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Sustainable phylloxera management - Phase II



FINAL REPORT to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: DPI 06/02

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Research Organisation: **DPI-Rutherglen**

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Sustainable Phylloxera Management– Phase II

A final report on project DPI 06/02

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June 2009

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ABSTRACT

The overall aim of this research project was to focus on the two most widely used management strategies, rootstocks and quarantine, available in Australia to minimise the risk of phylloxera spreading within and between quarantine regions. In addition where new phylloxera infestations occurred ensure appropriate strategies are utilised to ensure their containment. New developments during the project period included screening selected phylloxera genotypes against selected grapevine rootstocks (both commercially available and newly developed) using laboratory and glasshouse protocols, an improved dry heat disinfestation protocol and the integration of Keepad™ technology for workshop evaluation. In addition two phylloxera detections were made during the research period and these were monitored to add value to the research outputs. Awareness of industry and the scientific community both nationally and internationally, was enhanced through (i) participation in the Fourth International Grapevine Phylloxera Symposium and other national and international conferences, (ii) facilitation and participation in National Phylloxera Management Workshops, (iii) the project leaders role as Technical Adviser to the National Phylloxera Technical Reference Group and (iv) publication of results in industry and peer reviewed scientific journals.

EXECUTIVE SUMMARY

This report describes the research and extension activities of a GWRDC-funded project on phylloxera research and management conducted during the period July 2006-June 2009. Increased scientific knowledge of grapevine phylloxera, obtained during the project period was passed on to stakeholders all levels. The research activities conducted allowed the development of improved management practices particularly through an in-depth understanding of the interactions between phylloxera clonal lineages and rootstocks. Population monitoring at two new detection sites improved our understanding of the population dynamics and relative risk of dispersal of specific phylloxera genetic lineages.

Phylloxera monitoring allowed identification of high risk periods within phylloxera-infested vineyards in the Lancefield and Porepunkah regions. Phylloxera awareness activities were significantly enhanced during the course of the project through:

- a variety of extension activities including workshops, field days, seminars, industry and media articles, peer-reviewed scientific papers and presentation of research data at the Fourth International Grapevine Phylloxera Symposium,

- training for growers in the early detection and identification of field symptoms of phylloxera infestations and pre- and post-incursion options management through Annual Phylloxera Identification and Management Workshops,

- training of survey team participants from New South Wales, Queensland and South Australia in ground surveying techniques for early detection of phylloxera has also taken place,

- risk assessment providing an improved scientific and technical basis for the development of the National Phylloxera Management Protocols by the National Phylloxera Technical Reference Group,

- improved quarantine protocols for dry heat disinfestation and an improved awareness of the risks of grape phylloxera survival at low temperatures and in water and,

- an improved understanding of fundamental interactions between phylloxera genotypes and new and existing rootstocks, leading to improved confidence in developing future rootstock recommendations for Australian conditions.

INTRODUCTION

Project DPI 06/02– **Sustainable Phylloxera Management Phase II**

Time frame: *July 2006 to June 2009*

This is a report on phylloxera research, development and extension activities carried out from July 2006 to June 2009. The work was funded by the Grape and Wine Research and Development Corporation (GWRDC Project DPI 06/02), the Phylloxera and Grape Industry Board of South Australia and the Department of Primary Industries, Victoria (DPI).

Project Objectives:

The key project objectives were to:

1. Ensure rootstocks being used in the Australian viticulture industry have adequate resistance to a broad range of phylloxera clones and will remain a sustainable long-term management strategy for phylloxera under Australian conditions.

2. Understand and quantify the climatic and site related variables, which may influence phylloxera genotype-rootstock interactions.

3. Ensure recommendations are made to industry on appropriate rootstock selection in the event of outbreaks or resistance breakdown.

4. Increase biological understanding of the different phylloxera genotypes for risk assessment and quarantine stringency.

