A Scientific Basis for Risk Analysis of Grape phylloxera *Daktulosphaira vitifoliae* Fitch

Assoc. Professor Kevin S. Powell, Principal Research Scientist,
Catherine W. Clarke, Research Scientist

Agriculture Victoria Research, Department of Economic Development, Jobs, Transport and Resources, 124 Chiltern Valley Road, Rutherglen, Victoria 3685.



SUMMARY

The Phylloxeridae are a relatively small family, containing 75 species (Blackman and Eastop, 1984), which live on deciduous trees and perennial fruit crops. Grape phylloxera *Daktulosphaira vitifoliae* (Fitch) (Hemiptera, Phylloxeridae) is the most economically destructive and geographically widespread pest species of commercial grapevines, on which it is an obligate biotroph of *Vitis* species, occurring in almost all viticultural regions around the world (Powell, 2012). Grape phylloxera is internationally recognised as the world's worst insect pest of cultivated grapevines *Vitis* spp. (Vitaceae) (Powell, 2008). It can feed on both the roots and leaves of *Vitis* species depending on the genotype of host and insect. Root-feeding phylloxera cause most economic damage to *Vitis vinifera* L. and most research have focused on this part of the life-cycle. However leaf-galling phylloxera can also represent a significant risk in some grape-growing regions, especially where phylloxera resistant *Vitis* hybrids are grown.

Grape phylloxera is native to eastern North America where it coexists with native *Vitis* species (Wapshere and Helm, 1987). The *Vitis* species have been used for over 150 years in the form of phylloxera-resistant rootstock hybrids, as the main management option along with quarantine measures. Phylloxera was first reported in Europe in 1863 and caused significant economic damage as it spread through other continents during the late 19th century. In Australia, phylloxera was first detected in Geelong, Victoria, in 1877 and despite concerted efforts to eradicate the pest from the region, further detections followed in central and North East Victoria, New South Wales (NSW) and Queensland. Grape phylloxera is known to be present in designated Phylloxera Infested Zones (PIZ) in Victoria and NSW. No detections have yet been reported in other states and territories. This review examines the biotic and abiotic factors that influence the spread of grape phylloxera through both natural and human dispersal associated with viticultural and wine making practices. It highlights quarantine and management practices available to minimise the risk of entry, establishment, and spread; and knowledge gaps that should be addressed to minimise phylloxera risk to the Australian viticulture industry. The review provides up-to-date scientific knowledge that informs the development of Import Risk Analysis and Pest Risk Analysis documentation for grape phylloxera.

1. BACKGROUND

1.1. Purpose

This document is a risk analysis support paper on grape phylloxera and has been developed as a component of the project *Risks and Management of Exotic and Endemic Phylloxera* co-funded by Australian Grape and Wine Authority and the Department of Economic Development Jobs Transport and Resources. The scope of this document is to describe and review phylloxera risk vectors, pathways and knowledge gaps which are likely to influence the risk of entry, establishment and spread of exotic and endemic grape phylloxera in Australia.

1.2. Literature review

A comprehensive literature review was conducted focusing on nationally and internationally produced peer reviewed scientific papers, books, industry reports and personnel communication and observations.

1.3. Pest classification

The pest assessed is grape phylloxera (*Daktulosphaira vitifoliae*, Fitch). Grape phylloxera (Hemiptera, Phylloxeridae) was first described in 1855. Within Australia, 83 endemic strains are classified as a regulated biosecurity threat (PHA, 2009).

1.4 Taxonomy

1.4.1 Current name

Daktulosphaira vitifoliae (Fitch), 1856

1.4.2 Synonyms

Phylloxera vastatrix Planchon, 1868
Viteus vitifoliae (Fitch) 1867
Viteus vitifolii (Fitch) 1867

1.4.3. Common names

Grape phylloxera
Grapevine phylloxera
Filoxera
Vine louse

Reblaus
Vine fretter

1.5 Geographic distribution

Grape phylloxera is native to the northeast Rockies in North America. It has worldwide distribution covering most major grape-growing regions. It was first reported in Europe in 1863 and subsequently recorded in all major grape-growing regions of Europe. Grape phylloxera gained economic pest status in the late nineteenth century when it spread to Europe causing significant damage to highly susceptible ungrafted European grapevine, *Vitis vinifera* L. Considerable economic and social hardship occurred (Banerjee *et al*., 2007) initially in France, then western Europe where it spread to over 2.5 million hectares of grapevines (Bournier, 1976). It then spread to Australia, Russia, New Zealand, China, South Africa and South America (Crovetti and Rossi, 1987; Rossi, 1988) after it was inadvertently transferred on imported *Vitis* planting material (Buchanan, 1990; Mayet, 1890). However, in some countries, including China (Du *et al*., 2011), Australia (Powell, 2008), Russia (Frolov and David'yan, 2009) and Armenia (ARD, 2007), its distribution appears restricted only to some grape-growing regions. More recent detections include the Yarra Valley, in Australia in 2006; the Ararat Valley, Armenia in 2009 (V. Keushguerian, pers. comm.) and Hunan, Shaanxi and Liaoning provinces of China in 2006–2007 (Du *et al*., 2011). The reported areas of absence include Chile, Cyprus, Denmark, Estonia, Finland, Ireland, Latvia, Lithuania, Norway, Sweden and The Netherlands. However in some of these regions surveys to confirm the absence of phylloxera are unclear (EFSA, 2014).

1.6. Distribution in Australia

Historically, the first phylloxera detection in Australia was in Geelong in 1877 (Anon, 1878). This infestation was traced back to nursery material which was imported in 1875, although it may even have been introduced prior to this date (Buchanan, 1990). After conducting ground surveys to determine distribution, attempts were made to restrict movement and eradicate phylloxera from the infested area in Geelong. This was done by prohibiting the importation of vines and uprooting all grapevines within a 5km radius of infested vineyards. Vines were subsequently destroyed on around 2000 properties in Geelong as part of the attempted eradication

program. Although quarantine measures were introduced and phylloxera-resistant rootstocks recommended in the late 19th century, a number of detections subsequently followed in Victoria, NSW and Queensland. Consequently the eradication policy was deemed untenable. Over the last two decades, since 1991, phylloxera has spread in Victoria and resulted in the establishment of new PIZs in the Yarra Valley, Lancefield, King Valley and Upton and expansion of existing quarantine boundaries namely in the Nagambie and North East Victoria PIZ's (Vine Health Australia, 2017)**.**

2. FACTORS WHICH INFLUENCE THE RISK OF ENTRY, ESTABLISHMENT AND SPREAD

There are a number of biotic and abiotic factors which influence the potential for establishment and subsequent spread of phylloxera and these key factors are discussed below.

2.1 Biotic factors

2.1.2 Host plant range and damage

Grape phylloxera feeds only on *Vitis* species. It infests the root system and leaves of *Vitis* species forming galls. The European grapevine *V. vinifera* is highly susceptible to radicole (root-galling) grape phylloxera. Feeding on *V. vinifera* roots by grape phylloxera induces nodosities on non-lignified roots and tuberosities on older lignified roots (Figure 1). Root galling disrupts normal root function and limits water and nutrient uptake, resulting in reduced leaf area, premature senescence, yield decline and vine death in some instances (Stellwaag, 1928).

Gallicole (leaf-galling) grape phylloxera also occurs but is primarily associated with foliage of resistant rootstocks in abandoned habitats, nursery plantings and from rootstock suckers on grafted grapevines; and rarely leads to economic loss. Fully developed phylloxera-induced leaf galls have occasionally been reported, on *V. vinifera* in Europe and South America (Strapazzon and Girolami, 1983; Koennecke et al., 2011; Vidart et al., 2013) but not in Australia. However, in Australia partially developed leaf galls have been observed (K. Powell pers. observation) on ungrafted *V. vinifera* in a single vineyard located in Rutherglen and the King Valley in NE Victoria.

Despite recommendations being made in the late 19th century in Australia to use phylloxera resistant rootstocks, there has been limited use of resistant rootstocks and around 85% of grapevine plantings are currently on ungrafted *V. Vinifera,* which is highly susceptible to grape phylloxera. Phylloxera can also survive on the roots and leaves of American *Vitis* species or hybrids used as phylloxera 'resistant' grapevines. As only 15% of Australian vineyard plantings are on resistant rootstocks, the risk of spread of leaf-galling phylloxera is currently considered low. Although more commonly detected in wine grape vineyards, phylloxera does not discriminate between wine grapes, table grapes and dried grapes; and consequently has the potential to impact on all three sectors of the viticulture industry.

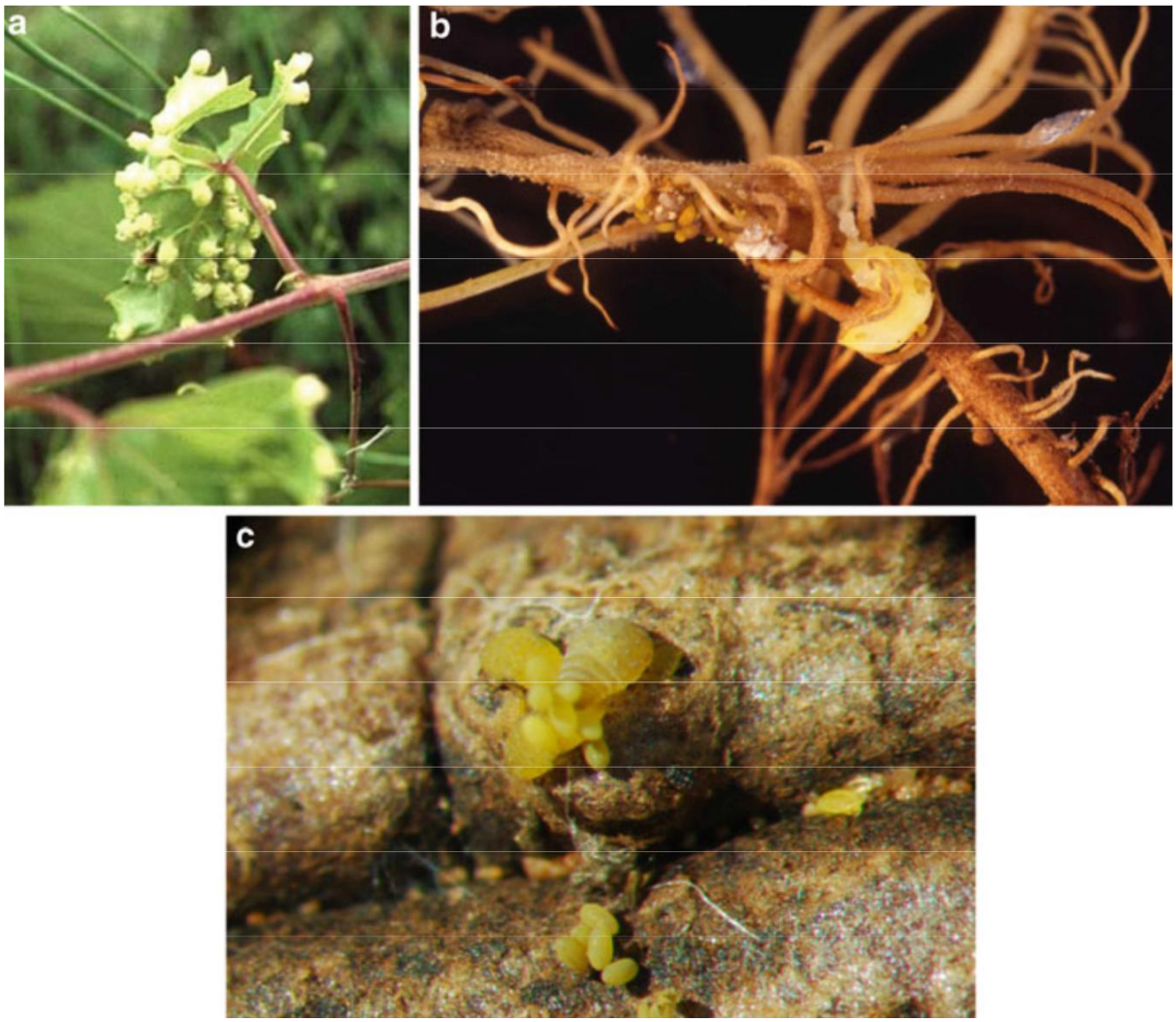


Figure 1. Three types of grape phylloxera induced galls (a) leaf galls on 'resistant' grapevine rootstock foliage, (b) *yellow hook* -shaped nodosities produced on both non-lignified roots of *Vitis vinifera* and *Vitis* sp., (c) swellings or tuberosities produced on *V. vinifera* (Source: Powell 2012).

2.1.3. Phylloxera life-cycle and genetics

2.1.3.1. Classical life-cycle

Grape phylloxera has a complex life-cycle, with a number of life-cycle variations (Forneck and Huber, 2009). Its classic life-cycle (Figure 2) has both sexual and asexual reproductive phases. It can feed and develop on roots and/or leaves of *Vitis* species in the spring and summer (Granett *et al*., 2001) when temperature and host plant phenological conditions are optimal (Omer *et al*., 2002). In autumn, alate adults are produced that have the potential, under suitable environmental conditions, to asexually produce non-feeding male and female sexuales that can mate and oviposit a single overwintering egg. A female fundatrix can then hatch from the sexual egg in spring and move onto *Vitis* leaves forming leaf galls to recommence an asexual reproduction cycle. First instars that hatch are referred to as 'crawlers' and they move onto the roots in early spring and summer.

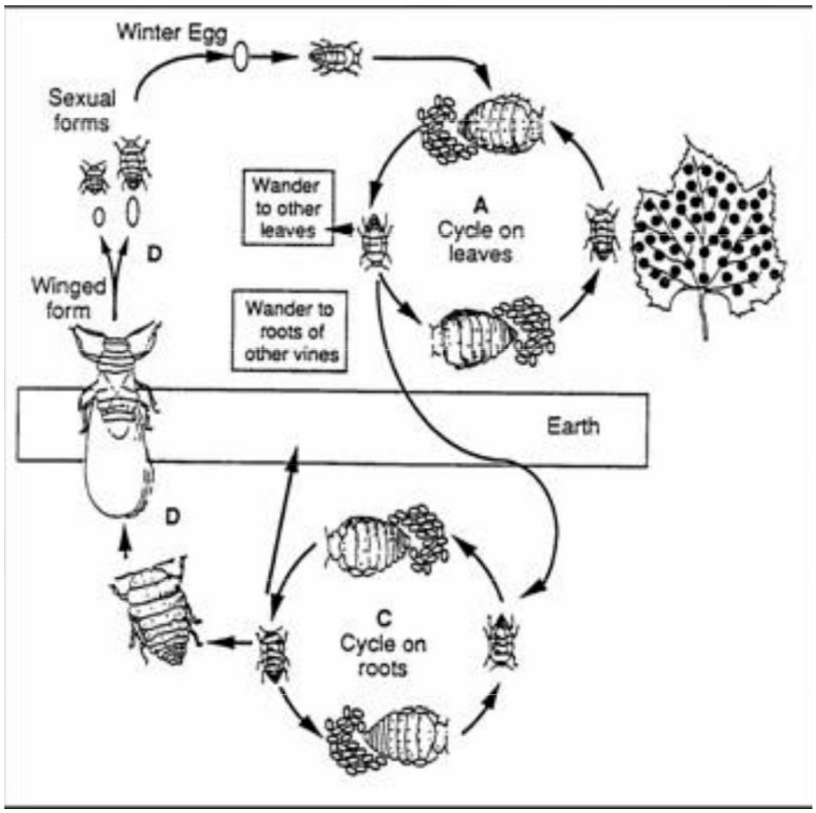


Figure 2. Classical life-cycle of grape phylloxera *(Adapted from: Buchanan, 1990).* A= Asexual summer leaf-galling life-cycle on American *Vitis* species or rootstock hybrids; B = Late summer first instars move to the root system; C= Asexual root-galling life-cycle on both European *V. vinifera* and American *Vitis* species; D= Commencement of sexual life cycle, where alate adult lays male and female eggs which hatch and mate, female produces winter egg to initiate leaf galling cycle in spring.

