young non lignified expanding root tips but no reduction in vine vigour, premature yellowing or tuberosity development on lignified roots (Buchanan & Hardie, 1978; Granett *et al.*, 2001; Granett *et al.*, 2007). However, rootstock screening conducted in glasshouse

conditions has recently confirmed the development of tuberosities on lignified roots of some rootstocks (Korosi *et al.*, 2007), further highlighting the complexity of phylloxera-host interactions.

Ground truthing

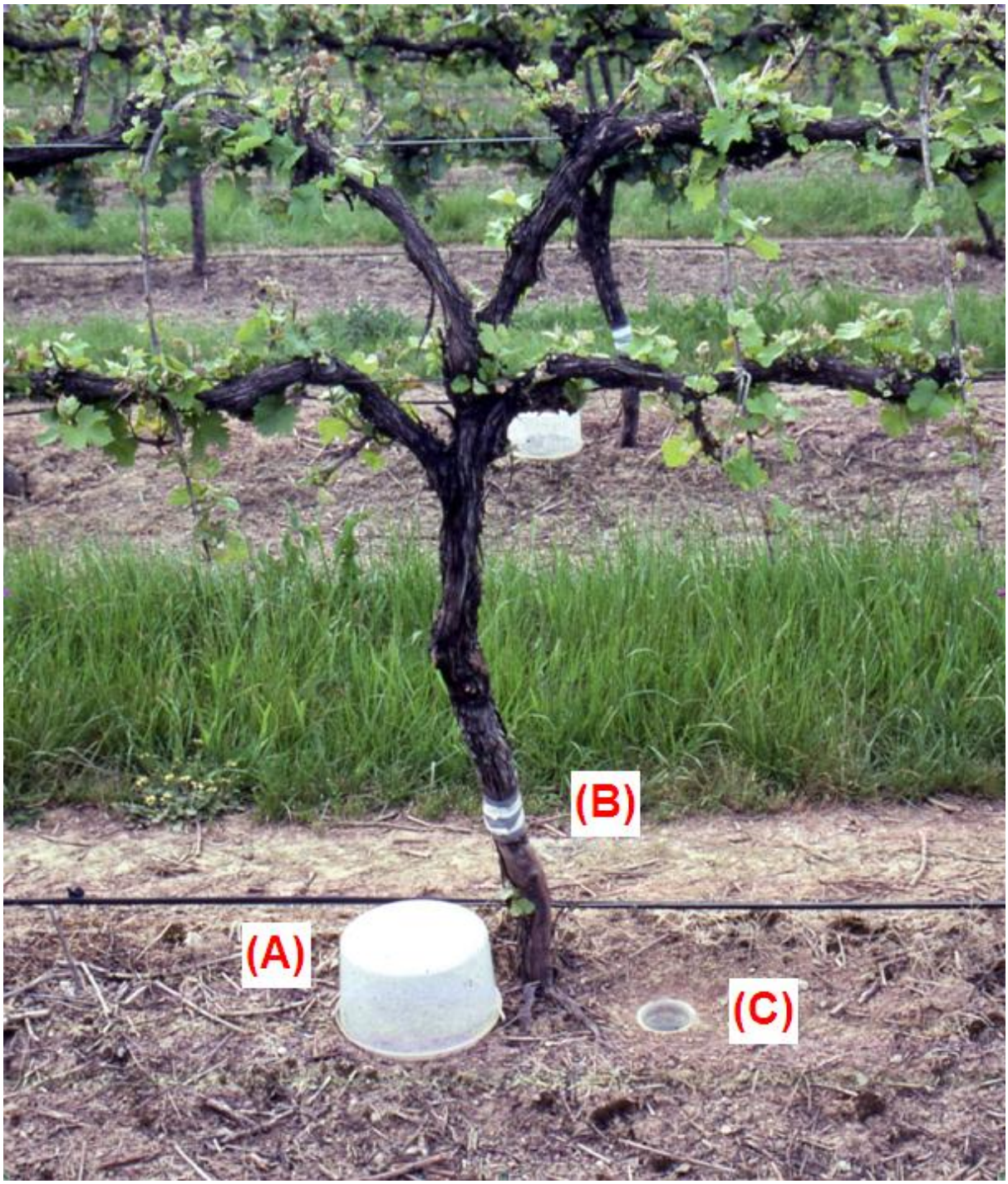
Currently conventional detection of phylloxera involves manual excavation and examination of the grapevine root system by ground survey teams for the presence of galls and phylloxera colonies, typically coinciding with peak phylloxera activity in the summer months. Ground surveys are costly and labour intensive and such activities require systematic implementation (NVHSC, 2009). A ground survey detection approach also has a number of disadvantages: (i) reliance on successful recognition of visual signs of phylloxera infestation both in foliage and on the root system which can be influenced by several site dependent variables (ii) it is usually economically unviable to sample every vine in a single vineyard or multiple vineyards as part of an area wide surveillance scheme and repeated annual surveys maybe required, (iii) surveys are climate dependent i.e. rain and high temperatures make soil excavation and identification of phylloxera on roots difficult and can, in the case of heat or drought, influence the degree of stress expressed in the canopy and also the abundance of phylloxera on the root system (iv) surveys are often conducted late to prevent spread as visual signs of decline typically do not manifest until at least two to three years after initial infestation and (v) low virulence genotypes or high virulence genotypes with low abundance often elicit no visual signs of decline at all (K. Powell, DPI Victoria, personal observation).

Novel detection and surveillance

Insect trapping

Insect emergence trapping methods were used initially as a tool to monitor population dynamics of different phylloxera strains (Figure 1) (Powell *et al.*, 2000; Herbert *et al.*, 2006). More recently the emergence trap technique has also being validated as a detection tool to monitor the spread of phylloxera populations above-ground and offer a potential method for area wide surveillance in the

future (Powell *et al.*, 2009) when combined with soil and/or vegetation mapping as part of a targeted surveillance strategy (Bruce *et al.*, 2009).



Spectral fingerprinting

The use of remote aerial and ground-based photography and spectral imaging for pest detection 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 ground surveys. It can provide area-wide coverage over short time periods, identify 'weak spots' for targeted ground surveys and allow temporal surveillance of known phylloxera infestations to determine rates of spread over consecutive seasons under different climatic and soil conditions.

There are two alternative spectral imaging approaches to phylloxera detection, multispectral and hyperspectral. Spectral fingerprinting as a means of evaluating canopy vigour and mapping patterns of leaf area as a tool in phylloxera detection has been examined (Wildman *et al.*, 1983) using multispectral colour-infra red (IR) aerial photography to observe phylloxera weak spots and predict its spread in infested *V. vinifera* vineyards. The multispectral imagery obtained allowed discrimination between phylloxera-damaged vines from that of oak-root fungus, *Amrillaria mellea* and Pierce's disease affected vines – both of which gave differing spectral signatures. Johnson *et al*. (1996) utilised near infra-red (NIR) aerial reflectance imagery in the Napa Valley, California to monitor a 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 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 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 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 temporal surveillance and ground truthing and would also be useful for temporal surveillance of known phylloxera-infested vineyards.

In contrast, hyperspectral imaging employs narrower and significantly more 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 phylloxera infestation could be detected (Renzullo *et al.*, 2004; Renzullo *et al.*, 2006). The study concluded that 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 warrrants further investigation as it may prove more effective than a multispectral approach.

Photosynthetic pigment fingerprinting

Symptoms of phylloxera presence on infested grapevines include reduced chlorophyll and increased photo-protective pigment concentration in leaves (Baldy *et al.*, 1996; Blanchfield *et al.*, 2006). 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 damaged root system (Blanchfield *et al.*, 2006). 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 integrated detection method combined with spectral imagery.

Chemical fingerprinting

Metabolomics methods and techniques 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 previously 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.

Metabolic changes induced in grapevines, as a result of phylloxera infestation, have focussed on feeding sites of both foliar (Warick & Hildebrant, 1966; Schaefer, 1972) and root-galling

phylloxera (Schaefer, 1985; Kellow *et al.*, 2004; Lawo *et al.*, 2011). Investigations of leaf gall tissue in culture 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 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 the development of a phylloxera-specific chemical fingerprint for detection purposes. In susceptible *V. vinifera* roots starch and amino acid levels change in the presence of phylloxera feeding but no evidence of a specific chemical defence response is evident (Kellow *et al.*, 2004; Du *et al.*, 2008). This is in contrast to the response in 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 by Lawo *et al.* (2011) through analysis of the volatile metabolome of the grapevine rootstock hybrid of *V. berlandieri* Planch. x *V. riparia* Fitch. The investigation identified 14 differentially expressed compounds with preliminary data suggesting the involvement of the mevalonate and/or alternative isopentenyl pyrophosphate, the phenylpropanoid and lipoxygenase plant defense related pathways stemming from phylloxera infestation.

Preliminary glasshouse and field studies examining metabolic profile shifts in areas remote (e.g. foliage *of V. vinifera*) from the point of root-feeding having been conducted using NMR methods (Tucker *et al.*, 2007). Principal component analysis of both mature and immature leaves sampled through selected vine growth stages indicated reasonable 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 extract was observed with phylloxera infestation (Tucker *et al.*, 2007). However, this appears to be a general defense response as Koussa *et al*. (2002) also observed an elevation in this ratio in *V. vinifera* infected with the fungal pathogen *Eutypa lata*. The spectra from phylloxera infested vines was 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 phylloxera-infested *V. vinifera* indicated an upregulation of the flavonoid compounds isorhamentin glycoside, rutin, kaempherol glycoside and quercetin glycoside, (Figure 2); (Benheim *et al.*, 2011). These compounds are involved in both passive and induced defensive 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 being directly attributable to 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 discovery and validation of biomarkers for phylloxera infestation compared to these stressors.

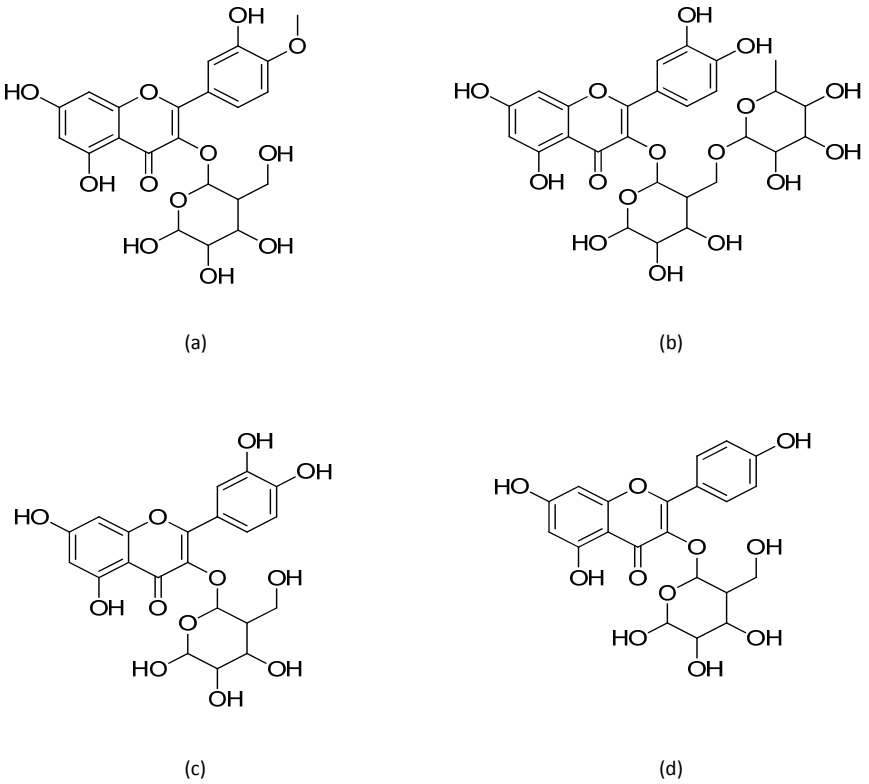


Fig. 2 Structures of previously identified flavonoid compounds from *V. vinifera* leaf extracts taken from phylloxera infested grapevines: (a) isorhamnetin glycoside (b) rutin (c) quercetin glycoside and (d) kaempherol glycoside. (Source: Benheim *et al.* 2011)

Molecular fingerprinting

Molecular methods for phylloxera detection have been explored (Herbert *et al.*, 2008b) with the development of a phylloxera-specific DNA soil probe. 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). Validation of a phylloxera-specific DNA probe as a detection tool has been undertaken under both field and laboratory conditions. Use of this technique is being studied to determine the optimal sampling strategy and to compare its efficacy with other detection techniques (Bruce *et al.*, 2011).