Overall Aim and Objectives:

The projects overall aim was to identify methods of spread of phylloxera between vineyards, further develop techniques to manage phylloxera within vineyards, and contribute to the prevention of future infestations. These objectives were met through:

- determining the relative risk of dispersal of clonal lineages at new infestation sites through above-ground monitoring of phylloxera, especially in regard to the number and survival of dispersive stages and genotype interactions during the spring, summer and autumn on ungrafted grapevines,

- optimising a dry heat treatment protocol for viticultural machinery to ensure a reduced risk of transfer of widespread phylloxera genotypes,

- facilitating and providing technical support for the validation, development and implementation of National Phylloxera Management Protocols particularly through provision of scientific support, as Technical Adviser to the National Phylloxera Technical Reference Group. In addition, assistance was provided to industry and state government personnel through National Phylloxera Management Workshops and ground survey training,

- and providing advice on rootstock management through rigorous evaluation of rootstocks, for phylloxera control, under controlled laboratory and glasshouse conditions. To collect data to allow the future development of sustainable rootstock recommendations for a range of phylloxera clonal lineages.

PROJECT AIMS AND OUTPUTS

The project objectives and outputs achieved are summarised as follows:

Objectives	**Outputs**
Knowledge transfer via two National Phylloxera Workshops per year	*Seventeen phylloxera workshops and information sessions were conducted over three years*
Information on phylloxera clones on selected rootstocks	*Information on the interactions between phylloxera genetic strains on selected grapevine root types quantified under (controlled conditions) laboratory and glasshouse. Five new rootstock hybrids and eight conventional rootstocks were screened against up to six phylloxera genotypes*
Published articles on phylloxera management	*Several published scientific article and industry articles were produced (Appendix 1)*
Monitoring of new infestations	*Two new infestations were monitored in two PIZs*
Scientific validation of National Phylloxera Management Protocols	*A modified heat treatment protocol for disinfestation of machinery developed and endorsed by the National Phylloxera Technical Reference group and National Vine Health Steering Committee*

CHAPTER 1

MONITORING OF NEW PHYLLOXERA INFESTATIONS

Objective: To characterise the genetics and seasonal abundance of grapevine phylloxera in a newly declared phylloxera-infested zone

Summary

Following the detection of phylloxera in commercial vineyards near Porepunkah (Site 1) and Lancefield (Site 2) in November 2007 and January 2008 respectively two field-based studies were conducted over a single grapevine growing season to monitor the population dynamics, relative dispersal risk and impact of two different phylloxera genotypes on ungrafted *Vitis vinifera* L. At site one G4 was the single predominant genotype, as confirmed by DNA typing, whereas at site two G1 phylloxera was the single predominant genotype Phylloxera abundance as assessed by the use of emergence traps at the soil surface was relatively high at Site 1 compared with Site 2. Seasonal abundance of both genotypes was comparable to previous studies conducted on G1 and G4 in North East Victoria (Powell et al., 2003).

Introduction

Porepunkah detection

In November 2007 grapevine phylloxera was detected in a commercial vineyard inside the existing North East Victoria phylloxera-infested zone (PIZ) near Porepunkah. On detection the phylloxera present was confirmed to be genotype G4 by DNA typing (Umina et al., 2007). A visual canopy assessment of potential spread of phylloxera within the infested block was made.

Methods

Site details

The study site was located in a commercial vineyard near Porepunkah, within the North East PIZ. Phylloxera was first detected in the vineyard on 1st November 2007. The phylloxera genotype within the study block was characterised, using microsatellite markers (Umina et al., 2007), as G4 which is the predominant genotype found in the Ovens Valley/King Valley region and one of the six clonal lineages previously recommended for further evaluation (Hoffman, 2003).

Experimental design

The vineyard block, chosen for the study, consisted of a 3ha block of 43 rows of ungrafted *V. vinifera* L. cv. Merlot planted in the early 1990s. In each of ten alternate parallel rows there were four 'monitor vines', therefore a total of 40 infested grapevines were selected to monitor above-ground phylloxera dispersal and below-ground phylloxera population dynamics using emergence traps.