2.1.3.2. Leaf galling phylloxera

In Europe and South America leaf-galling phylloxera (Figure 1), occasionally causes economic damage to *V. vinifera* (Koennecke *et al*., 2011; Molnár *et al*., 2009; Quaglia and Rossi, 1987; Raspi and Antonelli, 1987; Vidart *et al*., 2013). This occurs more commonly in abandoned vineyards, on nursery rootstocks plantings or on resistant rootstock suckers that emerge from below the graft union of grafted vines (Hellman, 2003). Phylloxera populations reach vast numbers in leaf galls and as such are primary sources for phylloxera infestations on leaves in neighbouring vineyards. Some leaf galling strains also have the ability to move to the roots and overwinter as first instars and remerge in the spring. In South America, leaf galling has led to severe defoliation of *V. vinifera* cultivars and vine death (Botton and Walker, 2009). The resistance among *V. vinifera* host plants changes by genotype (Konnecke *et al*., 2009; Vidart *et al*., 2013; Wegner-Kiss, 2007) and is also affected by environmental conditions and inoculum pressure. In Australia, leaf galling forms are limited to the North East Victoria PIZ where 34 leaf galling strains have been identified. There have been no reports of leaf-galling in Australia on *V. vinifera*, however the environmental triggers which could potentially change this host plant association have not yet been determined.

2.1.3.3 Root galling phylloxera

The distribution and frequency of phylloxera strains varies worldwide. In Australia, root-galling is the predominant cause of damage on *V. vinifera*. Root-galling can cause extensive damage to ungrafted *V. vinifera*. Virulence levels of particular root-galling phylloxera strains may vary on susceptible *V. vinifera* in the field and this can make detection difficult and it may take several years to detect an infestation. The difference in virulence of root-galling phylloxera strains on *V. vinifera*, may explain differences in patterns of field damage, phylloxera distribution and continued survival and reproduction on *V. vinifera* in some infested areas (Powell *et al*., 2003; Herbert *et al*., 2006).

2.1.3.4. Life-cycle of grape phylloxera in Australia

The classic life-cycle has a number of variations, and in Australia (and some other countries e.g. USA (California), China, New Zealand) asexual reproduction predominates and radicoles are more common than gallicoles (Figure 3). In contrast,

leaf-galling forms are reportedly more common than root-galling forms in southern Europe (Forneck *et al*., 2009). Sexual reproduction, if it occurs in Australia at all, is relatively rare (Corrie and Hoffman, 2004). Detecting sexual eggs laid by winged adults is particularly difficult as they are laid under the bark of grapevines. There have been no reports worldwide in the last 50 years (Forneck and Huber, 2009). Environmental conditions may influence the ability of winged phylloxera to lay eggs. Sexual eggs (laid by winged phylloxera) have been observed under laboratory conditions in Australia but not in the field (Powell pers. observ.).

In Australia, although radicoles predominate in all PIZ's gallicoles are also known to be present but only in a single PIZ, that of North East Victoria, which is the largest and oldest PIZ. This particular zone is regarded as the centre of genetic diversity of endemic phylloxera with 83 genetic strains known to be present. This genetic diversity predominates in key grape growing regions of North East Victoria including Rutherglen, Milawa and Glenrowan, where 'resistant' rootstocks are predominantly grown.

Knowledge gaps

Multiple field studies have examined the population dynamics of root-galling phylloxera in Australia (Powell *et al*., 2000; 2003; 2009; Herbert *et al*., 2006). However no studies on leaf-galling phylloxera population dynamics have been conducted, which leaves a substantial gap in our knowledge of risk of spread of these genetic strains under different environmental conditions.

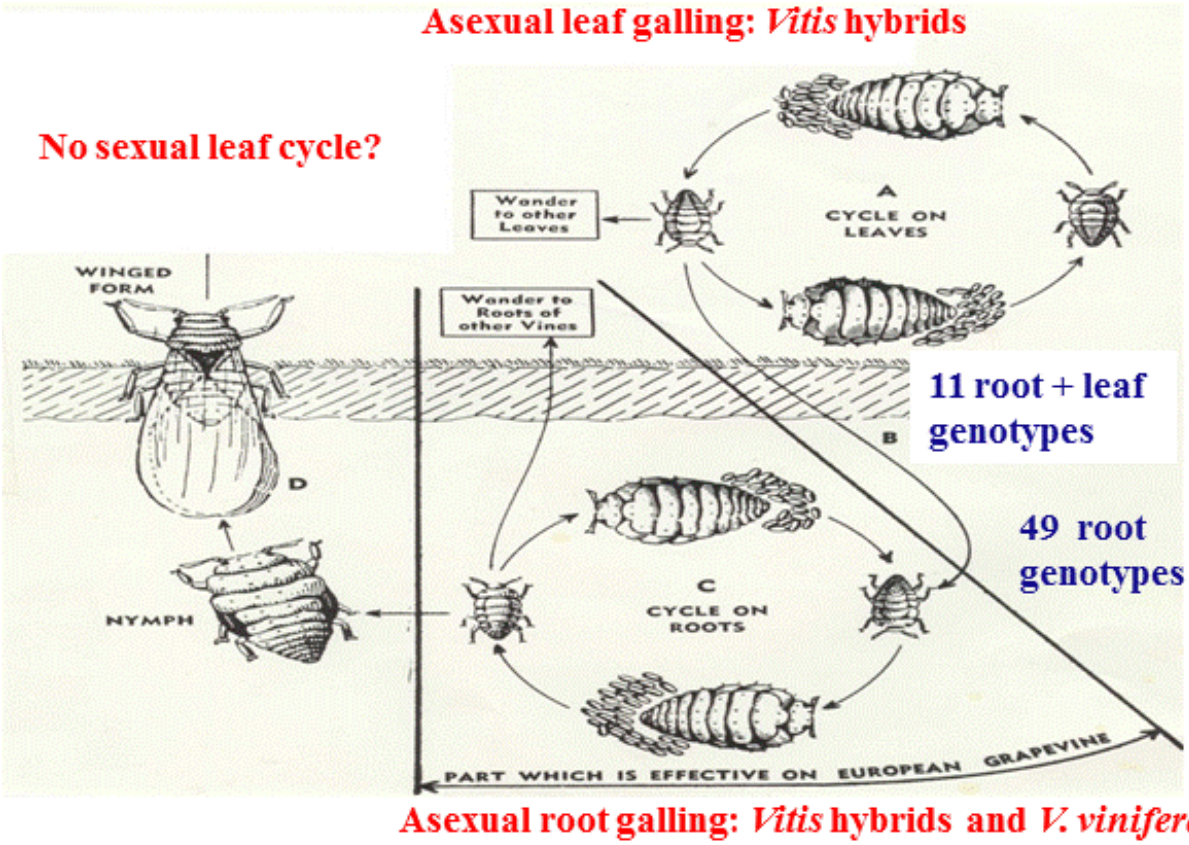


Figure 3. Adapted life-cycle of grape phylloxera in Australia

A= Distinct asexual leaf-galling life-cycle on American *Vitis* species or rootstock hybrids; B = some leaf galling genotypes move as first instars move to the root system; C= Asexual root-galling life-cycle on both European *V. vinifera* and American *Vitis* species; D= Alates develop and emerge to above-ground but no evidence of egg laying or sexual life cycle has been observed. The number of genotypes varies depending on the life-cycle and host range.

2.1.3.5. Phylloxera genotypes

Molecular markers have been used to characterise genetic diversity and geographic distribution of holocyclic (sexual) and anholocyclic (asexual) populations and elucidate the life-cycle of grape phylloxera using random amplified polymorphic DNA (RAPD) (Corrie *et al*., 1997; Fong *et al*., 1995; Kocsis *et al*.,1999; Yvon and Peros, 2003), amplified fragment length polymorphisms (AFLP) (Forneck *et al*., 2000, 2001b; Vorwerk and Forneck, 2007; Ritter *et al*., 2007) and microsatellite markers (Corrie *et al*., 2002).

Over the past 30 years, evidence for the existence of different phylloxera 'biotypes' has become more apparent because of differences in their performance on a range

of *Vitis* species and hybrids. New strains can evolve, such as biotype B (Granett *et al*., 1985), which overwhelmed the partially resistant rootstock genotype AXR#1 (Sullivan, 1996) in California. A wide-range of strains has been reported in Europe (Song and Granett, 1990; Forneck *et al*., 2001b; Yvon and Peros, 2003), Australasia (King and Rilling, 1985; Corrie *et al*., 1997, Corrie *et al*., 2002), Canada (Stevenson, 1970), South Africa (De Klerk, 1979) and the USA (Williams and Shambaugh, 1987; De Benedictis and Granett, 1992). From the studies using microsatellite markers, asexual reproduction appears to predominate in Europe (Vorwerk and Forneck, 2006), Australia (Corrie and Hoffmann, 2004; Umina *et al*., 2007), China (Sun *et al*., 2009) and California, United States (Lin *et al*., 2006).

2.1.3.6. Endemic and exotic strains

There are two genetically distinct groups of phylloxera of potential concern, exotic and endemic strains, in regards to risk of entry. Exotic strains are not present in Australia but could potentially be introduced on imported material, particularly grapevine products such as germplasm, grapevine cuttings and table grapes. The risk of their entry is relatively low provided pre-entry surveillance and quarantine procedures are adhered to. However should exotic strains enter Australia, the risk of establishment may be considered high (PHA, 2009) due to the relative proportion of ungrafted *V. vinifera* plantings in Australia and the possibility of introduction of highly virulent genetic strains. Once established genotypic screening would need to be conducted to ensure the correct rootstock selection for effective post-entry management. In contrast, some endemic strains are known to be present in all PIZs in high abundance and therefore represent a higher risk of transfer to PRZs or PEZs. The risk of entry needs to be controlled by effective implementation and utilisation of scientifically validated quarantine measures such as those represented in state and national phylloxera quarantine protocols.

2.1.3.6.1. Endemic phylloxera strains

In Australia, the first evidence of genetically diverse phylloxera strains was obtained using RAPD markers to compare phylloxera strains from three geographically distinct grape-growing regions the King Valley, Nagambie and Rutherglen (Corrie *et al*., 1997). Three root-feeding ‘biotypes’ were initially described (Corrie *et al*., 1998). More recently detailed surveys and the use of improved genetic markers have

subsequently identified 83 genotypes in Australia, including both radicole and gallicole forms, using six microsatellite mitochondrial DNA markers (Corrie *et al*., 2002; Corrie and Hoffmann, 2004, Umina *et al.,* 2007). All 83 genotypes are located in the oldest quarantine region the NE Victoria PIZ (Table 1). In all other PIZs and regions, namely Whitebridge, Maroondah, Upton, King Valley (which was later merged with the NE PIZ) and NSW PIZs, only a single genotype has been confirmed. Currently two radicole genotypes, G1 and G4, predominate in Australia and are described as 'superclones' (Umina *et al*., 2007). These superclones have a broader geographic distribution (for example G1 is found in all PIZs) and comparatively higher virulence levels. They have been identified in most of the phylloxera outbreaks occurring in Australia since 1994 with the exception of a single detection of G20 in the Buckland Valley (K.S. Powell, pers. observation).

Virulence levels (as assessed by relative population abundance) of endemic phylloxera strains may vary on susceptible *V. vinifera* as shown in laboratory, glasshouse and field trials that compared the performance of radicole phylloxera strains (Herbert *et al*., 2008, 2010; Trethowan and Powell, 2007; Korosi *et al*., 2009, 2011). The strains differ in fecundity, survivorship, development, virulence and damage levels and consequently risk of transfer. The two 'superclones' G1 and G4 display the highest virulence which can be 100-fold higher and damage levels causing death of ungrafted *V. vinifera* within a 3-5 year period. In contrast less virulent phylloxera genotypes may cause minimal damage over several decades (Figure 4).



Figure 4. Relative visible damage symptoms seen on ungrafted *Vitis vinifera* foliage from (a) a 5-year old infestation with G4, a highly virulent endemic phylloxera strain and (b) a 30-year old infestation with low virulent endemic phylloxera strain G19.

These results point to only some highly virulent phylloxera clones on *V. vinifera* in some environmental conditions that help to explain differences in patterns of field damage, phylloxera distribution and continued survival and production (Herbert *et al*., 2006; Powell *et al*., 2003). However other genotypes (e.g. G7, G19, G20 and G30) differ biologically in terms of their host-plant interactions and could still represent a high risk of transfer particularly in areas where rootstocks are predominantly planted. In addition, because these phylloxera genetic strains can reproduce on a broad range of 'resistant' rootstocks without doing notable damage, they could be more difficult to detect when reliant on visible symptoms.

Table 1. Summary of current known phylloxera genotypic and life-cycle geographic distribution in Australia

PIZ	**Radicoles**	**Gallicoles**	**Genotypes**
VICTORIA			
North East Victoria	Yes	Yes	83 genotypes (including G1, G4 and G20)
**King Valley*	*Yes*	*Yes*	*G4 and yet to be identified genotypes*
**Buckland Valley*	*Yes*	*No*	*G4, G20*
**Ovens Valley*	*Yes*	*No*	*G4*
Nagambie	Yes	No	G1
Whitebridge	Yes	No	G1
Upton	Yes	No	G1
Maroondah	Yes	No	G1
NEW SOUTH WALES			
Sydney	Yes	No	G1
Albury-Corowa	Yes	No	G1

* The King, Ovens and Buckland Valleys are cool climate regions of the NE Victoria PIZ. However, only two genotypes have been detected in these regions (G4 in the King and Ovens Valley and G4 and G20 in the Buckland Valley) as compared with the rest of the NE PIZ where 83 genotypes have been detected.

2.1.3.6.2 Exotic phylloxera genetic strains

Although there are 83 genetic strains known to occur in Australia, outside Australia more genetic strains are known to occur. For example, in Europe high genotypic diversity and predominant asexual reproduction on *V. vitifoliae* has been reported (Vorwerk and Forneck, 2006) with strains being predominantly leaf-galling. In Australia, focus on screening of rootstocks has been on known endemic root-galling genetic strains whereas individual strains or leaf-galling strains have been primarily investigated overseas. Therefore should exotic strains enter Australia, rootstock recommendations would be difficult to determine.

3. POTENTIAL FOR ESTABLISHMENT

The potential for phylloxera to establish in Australia will be determined by a number of factors i) Phylloxera life-stage- the first instar is the life-stage which actively seeks a food source and once established the nymph remains at its feeding site and develops into intermediate nymphal instars and then into adults. Adults and intermediate instars are relatively limited in their movement. ii) Temperature - Turley *et al.* (1996) noted that feeding does not occur until temperatures reach 18°C. In cold winter periods, this could potentially limit establishment iii) Soil properties - textural and chemical properties could potentially limit phylloxera establishment although most of the evidence for this assumption is either anecdotal or limited. Soil moisture is unlikely to limit phylloxera establishment as phylloxera can survive at both low and high humidity.

3.1 Biotic factors

The main biotic factors which are likely to influence establishment are the phylloxera genotype and the host plant rootstock..

3.1.2. Phylloxera genetic strain characteristics

Some endemic genetic strains, most notably G1 and G4 are described as ‘superclones’ and have a higher reproductive capacity, are more abundant in number than other strains and are more likely to establish on *V. vinifera*. The more virulent the genetic strain, the higher the abundance of phylloxera and the greater the risk of transfer and economic damage.