Soil sensing

Being predominantly subterranean 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 phylloxera (Nougaret & Lapham, 1928; de Klerk, 1972; King & Buchanan, 1986; Bruce *et al.*, 2009) and host-plant susceptibility. Phylloxera population dynamics are also influenced by soil temperatures with phylloxera unable to initiate feeding sites or develop beyond hatching at soil temperatures lower than 15-18°C (Helm *et al.*, 1991; Turley *et al.*, 1996).

Elevated soil electrical conductivity and aluminium exchange capacity are associated with areas of higher phylloxera abundance (Bruce *et al.*, 2009). The apparent electrical conductivity (ECa) is influenced by soil moisture, ionic content and salinity. 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) and soils with toxic levels have been found to be preferable to phylloxera establishment indicating a relationship between root phenology and phylloxera.

The study of soil interactions and how they may influence phylloxera establishment, development and dispersal affords great potential for the development of an *a priori* risk assessment matrix. This matrix would comprise of novel monitoring techniques such as emergence traps and DNA probes, which are capable of discerning regions of vineyards with greatest threat of infestation.

Quarantine

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

Risk vectors

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

First instar 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 illustrated an additional risk of unintentional phylloxera transfer during the harvest period (Korosi *et al.*, 2009), with possible transfer to post harvest material such as grapes and unfermented pomace or marc (a mixture of grape seeds, skins and stalks) (Powell, 2008).

Phylloxera are unable to survive composting of green waste and winery waste. Bishop *et al.* (2002) demonstrated 100% mortality of all phylloxera life-stages within three weeks in a commercial green waste composting process with temperature being the predominant factor influencing phylloxera mortality (Keen *et al.*, 2002). Korosi *et al.* (2009) recommending a period of at least four days composting of white grape marc (pomace) to remove any further risk of phylloxera transfer, based on mortality of first-instar life-stages. Furthermore, post-harvest red must fermentation (Deretic *et al.*, 2003) and fumigation of table grapes with sulphur dioxide (Buchanan, 1990) have also achieved 100% phylloxera mortality.

Quarantine boundaries

The rapid implementation of quarantine procedures upon detection of a phylloxera outbreak is crucial for the protection of other viticultural regions. Phylloxera-specific quarantine is practiced by relatively few grape-growing countries including China, Russia, the Netherlands, Armenia and Australia. One of the most detailed set of quarantine protocols has been developed in Australia where distribution of phylloxera has been limited to a few grape-growing regions (representing only 2% of production areas) despite the original detection of phylloxera dating back to 1877. Initial quarantine measures included the introduction of the Vine Disease Eradication Bill (1878) and the Vine Disease Act (1890). However, phylloxera remains a major threat to the Australian viticulture industry and National Phylloxera Management Protocols (NVHSC, 2009) for a range of risk vectors have been developed. Australian state legislation also exists and when a phylloxera infestation is reported outside existing quarantine boundaries a new phylloxera infested zone (PIZ), is declared which has a minimum five km boundary from the initial detection point (Buchanan, 1990).

Disinfestation techniques

Procedures for disinfestation of phylloxera from viticultural machinery, planting material, diagnostic materials, equipment and footwear employ either a heat-based or chemical-based treatment. 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 hours (Korosi *et al.*, 2009; NVHSC, 2009; Korosi *et al.*, 2011) and has recently been shown to be effective against at least two root-galling phylloxera strains (Korosi *et al.*, 2011). Heat treatment of soil samples for diagnostic purposes is also recommended where samples are dispatched from a phylloxera-infested region to a processing laboratory for general diagnostic testing (NVHSC, 2009).

Handheld horticultural equipment and footwear can be disinfested with a 2% NaOCl solution for a minimum of 30 seconds (recommended practice prior to entering and or leaving phylloxera infested vineyards) as this has been shown to be effective (Dunstone *et al.*, 2003). Disinfestation of planting material (vine cuttings) necessitates hot water treatment (EPPO, 2009; NVHSC, 2009; Powell *et al.*, 2009) or methyl bromide fumigation (Sakai *et al.*, 1985). Methyl bromide can however 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, due to its impact on the ozone layer, except for allowable exemptions including the Quarantine and Preshipment exemption, to eliminate quarantine pests. It is unlikely that methyl bromide will be used extensively in the future for 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 in the disinfestation of both grapes and 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 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 phylloxera disinfestation has yet to be fully exploited.

Disinfestation treatments for winery waste have also been developed including composting of grape pomace (Korosi *et al.*, 2009), fermentation of must (Buchanan *et al.*, 1996) and cold treatment of unfiltered white juice (K. Powell, DPI Victoria, unpublished data). Ultimately however effective such disinfestation treatments may be, they rely on adoption by the whole industry and any circumvention of the process is likely to lead to quarantine breakdown.

Alternative management

Post detection may offer a number of potential options for phylloxera management which could be implemented. Although removal of infested grapevines and replanting with phylloxera resistant rootstocks is the predominant method currently employed other options may potentially be useful in the short-term depending on the level of phylloxera infestation, genotypic characteristics of both host plant and phylloxera, extent of damage identified in the initial detection period and predicted rate of spread.

Biological control

Biological control of phylloxera has been subjected to limited research activity, compared 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 its successful application (Bernard *et al.*, 2007).

In 1873, Charles Riley identified and introduced a predatory mite *Tyroglyphus phylloxera* to France to control the spread of grape phylloxera (Riley, 1881; Gullan & Cranston, 2010). Riley's attempts were unsuccessful (Kirchmair *et al.*, 2009). There have been some historical reports of natural predators of grape phylloxera in the literature (Anon., 1881), including the millipede *Polyxenus lagarus* (Haller, 1878) and lacewings *Chrysopa* sp (Riley, 1875). However most only describe initial observations of predatory behaviour and their effectiveness in controlling large populations of phylloxera has been questioned (Mayet, 1890). No recent work on the efficacy of these predators has been published. More recently, Wheeler & Henry (1978) noted predatory behaviour of *Ceratocapsus modestus* targeting gallicole grape phylloxera. There followed other observations of the coccinellid *Scymnus cervicalis* Mulsant preying on gallicole grape phylloxera in the USA (Wheeler & Jubb, 1979). Predators have also been described for other phylloxerids (von Fulmek, 1857; Jancke, 1954).

Although published evidence of natural predators of 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 beneficial predators.

Entomopathogens

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 1930's (Glaser, 1932). Entomopathogenic nematodes have been tested against radicicolae grape phylloxera in laboratory-based trials (English-Loeb *et al.*, 1999) with marginal success. Of the nematodes examined, the Oswego strain of *Heterohabditis bacteriophora* Poinar (Hb Oswego) used in petri-dish trials reduced phylloxera populations up to 80% when compared to the experimental controls. In soil-cup trials the suppressive action of Hb Oswego was significant, but found to be dependent on a high levels of moisture (>13% wt:wt) and nematode density (>15,000/g soil). In addition, no evidence was observed as to the ability of Hb Oswego to reproduce once it had infected its phylloxera host and coupled with its application difficulties this rendered it commercially unviable.

Fungi

Entomopathogenic fungi have been widely used in agriculture, forestry and land management stemming from interest in 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, *Beauvaria bassiana* (Balsamo) Guillemin (white muscardine) and *Metarhizium anisopliae* (Metschnikoff) Sorokïn (green muscardine), have been the focus of research due to their broad-based distribution and being natural enemies of many insect species (Meyling & Eilenberg, 2007). *Beauvaria bassiana* has shown

successful 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 phylloxera control agent, with 80% of treated samples demonstrating an absence of fresh phylloxera infections, compared with untreated controls showing new nodosity formation and continued population increase. *In situ* examination of infected phylloxera to quantify infected insects is difficult because bioassays confirm that *M. anisopliae* kills and mummifies the insect (Kirchmair *et al.*, 2004; Kirchmair *et al.*, 2009). Field trials have used applications of a commercial formulation of *M. anisopliae,* Granmet® (Kwizda Agro GmbH, Austria & Agrifutur s.r.l., Italy). This treatment reduced population abundance of phylloxera two years post-application but reduced persistence of the treatment to negligible levels three years after application (Kirchmair *et al.*, 2007), thus indicating repeated applications would be required to reduce phylloxera to manageable levels. *Paecilomyces farinosus* (Holm & Gray) has also been tested against phylloxera (Goral *et al.*, 1975) with reported success, but has not been investigated further.

The selection of suitable virulent strains of entomopathogenic fungi, or their toxic metabolites, which may be specific to grape phylloxera is of upmost importance, yet the vast majority of literature has focussed purely on inoculation with these fungal agents as opposed to investigations into their ecology and specificity.

Chemical control

The existence of a chemical control option which is effective against both leaf-galling and root-galling phylloxera, despite several studies, still remains elusive. To-date a range of chemical options have been examined (Table 1). Although many insecticides have reported success in suppressing phylloxera populations, their registration as phylloxera control agents worldwide is limited. Only four insecticides: imidacloprid, acetamiprid, fenopropathrin and spirotetramat are currently registered against the foliar form in the USA (Johnson *et al.*, 2009), Europe and South Africa. In contrast as yet, no insecticide treatments for controlling either the root or leaf form of grape phylloxera have been registered for use in Australia.

Compound Class	Active Ingredient (Trade name)	Trial Location	Trial Type	Phylloxera Type	Source
	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 & Organophosphates	**Carbofuran**	USA, Australia	Field & Laboratory	Radicicolae	Rammer, 1980; Granett et al., 1986; Buchanan et al. 1990
	Fenamiphos	Germany, USA & Australia	Field	Radicicolae	Homeyer & Wagner, 1981; Buchanan et al. 1990; de Klerk, 1979
	Phosphorothioic acid	Canada	Field	Radicicolae	Stevenson, 1968
	o-isopropoxyphenyl methylcarbamate	Canada	Field	Radicicolae	Stevenson, 1968
	Disulfoton	South Africa & Canada	Field	Radicicolae	Stevenson, 1968; de Klerk, 1979
	Oxamyl	Australia	Field	Radicicolae	Buchanan and 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 & Laboratory	Radicicolae	Cox *et al., 1960*
	Endosulfan	USA & Canada	Field	Gallicolae	Stevenson, 1970; Williams, 1979
Neonicotinoids	**Thiamethoxam**	Australia & USA	Laboratory	Radicicolae	Granett et al., 2001; Nazer et al., 2006; Herbert et al., 2008
	Imidacloprid	South Africa, Jordan, USA and Australia	Field & Laboratory	Radicicolae & 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

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

Soil fumigants

In France in the 1870's the use of the soil fumigant carbon bisulphide (*aka* carbon disulphide CS_2), was first examined for phylloxera control. Initial trials of CS_2 proved unsuccessful as its application killed both the host plant and phylloxera. Subsequent trials at lower concentrations showed some initial control effect, but were found ineffective as phylloxera 'reappeared' most likely due to either hibernation on the roots or failure of CS_2 to effectively penetrate the full depth of the grapevine root zone. Phylloxera has been detected to depths of over 1m in the soil profile in Australia (Buchanan, 1990) and South Africa (de Klerk, 1974).