Phylloxera monitoring

A visual assessment of vine vigour was also made classifying vines as high or low canopy vigour to determine where to place insect traps (Fig. 1). To monitor above ground movement emergence traps were used. These consisted of 5.1 litre translucent plastic containers, inverted and secured adjacent to trunks of each 'monitor' grapevine with 3 small tent pegs (Fig. 2) as described in previous studies (Powell et al., 2000). Emergence trapping is a standard protocol developed by DPI-Rutherglen to assess above-ground phylloxera activity (and hence relative risk of transfer) and relative spatial and temporal distribution. The seasonal abundance of phylloxera dispersive stages moving from below the ground onto the soil surface was measured, over one growing season commencing 21st December 2007 and finishing on 23rd April 2008. Trapped insects were collected every 2 weeks by washing into plastic vials, with 100% ethanol. Trap samples were transported under quarantine conditions

to the DPI-Rutherglen quarantine laboratory and examined using a low power binocular microscope. Emergent phylloxera life-stage numbers, consisting of crawler and alate (winged adults) were recorded.



Fig. 1. Low and high vigour vines at Porepunkah field site



Fig. 2. Emergence traps adjacent to grapevine trunk

Results

Phylloxera emergence

Phylloxera dispersive stages, crawlers and alates, were monitored from 21st December 2007 until 23rd April 2008 (Fig. 3). Because phylloxera is not active above-ground during the July-September period in North east Victoria (Powell et al., 2003) no sampling was conducted during the winter months. During the season, crawlers were the predominant life-stage in trap samples and were first detected in emergence traps in mid-December, peaking in abundance in late January-early February with a mean of over 1200 crawlers per trap and were still detectable in late-April when sampling ceased. The maximum phylloxera collected for any one sampling period on a single vine was over 6000 in early February. At harvest (mid-March) although still present above-ground crawler numbers were relatively low (mean of >60 crawlers per trap) but some individual vines still had over 300 crawlers per trap during harvest. There was a large variation in trap numbers over the entire sampling period ranging from zero to over 13,000 per monitor grapevine (Fig. 4).

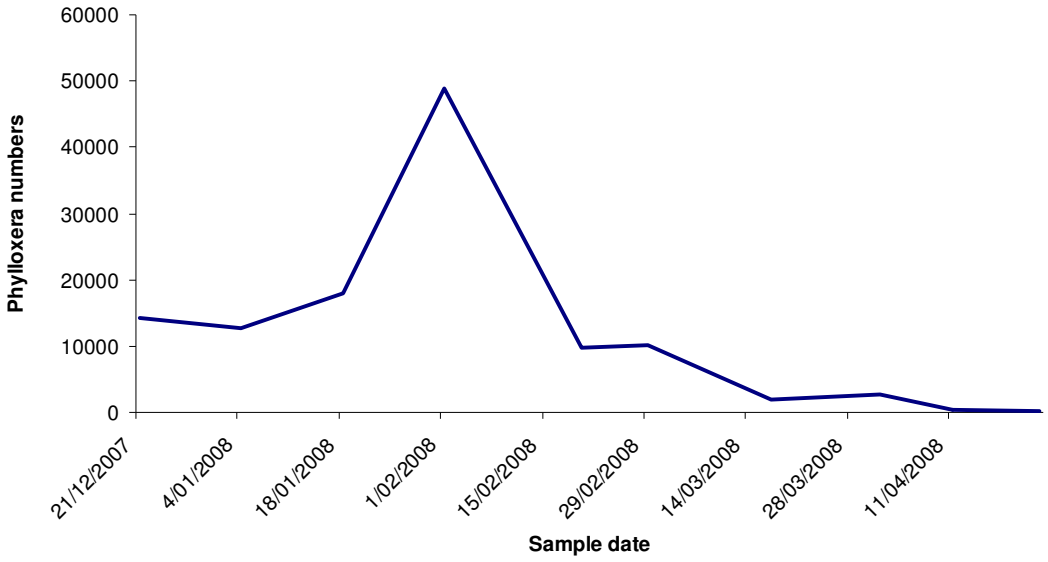


Fig. 3. Total phylloxera crawlers collected in emergence traps at the base of *Vitis vinifera* cv. Merlot, at the Porepunkah field site, over the 2007-2008 growing season, where n = 40 vines