3.1.2. Resistant rootstocks

Rootstocks from North American hybrids of *Vitis* species onto which *V. vinifera* cultivars are grafted are resistant or partially resistant to phylloxera. The use of rootstock, therefore, is the predominant and most successful management option to prevent economic damage from grape phylloxera.

Laboratory trials have shown that endemic phylloxera strains can establish on resistant rootstocks (Table 2). Thus, whilst the use of resistant rootstocks can reduce damage it does not necessarily eliminate the risk of phylloxera spread and quarantine measures will still need to be used. Use of selected rootstocks could however substantially reduce the risk of transfer particularly in areas where only single phylloxera strains are known to occur.

Table 2. A summary of overall rankings for rootstock resistance, partial resistance (tolerance) and susceptibility to radicole grape phylloxera based on either (a) *in vitro* excised root screening under laboratory conditions and (b) *in planta* screening under glasshouse conditions using six genetic strains (Adapted from Powell, 2012; Powell *et al.*, 2014).

Rootstock	**Genotype Bioassay**	**G1** a	**G1** b	**G4** a	**G4** b	**G7** a	**G7** b	**G19** a	**G19** b	**G20** a	**G20** b	**G30** a	**G30** b
Vitis vinifera	*V.vinifera*	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
Ramsey	*V. rupestris* x *V. candicans*	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**
Schwarzmann	*V. rupestris* x *V. riparia*	**R**	**R**	**R**	**R**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**
Borner	*V. riparia* x *V. cinerea*	**R**	**R**	**R**	**R**	**T**	**R**	**R**	**R**	**R**	**R**	**T**	**R**
110 Richter	*V. berlandieri* x *V. rupestris*	**T**	**T**	**T**	**T**	**T**	**R**	**T**	**R**	**T**	**T**	**T**	**R**
140 Ruggeri	*V. berlandieri* x *V. rupestris*	**T**	**R**	**T**	**T**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
1103 Paulsen	*V. berlandieri* x *V. rupestris*	**T**	**T**	**R**	**R**	**R**	**R**	**T**	**T**	**R**	**R**	**R**	**R**
420A	*V. berlandieri* x *V. riparia*	**R**	**T**	**R**	**R**	**R**	**R**	**R**	**T**	**R**	**T**	**R**	**R**
5BB Kober	*V. berlandieri* x *V. riparia*	**R**	**R**	**R**	**R**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**
101-14	*V. riparia* x *V. rupestris*	**R**	**R**	**R**	**R**	**T**	**R**	**R**	**R**	**R**	**T**	**R**	**R**
3309C	*V. riparia* x *V. rupestris*	**R**	**R**	**R**	**R**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**

Knowledge gaps

Although substantial screening for rootstock resistance to six endemic phylloxera strains has occurred, only ten commercially available rootstocks have been tested to date. It should also be noted that this screening has primarily been conducted under controlled conditions and field screening has been limited. Under controlled conditions, the influence of other factors on phylloxera establishment and interactions with the rootstock, such as soil type, temperature, and humidity have not been examined. Further screening of rootstocks for resistance against endemic strains particularly under different soil conditions is required.

3.2. Abiotic factors

The key environmental factors which may influence phylloxera establishment, survival and rate of spread are temperature, relative humidity and soil characteristics.

3.2.1. Temperature

The distribution of phylloxera worldwide includes all climatic zones where its host plant *Vitis* species is grown. It is present in regions with extreme temperature ranges, for example the Ararat Valley, Armenia where temperatures range from -30°C to 42°C (Armenian Travel Bureau, 2010) and cold regions of Russia including Moldova, Ukraine, in Rostov Region, Stavropol and Krasnodar Territories of Russia, in the Caucasus (Afonin *et al*., 2009). This broad temperature survival range of phylloxera would suggest that temperature is not a limiting factor in the potential for phylloxera survival in grape growing regions of Australia. Phylloxera has been detected in both cool and warm climate grape growing regions of Victoria and NSW.

Once phylloxera is established, it can survive harsh temperature regimes presumably due to its ability to overwinter in a dormant ‘crawler’ stage and its ability to move down the soil profile as far as the grapevine root system allows. In some studies in Australia and South Africa, phylloxera has been detected over 1 metre vertically down the root profile (Buchanan, 1990, De Klerk 1974). This ability to move down the root profile also highlights the difficulty in some instances of detecting the insect or controlling the insect through the use of systemic insecticides. Few life-table studies have been conducted to determine the impact of temperature on grape phylloxera development and geographic distribution and none have been determined for endemic phylloxera strains in Australia. Some studies to assess the impact of

temperature on radicole phylloxera have been conducted under laboratory and field conditions. Prior to the 'discovery' of biotypes in California, life-table studies were conducted using the excised root system on phylloxera populations sourced from the Napa Valley and Davis regions. Reproductive rate, generation time and finite rate of increase were all determined and compared on susceptible *V. vinifera* and resistant *Vitis* hybrids. Survivorship to adulthood, establishment and daily egg production was higher on susceptible *V. vinifera* cultivars (Granett *et al*., 1983).

A later study using excised roots demonstrated that for phylloxera 'biotype A', the optimal temperature range for nymphal survival was 21ºC to 28ºC, temperatures <16ºC and >36ºC reduced egg hatch though eggs hatched at >7ºC. At 32ºC development to adulthood ceased (Granett and Timper, 1987). In a second laboratory bioassay, again using 'biotype A', the threshold temperature for establishment of a feeding site was 18ºC (Turley *et al*., 1996). Makee (1984) using phylloxera of unspecified 'biotype' determined the optimal range for nymph development was between 15ºC and 30ºC and that optimal egg production was at 25ºC. Connelly (1995) showed that temperatures >16ºC were required under laboratory conditions for development, but overall laboratory bioassay results were not representative of field conditions for development.

Temperature thresholds have also been developed for foliar grape phylloxera reared on the resistant rootstocks 5BB Kober (Belcari and Antonelli, 1989) with optimal temperatures ranging between 25-30ºC. From field observations in California, Davidson and Nougart (1921) suggested that at <19ºC phylloxera hibernated, whilst Zhang *et al*. (2010) describe phylloxera first instars becoming active as ground temperatures exceed 17ºC. A study comparing phylloxera populations from four geographically distinct regions in the Pacific Northwest USA overall upper and lower temperature thresholds were <6ºC and >33ºC respectively (Fisher *et al*., 2003). Degree-day models have been developed for field populations of phylloxera in Italy (Belcari and Antonelli, 1989), USA (Turley *et al*., 1996; Sleezer *et al*., 2011) and Australia (Herbert *et al*., 2010), yet none of these models takes into account the combined influence that temperature, soil properties and vine genetic background may have on different phylloxera strains. Granett and Timper (1987) observed that different phylloxera 'biotypes' may have different optimal temperature ranges and this was confirmed in a recent study comparing the influence of

temperature and humidity on two selected radicole phylloxera clonal lineages (Korosi *et al*., 2012).

Knowledge gaps

Whilst life-table studies have been conducted to determine the influence of temperature on survival and development of phylloxera elsewhere, no such studies have been conducted on Australian endemic strains. This type of information is essential as a predictive tool to determine potential risks of phylloxera establishing in different regions. Disinfestation trials, using heat treatment, do however provide some preliminary indication that thermal limits for survival may differ between phylloxera endemic strains. As temperature is one of the key factors that influences insect development and feeding site establishment, further research is warranted. The only controlled studies conducted in Australia to examine the potential impact of temperature on phylloxera survival were restricted to G1 and G4 phylloxera. These two strains are genetically quite similar and it is unknown what impact temperature may have on other diverse genetically endemic strains.

Phylloxera, like most insect species, is highly sensitive to temperature. Temperature affects development, fecundity, generation times, population development and above and below ground risks of transfer. Knowledge of the insects sensitivity to temperature, particularly lethal limits (both high and low), informs distribution and establishment, population abundance and hence relative quarantine risks, and the efficacy of detection and disinfestation treatments.

3.2.2. Relative humidity

Although there is limited scientific literature on the effect of relative humidity (RH) on phylloxera some general trends have been observed. Buchanan (1990) compared phylloxera survival of a range of relative humidity 12-95% RH and a temperature range of 15-35°C. This study showed that relative humidity's <50% reduced survival and 95% RH was consistently better survival high at all temperatures. There was a significant interaction between RH and temperature. Hatching is relatively low at 35% and highest at 100% RH (Buchanan 1990). Development of all active life-stages is also improved as relatively humidity is increased above 35%. This study, however, was only conducted using a single phylloxera population sourced from Nagambie Victoria.

Further studies have been conducted examining the effects of RH and higher temperatures (Korosi *et al*., 2009; 2012). In the 2009, a study using G4 phylloxera showed that survival at 30% RH and 100% RH was dependent on temperature. When G1 and G4 phylloxera strains were compared (Korosi *et al*., 2012) both strains preferred higher RH but there were differences in survival between the two strains. This study was limited to temperatures of 30-45°C at 5°C increments.

Knowledge gaps

As the only studies to examine the potential impact of relative humidity on phylloxera survival were restricted to G1 and G4 phylloxera, a more comprehensive study should be conducted to compare a minimum of six endemic strains which represent a broader genetic diversity.

3.2.3. Carbon dioxide

Although there is limited information on the impact of atmospheric carbon dioxide ($CO_{2)}$ on grape phylloxera, Granett *et al*. (1991) when testing carbon dioxide as a potential fumigant for radicole phylloxera reported CO_2 in laboratory tests requires a 'high concentration and relatively long duration to be an effective control method. The authors, however, did not specify the CO_2 concentrations required. Atmospheric and soil CO_2 levels could potentially impact on phylloxera development and hence the level of risk of establishment and spread in future climates. A range of responses to elevated CO_2, including modified fecundity, population density and development rates, have been observed in several aphid species (Holopainen, 2002; Trebicki *et al*., in prep). Elevated CO_2 levels alter plant chemistry and root phenology by modifying the C:N ratios in plant sap, which could then impact secondary metabolite, carbohydrate and amino acid profiles (Bezemer and Jones, 1998), and increased cell production and cell expansion in plant roots (Lawler *et al*., 1997).

3.2.4. Soil characteristics

In contrast to gallicoles, radicole phylloxera spend much of their life-cycle below-ground although first instars and alates do emerge during spring and summer. Therefore the soil environment is likely to have a major influence on the insects' life-cycle and population dynamics. Soil properties including chemistry, texture, moisture, atmosphere and temperature are all likely to impact on phylloxera establishment,

population development and dispersal and also the development of the host-plant root system (Kühnelt, 1963). Control and management of radicole grape phylloxera, requires an understanding of how environmental factors impact on the insects' biology and interactions with the host plant below-ground. Soil temperature, humidity, organic matter, salinity and CO_2 content can affect root-feeding insect's survival and host plant location (Kühnelt, 1963; Gerson, 1996). Soil conditions can also influence the rooting depth of different *Vitis* species (Hellman, 2003) and consequently the potential depth of migration of radicole phylloxera within the soil profile. Radicole phylloxera have been observed on roots at soil depths of over 1 metre (De Klerk, 1974; Buchanan, 1990). The ability of radicole grape phylloxera first instars to move down the soil profile on the root system is likely to constrain the efficacy of some cultural, biological and chemical control options (Benheim *et al*., 2012) where soil interactions may occur and also influence the efficacy of detection systems.

3.2.4.1. Soil texture

Most observations on the influence of soil on phylloxera, are largely anecdotal and focus on textural properties particularly on the sand to clay ratio. In vineyard surveys conducted in South Africa, phylloxera predominated in soils with less than 65% sand content (De Klerk, 1972). In Canadian and Californian vineyards, phylloxera are reportedly more common on clay soils rather than loam and sandy-loam soils (Bioletti, 1901; Nougaret and Lapham, 1928; Stevenson, 1964). Ermolaev (1990) suggests that the amount of silicon in sandy soil may be an important factor in reducing phylloxera abundance.

3.2.4.2. Soil chemistry

The influence of soil properties on phylloxera is more complex than just soil texture. In Austrian vineyards, soils pH, carbonate content, organic carbon, and texture in combination influence phylloxera population abundance (Reisenzein *et al*., 2007). In contrast, a glasshouse study showed that similar soil properties had minimal impact on establishment of phylloxera populations sourced from the Pacific Northwest region of North America (Chitkowski and Fisher, 2005). This apparent discrepancy could be attributed to soil temperature, which in the glasshouse study were controlled, and phylloxera genetic strain which was not specified in either of the two studies mentioned above.

Under Australian field conditions, some chemical factors have been highlighted which may influence phylloxera abundance and dispersal (Powell *et al*.,2003). High levels of aluminium exchange capacity, toxic to grapevine roots, are related to higher phylloxera abundance (Powell, 2007). Aluminium is known to stress plant root systems inhibiting root growth and affecting root chemistry (Delhaize and Ryan, 1995; Dipierro *et al*., 2005).

The spatial distribution of radicole phylloxera has been linked to specific soil electrical conductivity ranges as assessed using EM38 sensors (Bruce *et al*., 2011). Apparent electrical conductivity (ECa) is related to soil texture and chemical properties including soil moisture, salinity and ionic content (Corwin and Lesch, 2005; Friedman, 2005). However the specific property or properties, related to ECa, which directly influence radicole phylloxera distribution in soil are yet to be determined. Other sensed data such as aerial or satellite acquired reflectance bioimagery, which focuses on stress symptoms in grapevine foliage, when combined with ECa data offers opportunities for integrated targeted phylloxera detection (Bruce *et al*., 2011).

Determining which soil properties influence phylloxera spatial distribution and emergence of dispersive life-stages is also important for optimising sampling strategies for newly emerging detection tools including a phylloxera specific molecular probe for soil samples (Herbert *et al*., 2008) and trapping techniques (Powell *et al*., 2009). Understanding the influence of soil properties on phylloxera could also lead to improved cultural management techniques.

Knowledge gaps

Studies conducted on the interactions between soil and phylloxera are very limited. If more basic information on the influence of soil physical and chemical factors were collected, this information could be combined with temperature data and used to develop phylloxera risk maps. This approach has been considered previously in Western Australia (Botha et al., 2007) but this approach does require more basic data before it could be used for predictive models.

4. FACTORS INFLUENCING PHYLLOXERA DISPERSAL

Natural and human assisted dispersal are essentially, two major pathways by which phylloxera could be spread within and outside a PIZ.

4.1 Natural dispersal

The risk of phylloxera spreading by natural means is limited by a number of factors including life-cycle mode and life-stage.

4.1.1 Phylloxera life-stage

There are five phylloxera life-stages - eggs, first instars, intermediate instars (which include second, third and fourth instars), winged adults (alates) and wingless (apterous) adults. However the relative mobility and natural dispersal ability of each stage differs.

4.1.1.1. Eggs

Phylloxera eggs, for both radicole and gallicole, are immobile and therefore have no natural dispersal ability. Eggs can be found below ground and on foliage depending on the life-cycle and can only be disperse by human assisted means or movement of vineyard equipment.

4.1.1.2. First instars

This life-stage of phylloxera is considered to be the highest risk for a number of reasons. Firstly, it is produced in relatively high abundance, both in the root-galling and leaf galling life-cycle, compared to other instars. Secondly, it is more mobile than other instars. Thirdly, its distribution above and below ground is broader than other life-stages. It is mobile within the soil profile, on the soil surface, within the grapevine canopy and can also be wind dispersed. This life-stage is asexual and hence a single crawler could potential start a new establishment. The crawler could then develop rapidly to produce an apterous adult capable of producing several hundred eggs. The first instar stage is the most abundant with high population levels found on the soil surface, foliage, and fruit throughout late spring and summer (King and Buchanan, 1986; Powell *et al*., 2000; Omer *et al*., 2002; Porten and Huber, 2003). Mobility and dispersal is assisted by increasing temperatures (Herbert *et al*., 2006) and changes in grapevine phenology.