An aqueous solution of sodium tetrathiocarbonate which releases CS_2 on breaking down in soil, has also been used with limited success in California for management of grape phylloxera (Weber *et al.*, 1996). However, CS_2 is a potent neurotoxin and is flammable and explosive in air at given concentrations and therefore its use for phylloxera control is currently considered impractical due to safety and environmental concerns.

Chemical insecticides

Carbamates and organophosphates

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) has been investigated as a potential control option for phylloxera. Granular formulations when applied to soils in California, USA significantly reduced phylloxera abundance (Rammer, 1980). The reduction, as determined through examination of infested excised roots removed from the field post-application was achieved regardless of soil texture, moisture and pH.

Population dynamics studies highlight the production of large numbers of first instars during the early spring following hibernation in the winter months. First instars have been shown to be the most susceptible life-stage in several insecticide trials (Rammer, 1980; Granett *et al.*, 1986; Buchanan & Godden, 1989; Herbert *et al.*, 2008a). Although when using carbofuran the mortality of first instars on untreated control vines was relatively high, data indicated a statistical trend toward

higher mortality on treated vines, with other life stages (egg, intermediate nymphs and adults) having a lower sensitivity to treatment. In addition, phylloxera that survived sub-lethal concentrations showed little if any deviation from standard developmental times, long-term survival and fecundity (Granett *et al.*, 1986). Field studies conducted in Australia confirmed carbofuran to be more effective than oxamyl, aldicarb and fenamiphos, significantly reducing the phylloxera population by up to 99% and improving grape yield and vine growth (Buchanan & Godden, 1989). However the effectiveness of carbofuran on plant response as a function of vine yield and vigour of phylloxera infested vines has not been directly correlated. Phosphorothioic acid (O,O-diethyl O-2 pyrazinal phosphorothioate) and o-isopropoxyphenyl methylcarbamate are widespread in their agricultural usage worldwide. Field studies using *V. labrusca* and *V. labrusca* x *V. riparia* hybrid cultivars demonstrated successful control of phylloxera with drench treatments of phosphorothioic acid and o-isopropoxyphenyl methylcarbamate (Stevenson, 1968). The effect on phylloxera was indirectly measured as a function of mean galls per dry weight of roots as compared to an untreated control, with both agents significantly reducing the mean gall/dry weight of roots. However, because these experiments did not include a phylloxera-susceptible ungrafted *V. vinifera* control there effectiveness in severely infested ungrafted vineyards is yet to be determined.

Phenamiphos (N-[ethoxy-(3-methyl-4-methylsulfanylphenoxy)phosphoryl]propan-2-amine) although primarily used as a nematicide, also has insecticidal properties. In its granular formulation field trials were conducted on phylloxera-infested ungrafted *V. vinifera* in Australia and South Africa (de Klerk, 1979; Buchanan & Godden, 1989). In both studies, it was ineffective at controlling radicicolae phylloxera. De Klerk (1979) cited minimal effect in the first season on ungrafted *V. vinifera*, followed by no observed impact for the second, third and fourth seasons of the trial. Buchanan and Godden (1989) observed similar results over four consecutive seasons.

Disulfoton (diethoxy-(2-ethylsulfanylethylsulfan)-sulfanylidenephosphorane) is a granulated systemic insecticide commonly used to control several Hemipteran pests (Hurej & Dutcher, 1994). Its efficacy against radicicolae phylloxera control was tested in South Africa (de Klerk, 1979) and caused substantial decreases in abundance after a single season but the effect was negated by the third season.

In Canada (Stevenson, 1968) when applied on phylloxera infested *Vitis* hybrids showed no observable effect on radicicolae abundance.

Oxamyl (N, N-dimethyl-2-methyl-carbamoyloximino-2-(dimethylthio) acetamide) a broad spectrum systemic insecticide with low residual effects is used in either granular or liquid formulations. In Australia under field conditions its affects on radicicolae phylloxera were tested using both soil incorporated granules and foliar applications (Buchanan & Godden, 1989). The granular formulation applied in spring showed reasonable effectiveness over the course of initial sampling periods, but failed to supress phylloxera populations sufficiently over the course of a season after a single treatment. Foliar treatments were applied six times during the season and displayed systemic activity, yet yielded comparable results to the granulated form with a noticeable reduction in vine vigour and yield, suggestive of phytotoxic effects (Buchanan & Godden, 1989).

Aldicarb (2-methyl-2-(methylthio) propanal O-(N-methylcarbamoyl) oxime) is a highly toxic carbamate nematicide, with secondary use for some insect orders including Hemiptera (BCS, 2011b). In Australia aldicarb presented similar results to that of oxamyl (Buchanan & Godden, 1989) indicating a dependence on multiple applications. It lacks residual activity against radicicolae phylloxera (Loubser *et al.*, 1992), and its potential use as a long-term solution in phylloxera management is also problematic due to a high acute mammalian toxicity level (Risher *et al.*, 1987) and high residue levels in grapes (Buchanan & Godden, 1989).

Sulphocarbonates

Historically sulphocarbonates have been trialled against phylloxera. On exposure to carbon dioxide in air and soil, sulphocarbonate salts break down into CS_2 and H_2S (hydrogen bisulphide), both of which had been proven harmful to phylloxera at specified application rates. Sulphocarbonate salts of sodium, barium and potassium were assessed for their effectiveness in France in 1874-75 (Ordish, 1987). Of the three compounds tested, potassium sulphocarbonate was the most successful with laboratory tests demonstrating that at high concentrations, phylloxera mortality reached up to 100% 15mins post-application. However, their use was deemed uneconomic due to the cost of large

quantities of water necessary to transport the salt to a sufficient soil depth to affect phylloxera on the grapevine root system (Ordish, 1987; Campbell, 2004).

Organochlorines

Hexachloroalkadienes are fumigant insecticides which have been tested for the control of the radicicolae grape phylloxera. Prinz (1964) reported 100% phylloxera control with one application over a period of three years. Slonovskii (1971) observed an additional two to four years of total control with a similar single application rate, resulting in improved vine vigour and yield (Slonovskii, 1971).

Hexachlorobutadiene (HCBD), has been investigated for phylloxera control in South Africa (de Klerk, 1979) and Russia (Litvinov, 1982). Phylloxera control levels varied for both ungrafted and grafted *V. vinifera* with no measurable improvements in yield and vigour as a function of treatment concentration. In 1975, 600-800 tonnes of HCBD were used specifically as a soil fumigant for phylloxera control in the former USSR (Brown *et al.*, 1975). Further observations to this effect were also noted in Argentina, where total control was reportedly obtained for two years at various application rates (Vega, 1972). However it should be noted that in all aforementioned cases the species of *Vitis* used or the phylloxera genetic clone in their respective trials was not reported.

Hexachlorocyclopentadiene (HCCPD) has been tested against radicicolae phylloxera in the field. HCCPD was found to be toxic toward phylloxera and was trialled for its phylloxera-suppression efficacy on *V. labrusca* (cv. Concord) vines in field and laboratory-based trials (Cox *et al.*, 1960). Field trials showed a high level of population suppression, recording a zero incidence of phylloxera in one instance.

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide) is highly toxic and persistent. Trials against radicicoliae phylloxera on *V. labrusca* x *V. riparia* hybrids (Stevenson, 1968) had no suppressive effect on phylloxera. Foliar sprays of endosulfan proved effective in controlling gallicolae phylloxera and reduced subsequent leaf-gall formation on three grafted grapevine cultivars (Stevenson, 1970). Similar investigations

showed a mean leaf gall reduction of 89% over three seasons on *V. labrusca* x *V. riparia* rootstock hybrids (Williams, 1979). Although the ability of endosulfan to suppress the gallicolae form of phylloxera is apparent evident, published evidence of its suppressive action against radicicolae forms of phylloxera on *V. vinifera* is lacking.

Historically DDT (1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane) has also been assessed for its efficacy against phylloxera (Simmons *et al.*, 1951). The use of DDT as an insecticide is now banned worldwide.

Acetylcholenisterase inhibitors

Acetylcholenisterase inhibitors, which include the neonicotinoids, prevent the rapid hydrolysis of acetylcholine (ACh), a neurotransmitter in nerve synapses which controls the motor division of the somatic nervous system. This results in excess ACh production causing accumulation at the nerve synapses and myoneural junctions, leading to convulsions, a loss of voluntary muscular action (tremors, twitching), paralysis and eventual death (Opperman & Chang, 1991; Herbert *et al.*, 2008a). The majority of carbamate and organophosphorous insecticides mentioned in the previous section also act as ACh inhibitors.

Thiamethoxam (3-[(2-chloro-1,3-thiazol-5-yl) methyl]-5-methyl-*N*-nitro-1,3,5-oxadiazinan-4-imine) and imidacloprid (*N*-[1-[(6-chloro-3-pyridyl) methyl]-4,5-dihydroimidazol-2-yl] nitramide) are both systemic neonicotinoids. Both of these upwardly and downwardly mobile insecticides have been shown to markedly suppress populations of phylloxera in some, but not all, field trials and also in laboratory and glasshouse trials (Botton *et al.*, 2004; Al-Antary *et al.*, 2008; Herbert *et al.*, 2008a; Johnson *et al.*, 2008).

In Brazil, field-based trials conducted on leaf-galling phylloxera damage on the rootsock 1103 Paulsen was reduced by 90% with applications of thiamethoxam and imidacloprid (Botton *et al.*, 2004). Thiamethoxam and imidacloprid were assessed for their efficacy at suppressing egg hatch rate and increasing first instar mortality of a highly virulent single clonal lineage (genotype G4) of

radicicolae phylloxera both *in vitro* and *in planta* on *V. vinifera* roots (Herbert *et al.*, 2008a). *In vitro* studies demonstrated a 30% decrease in egg hatch rate using imidacloprid with no significant decrease in hatch rate observed for thiamethoxam. However both thiamethoxam and imidacloprid increased crawler mortality with increasing concentration. *In planta* glasshouse trials demonstrated a significant decrease in phylloxera population on the root system. Insecticide treatments had no substantial influence on egg hatch, yet crawler and later life stage abundance was reduced by the treatments, with imidacloprid displaying superior effectiveness over thiamethoxam with more than 90% reduction in abundance of crawler and later life-stages over a 143 day infestation period. Both treatments also resulted in an increase in leaf area and root mass and hence an enhancement of overall grapevine vigour.

No field trials of these compounds have been undertaken against any single phylloxera clonal lineages to investigate their potential for phylloxera population suppression as a means of vine maintenance or effective quarantine control over newly infested vineyards. In addition, investigations into the effect of these insecticides on grape quality, potential residue levels, safety for consumption and any residual effects in the xylem of *V. vinifera* have yet to be conducted.