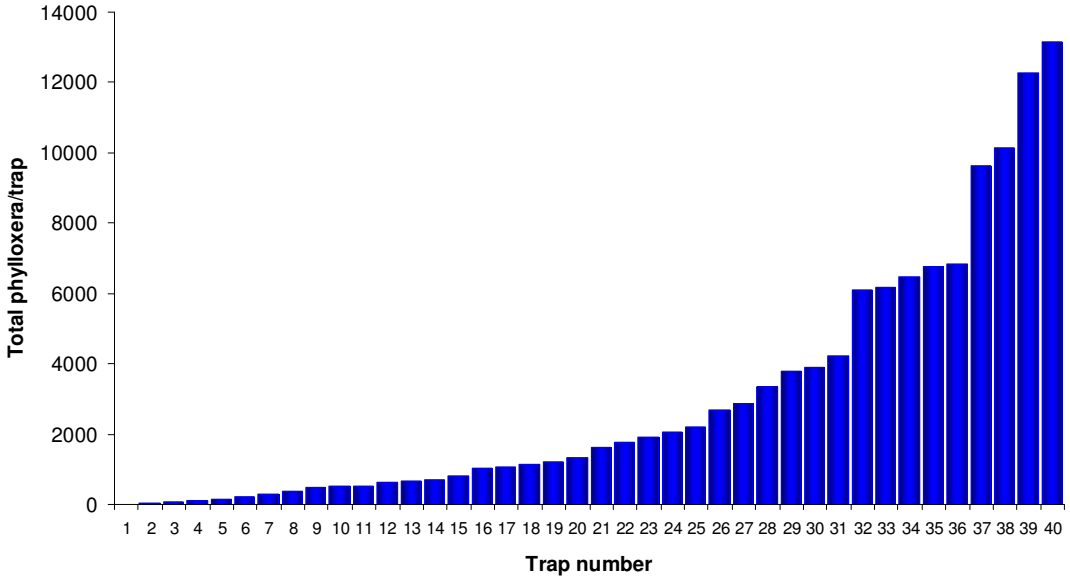


Fig. 4. Total phylloxera crawlers collected on individual emergence traps at the base of *Vitis vinifera* cv. Merlot, at the Porepunkah field site, over the 2007-2008 growing season

Conclusion

The phylloxera genotype G4 has been monitored previously under field conditions in the King Valley and Ovens valley on ungrafted *Vitis vinifera* (Powell et al., 2000; 2003a; 2006a; Herbert et al., 2006; Powell et al., 2009) and is known to produce relatively high population abundance under cool climate conditions. A relatively high population abundance, as assessed by above-ground trapping, was recorded at the Porepunkah site, in comparison with earlier studies conducted on the more geographically widespread genotype G1 which predominates in the Nagambie (Powell, 2003; 2006b), Yarra Valley (Powell, 2009), Lancefield (this chapter) and Upton regions (Powell et al., 2003), which indicates four possible inter-related scenarios. Firstly, G4 may prefer cooler climate regions. Evidence in support of this is provided in Chapter 4 which shows that lethal maximum temperatures for G4 differ to that of G1. Secondly, G4 may be more virulent than G1. In several laboratory and glasshouse studies (Chapter 2 and 3 this report; Powell 2006b) the development of G4 on *Vitis vinifera* does appear to be higher than G1 although not always significantly so. Thirdly G4 may require specific edaphic conditions in which to establish and proliferate (Powell, 2007). Evidence for this has been provided previously from field studies (Powell et al., 2003a, 2006) where G4 populations differ between regions and glasshouse studies (Powell, 2007).
Both G1 and G4 have been described as 'superclones' as they are more geographically widespread (Umina et al., 2007). However interestingly despite producing relatively high population numbers under field conditions compared to G1 (Powell et al., 2003), G4 is less geographically widespread. The overriding factor here maybe the relative risks and routes of transfer and establishment potential.