However, first instar grape phylloxera have limited natural dispersal ability (King and Buchanan, 1986) and are spread predominantly through human activity, transferred on viticultural machinery and equipment, footwear and clothing, as well as planting material, soil and some grape products (Deretic *et al*., 2003; Powell, 2008).

Population dynamics studies have confirmed the presence of first instars on both vine foliage and fruit throughout the growing season (Powell *et al*., 2000). This illustrates an additional risk of unintentional grape phylloxera transfer during the harvest period (Korosi *et al*., 2009), through transfer on mechanical harvesters (King and Buchanan, 1986), clothing of vineyard personnel involved in hand harvesting and on post-harvest material such as grapes and unfermented marc (Powell, 2008).

4.1.1.3. Intermediate instars

Natural dispersal of radicole intermediate instars is limited to the roots of infested grapevines and surrounding soil as they do not move from their food source. For galliciole intermediates they are enclosed within leaf galls and are therefore cannot disperse unless the leaf gall structure is damaged.

4.1.1.4. Apterous adults

Galliciole apterous (wingless) adults are enclosed within leaf galls and are therefore restricted within the gall and cannot disperse unless the leaf gall is damaged. Radicole apterous adults, although not enclosed by a gall, do not naturally disperse beyond the grapevine root on which they are feeding or surrounding soil.

4.1.1.5. Alate adults

Radicole phylloxera are not enclosed within galls and when the nutritional quality of roots is diminished or the phylloxera population becomes overcrowded, alate (winged) adults will develop. They can disperse both below- and above-ground. Alates have been recorded on multiple occasions in the summer months above ground in Australian vineyards (Powell. pers. observation). However alate phylloxera have limited survival capacity and are weak flyers. They have been recorded in aerial traps which indicates they can disperse by wind assistance. However unlike apterous adults they can only lay two types of egg a single male egg and a single female egg. These non-feeding sexual offspring mate and lay a single egg which develops into a fundatrix for the next season. There have been no confirmed sightings of a sexual egg being produced in Australia and the restricted genetic diversity of endemic strains makes it unlikely that sexual reproduction occurs. The risk therefore for alate adults contributing to dispersal, either by natural or human assisted means, and

subsequent establishment is considered very low. Alate adults have not been detected in leaf galls.

4.1.2. Phylloxera life-cycle

4.1.2.1. Root-galling phylloxera

The radicole egg stage is immobile and is found only on the root system or surrounding soil. There are six mobile stages of radicole phylloxera; first, second, third fourth instars, apterous adult and alate adult. All of these stages are mobile within the root zone of infested vines. Second to fourth instars and apterous adults once established stay on the root system and do not migrate above ground, they also may be found around the root zone when soil is disturbed through cultivation practices. When overcrowding or competition for roots increase and soil temperatures increase, first instars and alate adults can move (or emerge) to the soil surface in spring and summer. Alates are considered low risk under both Australian and European climatic conditions. Evidence for the sexual component of the life-cycle is limited (Corrie *et al*., 2002; Vorwerk and Forneck, 2006) and their relative abundance in comparison with first instars is low (Powell *et al*., 2000).

The first instars are the most active and mobile of grape phylloxera life-stages. This instar is called a 'crawler' because it crawls within the soil profile, on the soil surface, on the grapevine trunk, foliage and grape in search of a food source (Powell, 2006). It is also the most abundant life-stage and in some PIZs has been detected in relatively high numbers and therefore represent a high quarantine risk. In the King Valley, for example, emergence traps have detected >16,000 first instars emerging from a single grapevine over a 14 day period in summer (Powell. pers. observ). Due to its asexual lifestyle, a single crawler can potentially start a new infestation. Maximal distance of movement across the soil surface is 100 meters per season (King and Buchanan, 1986) although 1km per year has also been noted (Buchanan et al, 1998). Based on natural dispersal a minimum 5km buffer zone was original suggested for a PIZ, as it was believed that new phylloxera infestations may take up to 5km to detect (Buchanan *et al*., 1998). Alates are far less abundant and because they do not feed or reproduce, they represent a very low quarantine risk.

4.1.2.2. Leaf-galling phylloxera

Gallicole phylloxera life-stages are the same as for radicole phylloxera, however their habitat is primarily above-ground. There are some leaf-galling genetic strains which have the ability to migrate down to the roots from the leaves (Figure 3). Leaf-galling phylloxera has only been observed on resistant rootstocks in Australia. As less than 20% of vineyard plantings are grafted rootstocks, with good vineyard management of rootstock suckers (where galling occurs), the risk of dispersal of mobile stages is lower than for radicole. However, if leaf galls are present each leaf may have multiple leaf galls. In a single enclosed gall a single female apterous adult can produce several hundred eggs. When eggs hatch, the gall opens and several hundred phylloxera first instars emerge and disperse onto foliage grapes, trunks and the soil surface and down to the root system.

4.1.2.3. Wind-assisted dispersal

Phylloxera first instars and alates can be moved at least 30 metres in wind (Powell, 2000). It is also possible they may be dispersed for longer distances but this assumption has not been validated scientifically.

4.1.2.4. Water-assisted dispersal

The ability of mobile phylloxera life-stages to disperse by water run off or through water in irrigation piping has not been quantified. However it is feasible that the most active first instar stage could be dispersed in this manner through infested vineyards either directly in irrigation water or along dripper lines. Phylloxera first instars can survive for 7 to 21 days immersed in water depending on the temperature (Powell *et al*., 2014).

4.1.2.5. Animal-assisted dispersal

Although potentially feasible, there is no published scientific evidence to show that phylloxera is transported by either birds or animals.

4.2. Human assisted dispersal

Because natural dispersal is limited in its geographic range, the major risks of transfer is on human assisted vectors. Primary vectors include viticulture machinery, clothing and footwear, planting material, soil and certain grape products. The

presence of first instars on wine grapes at harvest (Powell, 2000) means they could be inadvertently transferred from an infested vineyard either in grape bins or to an uninfested vineyard on mechanical grape harvesters, or in clothing or footwear attached to vine yard workers, if quarantine precautions are not adhered to.

Other major sources of phylloxera movement are planting material and soils. Phylloxera can potentially be transported on grapevine planting material as rootlings or potted vines, which can harbour all phylloxera life-stages and represent a significant risk. Postharvest products such as grapes or unfermented marc (a mixture of grape seeds, skins and stalks), and other winery waste products are considered a risk as they can carry viable phylloxera first instars (Deretic *et al*., 2003; Korosi *et al*., 2008). A range of primary risks have been identified and a summary is provided for both root-galling and leaf-galling phylloxera (Tables 3 and 4). This list is by no means exhaustive but does represent the most common risks within the vineyard and winery and those for which some quarantine measures have already been developed (National Vine Health Steering Committee, 2009).

Table 3. Relative risk of radicicole phylloxera dispersal on different vectors. Disinfestation treatments are based on relative abundance, phylloxera life-stages, survival and establishment likelihood.

Risk pre-disinfestation	Predominant life-stage(s)
Grapevine Cuttings/Rootlings	All life-stages present
Soil	All life-stages present
Diagnostic samples	All life-stages present depending on type of diagnostic sample
Viticultural Machinery	First instar
Grape bins	First instar
Wine grapes	First instar
Table grapes	First instar
Footwear	First instar
Foliage	First instar
Clothing	First instar
Marc	First instar
Unfermented must	First instar
Unfiltered juice	First instar

NB. Alates have not been included as there is no evidence that sexual reproduction occurs in Australia and their abundance is relatively low compared with other life-stages.

Table 4. Relative risk of gallicole phylloxera dispersal on different vectors. Disinfestation treatment is based on relative abundance, life-stages, survival and establishment likelihood.

Risk	**Predominant Life-stage(s)**
Grapevine Cuttings/Rootlings	All life-stages present only if leaf galling strains migrate and establish on roots
Soil	All life-stages present only if leaf galling strains migrate and establish on roots
Diagnostic samples	All life-stages present depending on type of diagnostic sample
Viticultural Machinery	All life-stages potentially present but first instars most abundant
Wine grapes	First instar
Table grapes	First instar
Footwear	First instar
Foliage	First instar
Clothing	First instar
Marc	First instar
Unfermented must	First instar
Unfiltered juice	First instar

NB Overall the risk for leaf galling phylloxera is low due to limited geographic distribution of endemic gallicolae strains and presence only on rootstock leaves.

4.2.1. Grapevine and grape products

4.2.1.1. Vitis propagation material

Vitis species is the only known host of phylloxera. All life-stages of radicicole phylloxera can survive on root material in relatively high numbers and therefore, this pathway represents a particularly important risk of transfer. The relative abundance of phylloxera on *Vitis* planting will however depend on the interaction between the *Vitis* host parentage and the genotype of phylloxera. *Vitis vinifera* can host at least six genotypes of endemic phylloxera in Australia. In addition, all 83 genotypes have been detected on at least one type of resistant *Vitis* rootstock. The dormancy of grapevine rootlings or cuttings will influence what life-stages may be present. On dormant material first instars are in hibernation mode, whereas on vital material all life-stages are known to be present. The movement of *Vitis* propagation material is prohibited from PIZs (National Vine Health Steering Committee, 2009).

Knowledge gaps

Whilst it is assumed that all endemic strains of grape phylloxera will survive on *V. vinifera,* this has not yet been fully elucidate. Data on suitability of ungrafted *V. vinifera* and commercially available *Vitis* hybrids as a host for all 83 known endemic genotypes of phylloxera is limited. More field surveys are required to be able to map out the full genetic diversity of endemic strains and determine their distribution.

4.2.1.2. Grapes – for consumption or processing

Phylloxera does not actually feed or develop on grapes. However field studies conducted in Australia, have shown that phylloxera first instars can be found on grape bunches during the harvest period (Powell 2000) and consequently represent a risk (National Vine Health Steering Committee, 2005).

4.2.1.3. Grapevine foliage

Some gallicole genetic strains of phylloxera first instars can initiate leaf galls on *Vitis* species, develop adults on grapevine leaves studies conducted in Australia have determined that phylloxera first instars can be found on grape bunches during the harvest period (Powell et al., 2000; Deretic et al., 2003).

4.2.1.4. Crushed and destemmed grapes

As phylloxera first instars can survive transportation in grape bins, the next stage in the post-harvest treatment after grape delivery at the winery is crushing and destemming. Phylloxera crawler survival has been assessed following mechanical crushing and destemming of two grape varieties Semillion and Shiraz (Deretic et al., 2003; Powell et al 2003). Up to 34% survived the crushing treatment and 3.5% were present on the stems post-treatment. No significant effect of grape variety was recorded. This study highlighted the risk of phylloxera presence in grape must and marc. Powell (2017) showed that phylloxera first instar nymphs are killed in homogenised grape samples at three homogeniser settings when subjected to replicated treatments for 10, 20 and 33 seconds.

Knowledge gaps

The survival of phylloxera on crushed grapes has only been assessed for first instar phylloxera. Intermediate instars and adults are bigger in size and could potentially escape blades during the homogenisation process. The latter aspect warrant further investigations.

4.1.3.5. Pressed grapes

White must is usually pressed after crushing. Research work conducted on a single root-galling endemic strains has shown that 83% survival is possible for first instars (Deretic *et al*., 2003), with 73% passing through to juice and 10% remaining alive in the pressed solids.

Knowledge gaps

The survival of phylloxera on pressed grapes has only been assessed for single genetic strain and limited grape varieties.

4.1.3.6. Grape must

Grape must is the juice of crushed grape berries and it may include skins, seeds, pulp, stems and leaves. As some phylloxera first instars can survive in must after 24 hours, there is a risk of phylloxera being transferred in unfermented must (Deretic *et al*., 2003).

Knowledge gaps

The survival of phylloxera in grape must has only been assessed for single genetic strain and limited grape varieties.

4.1.3.7. Grape marc

Marc is the solid residues remaining after pressing of must. It can contain skins, seeds and stems. Phylloxera has been shown to survive in unfermented marc (Deretic *et al*., 2003).

Knowledge gaps

The survival of phylloxera on grape marc has only been assessed for single genetic strain and limited grape varieties.

4.1.3.8. Grape juice

A proportion of phylloxera active life-stages can survive post-harvest grape processing including crushing, desteming and pressing and so can be present in juice. Processing of juice including filtering, fermentation and cold treatment can impact on phylloxera risk (see section 5).

Knowledge gaps

The survival of phylloxera in grape juice has only been assessed for single genetic strain and limited grape varieties.

4.1.4. Clothing and footwear

First instars are the active stages of phylloxera and have been shown to be present on the soil surface, the grapevine trunk, foliage and grapes. There is, therefore, a high likelihood that phylloxera will be present on footwear and clothing particularly in the spring and summer months when above-ground populations of phylloxera are abundant (Powell *et al*., 2000; 2003; Herbert *et al*; 2006). The relative abundance of phylloxera on clothing is seasonally dependant and also linked to the life-cycle and genetics of the insect.

Knowledge gaps

Despite the risk of transferring first instar phylloxera, there has not been an effective and safe disinfestation procedure developed for clothing. Footwear disinfestation protocols are discussed in section 5.2.5 below.

4.3. Viticulture machinery and vehicles

4.3.1. Grape bins

Phylloxera first instars have been shown to be present at relatively low levels on both grapes and foliage at harvest time (Powell *et al*., 2000) thus highlighting to potential transfer to winery receival areas on grapes in grape bins. Studies on the survivability of grape phylloxera first instars in grape bins has been limited to two Australian studies which examined G4 phylloxera survival on red and white grapes (Deretic *et al*., 2003; Powell *et al*., 2003). These studies indicated on average 70% survival at 15-25°C for at least 16 hours and highlighted the risk of phylloxera transfer in transported grape bins.

Knowledge gaps

The survival of phylloxera in grape bins has only been assessed for a single genetic strain and limited grape varieties.

4.3.2. Grape harvesters

A single study has been conducted which quantified the presence of phylloxera first instars on grape harvesters (King and Buchanan, 1986). The likelihood of active phylloxera life-stages on mechanical harvesters and other machinery is high as phylloxera first instars have been detected on harvested grapes and foliage at harvest (Powell, 2000). However the relative abundance of first instars in the canopy is likely to be determined by phylloxera genetics and site related biotic and abiotic factors.

4.3.3. Soil

Survival of phylloxera in soil, which may or may not contain remnants of grapevine roots, will be determined by a combination of factors. The length of time that phylloxera can survive in the absence of food, the viability of root material, the temperature and moisture content of the soil. At 25°C phylloxera can survive without

food for up to seven days (Kingston *et al*., 2009). However at 10°C in water, phylloxera can survive for up to 21 days (Powell *et al*., 2014).

Knowledge gaps

To determine the impact of temperature on endemic strains in soil, a more comprehensive study should be conducted under controlled conditions to compare a minimum of six endemic strains which represent a broader genetic diversity.

5. RISK REDUCTION OPTIONS

There are a number of risk reduction options for grape phylloxera. However the three primary options are early detection, the use of phylloxera resistant rootstocks and quarantine. Other options such as cultural, biological and chemical control have been reviewed in several recent publications (Benheim *et al*., 2012; Powell, 2012).

5.1. Resistant rootstocks

The native range of grape phylloxera is restricted to eastern North America in regions where *V. riparia* Michaux is distributed. Grape phylloxera was inadvertently transferred to Europe and other grape-growing regions on wild American *Vitis* spp. (Gale, 2003). The natural tolerance of American *Vitis* spp. has been exploited for over 150 years in breeding programs where *V. vinifera*, which possesses characteristics important for fruit quality, is grafted on to the root system of phylloxera-tolerant hybrids. A range of rootstock hybrid combinations are commercially available and new hybrids are under development along with different approaches around the world to screen for phylloxera resistance.