Spirotetramat, a relatively new systemic insecticide, 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, 2008; van Steenwyk *et al.*, 2009; Johnson *et al.*, 2010; Sleezer *et al.*, 2011). Spirotetramat has also recently been registered for use against grape phylloxera in Canada (BCS, 2011a).

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 gallicolae phylloxera are frequently observed to have high efficacy. In direct contrast to radicicolae phylloxera, gallicolae phylloxera are completely exposed on the leaf surface and form fibrous galls that offer limited protection from predators and application of foliar sprays. The chemical management of radicicolae phylloxera presents a considerably more complex challenge which can be placed into five distinct categories: (i) insecticide mobility (ii) insect habitat (iii) environmental interactions (iv) host plant phenological traits and (v) environmental toxicity insecticide withdrawal.

(1) Insecticide mobility

The efficacy of chemical insecticides against both radicicole and gallicolae grape phylloxera requires careful selection and depends greatly on many factors. The agent(s) must be ideally systemic, have adequate interaction between it and the insect, long-lasting residual activity in the soil, and both upward and downward mobility (Granett *et al.*, 2001; Herbert, 2005; Powell, 2008).

(2) Insect habitat

Radicicolae phylloxeras' subterranean habitat affords a natural protection from non-systemic foliar sprays and low-dispersive soil drench treatments. Phylloxera reproduce rapidly, have a high fecundity, with many overlapping generations and have been found to survive several metres into the soil (de Klerk, 1974; Buchanan, 1990). The variation in distribution and concentration of roots as a function of vine type and soil composition can lead to a greater potential for phylloxera dispersal in and around the root zone and above-ground (Powell, 2008). An effective chemical management solution would need to incorporate a high level of dispersion, be sufficiently downwardly mobile with high level diffusion into the soil in order to interact with maximum numbers of phylloxera. In addition, it must target specific developmental stages. First instars, were found to be more susceptible to carbofuran than that of egg, intermediate nymphal instars and adult stages (Granett *et al.*, 1986). Since first instars represent the most active and mobile life-stage, through optimal application timing the effective suppression of this dispersive stage would markedly aid in lowering the risk of infestation of either neighbouring vines or uninfested vineyards and reducing economic damage. Previous reports suggested that radicicolae phylloxera populations are more easily established in clay loam soils (Nougaret & Lapham, 1928; Granett & Timper, 1987). As a result, the crucial phylloxera-insecticide interaction may be severely hindered through surface applied agents due to restrictions in depth penetration, and non-uniform distribution of the insecticide (Hathaway, 1999). However, recent studies (Chitikowski & Fisher, 2005) indicated that although some soil compositions may hinder population growth, the establishment of phylloxera colonies is independent of all soil composition.

(3) Environmental interactions

Environmental factors such as temperature (both in soil and air), soil type, rainfall and humidity play fundamental roles in insecticide efficacy. For instance, soil composition (specifically, soil layers; e.g. silt, sand and clay) has been noted to affect the bioactivity of the insecticides heptachlor, DDT, diazinon, parathion and diethylcarbamazine (Harris, 1966). Chlorinated hydrocarbons such as DDT and dieldrin have been observed to 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 the organophosphorous compounds chlorpyriphos, quinalphos and endolsulfan (Satpute *et al.*, 2007). In the case of endosulfan, increased toxicity was achieved by combination of high relative humidity and temperature.

(4) Host plant toxicity

Phytotoxicity is a common secondary effect imparted by numerous insecticides, commonly resulting in reduction of yield, damage to foliage and shoots (e.g. leaf burn) (Boutin, 2002). Although there is limited evidence to conclude that commonly used insecticides in viticulture are phytotoxic the organocarbamates, carbofuran and aldicarb, have been shown to exhibit phytotoxic effects on a range of crops (Singh & Maheshwari, 1989). Endosulfan is phytotoxic to several sulphur-sensitive grapevine cultivars (Johnson *et al.*, 2009). Exposure of grapes (and other crops) to chemical treatments in agricultural practice has resulted in the establishment of Maximum Residue Limits (MRLs) for agrochemicals, set through government legislation in numerous countries (AWRI, 2011).

(5) Environmental toxicity and insecticide withdrawal

The environmental toxicity of some synthetic insecticides has been well documented and has lead to numerous 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, the Australia placed a total ban on the use of endosulfan due to health and environmental concerns (Cubby, 2010). For example endosulfan is well recognized as an endocrine system disruptor, with links to juvenile attention deficit disorder and Parkinson's disease in men (Dayton, 2010).

In some countries other insecticides previously tested against phylloxera are also either banned, under restricted use or under review for potential withdrawal including fenamiphos (APVMA, 2003; EPA, 2008), disulfoton (EPA, 2006; APVMA, 2011); aldicarb and HCCPD derivatives.

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

Cultural management

Cultural management of grape phylloxera has been explored as an alternative management method to the use of rootstocks, in light of some reports of a failure of rootstock resistance to phylloxera (Porten *et al.*, 2000; Granett *et al.*, 2001). One of the earliest examples of cultural management was flooding of vineyards during the winter months as a potential option for eradication (Riley, 1875; Hilgard, 1876). This submersion method caused a noticeable increase in the vigour of all plants, yet could only work on non-permeable soils. Flooding is also highly uneconomical, as it requires access to large quantities of fresh water and subsequently fertilizer to replace the water-soluble nutrients drawn away during the treatment. Flooding is still used as a means of controlling phylloxera numbers in southern France (Campbell, 2004). However, in laboratory trials phylloxera first instars and eggs have been shown to survive for up to 10 days when submersed in water (Korosi *et al.*, 2009), indicating they are quite resilient to submersion. Efficacy of submersion treatment is also influenced

by temperature and phylloxera life-stage with temperatures of ≤5°C reducing survival of egg and crawler stages (Korosi *et al.*, 2009).

Soil type has been shown to have an effect on the establishment and population dynamics of phylloxera. Soil characteristics such as low aluminium exchange capacity and acidic pH are associated with high phylloxera abundance above and below ground 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 phylloxera infestation enhanced as a function of low nutrient availability (i.e. phosphorous, magnesium, copper, zinc and potassium) (Reisenzein *et al.*, 2007).

Soil amendments such as composted green waste (Powell *et al.*, 2007b) and composted winery waste mulches (Powell *et al.*, 2007a) have been investigated as potential phylloxera management options in vineyards in Australia. The annual application of composted green waste over three consecutive growing seasons resulted in a significant increase in the abundance and dispersal of above-ground first instar phylloxera, with no improvement to vine vigour, grape yield and quality or pruning weight. Some formulations of composted winery waste consisting of grape marc or pomace were shown to considerably reduce phylloxera emergence above ground when compared to untreated vines (Powell *et al.*, 2007a). Both studies had limited effect on first instar and total life-stage abundance below-ground when compared to controls but have implications for increasing the risk of phylloxera transfer above-ground on viticultural machinery and vineyard personnel clothing.

In a survey comparing conventionally managed vineyards (CMV) and organically managed vineyards (OMV) CMV's showed a strong correlation between phylloxera population density and root necrosis initiated by secondary fungal pathogens (Granett *et al.*, 2001), whereas OMV's showed reduction in necrosis with similar phylloxera population numbers as those observed in the conventional farming study. This disparity was possibly attributed to either microbial ecology, pathogen suppression by discrete soil characteristics or the induction of systematic acquired resistance which if validated, could be of great significance in understanding the intrinsic defence mechanisms of *Vitis* towards grape phylloxera.

Genetically modified vines

Although several opportunities exist for the development of genetically modified grapevines for pest resistance (Viss & Driver, 1996), this research area is still largely unexplored in the case of insect pests of the grapevine. 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 limited research has focussed on the potential modification of grapevine to resist 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 sites in the insects gut and also have feeding deterrent properties (Kingston *et al.*, 2005; Powell, 2008). One particular group of lectins, which are mannose-binding, includes the 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) which 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 phylloxera are feasible, following the development

of *in vitro* artificial diet systems for both gallicolae (Forneck & Wöhrle, 2003) and radicolae phylloxera (Kingston *et al.*, 2007), but no progress in this area has been reported.

A single *in vitro* study using transgenic *Vitis* species was conducted to assess inducible defence against radicicolae grape phylloxera (Franks *et al.*, 2006). Three *Sorghum bicolour* genes expressing biosynthesis of cyanogenic glycoside were transferred to *V. vinifera* L., producing one hairy root transgenic line capable of releasing cyanide on maceration. Both cyanogenic and acyanogenic lines proved unsuccessful in reducing phylloxera development. The successful application of transgenic technologies to phylloxera remains very much uncharted territory and requires significant attention particularly as grape phylloxera is a monophagous pest and potentially more amenable to this type of application than 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 and, in the case of aphids, jasmonic acid and salicylic acid pathways have been shown to be involved (Moran & Thompson, 2001; de Ilarduya *et al.*, 2003; Girling *et al.*, 2008) in resistance. Jasmonic acid has been implicated as a potential resistance mechanism for 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 above-ground foliage pests (Powell & Pickett, 2003) pests rather than below-ground herbivores, such as radicicolae phylloxera. The pathways which may be induced when phylloxera attacks the root system have received minimal attention. Exploiting natural plant defence systems may provide a future opportunity to enhance resistance to grape phylloxera through both conventional breeding and introducing foreign genes through genetic manipulation. However because of the genetic diversity of grape phylloxera some resistance mechanisms may only be effective against some phylloxera 'biotypes'.

Eradication

There are no countries in the world where phylloxera has been successfully eradicated. Risks of reintroduction are inevitable if quarantine procedures are not strictly adhered to. Following the discovery of 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 phylloxera was found in the Bendigo district, and again a policy of eradication was followed. During the late 1890s, however, phylloxera was discovered at Heathcote in the Goulburn Valley, in replanted vineyards at Geelong, and in the Rutherglen district (Buchanan *et al.*, 2011). In Australia all eradication attempts proved ultimately unsuccessful (Buchanan & Hardie, 1978).

Removal of grapevines from infested regions was also the predominant recommended 'eradication' method in Europe (Börner & Schilder, 1934). In China phylloxera was first reported in 1893 and reportedly 'disappeared' during the Cultural Revolution, when vineyards were removed and replaced by food crops, but remerged in several provinces during 2006-2007 (Du *et al.*, 2011). All attempts at eradicating grape phylloxera worldwide have thus far been unsuccessful.

Any attempt at eradication of phylloxera involve a concerted multidisciplinary research approach to understand the fundamental interactions between environment, pest and host-plant are researched and clearly understood and implementation of quarantine protocols to prevent reinsfestation.

Concluding remarks

A range of alternative approaches for phylloxera control are potentially available or at the very least warrant further investigation before they can be utilised in an integrated management approach. Chemical treatments in all forms have been thus far unable to create an effective alternative for phylloxera control particularly in ungrafted *V. vinifera* vineyards. Although insecticides such as imidacloprid and spirotetramat have shown suppression of phylloxera in laboratory, glasshouse and field based trials(Herbert *et al.*, 2008a), the use of such agents under field conditions introduces

multiple variables which impact on their efficacy. The subterranean habitat of phylloxera in conjunction to consideration of soil type, climatic conditions, vine cultivar, method and rates of application all bear great influence over the degree of suppression attained. The final, and arguably, most important factor is the environmental and personal impact of insecticides. Sulphocarbonates, hexchlorobutadiene and endosulfan have all proven effective against phylloxera under field conditions, yet due to very high toxicity, carcinogenicity and phytotoxicty respectively, their agricultural use is now greatly restricted. An effective chemical treatment against radicicolae grape phylloxera requires excellent chemical-phylloxera interaction, a systemic mode of activity, downward mobility, ease of diffusion through the soil and have high residual activity which would not impact on the final commodity product. Currently, a fully effective chemical-based mode of phylloxera control is unavailable.