There is no consistent approach to screening for phylloxera resistance both internationally and in Australia. Available screening methods have included laboratory bioassays (both excised roots and tissue culture) (Forneck *et al*., 2001; Kellow *et al*., 2002; Makee *et al*., 2004), potted grapevines in glasshouses, and field screening. While the genotype of the resistant hybrid is usually well characterized, the genetic characteristics of phylloxera, with few notable exceptions, are rarely considered in any screening program. A three-phase screening system (Powell, 2006) has been developed in Australia, whereby single clonal lineages of phylloxera are screened to determine resistance levels to both ‘susceptible’ *V. vinifera* and grafted American *Vitis* hybrids. This triphasic approach is used rather than a single

system to account for variability in the host plant response to phylloxera infestation in the three phase screening systems used. The three screening systems are excised root bioassays (Granett *et al*., 1983), potted grapevine (Korosi et al. 2007), and field monitoring (Trethowan and Powell, 2007). This screening process has allowed recommendations to be made on resistance and tolerance of rootstocks to endemic phylloxera strains (Table 3). By effectively screening against these strains, targeted replanting in regions where single genetic strains are detected can be recommended.

5.2. Quarantine and disinfestation

The National Phylloxera Management Protocol (NPMP) was developed by the National Vine Health Steering Committee (National Vine Health Steering Committee, 2009) based on an interim protocol originally established in 1997 which was subsequently revised and updated. The NPMP provides definitions of phylloxera management zones (Table 5) and also recommended movement procedure and disinfestation protocols for a range of risk vectors which could transfer phylloxera. The NPMP does however not replace any state government legislation or regulatory requirements.

5.2.1. Quarantine Zones

Grape phylloxera-specific quarantine within countries is practiced by relatively few grape-growing countries including China, Russia, the Netherlands, Armenia, Cyprus, and Australia. The different types of phylloxera quarantine zone in Australia are shown in Table 5. Upon detection of a grape phylloxera infestation outside an existing PIZ, legislation requires the declaration of a grape phylloxera infested zone (PIZ) with a minimum 5 km boundary from the initial detection point (Buchanan, 1990). The basis of the quarantine zone is around the limits of natural dispersal of the insect. Based on phylloxera natural dispersal, a minimum of 5km buffer zone was original suggested for a PIZ as it was believed that new phylloxera infestations may take up to 5 years to detect and natural dispersal in one year could be up to 1km (Buchanan *et al*., 1998). However at the time this assumption was made there was no knowledge of the range of genetic diversity of endemic strains or that their virulence levels differ making them more difficult to detect by visual symptoms alone.

Table 5. Definitions of grape phylloxera-specific quarantine zones in Australia (modified after National Vine Health Steering Committee, 2009).

Quarantine zone status	Description
Phylloxera infested zone (PIZ)	A PIZ contains vineyards known to be infested with phylloxera or to have been infested with phylloxera. Its outer boundary must be a minimum of 5 km from any infested vineyard. The boundary of the PIZ may be defined by local government boundaries or other landmarks, provided they are no closer than 5 km from the edges of any known infested vineyards.
Phylloxera risk zone (PRZ)	The boundaries of a PRZ are determined by default. They include all areas not defined as a PIZ or PEZ.
Phylloxera exclusion zone (PEZ)	A PEZ is an area that has been established by historical information and/or a survey program as not being infested by phylloxera. A PEZ is governed by appropriate regulations to control the movement of grapevine material, specified grape products and vineyard equipment into the area.

5.2.2. Disinfestation treatments

Both recommended and potential disinfestation treatments for phylloxera are primarily based on temperature and chemical treatments.

5.2.2.1. Heat treatment

Heat treatment as a disinfestation treatment for phylloxera is based on exploiting the thermal limits (both hot and cold) for phylloxera survival in either wet or dry heat.

5.2.2.2. Hot water treatment

Hot water treatment is recommended for disinfestation of rootlings and cuttings (NPMP Procedure B; National Vine Health Steering Committee, 2009) and some viticulture equipment (such as grape bins) (NPMP Procedure G; National Vine Health Steering Committee, 2009).

5.2.2.2.1. Propagation material

The protocol for disinfestation of cuttings/ rootlings and some diagnostic samples is immersion in hot water at either 54°C + 1°C for 5 minutes or 50°C + 1°C for 30 minutes (NPMP; Procedure B; National Vine Health Steering Committee, 2009). Recent studies in Australia showed that the hot water immersion as currently recommended was 100% effective for disinfestation of first instars across all six genetic strains (Powell, 2017). This disinfestation protocol, although similar, differs from some overseas recommendations and published scientific data. For example in Europe a primary hot water treatment of 43°C for 5 min, followed by a second immersion at 52°C for 5 min is recommended for phylloxera and other external pests (EPPO, 1990). In Japan 45°C for 20 min has been reported to be effective (Sakai *et al*., 1985). Recent studies in Australia showed that some genetic strains survived when first instars were immersed in water heated to 45^{o}C for 5 minutes (Powell, 2017). There appears to be no international consistency regarding hot water treatment of grapevine roots for phylloxera. In Australia a study using a phylloxera population sourced from leaf galls showed that a hot water immersion treatment of 52°C for 5 minutes was 100% effective against active life-stages of phylloxera (Buchanan *et al*., 1998).

Knowledge Gaps

Although there are two recommended hot water treatments for grapevine cuttings and rootlings, neither treatment has not been fully scientifically validated against eggs and intermediate stages of diverse endemic strains of phylloxera in Australia. One study using phylloxera, obtained from leaf galls in Rutherglen, showed that 52°C HWT for 5 minutes achieved 100% phylloxera mortality (Buchanan *et al*., 1998). The treatment was effective against all phylloxera life-stages, including egg and all active life-stages. However the genetic strain of phylloxera used in the study was undetermined and it is likely to be rare in its geographic distribution as leaf

galling phylloxera have only been detected in the NE PIZ. The thermal limits for survival of phylloxera in hot water are unclear particularly for the different genetic strains and life-stages. The two recommendations, 54°C + 1°C for 5 minutes or 50°C + 1°C for 30 minutes, need to be tested against other life-stages of phylloxera of diverse endemic genetic strains.

5.2.2.2.2. Grape bins

The recommended disinfestation treatment for vineyard equipment (NPMP Procedure G; National Vine Health Steering Committee, 2009) such as grape bins is full immersion of the equipment in water at 70°C. The equipment must be held in the water for at least 2 minutes after the temperature has reached 70°C. No countries, other than Australia, use hot water treatment for phylloxera disinfestation of vineyard equipment.

The HWT recommendation of 70°C for 2 minutes for viticulture equipment was validated in a more recent study. Results showed that a minimum of 50°C for 1 minute achieved 100% mortality of first instar stages of six diverse endemic root-galling phylloxera strains (Powell, 2017). These results show that the recommendation could potentially be reduced in temperature and duration.

Knowledge gaps

There is a high risk that other phylloxera life stages are transferred through grapevine rootlings and cuttings as they would be attached to a food source. Although results obtained for first instars phylloxera of six endemic root-galling phylloxera strains were effective, older life stages would need to be investigated as they may be much more resistant to heat due to their larger sizes compared to first instar stages.

5.2.2.3. Steam Treatment

Steam treatment is one of the recommended disinfestation protocols for grape phylloxera to control the movement of phylloxera on vineyard equipment out of a PIZ or PRZ (NPMP Procedure G; National Vine Health Steering Committee, 2009). The disinfestation procedure states that *steam when applied must be >100°C as indicated by a jet of clear invisible steam between the steam out let and the visible condensate cloud*. It also states that *steam must contact all surfaces until the surface*

is left dry, not wet with condensate. The efficacy of steam treatment towards grape phylloxera was validated scientifically against six endemic strains. Results indicated that steam application at >80°C for a minimum of 10 seconds achieves 100% first instar mortality (Powell, 2017). The study, however, showed that temperature was reduced as the distance from source of steam increased. This finding suggests that steam should contact all surfaces and be applied as close to the surface being disinfested as possible.

Knowledge gaps

The pressure of any steam cleaner will also need to be taken into consideration to fully determine steam disinfestation efficacy. It should also be noted that many vineyard/winery operators use hot water pressure washers which are not designed to produce steam and hence temperatures may be <100°C (R. Fabis, pers. communication). The efficacy of hot water pressure steamers as an alternative disinfestation treatment therefore needs to be determined.

5.2.2.4. Dry heat treatment

The National Phylloxera Management Protocols (NPMP Procedure G; National Vine Health Steering Committee, 2009) recommends a disinfestation protocol for use against phylloxera on vineyard machinery, particularly grape harvesters. The protocol recommends *dry heat treatment at 45ºC for 75 minutes or 40ºC for 120 minutes when moving vineyard equipment out of a PIZ or PRZ*. There are two recommendations for dry heat treatment in the NPMP (NPMP Procedure G; National Vine Health Steering Committee, 2009) one for machinery and one for diagnostic soil samples (NPMP Procedure C; National Vine Health Steering Committee, 2009). The recommendation for dry heat treatment of machinery is based on two studies conducted in Australia**.** Buchanan (1990) compared phylloxera survival over a temperature range of 15-40ºC and assessed survival at 5ºC increments. This study showed that survival was highest at 15-20ºC and declined markedly from 30-35ºC and no phylloxera survived after 60 minutes at 40-45ºC. This study however was only conducted using a single phylloxera population sourced from Nagambie Victoria. Further studies examining the effects of high temperatures on two endemic strains were conducted by Korosi *et al*., (2009; 2012). In the 2009 study, G4 phylloxera survival showed a similar trend to Buchanan's study with temperatures of

40ºC and 45ºC markedly reducing survival compared with 30-35ºC. After 45 at 75 minutes 100% mortality was achieved. Korosi *et al*. (2012) compared G1 and G4 phylloxera strains and showed that both strains had reduced survival as temperature reached 40-45ºC but there were differences in survival between strains. Both G1 and G4 reached 100% mortality at 75 minutes but G4 survived for longer at 40ºC than G1 and the survival was influenced by humidity. This study was limited to temperatures of 30-45ºC. Powell (2017) recently validated the efficacy of the dry heat treatments against six diverse phylloxera strains and showed that 45ºC for 75 minutes is effective against all the six strains. However, 40ºC for 120 minutes was only 100% effective against four of the six strains.

Knowledge gaps

Although results have only been obtained for first instar phylloxera across six endemic strains, a study on the survival of older life stages that would reach adulthood and lay viable eggs, is warranted.

5.2.2.5. Cold air temperature

There are a number of cold disinfestation treatments for phylloxera (NPMP Procedure C; National Vine Health Steering Committee, 2009) in relation to the movement of diagnostic samples from a PIZ or PRZ into a PRZ or PEZ. The cold treatments include (i) freezing to -18°C for 24 hours and packed in dry ice for transport (ii) freezing and transferred under liquid nitrogen at -196°C and (iii) freeze drying. Some of these treatments have been tested against root-galling phylloxera in Australia, although the genetic strain was unknown.

The treatment recommendation of -18°C for 24 hours is likely to be effective as it is likely to cause cell damage, although the temperature may require some modification as -16°C for 24 hours has been shown to be effective (Buchanan *et al*., 1998). The freezing of phylloxera in liquid nitrogen is also likely to be effective as Buchanan *et al*. (1998) showed no active phylloxera life-stage survival in liquid nitrogen after 5 minutes at -196°C.

Powell (2017) validated the disinfestation recommendation of -20ºC for an exposure period of 24 hours. 100% mortality was achieved across six diverse genetic strains. Further, no first instars survived at -20°C for 12 hours. The same

study also showed that egg hatch does not occur when subjected to -20°C for 12 and 24 hours.

5.2.2.6 Cold water treatment

The duration of phylloxera survival in water is influenced by the water temperature. Although water (with the exception of hot water treatment) is not used as a disinfestation treatment, it is important to understand the survival limits of phylloxera in water, as water is sometimes used in wash down and spillage procedures and in some countries as a flooding option in vineyard management. Studies of phylloxera survival when immersed in water have been conducted in Australia under controlled conditions at temperatures ranging between 2° to 25°C. At 25°C phylloxera first instars can survive for up to 8 days (Kingston *et al*., 2009). As the temperature is lowered to 10°C, phylloxera can survive for 21 days (Powell *et al*., 2014). This lower temperature presumably reduces metabolic activity and induces first instar phylloxera to hibernation mode. Survival is reduced to 9 and 7 days as the temperature is reduced to 5°C and 2°C, respectively (Powell *et al*., 2014). These published data from a single phylloxera endemic strain do indicate that phylloxera is resilient to immersion in water, at least in the short term of 1-3 weeks, and the use of cold-ambient water as a disinfestation treatment is likely to be ineffective.

5.2.2.7. Chemical disinfestation

There are a range of recommended chemical disinfestation procedures for phylloxera.

5.2.2.7.1 Sodium hypochlorite

First instar life-stage of phylloxera are the most active and known to be present on the soil surface. The recommended disinfestation protocol for the movement of vineyard visitors in relation to footwear out of a PIZ or PRZ is to scrub boots and dip in 2% active sodium hypochlorite (NaOCl) for 30 seconds followed by a thorough rinse in clean water (NPMP Procedure H; National Vine Health Steering Committee, 2009). The cleaning procedure is aimed at removing mud and recommends water and detergent. The detergent is advised merely as a cleaning agent as it has been shown to have minimal effect on phylloxera survival, although the type of detergent to be used is unspecified (Buchanan *et al*., 1998).

Sodium hypochlorite (the active ingredient of household bleach) was initially used as a research disinfestation treatment purely to clean phylloxera eggs of potential microbial/fungal contaminants so that the viable eggs could be used to rear phylloxera in *in vitro* tissue cultures (Pelet *et al*., 1960; Askani and Beiderbeck, 1991). In a Californian study 1-7 day old phylloxera eggs, sourced form mixed populations, survived immersion in 5.25% a.i. sodium hypochlorite for at least 7 minutes (Grzegorcyzk and Walker, 1997). Significant reduction in egg hatching only began to occur when immersed in 3.94% sodium hypochlorite for 5-7 minutes.

A study using an unknown population of phylloxera sourced from Nagambie indicated that a minimum of 2% sodium hypochlorite was required to achieve 100% first instar mortality. Recent studies have focused on comparing the efficacy of sodium hypochlorite as a suitable footwear disinfestation treatment against selected endemic strains of radicole grape phylloxera. Results indicated that effective disinfestation is influenced by sodium hypochlorite concentration, duration of treatment and the Phylloxera endemic strain (Clarke et al., 2017). First instars of six different phylloxera strains survived immersion in 2% sodium hypochlorite at 30 seconds (the current recommended treatment) and at 4% sodium hypochlorite for at least 60 seconds when a post- disinfestation water rinse treatment was applied. Results further showed that first instars that survived treatments in sodium hypochlorite established feeding sites and reproduced (Clarke et al., 2017). Though surviving first instars can re-establish on grapevine roots, their development is affected and they are unable to produce eggs at the higher sodium hypochlorite concentration and immersions time. For 100% first instar mortality, results showed that 2% sodium hypochlorite treatment for 30 seconds is required providing a water rinse is not applied post-treatment.

Knowledge Gaps

The efficacy of sodium hypochlorite appears to differ significantly between endemic phylloxera strains. The challenge with sodium hypochlorite, however, is that of odour and residues and subsequent damage to footwear. Because of this limited efficacy and potential OHS considerations, alternative disinfestation approaches for footwear should be examined.

5.2.2.7.2. Ethanol

A solution of 70% ethanol is recommended as a fixative for phylloxera disinfestation during movement of diagnostic samples from a PIZ or PRZ into a PRZ or a PEZ (NPMP Procedure C; National Vine Health Steering Committee, 2009).