Cultural management strategies are classically used as control methods rather than for prevention or eradication. Such strategies aim at creating crop habitats that interfere with or disable the reproductive potential of pests through an understanding of their life-cycle and biological requirements and how they relate to cultural management practice. 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 phylloxera resistant rootstocks currently presents a very effective means of long-term management following a phylloxera outbreak and as protection in case of phylloxera incursion. However, reports of collapse in rootstock resistance to phylloxera are increasing (Porten *et al.*, 2000; Granett *et al.*, 2001). The continued development of highly resistant rootstock hybrids, either through conventional breeding or genetic modification, and a coordinated international approach to phylloxera resistance screening is required. Conventional breeding needs to consider the broad genetic diversity of phylloxera to ensure 'biotypes' do not occur which could result in future rootstock failures. The breeding of genetically modified rootstocks presents a significant opportunity to develop 'broad spectrum' and genotype-specific rootstocks possessing genetic traits that render them unfavourable hosts for grape phylloxera. In addition, the

potential risk of collapse of resistance to grape phylloxera in engineered rootstocks would be lessened in some phylloxera-infested regions due to the presence of single genetic strains or a low incidence of recombinant genotypes which lack sexual reproduction among grape phylloxera populations.

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 radicicolae grape phylloxera the range of potential predatory insects may be restricted to fungi, nematodes and other soil-borne pathogens. Although some successes have been observed using entomopathogenic fungi (Kirchmair *et al.*, 2004), the use of biological control for grape phylloxera control is a significantly promising area still necessitating further research.

The continued development of early detection techniques such as the DNA soil probe and, spectrochemical fingerprinting for phylloxera infestation and the use of metabolomics in chemical biomarker discovery are an important future research focus. These technologies may ultimately be included in an integrated approach for rapid detection and rapid management intervention to reduce the risk of further quarantine breakdown and minimise economic loss.

Acknowledgements

The authors acknowledge the support of the Grape and Wine Research and Development Corporation, Dried Fruits Australia, the Phylloxera and Grape Industry Board of South Australia and the Department of Primary Industries, Victoria who provide funding for phylloxera research activities in Australia.

References

Al-Antary T. M., Nazer I. K., Qudeimat E. A. (2008) Population trends of grape phylloxera, *Daktulosphaira* (*Vites*) *vitifoliae* Fitch. (Homoptera: Phylloxeridae) and effect of two insecticides on its different stages in Jordan. *Jordan Journal or Agricultural Science,* **4**, 343-349.

Al-Bachir M. (1999) Effect of gamma irradiation on storability of two cultivars of Syrian grapes (*Vitis vinifera*). *Radiation Physics and Chemistry,* **55**, 81-85.

Anon. (1881) *Annual Report of the Board of State Viticultural Commissioners, California*. Ed E. Bosque. San Francisco, California.

APVMA (2003) *Fenamiphos* [WWW Document]. URL. http://www.apvma.gov.au/products/review/current/fenamiphos.php [Accessed on 2 March 2011].

APVMA (2011) *Priority 4 Chemicals nominated for review* [WWW Document]. URL. http://www.apvma.gov.au/products/review/nominated/priority_4.php [Accessed on 11 April 2011].

Atkins S. D., Manzanilla-López R. H., Franco J., Peteira B., Kerry B. R. (2005) A molecular diagnostic method for detecting *Naccobus* in soil and in potato tubers. *Nematology,* **7**, 193-202.

AWRI (2011) *Maximum Residue Limits (MRLs).* [WWW Document]. URL. http://www.awri.com.au/industry_support/viticulture/agrochemicals/mrls/ [Accessed on 7 April 2011].

Baldy R., De Benedictis J., Johnson L., Weber E., Baldy M., Osborn B., Burleigh J. (1996) Leaf colour and vine size are related to yield in a phylloxera-infested vineyard. *Vitis,* **35**, 201-205.

Barlow F., Hadaway A. B. (1955) Studies on aqueous suspensions of insecticides. Part V. The sorption of insecticides by soils. *Bulletin of Entomological Research,,* **46**, 547-559.

BCS (2011a) *Movento* [WWW Document]. URL. http://www.bayercropscience.ca/products/insecticides/movento/ [Accessed on 7 April 2011].

BCS (2011b) *Aldicarb* [WWW Document]. URL. http://compendium.bayercropscience.com/BAYER/CropScience/CropCompendium/BCSCropComp.nsf/id/aldicarb.htm [Accessed on 7 April 2011].

Benheim D., Rochfort S., Ezernieks V., Powell K., Korosi G. A., Robertson E., Potter I. D. (2011) Early detection of grape phylloxera (*Daktulosphaira vitifoliae* Fitch) infestation through identification of chemical biomarkers. *Acta Horticulturae,* **904**, 17-24.

Bernard M., Horne P. A., Papacek D., Jacometti M. A., Wratten S. D., Evans K. J., Herbert K. S., Powell K. S., Rakimov A., Weppler R., Kourmouzis T., Yen A. L. (2007) Guidelines for environmentally sustainable winegrape production in Australia: IPM adoption self-assessment guide for growers. *Australian and New Zealand Grapegrower and Winemaker*, 24-27.

Blanchfield A. L., Robinson S. A., Renzullo L. J., Powell K. S. (2006) Phylloxera-infested grapevines have reduced chlorophyll and increased photoprotective pigment content - can leaf pigment composition aid pest detection? *Functional Plant Biology,* **33**, 507-514.

Börner C. F., Schilder A. (1934) Die verbreitung der Reblaus in Deutchland nach dem Stande des Jahres. *Revue of Applied Entomology,* **A22**, 629.

Botton M., Ringenberg R., Zanardi O. (2004) Chemical control of grape phylloxera *Daktulosphaira vitifoliae* (Fitch, 1856) leaf form (Hemiptera: Phylloxeridae) on vineyards. *Ciencia Rural,* **34**, 1327-1331.

Boubals D. (1966) Etude de la distribution et des cause de la resistance au Phylloxera radicicole chez les Vitacees. *Annales de l'Amelioration des Plantes,* **16**, 145-184.

Boutin C. (2002) Phytotoxicity. *In: Encyclopedia of Pest Management*, Ed D. Pimentel. CRC Press.

Brown S. L., Chan F. Y., Jones J. L., Liu D. H., McCaleb K. E., Mill T., Sapios K. N. (1975) *Research program on hazard priority ranking of manufactured chemicals,* Menlo Park, California: Stanford Research Institute.

Bruce R. J., Lamb D. W., Mackie A. M., Korosi G. A., Powell K. S. (2009) Using objective biophysical measurements as the basis of targeted surveillance for detection of grapevine phylloxera *Daktulosphaira vitifoliae* Fitch: Preliminary findings. *Acta Horticulturae,* **816**, 71-79.

Bruce R. J., Hoffmann A. A., Runting J., Lamb D., Powell K. S. (2011) Towards improved early detection of grapevine phylloxera (*Daktulosphaira vitifoliae* Fitch) using a risk-based assessment. *Acta Horticulturae,* **904**, 123-131.

Buchanan G. A., Hardie W. J. (1978) Phylloxera: The implications of D.C. Swan's observations for viticulture in Victoria. *Journal of the Australian institute of Agricultural Science,* **June**, 77-81.

Buchanan G. A. (1987) The distribution of grape phylloxera, *Daktulosphaira vitifolii* (Fitch), in central and north-eastern Victoria. *Australian Journal of Experimental Agriculture,* **27**, 591-595.

Buchanan G. A., Godden G. D. (1989) Insecticide treatments for control of grape phylloxera (*Daktulosphaira vitifolii*) infesting grapevines in Victoria, Australia. *Australian Journal of Experimental Agriculture,* **29**, 267-271.

Buchanan G. A. (1990) *The Distribution, Biology and Control of Grape Phylloxera, Daktulosphaira vitifolii (Fitch), in Victoria.* PhD Thesis, La Trobe University, Melbourne, 177pp.

Buchanan G. A., Corrie A., Whiting J. R., Brown D. (1996) *Management of Grape Phylloxera in Australia*. p. 100. Victoria, Australia: Department of Natural Resources and Environment.

Buchanan G. A., Powell K. S., Loch A., Learmonth S. (2011) Grape pests. *In: Viticulture Practices*, Eds P. R. Dry and B. Coombe. Winetitles, Adelaide, Australia (*In Press*).

Campbell C. (2004) *Phylloxera: How Wine was Saved for the World,* London: Harper Collins.

Carrillo L., Martinez M., Alvarez-Alfageme F., Castañera P., Smagghe G., Diaz I., Ortego F. (2011) A barley cysteine-proteinase inhibitor reduces the performance of two aphid species in artificial diets and transgenic *Arabidopsis* plants. *Transgenic Research,* **20**, 305-319.

Chitikowski R. L., Fisher J. R. (2005) Effect of soil type on the establishment of grape phylloxera colonies in the Pacific Northwest. *American Journal of Enology and Viticulture,* **56**, 207-210.

Coombe B. G. (1963) Phylloxera and its Relation to South Australian Viticulture. *Technical Bulletin, Department of Agriculture, South Australia*, 1-57.

Corredor M., Davila A. M., Gaillardin C., Casaregola S. (2000) DNA probes specific for the yeast species *Debaryomyces hansenii*: Useful tools for rapid identification. *FEMS Microbiology Letters,* **193**, 171-177.

Corrie A. M., Crozier R. H., van Heeswijck R., Hoffman A. A. (2002) Clonal reproduction and population genetic structure of grape phylloxera, *Daktulosphaira vitifoliae*, in Australia. *Heredity,* **88**, 203-211.

Corrie A. M. (2003) *Genetic Structure of Grape Phylloxera Populations in Australia.* PhD Thesis, La Trobe University, Melbourne, 135 pp.

Corrie A. M., van Heeswijck R., Hoffman A. A. (2003) Evidence for host-associated clones of grape phylloxera *Daktulosphaira vitifoliae* (Hemiptera: Phylloxeridae) in Australia. *Bulletin of Entomological Research,* **93**, 193-201.

Cox J. A., Van Geluwe J., Lawatsch D. (1960) Hexachlorocyclopentadiene: A promising new insecticide for the control of the root form of the grape phylloxera. *Journal of Economic Entomology,* **53**, 788-790.

Cubby B. (2010) Australia joins other countries is banning endosulfan. *The Sydney Morning Herald*, October 30 2010.

DAFWA (2006) *Fact Sheet: Grape phylloxera Daktulosphaira vitifoliae - Exotic threat to Western Australia* [WWW Document]. URL. http://www.agric.wa.gov.au/objtwr/imported_assets/content/pw/ins/pp/hort/fs00200.pdf [Accessed on 8 April 2011].

Davidson W. M., Nougaret R. L. (1921) The grape phylloxera in California. *United States Department of Agriculture Bulletin,* **903**, 1-127.