Ethanol has been used in research to surface sterilise phylloxera eggs for using in tissue culture with phylloxera eggs surviving immersion in 50% ethanol for 7 minutes (Forneck et al., 1996) and 70% ethanol for at least one minute (Kellow *et al*., 1998). Some phylloxera eggs survived and hatched when immersed in 100% ethanol for seven minutes (Grzegorcyzk and Walker, 1997). This indicates that phylloxera eggs are relatively resilient to ethanol treatment unless immersed for relatively long periods. Ethanol would hence act as a fixative rather than a disinfestation treatment.

Phylloxera first instars are considered a higher risk of spread due their active mobility. Because of their relatively soft bodied cuticle compared to eggs, it is often assumed that they would be less resilient to ethanol treatment. In one Australian study, using a leaf galling phylloxera population, phylloxera survival albeit low, was recorded when immersed in 70% ethanol for 5 minutes (Buchanan *et al*., 1998).

Knowledge gaps

The efficacy of using ethanol as a potential disinfestation treatment has not been fully explored and requires further validation to determine the duration of immersion and concentration required to provide 100% mortality of active life-stages of endemic phylloxera strains.

5.2.2.7.3. Sulphur

The use of sulphur pads, containing a minimum 970g/kg sodium metabisulphite is a recommended disinfestation procedure for the movement of table grapes out of a PIZ into a PRZ or a PEZ (NPMP Procedure F; National Vine Health Steering Committee, 2009) and also out of a PRZ and into a PEZ (NPMP Procedure F1; National Vine Health Steering Committee, 2009). This recommendation is based on research conducted in Australia (Buchanan, 1990). Sodium metabisulphite liberates sulphur dioxide when in contact with water or high humidity. Tests conducted at 5°C and 95% relative humidity showed that after 36 hours sulphur dioxide released from sulphur pads was highly effective achieving 100% phylloxera mortality in 10kg table

grape consignments. If the sulphur dioxide concentration reached 12ppm, 100% phylloxera mortality was achieved after 24 hours.

Buchanan (1990) also raised the possibility of utilising sulphur dioxide as a potential disinfestation treatment for the movement of harvested wine grapes. The movement of fresh wine grapes from a PIZ to a PEZ is currently prohibited (National Vine Health Steering Committee, 2009). The movement of fresh wine grapes out of a PRZ into a PEZ is only allowed following adherence to specified transport procedures at the source vineyard and receiving winery quarantine requirements (NPMP Procedure A; National Vine Health Steering Committee, 2009). Sulphur dioxide is also used in the wine making process usually in the form of sodium metabisulphite. Its efficacy against phylloxera in white juice is, however, limited (Powell *et al*., 2014).

Knowledge gaps

Buchanan (1990) used an unspecified root galling grape phylloxera population (although likely to be G1 phylloxera as it was sourced form Nagambie where only a single strain is known to be present). In addition, the same study used insects with a food source (root nodosities) whilst they underwent SO_2 treatment. The presence of a root source could be considered a confounding variable in the scientific validation of this procedure. Moreover, roots are unlikely to be present in a table grape consignment.

No further studies have been conducted against Australian endemic strains of phylloxera or against phylloxera in table grape or wine grape consignment conditions (i.e. in the absence of nutrition). Further studies would also need to compare the efficacy of sulphur pads against both leaf-galling and root-galling phylloxera.

5.2.2.8. Grape Juice

Although juice is not regarded as a disinfestation treatment, some juice does seem to reduce phylloxera survival. Despite the fact that there is a risk of transferring phylloxera in juice, there are no robust studies to show survival of phylloxera in Australia and overseas. In Australia, studies have been limited to testing with a single root-galling phylloxera strain. Overall in these studies, first instar phylloxera survived in juice and the type of juice and duration of immersion influenced the level of survival. At temperatures of between 15-20°C, G4 phylloxera survived for at least

16 hours in clarified white juice, unclarified white juice and red juice (Deretic *et al*., 2003). Further studies conducted at a lower temperature range of 2-10°C showed survival of between 3-7 days in unclarified white juice. With further modification of juice Baume pH 3.0-3.5 at 11-14°C, sulphur concentration had minimal impact on phylloxera survival. However, studies by Powell *et al*., (2014) have shown that phylloxera first instars survive for shorter periods in white juice than water indicating that some yet undetermined chemical or physical property of juice may be affecting the insect.

In order to move juice from a PIZ or PRZ (Phylloxera Risk Zone) to a PEZ, the existing disinfestation regulations (Procedure D; National Vine Health Steering Committee, 2009) are that juice must be either fermented for at least 72 hours or filtered through a 50 micron filter. The fermentation component of this protocol is based on experimental data originally developed using red juice (Buchanan *et al*., 1998). White juice is processed differently to red juice, and has a number of different physiochemical properties to red juice and undergoes a range of treatments prior to filtering (Rankine, 1995). At various stages in the white wine-making process, sulphur dioxide as sodium metabisulphite, is generally added. White juice also undergoes a cold treatment process. The impact of sulphur dioxide content, in white juice, on phylloxera mortality has been examined under cold temperature conditions using a single endemic radicole phylloxera strain (Powell, 2012; Powell *et al*., 2014). When sodium metabisulphite is added to water there is significant effect on phylloxera mortality. In contrast when sodium metabisulphite is added to white juice there is no significant effect on mortality (K. Powell, unpubl. data). This is likely due to some other physiochemical properties of white juice which increases phylloxera mortality and hence masks any effect of sulphur.

Knowledge gaps

From preliminary data on the impacts of white juice against phylloxera there appears to be anecdotal evidence that either physical or chemical properties of juice maybe reducing phylloxera survival. Further investigations into which property of juice is having a detrimental effect are warranted.

5.2.2.9. Fumigation

Methyl bromide fumigation is the only fumigation procedure currently recommended in the NPMP and is used for movement of table grapes out of a PIZ into a PRZ or a PEZ (NPMP Procedure F; National Vine Health Steering Committee, 2009). Although this treatment is rarely used domestically, the recommended treatments are shown in Table 6.

Table 6. Recommended methyl bromide treatments for grape phylloxera *(adapted from* National Vine Health Steering Committee, 2009*)*.

Fruit pulp temperature	Dosage rate (g/m3)	Time (Hours)	Dosage at 30 mins (75%)	Dosage at 2 hrs (60%)
>21°C	32	2	24g/m^3	20g/m^3
≥15.5°C and <21°C	40	2	30g/m^3	24g/m^3
≥10°C and < 15.5°C	48	2	36g/m^3	29g/m^3

A single scientific study has reported the effectiveness of methyl bromide fumigation for controlling grape phylloxera on grapevine root material. The most effective Methyl bromide treatment was at 24 g/m^3 for 3 hours (Sakai *et al*., 1985).

Knowledge gaps

No studies have examined the efficacy of methyl bromide or other potential fumigants against Australian endemic strains of phylloxera or phylloxera in table grape consignments (i.e. in the absence of a root source for feeding).

5.2.2.10. Irradiation

Gamma and microwave radiation are two irradiation procedures that have been tested for phylloxera disinfestation.

5.2.2.10.1. Gamma irradiation

In a single study conducted in Syria (Makee *et al*., 2008), phylloxera eggs and mobile life-stages on grapevine root pieces were exposed to different doses of gamma irradiation. As the irradiation dose increased, phylloxera mortality increased proportionately accompanied with reduced fecundity and egg hatch. A dose of 50 Gy-irradiation was the most effective treatment in reducing egg production and egg hatching although 100% mortality was not achieved.

5.2.2.10.2. Microwave irradiation

Although not a recommended disinfestation procedure, the potential effects of microwave irradiation have been tested against a single endemic phylloxera strain. The rationale for testing was to determine whether microwave irradiation could be used as an effective and more rapid alternative disinfestation protocol for diagnostic samples, such as foliage or soil. In the study, the efficacy of microwave radiation on phylloxera in soil samples was influenced by soil type (Powell, 2003). Although microwave radiation reduced phylloxera survival, 10-30% first instars survived treatments after 4 minutes (Powell, 2003). In contrast microwave irradiation of leaf samples was more effective and 100% first instar mortality was achieved after 40 seconds. Post treatment morphological and physiological changes to the leaf sample were visible.

Knowledge gaps

Gamma irradiation is not one of the recommended disinfestation treatments for phylloxera in Australia, although there may be some potential to explore its use for disinfestation of propagation material or table grapes. No irradiation studies have been conducted against Australian endemic strains of phylloxera or against phylloxera in table grape consignment conditions (i.e. in the absence of a root source for feeding).

Microwave irradiation is also not one of the recommended disinfestation treatments for phylloxera in Australia. There may be some potential to explore its use for more rapid disinfestation for some diagnostic materials provided the treatment does not damage the integrity of the sample.

5.2.2.11. Fermentation

The movement of must has a disinfestation procedure which states that at least 72 hours of fermentation is required for the product to be moved out of a PIZ or PRZ into a PEZ (NPMP Procedure D; National Vine Health Steering Committee, 2009). There have been four studies conducted in Australia regarding survival of phylloxera in must (Buchanan *et al*., 1998) and no published studies overseas. The duration of immersion required for effective disinfestation of phylloxera differs depending on grape variety and addition of sodium metabisulphite. Phylloxera survived in immersion in Sultana must for at least 15 hours (Buchanan *et al*., 1998). Some root-galling phylloxera active life-stages survived 36 hours in fermenting Merlot must, but did not survive after 72 hours immersion. Eggs of leaf-galling phylloxera hatched and survived as first instars after 24 hours immersion in Malbec must. A further study showed that eggs are more resistant to immersion in must and survived after 48 hours in red must, whereas first instars did not survive immersion.

Knowledge Gaps

The experimental work on survival of phylloxera in must were conducted over 20 years ago when the extent of genetic diversity of endemic strains in Australia was unknown. As virulence levels of these genetic strains, particularly the more damaging root-galling strains is known to vary, it would be pertinent to revalidate the protocol to ensure its efficacy against diverse range of endemic genetic strains.

5.2.2.12. Composting

The disinfestation treatment for marc is either fermentation for 72 hours or composting or pasteurisation as per Australian Standard AS 4454. Three published studies, all conducted in Australia, have examined the survival and risk of transfer of grape phylloxera in both composted marc (Powell *et al*., 2007; Korosi *et al* 2009;) and composted green waste (Bishop *et al*., 2002; Keen *et al.,* 2002). In all studies only a single root-galling endemic phylloxera strain was tested. In a study conducted in a commercial composting facility, phylloxera survival was assessed in green waste using a windrow composting process, and a Vertical Compositing process both of which adhered to Australian Standard AS 4454 (Bishop *et al*., 2002; Keen *et al*., 2002). Temperatures in both processes reached lethal limits resulting in complete phylloxera mortality. In a latter study using commercial winery waste, without

windrowing, phylloxera first instars survived for a maximum of four days (Korosi *et al*., 2009) and mortality was attributed to a combination of temperature, depth of burial in the compost pile and lack of an available food source for the insect.

5.3. Cultural control

Cultural control options which could involve modification of the soil environment to deter phylloxera establishment or the risk of spread have been largely unexplored although further research is warranted especially for root-galling phylloxera. Knowledge of how the soil environment impacts on phylloxera distribution could also be beneficial in determine the risk of establishment and spread both at a vineyard and area wide scale.

Opportunities for controlling phylloxera through soil management such as tillage or through soil amendments, such as organic or physical mulches, have also received surprisingly limited research attention. Organic mulch applications can be used to modify physical and textural properties of the soil, making conditions more or less conducive to phylloxera establishment and survival on the root system or they may directly affect the insects' ability to disperse through the soil to reach the soil surface or the grapevine roots. Contrasting results have been reported using this approach. In a Californian study, in which organically managed vineyards and conventionally managed phylloxera-infested vineyards were surveyed, a lower incidence of phylloxera-associated damage was reported in organically managed vineyards (Lotter *et al*., 1999). In two 3-year field studies conducted in Australia, composted green waste mulch applications increased the abundance and dispersal of phylloxera above-ground, whilst grape-pomace mulch formulations reduced phylloxera population levels under field conditions (Powell *et al*., 2006; 2007). In Germany, reduced phylloxera abundance was observed when spruce sawdust was applied as mulch, but whether this was due to changed physical or changed microbial properties of the soil was unclear (Huber *et al*., 2003). Although mulch formulation is likely to be an important factor in the efficacy of such treatments for phylloxera management, soil properties may influence their efficacy. Studies in Europe have indicated that some vineyard soils may have suppressive properties for phylloxera establishment (Huber *et al*., 2009).

5.3.1. Organic mulches

Composted material, both green waste and marc, have been trialled as mulches in phylloxera-infested vineyards in Europe, USA and Australia (Lotter *et al*., 1999; Powell *et al*., 2006; 2007) and compost formulation appears to determine whether the risk of transfer is increased or decreased.

5.3.1.1. Composted green waste

In a three year field trial, conducted in the King Valley, Victoria, composted green waste was applied as a mulch to phylloxera-infested grapevines. The mulch application increased the abundance of emerging first instars on the soil surface and on grapevine trunks, and created a higher risk of transfer than grapevines with no mulch treatment applied (Powell *et al*., 2006; 2007a).

5.3.1.2. Composted winery waste

In a three-year field trial, conducted in Nagambie, Victoria, composted winery waste in three different formulations was applied as a mulch to phylloxera-infested grapevines. Mulch formulations containing marc decreased the abundance of emerging first instars on the soil surface and on grapevine trunks, and therefore reduced the risk of transfer than grapevines with no mulch treatment applied (Powell *et al*., 2007b). In a Californian study, Lotter *et al* . (1999) reported reduced phylloxera damage in organically managed vineyards where mulches were applied.

5.3.2. pH modification

The effects of pH on grape phylloxera have been recently examined because any potential effects could potentially influence phylloxera distribution in soils or survival in liquids of different pH range (e.g. juice, water). In a four year study in Austrian vineyards, phylloxera infestation levels were negatively correlated with soil pH with higher infestation levels in acidic compared to alkaline soils (Reisenzein *et al*., 2007). In Australia, phylloxera has been detected primarily in weak to strongly acidic soils of pH 4.7-6.9 (Powell, 2006, 2007) and in more alkaline soils (pH 7.0-9.0) at depth (>80cm) in some phylloxera infested areas such as Rutherglen. In the latter areas, phylloxera populations although present are in lower abundance than in acidic soils. However, it has not been confirmed whether soil alkalinity is the primary factor influencing the low abundance or some other site-related factors. A recent study

(Powell, 2012) has shown that pH has no significant impact on phylloxera survival in liquids. Phylloxera survives well in both white juice (with a pH range of 3.0-3.7) and water (variable pH range up to 7.0). Phylloxera appears to thrives in acidic liquid environments and also prefers an acidic diet of around 4.0 pH compared to aphids which prefer a weak acidic to neutral diet (pH 6.5 – 7.0) (Kingston *et al*., 2007).

5.3.3. Salinity and sodicity

In Australia, phylloxera has been detected primarily in soils with very low to low salinity ratings and non-sodic soils (Powell, 2006). The influence of salinity on phylloxera establishment has not been determined. At a depth of >95cm, however, soils become strongly sodic in some phylloxera infested vineyards such Rutherglen region. In these areas, phylloxera populations, although present, are in low abundance than in non-sodic soils.

Knowledge gaps

It is unclear whether salinity or sodicity influences phylloxera establishment or survival.

5.3.4. Soil chemistry

A number of soil chemical characteristics have been studied to determine their relationship with phylloxera abundance. Reisenzein *et al*. (2007) showed a significant positive relationship between phylloxera abundance and organic carbon in field studies in Austrian vineyards. Similarly high phylloxera abundance has been noted in both laboratory and field studies conducted in King Valley, Victoria (Powell *et al*., 2003; 2007). Reisenzein et al (2007) also reported a highly significant relationship between phylloxera abundance and the ratio of Potassium and Magnesium, this was also observed by Powell (2007) in Australian phylloxera-infested soils. Australian vineyard soils are renowned for their high potassium levels (Whiting, 2013).

5.4. Biological control

Options for the use of biological agents, either natural or introduced, for reducing the risk of spread of grape phylloxera are surprisingly poorly exploited despite the range of potential opportunities that exist for either a classical or inundative biological

control approach. The use and manipulation of natural enemies to control phylloxera has rarely been examined and this may in part be due to the somewhat complex life-cycle of this pest that includes both root-feeding and foliar feeding habitats. Overseas, some natural above-ground predators of phylloxera have been reported (Dalmasso, 1956; Gorkavenko, 1976; Jubb and Masteller, 1977; Wheeler and Henry, 1978; Wheeler and Jubb, 1979), but none have been reported in Australia. Below ground, potential control agents for root-feeding phylloxera include the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar (English-Loeb *et al*., 1999) and several entomopathogenic fungi including *Beauveria bassiana* (Balsamo) Vuillemin (Granett *et al*., 2001), *Metarhizium anisopliae* (Metshnikoff) Sorokin (Kirchmair *et al*., 2004; Huber and Kirchmair, 2007) and *Cephalosporium* spp. (Vega, 1956) and a predatory mite *Tyroglyphus phylloxerae* Riley (van Driesche and Bellows, 1996).

5.5. Chemical control

An effective chemical control option against both leaf-galling and root-galling grape phylloxera is, despite several studies (Benheim *et al*., 2012; Powell, 2012), still not available in Australia. Ideally, an effective chemical would need to be systemic which is both upwardly and downwardly mobile and have high efficacy. The product should also be able to control multiple overlapping populations of the insect. The chemical management of root-galling grape phylloxera poses a complex challenge as it needs to address issues of the phylloxera life-cycle, potential resistance of genetic strains, soil and environmental, economic viability, environmental impacts and host-plant residues.

5.6. Detection

Several factors can affect the establishment and development of grape phylloxera in vineyards and the development of cost effective early detection methods are paramount to reducing the risk of phylloxera spread. Several factors will influence the efficacy of any detection approach including the insect's life cycle, the insect and host plant genetic characteristics, soil properties and environmental conditions. Currently, root-galling phylloxera are of primary concern in Australia and a range of novel detection approaches have either been developed or are under development as relatively simple user-friendly trapping techniques (Powell *et al*., 2009) compared

to more complex molecular, chemical and spectral fingerprinting techniques (see Powell *et al*., 2007; Benheim *et al*., 2012). However, because of the complexity of the interactions involved, it is unlikely that a single untargeted detection technique will be successful. It is more likely that an integrated targeted approach to detection will succeed in the long term (Powell *et al*., 2014).

6. FUTURE CLIMATE

One as yet unquantified risk for phylloxera distribution is climate change. Under future climate change scenarios, it is predicted that suitable climatic regions for growing *Vitis* species will change in geographic distribution both internationally (Jones, 2007) and within Australia (Webb *et al*., 2010). This may also influence the distribution of grape phylloxera exotic and endemic strains. Even though grape phylloxera is currently established in almost all grape-growing countries some countries, like Australia, still have limited distribution. The direct impact of elevated temperature, reduced water availability and elevated carbon dioxide on phylloxera development and population abundance has not yet been examined in any detail. However, it is quite feasible that phylloxera strain distribution will change. Indirect changes could also potentially influence phylloxera through phenological changes in the host plant root biomass, root phenology, and nutritional quality, as has been suggested for other root-feeding aphids (Salt *et al*., 1996).

7. CONCLUSION

Unlike some major grape-growing regions of the world like Europe where barriers to phylloxera movement are relatively ineffective (EFSA, 2014) and spread is considered highly likely particularly on planting material, Australia is one of the few countries where widespread use of ungrafted *V. vinifera* is still common and phylloxera is relatively contained primarily through the use of quarantine protocols. Despite this, phylloxera has spread to new regions in Victoria over the last two decades. The reasons for this spread are unclear but may be related to a combination of complacency, changes in the industry and climate change. Our increased understanding of the genetic diversity and distribution of endemic populations will help in the future management of phylloxera. However there are a number of knowledge gaps in the literature and it is apparent that several factors are likely to influence the risk of phylloxera establishment, development, abundance and

dispersal. Two overriding factors which will influence future outbreaks are the genetic background of the host and the pest, which will influence the level of interaction and damage, but also the interactions between host plant, pest and the environment. In general terms, ungrafted *V. vinifera* is more susceptible to damage from all endemic strains of phylloxera than grafted rootstocks. Rootstocks still pose a quarantine risk as some sustain phylloxera development. In Australia 83 phylloxera genetic strains have been identified to date which adds the complexity of risk.

The emergence of 'biotypes' in the 1980s, notably Biotype' B' caused considerable economic loss for the Californian wine industry and this has led to an increased research focus on understanding interactions between phylloxera strains and rootstocks and a better understanding of life cycle variations. This research was aided by improved DNA typing techniques over the last 20 years. One major advancement in our knowledge of the genetic diversity in Australia has been the identification of 83 genotypes. In particular, the highly virulent superclones, G1 and G4, are more geographically widespread and appear to pose a higher risk of spread under current environmental conditions. The resilience of resistant rootstocks as the primary phylloxera management option could be challenged in the future by host-plant interactions with diverse endemic strains of grape phylloxera and by potential impacts of climate change on both grapevine and grape phylloxera distribution. Undoubtedly the use of a genomics approach will further advance our knowledge of the genetic basis of resistance more rapidly in the future as genomic sequences from both *Vitis* species and *D. vitifoliae* are being mapped (Delmotte *et al*., 2011, 2014; Jaillon *et al*., 2007). This knowledge should greatly improve the future management of the pest.

As well as these biotic and genetic factors, abiotic factors also influence the level of risk posed by phylloxera. The main factors are edaphic factors such as soil texture and chemistry and also environmental extremes particularly temperatures of <2°C and >40°C. Knowledge of the influence of temperature extremes is primarily useful in developing robust disinfestation treatments based on extreme heat but could also be used to map potential distribution. Our knowledge on the influence of soil factors, albeit limited, does allow us to cautiously make general assumptions on the level of risk of some soil types. In addition, this knowledge assists in developing targeted detection approaches for area wide surveillance of radicole phylloxera

because distribution, abundance and potential establishment can be influenced by soil properties.

There are no reports where grape phylloxera has been successfully eradicated (Morgan *et al*., 1973) worldwide, and the likelihood of phylloxera eradication in Australia is extremely low as this pest has been established since 1875 and important gaps in our fundamental knowledge are apparent. Any serious attempt at eradication of grape phylloxera would require a concerted, multidisciplinary approach, ensuring that fundamental interactions between environment, pest, and host-plant are researched and clearly understood, and that stringent quarantine protocols are appropriately implemented to prevent reinfestation. Knowledge gaps if addressed, particularly through international collaboration would markedly improve future management options.

8. REFERENCES

Afonin, A.N., Greene, S.L., Dzyubenko N.I., Frolov A.N. (2009) *Interactive Agricultural Ecological Atlas of Russia and Neighbouring Countries. Economic Plants and their Diseases, Pests and Weeds*. http://www.agroatlas.ru/en/content/pests/Viteus_vitifolii/

Andrews, K.B., Kemper, D., Powell, K.S., Cooper, P.D. (2012). Spatial trade-offs in the digestive and reproductive systems of grape phylloxera. Australian Journal Zoology 59, 392–399.

Anon. (1878). The Phylloxera at Geelong. *South Australian Register.* Adelaide, SA. 3.

ARD (Agriculture and Rural Development). (2007). *Armenia managing food safety and agricultural health: an action plan*. Agriculture and Rural Development Department and Europe and Central Asia Region/The World Bank/Zangak-97 Publishing House, Washington/Yerevan.

Armenian Travel Bureau. (2010). Climate in Armenia. http://www.atb.am/en/armenia/nature/climate/

Belcari, A., Antonelli, R. (1989). The influence of temperature on the development of pre-imaginal stages of *Viteus vitifoliae* (Fitch) (Rhynchota–Phylloxeridae). 3. Duration of larval development in epigeous generations at constant temperatures. In: *Influence of environmental factors on the control of grape pests, diseases and weeds*, Proceedings of Meeting European Community Experts Group, pp. 115–124.

Benheim, D., Rochfort, S., Robertson, E., Potter, I.D., Powell, K.S. (2012). Grape phylloxera (*Daktulosphaira vitifoliae*) - a review of potential detection and alternative management options. *Annals of Applied Biology* 161, 91–115.

Bezemer, T.M., Jones, T.H., (1998). Plant–insect herbivore interactions in elevated atmospheric CO_2: quantitative analyses and guild effects. *Oikos* 82, 212–222.

Bioletti, F.T., (1901). The phylloxera of the vine. Univ. Calif. Coll. Agric. Bull. 131, 13–28. Bishop, A.L., Powell K.S., Gibson, T.S., Barchia, I.M., Wong, P.T.W. (2002). Mortality of grape phylloxera in composting organics. *Australian Journal of Grape and Wine Research* 8, 48-55.

Blackman, R.L., Eastop, V.F. (1984). *Aphids on the World's Crops: An Identification and Information Guide*. John Wiley, Chichester, UK, p. 465.

Botha, J.h., Stephenson, B.E, Tille. P.J. (2007). Phylloxera and its threat to Western Australia. *Acta Horticulturae* 733, 101-106.

Botton, M., Walker, M.A. (2009). Grape Phylloxera in Brazil. Acta Horticulture 816, 39–40.

Bournier, A. (1976). Grape insects. *Annual Revue Entomology* 22, 355–376.

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

Buchanan, G.A. (1990). The distribution, biology and control of grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), in Victoria. PhD Thesis, La Trobe University, Melbourne, Australia.

Buchanan, G., Corrie, A.C., Whiting, J., Brown, D. (1998). Management of grape phylloxera in Australia. GWRDC and HRDC Report.

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.

Clarke, C.W., Wigg, F., Norng, S., Powell, K.S. (2017). Effectiveness of sodium hypochlorite as a disinfestation treatment against genetically diverse strains of grape phylloxera *Daktulosphaira vitifoliae* Fitch (Hemiptera: Phylloxeridae). Australian Journal of Grape and Wine Research, doi: 10.1111/ajgw.12288.

Corrie, A.M., Hoffmann, A.A. (2004). Fine-scale genetic structure of grape phylloxera from the roots and leaves of *Vitis. Heredity* 92, 118–127.

Corrie, A.M., Buchanan, G., Van Heeswijck, R. (1997). DNA typing of populations of phylloxera (*Daktulosphaira vitifoliae* (Fitch)) from Australian vineyards. *Australian Journal of Grape and Wine Research* 3, 50–56.

Corrie, A., Kellow, A.V., Buchanan, G.A., Van Heeswijck, R. (1998). Phylloxera biotypes in Australia*. Australian and New Zealand Grapegrower and Winemaker* 417, 32–36.

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

Corwin, D.L., Lesch, S.M. (2005). Apparent soil electrical conductivity measurements in agriculture. *Computers and Electronics in Agriculture* 46, 11–43.

Crovetti, A., Rossi, E. (1987). Field and laboratory observations on some eco–ethological aspects of the grape phylloxera (*Viteus vitifoliae* (Fitch)). Influence of environmental factors on the control of grape pests, diseases and weeds. Proceedings of Meeting EC Experts' Group, Thessalonika, 6–8 October 1987, Commission of the European Communities, A.A. Balkema, Rotterdam, The Netherlands.

Dalmasso, G. (1956). Lutte contre le phylloxéra (à l'exclusion de l'emploi des porte-greffes résistants). Rapport général pour l'Europe.*Bull de l'Off Int du Vin* 29,5–30.

Davidson, W.M., Nougaret, R.L. (1921). The grape phylloxera in California. *USDA Bulletin* 903, 1–127.

De Benedictis, J.A., Granett, J. (1992). Variability of responses of grape phylloxera (Homoptera, Phylloxeridae) to bioassays that discriminate between California biotypes. *Journal of Economic Entomology* 85, 1527–1534.

De Klerk, C.A. (1972). Occurrence 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 occurrence 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.

Delmotte. F., Forneck, A., Powell, K.S., Rispe, C., Tagu, D. (2011). Proposal to sequence the genome of the grape phylloxera (*Daktulosphaira vitifoliae* Fitch). http://www.aphidbase.com/var/aphidbase/storage/htmlarea/2965/file/White-Paper-Phylloxera_25may2011-1.pdf.

Delmotte. F., Rispe, C., Papura, D., Legeai, F., Jacquery, J., Breteaudeau, A., Forneck, A., Powell, K.S., Tagu, D. (2014). The grape phylloxera genome sequencing project. *Acta Horticulturae* 1045, 15-20.

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

Dipierro, N., Mondelli, D., Paciolla, C., Brunetti, G., Dipierro, S. (2005). Changes in the ascorbate system in the response of pumpkin (*Cucurbita pepo* L.) roots to aluminium stress. *Journal of Plant Physiology* 162, 529–536.

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* 17, 291–297.

EFSA PLH Panel (European Food Safety Authority Panel on Plant Health). 2014. Scientific opinion on the risk to plant health posed by *Daktulosphaira vitifoliae* (Fitch) in the EU territory, with the identification and evaluation of risk reduction options. *EFSA Journal* 12(5), 3678, 67pp.

English-Loeb, G., Villani, M., Martinson, T., Forsline, A., Consolie, N. (1999). Use of entomophagic nematodes for control of grape phylloxera (Homoptera: Phylloxeridae): a laboratory evaluation. *Biological Control* 28, 890–894.

Ermolaev, A.A. (1990). Resistance of grape phylloxera on sandy soils. *Agrokhimiya* 2, 141– 142.

Fong, G., Walker, M.A., Granett, J. (1995). RAPD assessment of California phylloxera diversity. *Molecular Ecology* 4, 459–464.

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.

Forneck, A., Walker, M.A., Blaich, R. (2000). Genetic structure of an introduced pest, grape phylloxera (*Daktulosphaira vitifoliae* Fitch), in Europe. *Genome* 43, 669–678.

Forneck, A., Walker, M.A., Blaich, R., Yvon, M., Leclant, F., 2001. Interaction of phylloxera (*Daktulosphaira* vitifoliae Fitch) with grape (*Vitis* spp.) in simple isolation chambers. *American Journal of Enology and Viticulture* 52, 28–34.

Friedman, S.P., 2005. Soil properties influencing apparent electrical conductivity, a review. *Computers and Electronics in Agriculture* 46, 45–70.

Frolov, A.N., David'yan, G.E. (2009). Pests *Viteus vitifolii* Fitch – Grape Phylloxera. Interactive agricultural ecological atlas of Russia and neighbouring countries. Economic plants and their diseases, pests and weeds. http://www.agroatlas.ru/en/content/pests/Viteus_vitifolii

Gale, G. (2003). Saving the vine from Phylloxera: a never-ending battle. In: Sandler, M., Pinder, R. (eds) *Wine: a Scientific Exploration*. Taylor and Francis, London, pp 70–91

Gerson, U. (1996). Arthropod root pests. In: Waisel, Y., Eshel, A., Kafkafi, U. (Eds.), *Plant Roots: The Hidden Half*, 2nd ed. Marcel Dekker Inc., New York, pp. 797–809.

Gorkavenko, E.B. (1976), Entomophages of grape phylloxera and their role in reducing of the pest population in the southern areas of Ukraine. *Tr Vses Nauchno-issled Inst Zashch Rast* 46, 88–97.

Granett, J., Bisabri-Ershadi, B., Carey, J. (1983). Life-tables of phylloxera on resistant and susceptible grape rootstocks. *Entomologia Experimentalis et Applicata* 34,13–19

Granett, J., Timper, P., Lider, L.A. (1985). Grape phylloxera (*Daktulosphaira vitifoliae*) (Homoptera: Phylloxeridae) biotypes in California. *Journal of Economic Entomology* 78, 1463–1465.