Dayton L. (2010) US bans health risk pesticide: Endosulfan. *The Australian*, 26 June 2010.

de Ilarduya O. M., Xie Q., Kaloshian I. (2003) Aphid-induced defense responses in Mi-1-mediated compatible and incompatible tomato interactions. *Molecular Plant-Microbe Interactions,* **16**, 699-708.

de Klerk C. A. (1972) Research note: Occurence and distribution of the vine Phylloxera *Phylloxera vitifoliae* (Fitch), in the Olifants River irrigation area, Northwest Cape Province. *Phytophylactica,* **4**, 25-26.

de Klerk C. A. (1974) Biology of *Phylloxera vitifoliae* (Fitch) (Homoptera: Phylloxeridae) in South Africa. *Phytophylactica,* **6**, 109-118.

de Klerk C. A. (1979) An investigation of two morphometric methods to test for the possible occurance of morphologically different races of *Daktulosphaira vitifoliae* (Fitch) in South Africa. *Phytophylactica,* **11**, 51-52.

Delhaize E., Ryan P. R. (1995) Aluminium toxicity and tolerance in plants. *Plant Physiology,* **107**, 315-321.

Deretic J., Powell K., Hetherington S. (2003) Assessing the risk of phylloxera transfer during post-harvest handling of wine grapes. *Acta Horticulturae,* **617**, 61-66.

Dry P. R., Loveys B. R. (1999) Grapevine shoot growth and stomatal conductance are reduced when part of the root system is dried. *Vitis,* **38**, 151-146.

Du Y. P., Wang Z. S., Yang Y., Zhao H., Zhai H., Wang Z. Y. (2008) Nodosity formation and nutrition consumption in grape cultivars with different phylloxera resistance and infested by grape phylloxera. *Acta Entomologica Sinica,* **51**, 1050-1054.

Du Y. P., Wang Z. S., Zhai H. (2011) Grape root cell features related to phylloxera resistance and changes of anatomy and endogenous hormones during nodosity and tuberosity formation. *Australian Journal of Grape and Wine Research,* **12**, 291-297.

Dunstone R. J., Corrie A. M., Powell K. S. (2003) Effect of sodium hypochlorite on first instar phylloxera (*Daktulosphaira vitifoliae* Fitch) mortality. *Australian Journal of Grape and Wine Research,* **9**, 107-109.

Edwards J., Norng S., Powell K. S., Granett J. (2007) Relationships between grape phylloxera abundance, fungal interactions and grapevine decline. *Acta Horticulturae,* **733**, 151-158.

English-Loeb G., Vitllani M., Martinson T., Forsline A., Consolie N. (1999) Use of entomopathogenic nematodes for control of grape phylloxera (Homoptera: Phylloxerideae) : A laboratory evaluation. *Environmental Entomology,* **28**, 890-894.

EPA (2006) *Registration eligibility decision for disulfoton* [WWW Document]. URL. http://www.epa.gov/opp00001/reregistration/REDs/disulfoton_red.pdf [Accessed on 11 April 2011].

EPA (2008) *Fenamiphos facts* [WWW Document]. URL. http://www.epa.gov/pesticides/reregistration/REDs/factsheets/fenamiphos_ired_fs.htm. [Accessed on 11 April 2011].

EPPO (1990) Data sheets on quarantine pests: *Viteus vitifoliae*. European and Mediterranean Plant Protection Organization (EPPO).

EPPO (2009) Hot water treatment of grapevine to control *Viteus vitifoliae*. *European and Mediterranean Plant Protection Organization (EPPO) Bulletin,* **PM 10/16**, 484-485.

Fitzgerald G. J., Maas S. J., Detar W. R. (2004) Spider mite detection and canopy component mapping using hyperspectral imagery and spectral mixture analysis. *Precision Agriculture,* **5**, 275-289.

Forneck A., Wöhrle A. (2003) A synthetic diet for phylloxera (*Daktulosphaira vitifoliae* Fitch). *Acta Horticulturae,* **617**, 129-134.

Forneck A., Huber L. (2009) (A)sexual reproduction - a review of life cycles of grape phylloxera, *Daktulosphaira vitifoliae*. *Entomologia Experimentalis et Applicata,* **131**, 1-10.

Franks T. K., Powell K. S., Choimes S., Marsh E., Iocco P., Sinclair B. J., Ford C. M., van Heeswijck R. (2006) Consquences of transferring three sorghum genes for secondary metabolite (cyanogenic glucoside) biosynthesis to grapveine hairy roots. *Transgenic Research,* **15**, 181-195.

Frazier P., Whiting J., Powell K. S., Lamb D. (2004) Characterising the development of grape phylloxera infestation with multi-temporal near-infrared aeriel photography. *Australian and New Zealand Grapegrower and Winemaker,* **32nd Annual Technical Issue**, 133-142.

Gale G. (2002) Saving the Vine from Phylloxera: A Never-Ending Battle. *In: Wine: A Scientifc Exploration*, pp. 70-91 Eds Sandler and R. Pindler. London: Taylor and Francis.

Gatehouse A. M. R., Down R. E., Powell K., Sauvion N., Rahbe Y., Newell C. A., Merryweather A., Hamilton W. D. O., Gatehouse J. A. (1996) Transgenic potato plants enhanced resistance to the peach-potato aphid *Myzus persicae*. *Entomologica Experimentalis et Applicata,* **00**, 1-13.

Gerolt P. (1961) Investigation into the problem of insecticide sorption by soils. *Bulletin of the World Health Organization,* **24**, 577-591.

Ghumare S., Mukherjee S., Sharma R. (1989) Effect of rutin on the neonate sensitivity, dietary utilization and mid-gut carboxylesterase activity of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). *Proceedings of Animal Sciences,* **98**, 399-404.

Girling R. D., Madison R., Hassall M., Poppy G. M., Turner J. G. (2008) Investigations into plant biochemical wound-response pathways involved in the production of aphid-induced plant volatiles. *Journal of Experimental Botany,* **59**, 3077-3085.

Glaser R. W. (1932) Studies on *Neoaplectana glaseri*, a nematode parasite of the Japanese beetle (*Popillia japonica*). *New Jersey Department of Agriculture Circular,* **37**, 21.

Goral V. M., Lappa N. V., Gorkavenko E. B., Bolko O. O. (1975) Interrelations between root phylloxera and certain muscardine fungi. *Zakhist Roslin,* **22**, 30-36.

Gorkavenko E. B. (1976) Natural enemies of grapevine phylloxera and their role in reducing the pest populations in the southern Ukraine. *Zashchita Rastenii,* **46**, 88-97.

Granett J., Timper P., White J. (1986) Grape phylloxera, *Daktulosphaira vitifoliae* (Homoptera:Phylloxeridae), susceptibility to Carbofuran: Stage and clonal variability. *Journal of Economic Entomology,* **79**, 1097-1099.

Granett J., Timper P. (1987) Demography of grape phylloxera, *Daktulosphaira vitifoliae* (Homophtera: Phylloxerideae), at different temperatures. *Journal of Economic Entomology,* **80**, 327-329.

Granett J., Benedictis De J., Wolpert J., Weber E., Goheen A. C. (1991) Phylloxera on the rise: Deadly insect pest poses increases risk to North Coast vineyards. *California Agriculture,* **45**, 30-32.

Granett J., Walker A., De Benedictis J., Fong G., Lin H., Weber E. (1996) California grape phylloxera more variable than expected. *California Agriculture,* **50**, 9-13.

Granett J., Kocsis L. (2000) Populations of grape phylloxera gallicoles on rootstock foliage in Hungary. *Vitis,* **39**, 37-41.

Granett J., Walker M. A., Kocsis L., Omer A. D. (2001) Biology and management of grape phylloxera. *Annual Review of Entomology,* **46**, 387-412.

Granett J., Walker M. A., Fossen M. A. (2007) Association between grape phylloxera and strongly resistant rootstocks in California: Bioassays. *Acta Horticulturae,* **733**, 23-31.

Gullan P. J., Cranston P. S. (2010) *The Insects: An Outline of Entomology,* Oxford, England: Wiley-Blackwell Publishing.

Haller G. (1878) *Die Kleinen Feinde der Phylloxera: Studien zu Ehren des Congresses Deutscher Oenologen in Freiburg,* Heidelberg, Germany.

Hardie W. J., Considine J. A. (1976) Response of grapes to water-deficit stress in particular stages of development. *American Journal of Enology and Viticulture,* **27**, 55-61.

Harris C. R. (1964) Influence of soil type and soil moisture on the toxicity of insecticides in soils to insects. *Nature,* **202**, 724.

Harris C. R. (1966) Influence of soil type on the activity of insecticides in soil. *Journal of Economic Entomology,* **59**, 1221-1224.

Hathaway S. (1999) Chemical control of phylloxera. *Australian Viticulture,* **July/August**, 40.

Helm K. F. (1983) Phylloxera in Australia. *Australian Grapegrower and Winemaker,* **November**, 16-20.

Helm K. F., Readshaw J. L., Cambourne B. C. (1991) The effect of drought on populations of phylloxera in Australian vineyards. *Wine Industry Journal,* **August**, 195-202.

Herbert K., Powell K., Hoffmann A., Parsons Y., Ophel-Keller K., van Heeswijck R. (2003) Early detection of phylloxera - present and future directions. *Australian and New Zealand Grapegrower and Winemaker,* **463a**, 93-96.

Herbert K. (2005) *The Early Detection and Alternative Management of Phylloxera in Ungrafted Vineyards of South-Eastern Australia.* PhD Thesis, La Trobe University, Melbourne, 179 pp.

Herbert K. S., Hoffmann A. A., Powell K. S. (2006) Changes in grape phylloxera abundance in ungrafted vineyards. *Journal of Economic Entomology,* **99**, 1774-1783.

Herbert K. S., Hoffmann A., Powell K. S. (2008a) Assaying the potential benefits of thiamethoxam and imidacloprid for phylloxera suppression and improvements to grapevine vigour. *Crop Protection,* **27**, 1229-1236.

Herbert K. S., Powell K. S., McKay A., Hartley D., Herdina, Ophel-Keller K., Schiffer M., Hoffmann A. (2008b) Developing and testing a diagnostic probe for grape phylloxera applicable to soil samples. *Journal of Economic Entomology,* **101**, 1934-1943.

Hilder V. A., Powell K., Gatehouse A. M. R., Gatehouse J. A., Gatehouse L. N., Shi Y., Hamilton W. D. O., Merryweather A., Newell C. A., Timans J. C., Peumans W. J., Damme v. E., Boulter D. (1995) Expression of snowdrop lectin in transgenic tobacco plants results in added in protection against aphids. *Transgenic Research,* **4**, 18-25.

Hilgard E. (1876) The phylloxera or grapevine louse and remedies for its ravages. *Bulletin of University of California, Supplement 1,* **23**, 1-25.

Hossain M. A., Maiti M. K., Basu A., Sen S., Ghosh A. K., Sen S. K. (2006) Transgenic expression of onion leaf lectin gene in Indian mustard offers protection against aphid colonization. *Crop Science,* **46**, 2022-2032.

Hurej M., Dutcher J. (1994) Effect of esfenvalerate and disulfoton on the behavior of the blackmargined aphid, black pecan aphid, and yellow pecan aphid (Homoptera: Aphididae). *Journal of Economic Entomology,* **87**, 187-192.

Hussain S., Makhdoom R., Husnaın T., Saleem Z., Riazuddin S. (2008) Toxicity of snowdrop lectin protein towards cotton aphids *Aphis gossypii* (Homoptera, Aphididae). *Journal of Cell and Molecular Biology,* **7**, 29-40.

Jancke G. (1954) Larven von *Scymnus auritus* Thunbg. als Eiparasiten an *Phylloxera foàae* CB. *Journal of Pest Science,* **27**, 29-30.

Johnson D. T., Lobitz B., Armstrong R., Baldy R., Weber E., Benedictis De J., Bosch D. (1996) Airborne imaging aids vineyard canopy evaluation. *California Agriculture,* **50**, 14-19.

Johnson D. T., Lewis B., Sleezer S. (2008) Chemical evaluation and timing of applications against foliar form of grape phylloxera, 2006. *ESA Arthropod Management Tests,* **33**, C11.

Johnson D. T., Lewis B., Sleezer S. (2009) Efficacy of insecticides against foliar form of grape phylloxera, 2008. *ESA Arthropod Management Tests,* **34**, C14.

Johnson D. T., Lewis B., Harris J., Allen A., Striegler R. K. (2010) Management of grape phylloxera, grape berry moth and Japanese beetles. Proceedings of the Symposium on Advances in Vineyard Pest Management. Midwest Grape and Wine Conference, February 6–8, 2010, Midwest Grape and Wine Conference, Osage Beach, Mo.

Keen B. P., Bishop A. L., Gibson T. S., Spohr L. J., Wong P. T. W. (2002) Phylloxera mortality and temperature profiles in compost. *Australian Journal of Grape and Wine Research,* **8**, 56-61.

Kellow A. V. (2000) *A Study of the Interaction Between Susceptible and Resistant Grapevines and Phylloxera.* PhD Thesis, University of Adelaide, 183 pp.

Kellow A. V., Sedgley M., van Heeswijck R. (2004) Interaction between *Vitis vinifera* and grape phylloxera: changes in root tissue during nodosity formation. *Annals of Botany,* **93**, 581-590.

King P. D., Buchanan G. A. (1986) The dispersal of phylloxera crawlers and spread of phylloxera infestations in New Zealand and Australian vineyards. *American Journal of Enology and Viticulture,* **37**, 26-33.

Kingston K., Powell K., Cooper P. (2005) Investigating the digestive function and feeding behaviour of grape phylloxera. *Comparative Biochemistry and Physiology,* **141A**, S116.

Kingston K., Powell K. S., Cooper P. (2007) Grape phylloxera: New investigations into the biology of an old pest. *Australian and New Zealand Grapegrower and Winemaker,* **521**, 12-17.

Kirchmair M., Huber L., Porten M., Rainer J., Strasser H. (2004) *Metarhizium anisopliae*, a potential agent for the control of grape phylloxera. *Biocontrol,* **49**, 295-303.

Kirchmair M., Hoffmann M., Neuhauser S., Strasser H., Huber L. (2007) Persistence of GRANMET®, a *Metarhizium anisopliae*-based product, in grape phylloxera-infested vineyards. *IOBC WPRS Bulletin,* **30**, 137-142.

Kirchmair M., Neuhauser S., Strasser H., Voloshchuk N., Hoffmann M., Huber L. (2009) Biological control of grape phylloxera - a historical review and future prospects. *Acta Horticulturae,* **816**, 13-17.

Korosi G., Trethowan C., Powell K. (2009) Reducing the risk of phylloxera tansfer on viticultural waste and machinery. *Acta Horticulturae,* **816**, 53-62.

Korosi G. A., Trethowan C. J., Powell K. S. (2007) Screening for grapevine rootstock resistance to phylloxera genotypes from Australian vineyards under controlled conditions. *Acta Horticulturae,* **733**, 159-166.

Korosi G. A., Mee P. T., Powell K. S. (2011) Influence of temperature and humidity on mortality of grapevine phylloxera *Daktulosphaira vitifoliae* clonal lineages – a scientific validation of a disinfestation procedure for viticultural machinery. *Australian Journal of Grape and Wine Research*, DOI: 10.1111/j.1755-0238.2011.00168.x.

Larsson S., Lundgren L., Ohmart C. P., Gref R. (1992) Weak responses of pine sawfly larvae to high needle flavonoid concentrations in scots pine. *Journal of Chemical Ecology,* **18**, 271-282.

Lawo N. C., Weingart G. J. F., Schuhmacher R., Forneck A. (2011) The volatile metabolome of grapevine roots: First insights into the metabolic response upon phylloxera attack. *Plant Physiology and Biochemistry,* **49**, 1059-1063.

Lima M. R. M., Felgueiras M. L., Gracxa G., Rodrigues J. E. A., Barros A., Gil A. M., Dias A. C. P. (2010) NMR metabolomics of esca disease-affected *Vitis vinifera* cv. Alvarinho leaves. *Journal of Experimental Botany,* **61**, 4033-4042.

Litvinov P. I. (1982) Use of hexachlorobutadiene for phylloxera control in vineyards. *Khimiya v Sel'skom Khozyaistve,* **1**, 29-31.

Loubser J. T., van Aarde I. M. F., Hoppnerl G. F. J. (1992) Assessing the control potential of Aldicarb against grapevine phylloxera. *South African Journal of Enology and Viticulture,* **13**, 84-86.

Madani M., Subbotin S. A., Moens M. (2005) Quantitative detection of the potato cyst nematode, *Globodera pallida*, and the beet cyst nematode, *Heterodera schachtii*, using real-time PCR with SYBR green I dye. *Molecular and Cellular Probes,* **19**, 81-86.

Makee H., Charbaji T., Idris I., Ayyoubi Z. (2008) Effect of gamma irradiation on survival and reproduction of grape phylloxera *Daktulosphaira vitifoliae* (Fitch). *Advances in Horticultural Science,* **22**, 182-186.

Mayet V. (1890) *Les insectes de la vigne,* Montpellier: Bibliothèque du Progrès Agricole et Viticole.

Meyling N. V., Eilenberg J. (2007) Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: Potential for conservation biological control. *Biological Control,* **43**, 145-155.

Miao J., Wu Y., Xu W., Hu L., Yu Z., Xu Q. (2011) The impact of transgenic wheat expressing GNA (Snowdrop lectin) on the aphids *Sitobion avenae*, *Schizaphis graminum*, and *Rhopalosiphum padi*. *Environmental Entomology,* **40**, 743-748.

Miller G. T. (2004) *Sustaining the Earth,* Pacific Grove, California: Thompson Learning, Inc.

Molnár J. G., Németh C. S., Májer J., Jahnke G. G. (2009) Assessment of pyhylloxera leaf galling incidence on European grapevines in Badacsony Hungary. *Acta Horticulturae,* **816**, 97-104.

Moran P. J., Thompson G. A. (2001) Molecular responses to aphid feeding in *Arabidopsis* in relation to plant defense pathways. *Plant Physiology,* **125**, 1074-1085.

Mordkovich Y. B., Chernej L. B. (1994) Overcoming of methyl bromide phytotoxic effect on grape planting material disinfected against phylloxera. Sel'skokhozyajstvennaya biologiya. *Seriya Biologiya rastenij,* **3**, 128-133.

Nauen R., Reckmann, U., Thomzik, J. and Thielert, W. (2008) Biological profile of spirotetramat (Movento ®) - a new two-way systemic (ambimobile) insecticide against sucking pest species. *Bayer CropScience Journal,* **61**, 245-252.

Nicol J. M., Stirling, G.R., Rose, B.J., May, P., and van Heeswijck, R. (1999) Impact of nematodes on grapevine growth and productivity: current knowledge and future directions, with special reference to Australian viticulture. *Australian Journal of Grape and Wine Research,* **5**, 109-127.

Nougaret R. L., Lapham M. H. (1928) A study of phylloxera infestation in California as related to types of soils. *United States Department of Agriculture Technical Bulletin,* **20**, 1-39.

NVHSC (2009) National Phylloxera Management Protocol p. 32. National Vine Health Steering Committee, Australia.

Omer A., Grannett J., Walker M. A. (2002) Influence of plant growth stage on grape phylloxera (Homoptera: Phylloxeridae) populations. *Environmental Entomology,* **31**, 120-126.

Omer A. D., Granett J., De Benedictis J., Walker M. A. (1995) Effects of fungal root infections on the vigor of grapevines infested by root-feeding grape phylloxera. *Vitis,* **34**, 165-170.

Omer A. D., Thaler J. S., Granett J., Karban R. (2000) Jasmonic acid induced resistance in grapevines to a root and leaf feeder. *Entomological Society of America,* **93**, 840-845.

Onyilagha J. C., Lazorko J., Gruber M. Y., Soroka J. J., Erlandson M. A. (2004) Effect of flavonoids on feeding preference and development of the crucifer pest *Mamestra configurata* Walker. *Journal of Chemical Ecology,* **30**, 109-124.

Ophel-Keller K., Engel B., Heinrich K. (1995) Specific detection of *Gaeumammomyces gramminis* in soil using polymerase chain reaction. *Mycological Research,* **99**, 1385-1390.

Opperman C. H., Chang S. (1991) Effects of aldicarb and fenamiphos on acetylcholinesterase and motility of *Caenorhabditis elegans*. *Journal of Nematology,* **23**, 20-27.

Ordish G. (1987) *The Great Wine Blight,* London: Sidgwick & Jackson Limited.

Pope R. B. (1957) The role of aerial photography in the current balsam woolly aphid outbreak. *Forestry Chronicle,* **September**, 263-264.

Porten M., Schmid J., Rühl E. H. (2000) Current problems with phylloxera on grafted vines in Germany and ways to fight them. *In: Proceedings of the International Symposium on Grapevine Phylloxera Management*, pp. 89-98 Eds K. S. Powell and J. Whiting. Melbourne: Department of Natural Resources and Environment.

Porten M., Huber L. (2003) An assessment method for the quantification of *Daktulosphaira vitifoliae* (Fitch) (Hem., Phylloxeridae) populations in the field. *Journal of Applied Entomology,* **127**, 157-162.

Powell K. S., Brown D., Dunstone R., Hetherington S., Corrie A. (2000) Population dynamics of phylloxera in Australian vineyards and implications for management. *In: Proceedings of the International Symposium on Grapevine Phylloxera Management*, pp. 7-19 Eds K. Powell and J. Whiting. Melbourne: Department of Natural Resources and Environment.

Powell K. S., Slattery W. J., Deretic J., Herbert K., Hetherington S. (2003) Influence of soil type and climate on the population dynamics of grapevine phylloxera in Australia. *Acta Horticulturae,* **617**, 33-41.

Powell K. S., Burns A., Norng S., Granett J., McGourty G. (2007a) Influence of composted green waste on the population dynamics and dispersal of grapevine phylloxera *Daktulosphaira vitifoliae*. *Agriculture, Ecosystems and Environment,* **119**, 33-38.

Powell K. S., Trethowan C. J., Blanchfield A., Norng S. (2007b) Composted winery waste and its influence on grape phylloxera in ungrafted vineyards. *Acta Horticulturae,* **733**, 143-149.

Powell K. S. (2008) Grape Phylloxera: An Overview. *In: Root Feeders: An Ecosystem Perspective*, pp. 96-114 Eds S. N. Johnson and G. M. Murray. CAB International.

Powell K. S., Korosi G. A., Mackie A. M. (2009) Monitoring grape phylloxera populations using simple non-destructive trapping systems. *Acta Horticulturae,* **816**, 29-34.

Powell W., Pickett J. A. (2003) Manipulation of parasitoids for aphid pest management: progress and prospects. *Pest Management Science,* **59**, 149-155.

Rahbe Y., Deraison C., Bottino M., Girard C., Nardon C., Jouanin L. (2003) Effects of the cysteine protease inhibitor oryzacystatin (OC-I) on different aphids and reduced performance of Myzus persicae on OC-I expressing transgenic oilseed rape. *Plant Science,* **164**, 441-450.