Granett, J., De Benedictis, J.D., Walker, M.A., Wolpert, J., Weber, E. (1991). Spread and Control of Biotype B Phylloxera. American Vineyard Foundation, California. http://www.avf.org/article.html?id¼3600.

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

Hellman, E.W. (2003). Grapevine structure and function. In: Hellman, E.W. (Ed.), *Oregon Viticulture.* Oregon State University Press, Corvallis, Oregon, pp. 5–19.

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

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

Herbert, K.S., Umina, P.A., Mitrovski, P.J., Powell, K.S., Viduka, K., Hoffmann, A.A. (2010). Clone lineages of grape phylloxera differ in their performance on *Vitis vinifera*. *Bulletin of Entomological Research* 100, 671–678.

Holopainen, J.K., 2002. Aphid response to elevated ozone and CO2. *Entomologia Experimentalis et Applicata* 104, 137–142.

Huber, L., Eisenbeis, G., Porten, M., Rűhl, E.H. (2003). The influence of organically managed vineyard-soils on the phylloxera-populations and vigour of grapevines. *Acta Horticulturae* 617, 55–59.

Huber, L., Kirchmair, M. (2007). Evaluation of efficacy of entomopathogenic fungi against small scale grape-damaging insects in soil – experiences with grape phylloxera. *Acta Horticulturae* 633,167–171.

Huber, L., Eisenbeis, G., Porten, M., Ruhl, E.H. (2003). The influence of organically managed vineyard soils on the phylloxera populations and the vigour of grapevines. *Acta Horticulturae* 617, 55–59.

Huber, L., Hoffmann, M., Rűhl, E.H., Kirchmair, M. (2009). Disease supressiveness of vineyard soils infested with grape phylloxera. *Acta Horticulturae* 816, 41–51.

Jaillon, O., Aury, J.M., Noel, B., Policriti, A., Clepet, C., Casagrande, A., et al., (2007). The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* 449, 463–467.

Jones, G.V. (2007). Climate change: observations, projections, and general implications for viticulture and wine production. In: Essick, E., Griffin, P., Keefer, B., Miller, S., Storchmann, K. (Eds.), *Economics Department Working Paper No. 7*, Whitman College, Department of Geography, Southern Oregon University, Ashland, pp. 1–14.

Jubb, G.L, Masteller, E.C. (1977). Survey of arthropods in grape vineyards of Erie County, Pennsylvania: Neuroptera. *Environmental Entomology* 6, 419–428.

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., McDonald, G., Corrie, A.M., van Heeswijck, R. (2002). *In vitro* assessment of grapevine resistance to two populations of phylloxera from Australian vineyards. *Australian Journal of Grape and Wine Research* 8, 109–116.

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.

King, P.D., Rilling, G. (1985). Variations in the galling reaction of grapevines, evidence of different phylloxera biotypes and clonal reaction to phylloxera. *Vitis* 24, 32–42.

Kingston, K.B., Powell, K.S., Cooper, P.D. (2009). Grape phylloxera, new investigations into the biology of an old grapevine pest. *Acta Horticulture* 816, 63–70.

Kirchmair, M., Huber, L., Rianer, J., Strasser, H. (2004). *Metarhizium anisopliae*, a potential biological control agent against grape phylloxera. *BioContro*l 49, 295–303.

Kocsis, L., Granett, J., Walker, M.A., Lin,H., Omer, A.D. (1999).Grape phylloxera populations adapted to *Vitis berlandieri* x *V. riparia* rootstocks. *American Journal of Enology and Viticulture* 50, 101–107.

Koennecke, T., Aigner, C., Specht, S., Lawo, N.C., Forneck, A. (2011). A stepwise assessment of *Daktulosphaira vitifoliae* infested grapevines in a Viennese vineyard site. *Acta Horticulture* 904, 59–62.

Korosi, G.A., Trethowan, C.J., Powell, K.S. (2007). Screening for rootstock resistance to genotypes from Australian vineyards under controlled conditions. *Acta Horticulture* 733, 159–165.

Korosi, G.A., Carmody, B., Powell, K.S. (2011). Rootstock screening for phylloxera resistance under controlled conditions using selected phylloxera clonal lineages. *Acta Horticulture* 904, 33–39.

Korosi, G.A., Trethowan, C.J., Powell, K.S. (2009). Reducing the risk of phylloxera transfer on viticulture waste and machinery. *Acta Horticulture* 816, 53-62.

Korosi, G.A., Mee P.T., Powell, K.S. (2012). Influence of temperature and humidity on mortality of grapevine phylloxera *Daktulosphaira vitifo*liae clonal lineages: a scientific validation of a disinfestation procedure for viticultural machinery. *Australian Journal of Grape and Wine Research* 18, 43-47.

Kűhnelt, W. (1963). Soil-inhabiting Arthropoda. *Annual Review of Entomology* 8, 115–136. Lawler, I.R., Foley, W.J., Woodrow, I.E., Cork, S.J. (1997). The effects of elevated CO_2 atmospheres on the nutritional quality of *Eucalyptus* foliage and its interaction with soil nutrient and light availability. *Oecologia* 109, 59–68.

Lin, H., Walker, M.A., Hu, R., Granett, J. (2006). New simple sequence repeat loci for the study of grape phylloxera (*Daktulosphaira vitifoliae*) genetics and host adaptation. *American Journal of Enology and Viticulture* 57, 33–40.

Lotter, D.W., Granett, J., Omer, A.D., (1999). Differences in grape phylloxera-related grapevine root damage in organically and conventionally managed vineyards in California. *Horticultural Science* 34, 1108-1111.

Makee, H. (2004). Factors influencing mortality, fecundity and fertility of grape phylloxera (*Daktulosphaira vitifoliae* Fitch). *Vitis* 43, 49-50.

Makee, H., Charbaji, T., Ayyoubi, Z., Idris, I. (2004). Evaluating resistance of some rootstocks to grape phylloxera with *in vitro* and excised root testing systems. *In vitro Cellular and Developmental Biology - Plant* 40, 225–229

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*. Masson, Montpellier, p. 528.

Molnár, J.G., Neme´th, Cs., Ma´yer, J., Jahnke, G.G. (2009). Assessment of leaf-galling incidence on European grapevines in Badascony, Hungary*. Acta Horticulture* 816, 97– 104.

Morgan C.V.G., Procter P.J., Vielvoye J. (1973). Occurrence of and attempts to eradicate grape phylloxera (Homoptera: Phylloxeridae) in British Columbia. *Journal of the Entomological Society of British Columbia* 70, 11–12.

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

National Vine Health Steering Committee (2005). *Pest risk analysis for the establishment of a new infestation of phylloxera in Australia as a result of harvest processes*. p19.

National Vine Health Steering Committee (2009). *National Phylloxera Management Protocol.* National Vine Health Steering Committee, Australia, 32pp.

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

PHA (Plant Health Australia). (2009) *Industry Biosecurity Plan for the Viticulture Industry* (Version 2.0). Plant Health Australia, Canberra, ACT.

Powell, K.S. (2000). *Management of Grape Phylloxera in South-East Australia Phase I and Phase II.* GRDC/NRE Report. p171.

Powell, (2003). *Targeted Phylloxera Management Options*. Final Report DNR 00/3. GRWDC. p165.

Powell, K.S. (2006). *Sustainable Long Term Strategies for Phylloxera Management under Australian Conditions*. GWRDC/DPI Report. p160.

Powell, K.S. (2007). *Optimising Detection and Management Methods for Soilborne Pest Incursions.* GWRDC/DPI Final Report. p113.

Powell, K.S. (2008). Grape phylloxera: an overview. In: Johnson, S.N., Murray, P.J. (Eds.), *Root Feeders: An Ecosystem Perspective.* CAB International, Oxon, pp. 96–114.

Powell, K.S. (2012). A holistic approach to future management of grapevine phylloxera. In: Bostanian, N., Vincent, C., Isaacs, R. (Eds.), *Arthropod Management in Vineyards: Pests, Approaches and Future Directions.* Springer Science and Business Media BV, London, UK, pp. 239–251.

Powell, K.S. (2017). Risks and Management of Endemic and Exotic Phylloxera. DEP 1301 AGWA Final Report p 10

Powell, K.S., Korosi, G.A. (2014). 'Taking the Strain' selecting the right rootstock to protect against endemic phylloxera strains. *Acta Horticulture* 1045, 99-108.

Powell, K.S., Cooper, P.D., Forneck, A. (2013). Chapter Four - The Biology, Physiology and Host–Plant Interactions of Grape Phylloxera *Daktulosphaira vitifoliae*. *Advances in Insect Physiology*. Johnson, S.N., Hintpold, I., Turlings, C.J.T. Academic Press. Volume 45, 159-218.

Powell, K.S., Bruce, R.J., Korosi, G.A. (2014). Assessing the risk of phylloxera survival during white grape processing. *Acta Horticulture* 1045, 49-58.

Powell, K.S., Herbert, K.S. Hoffman, A.A.. (2007). Grapevine phylloxera - opportunities for monitoring and early detection using non-destructive techniques. *Australian Viticulture* 50-53.

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

Powell, K.S., Trethowan, C.J., Blanchfield, A.L., Norng, S. (2007). Composted winery waste and its influence on grape phylloxera in ungrafted vineyards. *Acta Horticulture* 733, 143–150.

Powell, K.S., Brown D., Dunstone, R., Hetherington, S., Corrie, A. (2000). Population dynamics of phylloxera in Australian vineyards and implications for management. In: Powell K.S. and Whiting, J. (eds.), *Proceedings of the International Symposium on Grapevine Phylloxera Management*, January 21st 2000, Melbourne, Australia. DNRE Rutherglen.

Powell, K.S., Burns, A., Norng, S., Granett, J., McGourty, G. (2006). 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., 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., Korosi, G.A., Clingeleffer, P., Walker, R., Thomas, M.R., Smith, H., Smith, B. (2013). Genetic markers for phylloxera resistance: preliminary observations using two endemic phylloxera genetic strains. *VIth International Phylloxera Symposium*, Bordeaux-France, August 28-30, 2013. INRA, Bordeaux. p. 28.

Quaglia, F., Rossi, E. (1987). Susceptibility of some cultivars of ungrafted European grape– vines to the foliar attack of the grape phylloxera (*Viteus vitifoliae* (Fitch)), observations of the triennium 1982–1984. In*: Integrated Pest Control in Viticulture*. *Proceedings of a Meeting of the EC Experts Group, Portoferraio. Commission of the European Communities.* A. A. Balkema, Rotterdam, The Netherlands.

Raspi, A., Antonelli, R. (1987). Grape phylloxera (*Viteus vitifoliae* (Fitch)) infestation on American vine leaves in a nursery at San Pietro a Grado (Pisa) during the years 1982– 1983. In: *Integrated Pest Control in Viticulture. Proceedings of a Meeting of the EC Experts Group*, Portoferraio. Commission of the European Communities. A. A. Balkema, Rotterdam, The Netherlands.

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

Ritter, A., Vorwerk, S., Blaich, R., Forneck, A. (2007). Adaptational potential of grape phylloxera (*Daktulosphaira vitifoliae*) clonal lineages. *Mitt. Klosterneuburg* 57, 116– 122.

Rossi, E. (1988). La filossera della vite. *Info. Fitipat.* 9, 17–22.

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 vitifolia* Fitch. *Research Bulletin of the Plant Protection Service Japan* 21, 67-69.

Salt, D.T., Fenwick, P., Whittaker, J.B. (1996). Interspecific herbivore interactions in a high CO2 environment: root and shoot aphids feeding on Cardamine. *Oikos* 77, 326–330.

Song, G.-C., Granett, J. (1990). Grape phylloxera (Homoptera, Phylloxerideae) biotypes in France. *Journal of Economic Entomology* 83, 489–493.

Stellwaag, F. (1928). *Die Weinbauinsekten der Kulturelander*. Paul Parey, Berlin.

Strapazzon, A., Girolami, V. (1983). Foliar phylloxera infestations (*Viteus vitifoliae* (Fitch)) with completion dell'olociclo on *Vitis vinifera* (L.) engaged. *Redia* 66, 179–194.

Stevenson, A.B. (1964). Seasonal history of root-infesting *Phylloxera vitifoliae* (Fitch)(Homoptera: Phylloxeridae) in Ontario. *Canadian Entomology* 96, 979–987.

Stevenson, A.B. (1970). Strains of the grape phylloxera in Ontario with different effects on the foliage of certain grape cultivars. *Journal of Economic Entomology* 63, 135–138.

Sullivan, V. (1996). New rootstocks stop vineyard pest for now. *Californian Agriculture* 50, 7– 8.

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

Trębicki, P., Vandegeer, R., Powell, K.S., Bosque-Perez, N., Freeman, A., Yen, A., Fitzgerald G.J., Luck, J. (2015). The impact of elevated CO_2 and *Barley yellow dwarf virus* on *Rhopalosiphum padi* performance and feeding behavior and host plant chemistry. *In prep.*

Trethowan, C.J., Powell, K.S. (2007). Rootstock-phylloxera interactions under Australian field conditions*. Acta Horticulture* 733, 115–122.

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 Horticulture* 733, 183–195.

van Driesche, R.G., Bellows, T.S. (1996). Biological control. Chapman and Hall, New York. Vega, J. (1956) Lutte contre le phylloxera (à l'exclusion de l'emploi des porte-greffes résistants). Rapport général pour l'Amérique latine. *Bull de l'Off Int du Vin* 29, 31–42.

Vidart, M.V., Mujica, M.V., Bao, L., Duarte, F., Bentancourt, C.M., Franco, J., Scatoni, I.B. (2013). Life history and assessment of grape phylloxera leaf galling incidence on Vitis species in Uruguay. *SpringerPlus* 2, 181.

Vine Health Australia (2017). Phylloxera management zones in Australia. http://www.vinehealth.com.au/biosecurity-in-practice/maps/phylloxera-management-zones/ Accessed 6/9/17

Vorwerk, S., Forneck, A. (2006). Reproductive mode of grape phylloxera (*Daktulosphaira vitifoliae*, Homoptera, Phylloxeridae) in Europe, molecular evidence for predominantly asexual populations and a lack of gene flow between them. *Genome* 49, 678–687.

Vorwerk, S., Forneck, A. (2007). Analysis of genetic variation within clonal lineages of grape phylloxera (*Daktulosphaira vitifoliae* Fitch) using AFLP fingerprinting and DNA sequencing. *Genome* 50, 660–667.

Wapshere, A.J., Helm, K.F. (1987). Phylloxera and *Vitis*: an experimentally testable coevolutionary hypothesis. *American Journal of Enology and Viticulture* 38, 216–222.

Wegner–Kiss, G. (2007). Der reblaus einhalt gebieten. Badische Winzer. May, 24–26. Wheeler, A.G., Henry, T.J. (1978). *Ceratocapsus modestus* (Hemiptera: Miridae), a predator

of grape phylloxera: seasonal history and description of fifth instar. *Melsheimer Entomol Ser* 25, 6–10.

Wheeler, A.G., Jubb, G.L. (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). *Coleopterist Bulletin* 33, 199–204

Whiting, J. (2013). *Rootstock Breeding and Associated R & D in the Viticulture and Wine Industry.* GWRDC Report GWR 1009, 1-54.

Williams, R.N., Shambaugh, G.F. (1987). Grape phylloxera (Homoptera: Phylloxeridae) biotypes confirmed by electrophoresis and host susceptibility. *Annals of the Entomology Society of America* 81, 1–5.

Yvon, M., Peros, J.P. (2003). Variation in aggressiveness and genetic diversity of grape phylloxera in southern France. *J. Int. Sci. Vigne Vin.* 37, 77–84.