Rammer I. A. (1980) Field studies with carbofuran for control of the root form of the grape phylloxera. *Journal of Economic Entomology,* **73**, 327-331.

Rao K. V., Powell K. S., Rathore K. S., Bown D. P., Spence J., Bharathi M., Gatehouse A. M. R., Gatehouse J. A., Hodges T. K. (1998) Expression of Snowdrop lectin (GNA) in the phloem of transgenic rice plants confers resistance to rice brown planthopper. *Journal of Insect Physiology*, 1-24.

Rasmussen O. F., Reeves J. C. (1992) DNA probes for the detection of plant pathogenic bacteria. *Journal of Biotechnology,* **25**, 203-220.

Reisenzein H., Baumgarten A., Pfeffer M., G A. (2007) The influence of soil properties on the development of grape phylloxera populations in Austrian viticulture. *Acta Horticulturae,* **733**, 13-23.

Renzullo L., Held A., Powell K., Blanchfield A. (2004) Remote sensing phylloxera infestation: current capabilities and future possibilities for early detection. *Australian and New Zealand Grapegrower and Winemaker,* **32nd Annual Technical Issue**, 126-130.

Renzullo L. J., Blanchfield A. L., Powell K. S. (2006) A method of wavelength selection and spectral discrimination of hyperspectral reflectance spectrometry. *IEEE Transacations on Geoscience and Remote Sensing,* **44**, 1986-1994.

Riley C. V. (1875) The grape phylloxera *Phylloxera vastatrix* Planchon. *In: Sixth Annual Report on the Noxious, Beneficial, and Other Insects of the State Of Missouri*, pp. 57-73.

Riley C. V. (1881) General index and supplement to the nine reports on the insects of Missouri *United States Entomological Bulletin,* **6**.

Risher J. F., Mink F. L., Stara J. F. (1987) The toxicologic effects of the carbamate insecticide aldicarb in mammals: A review. *Environmental Health Perspectives,* **72**, 267-281.

Rodriguez-Perez J. R., Plant R., Lambert J. J., Smart D. (2011) Using apparent soil electrical conductivity to characterize vineyard soils of high clay content. *Precision Agriculture*, 1-20.

Sadeghi A., Broeders S., De Greve H., Hernalsteens J. P., Peumans W. J., Van Damme E. J., Smagghe G. (2007) Expression of garlic leaf lectin under the control of the phloem-specific promoter Asus1 from *Arabidopsis thaliana* protects tobacco plants against the tobacco aphids (*Myzus nicotianae*) *Pest Management Science,* **63**, 1215-1223.

Saha P., Majumder P., Dutta I., Ray T., Roy S. C., Das S. (2006) Transgenic rice expressing *Allium sativum* leaf lectin with enhanced resistance against sap-sucking insect pests. *Planta,* **223**, 1329-1343.

Sakai H., Tsutsumi Y., Kawai A., Sato S., Takano T., Takahashi T. (1985) Methyl bromide fumigation and hot water treatment of grapevine stocks against the grape phylloxera, *Viteus vitifoliae* Fitch. *Research Bulletin of the Plant Protection Service Japan,* **21**, 67-69.

Satpute N. S., Deshmukh S. D., Rao N. G., Tikar S. N., Moharil M. P., Nimbalkar S. A. (2007) Temperature-dependent variation in toxicity of insecticides against *Earias vitella* (Lepidoptera: Noctuidae). *Journal of Economic Entomology,* **100**, 357-360.

Sauvion N., Rahbe Y., Peumans W. J., van Damme E. J. M., Gatehouse J. A., Gatehouse A. M. R. (1996) Effects of GNA and other mannose-binding lectins on development and fecundity of the peach-potato aphid *Myzus persicae*. *Entomologia Expermentalis et Applicata,* **79**, 285-293.

Sayler G. S., Layton A. C. (1990) Environmental application of nucleic acid hybridisation. *Annual Review of Microbiology,* **44**, 625-648.

Schaefer H. (1972) Metabolic differences between phylloxera-infested and healthy grape leaves. *Phytopathologische Zeitschrift,* **75**, 285-314.

Schaefer H. (1985) Metabolic differences between phylloxera root galls and healthy grapevine roots. *Wein-Wissenschaft,* **40**, 219-227.

Schmid J., Manty F., Rühl E. H. (2003) Experience with phylloxera tolerant and resistant rootstocks at different vineyard sites. *Acta Horticulturae,* **617**, 85-93.

Shi Y., Wang M. B., Powell K. S., Van Damme E., Hilder V. A., Gatehouse A. M. R., Boulter D., Gatehouse J. A. (1994) Use of the rice sucrose synthase-1 promoter to direct phloem-specific expression of β-glucuronidase and snowdrop lectin genes in transgenic tobacco plants. *Journal of Experimental Botany,* **45**, 623-631.

Simmons P., Barnes D., Snyder M. (1951) Control of grape phylloxera. *Wines and Vines*, 22-23.

Singh S. P., Maheshwari D. K. (1989) Effect of GA3 on the phytotoxicity of aldicarb and carbofuran on seedling growth in *capsicum frutescens* var. California Wonder and rate of root knot nematode infestation. *Journal of Phytopathology,* **127**, 158-168.

Sleezer S., Johnson D., Lewis B., Goggin F., Rothrock C., Savin M. (2011) Grape phylloxera, *Daktulosphaira vitifoliae* (Fitch) seasonal biology, predictive model, and management in the Ozarks region of the United States. *Acta Horticulturae,* **904**, 151-156.

Slonovskii I. F. (1971) Effect of hexachlorobutadiene on phylloxera growth and productivity of grape vine. Immunitet vinograda filloksere Bor'ba Nei *Chemical Abstracts,* **76**, 18-34.

Smith D., Smith I., Collett N., Elms S. (2008) Forest health surveillance in Victoria. *Australian Forestry,* **71**, 188-195.

Stevenson A. B. (1968) Soil treatments with insecticides to control the root form of grape phylloxera. *Journal of Economic Entomology,* **61**, 1168-1171.

Stevenson A. B. (1970) Endosulfan and other insecticides for control of the leaf form of the grape phylloxera in Ontario. *Journal of Economic Entomology,* **63**, 125-128.

Sun Q., Chen Y., Wang H., Downie D., Zhai H. (2009) Origin and genetic diversity of grape phylloxera in China. *Acta Entomologica Sinica,* **52**, 885-894.

Tran P., Cheesbrough T. M., Keickhefer R. W. (1997) Plant proteinase inhibitors are potential anticereal aphid compounds. *Journal of Economic Entomology,* **90**, 1672-1677.

Trebicki P., Harding R. M., Powell K. S. (2009) Anti-metabolic effects of *Galanthus nivalis* agglutinin and wheat germ agglutinin on nymphal stages of the common brown leafhopper using a novel artificial diet system. *Entomologia Experimentalis et Applicata,* **131**, 99-105.

Treutter D. (2006) Significance of flavonoids in plant resistance: A review. *Environmental Chemistry Letters,* **4**, 147-157.

Tucker D. J., Lamb D. W., Powell K. S., Blanchfield A. L., Brereton I. M. (2007) Detection of phylloxera infestation in grapevines by NMR methods. *Acta Horticulturae,* **733**, 173-182.

Turley M., Granett J., Omer A. D., De Benedictis J. (1996) Grape phylloxera (Homoptera: Phylloxerideae) temperature threshold for establishment of feeding sites and degree-day calculations. *Environmental Entomology,* **25**, 842-847.

Umina P. A., Corrie A. M., Herbert K. S., White V. L., Powell K. S., Hoffmann A. A. (2007) The use of DNA markers for pest management - clonal lineages and population biology of grape phylloxera. *Acta Horticulturae,* **733**, 183-195.

UNEP (2000) *Montreal Protocol on Substances that Deplete the Ozone Layer, 2000*. UNEP Ozone Secretariat, United Nations Environment Programme.

UNEP (2008) IPPC Recommendation: Replacement or Reduction of the Use of Methyl Bromide as a Phytosanitary Measure. United Nations Environment Programme. *Recommendation for Implementation of the International Plant Protection Convention (IPPC), at the 3rd Session of the Commission on Phytosanitary Measures (CPM-3) of the IPPC,* **UNEP/OzL.Pro.WG.1/28/INF4**.

van Heeswijck R., Bondar A., Croser L., Franks T., Kellow A., Powell K. (2003) Molecular and cellular events during the interaction of phylloxera with grapevine roots. *Acta Horticulturae,* **617**, 13-16.

van Steenwyk R. A., Varela L. G., Ehlhardt M. (2009) Insecticide evaluations for grape phylloxera with foliar applications of Movento. Abstracts 83rd Orchard Pest and Disease Management Conference, Hilton Hotel, Portland, Oregon, Washington State University.

Vega E. G. (1972) Control de la filoxera de la vid con hexachlorobutadiene. *India,* **290**, 15-18.

Viss W. J., Driver D. J. (1996) Key grapevine pests and diseases in North America and resistance through genetic engineering. *Brighton Crop Protection Conference - Pest and Diseases*. pp. 125-130.

von Fulmek L. (1857) Insekten als Blattlausfeinde? Kritiscb-statistisdte Sichtung (Nadt dem Manuskript: Weh.Wirte-Index dee Paraslteninsekten von Insekten). *Annalen Des Naturhistorischen Museums in Wien,* **61**, 110-227.

Walker A., Granett J., Omer A. D., Lin H., Kocsis L., Forneck A., Porten M. (1998) Are Phylloxera feeding on 5C rootstock in Europe? *Practical Vineyards and Wineries*, 21-26.

Wapshere A. J., Helm K. F. (1987) Phylloxera and *Vitis*: An experimentally testable coevolutionary hypothesis. *American Journal of Enology and Viticulture,* **38**, 216-220.

Warick R. P., Hildebrant A. C. (1966) Free amino acid contents of stem and phylloxera gall tissue cultures of grape. *Plant Physiology,* **41**, 573-578.

Weber E., De Benedictis J., Smith R., Granett J. (1996) Enzone does little to improve health of phylloxera-infested vineyards. *California Agriculture,* **50**, 19-23.

Wheeler A. G., Jr, Henry T. J. (1978) *Ceratocapsus modestus* (Hempitera: Miridae), a predator of grape phylloxera: Seasonal history and description of fifth instar. *Melsheimer Entomological Series,* **1980**, 6-10.

Wheeler A. G., Jr, Jubb G. L., Jr (1979) *Scymnus cervicalis* Mulsant, a predator of grape phylloxera, with notes on *S. brullei* Mulsant as a predator of woolly aphids on elm (Coleoptera: Coccinellidae). *Coleopterists Bulletin,* **33**, 199-204.

Wildman W. E., Nagaoka R. T., Lider L. A. (1983) Monitoring spread of grape phylloxera by color infrared aerial photography and ground investigation. *American Journal of Enology and Viticulture,* **34**, 83-94.

Williams R. N. (1979) Foliar and subsurface insecticidal applications to control aerial form of the grape phylloxera. *Journal of Economic Entomology,* **72**, 407-410.

Witt A. K. H., Van de Vrie M. (1985) Gamma radiation for post-harvest control of insects and mites in cut flowers. *Mededelingen van de Faculteit Landouwwetenschappen, Rijksuniversiteit Gen,* **50**, 697-704.

Yang Z., Rao M. N., Kindler S. D., Elliot N. C. (2004) Remote sensing to detect plant stress, with particular reference to stress caused by the greenbug: A review. *Southwestern Entomologist,* **29**, 227-236.