Recommendations

This study confirms that in the cooler climate regions when the phylloxera genotype G4 establishes it causes economic damage and spreads within the vineyard relatively quickly when compared to other phylloxera genotypes previously studied. Because of the very high

numbers encountered during monitoring of this pest over even a single season it is clear the risks of transfer are great and quarantine protocols should be strictly adhered to avoid further transfer of this genotype to neighbouring regions. In addition rootstocks should be planted to reduce levels of the insect in infested vineyards. Selection of appropriate rootstocks should be based on the interaction of the specific phylloxera clonal lineage G4 with the rootstock (Chapters 2 and 3).

It is very important to determine when peaks in phylloxera abundance occur above-ground as this represents the period of relatively high risk of transfer. Minimising vehicle and personnel movement during this period and adherence to quarantine protocols (NVHSC, 2008) is strongly advised to avoid transferring phylloxera to uninfested grapevine blocks within or outside the infested block. It was notable in this study that despite relatively low phylloxera abundance both during and after harvest phylloxera crawlers were still present above-ground therefore still representing a significant risk of transfer.

Monitor new outbreaks of phylloxera should be instigated as soon after detection as possible to inform the decision-making process and allow the affected grower to optimise management strategies and timing of implementation based on site variables and phylloxera genetics.

Introduction

Lancefield detection

Grapevine phylloxera was detected at a commercial vineyard near Lancefield on 7th January 2008 by Victorian Plant Standards officers. Samples where collected and confirmed as being grapevine phylloxera. Additional samples were collected for DNA typing, processed by DPI-Rutherglen and DNA typed by CESAR, Melbourne (Umina et al., 2007). The genetic strain identified on the property was confirmed as genotype G1. G1 has been identified to-date in all Phylloxera Infested Zones (PIZ) within Australia (Powell 2008) with the exception of the King Valley region of the North East PIZ and the Mooroopna PIZ. It is therefore the most commonly distributed phylloxera genotype found to-date in South Eastern Australia. As

this was a new detection, in a previously uninfested grape-growing region of Victoria, this provided a unique opportunity to conduct research activity on this genotype in a new region. Following the initial detection the area surrounding the vineyard was incorporated into a newly designated Whitebridge PIZ (DPI, 2007).

Methods

There were five research components conducted at Site 2 and each of these will be described briefly as follows and timetable for conduction and completion of work by collaborating agencies described (Table 1). In addition, as a precursor to a more detailed early detection research project currently being conducted (DPI 08/01), a variety of remote sensing applications were utilised to collect data for future evaluation of the efficacy of integrating early detection technologies.

Table 1. Timetable for Research Activities at Site 2.

Research Activity	*Commenced*	*Data Collection Completed*
DNA typing[1]	January 2008	January 2008
Emergence trapping	14 January 2008	20 May 2008
Ground Survey[2]	February 2008	February 2008
EM38 survey[3]	May 2008	May 2008
Greenseeker Spectral Survey[4]	March 2008	May 2008

Collected in collaboration with: 1 CESAR, University of Melbourne; 2 Plant Standards Branch; 3 University of New England; 4 WineNet

Ground survey - baseline information

In February 2008 a Victorian Plant Standards ground survey team conducted a survey of the entire property using the standard survey procedure. This procedure was in addition to the original survey conducted in January 2008 to confirm the presence of the insect. Blocks where phylloxera was detected by the survey team were tagged with flagging tape to indicate to the grower where phylloxera had been found. The survey indicated that at least 6 blocks

(representing at least 15ha) on the Eastern side of the 30 ha property were infested with phylloxera.

Emergence trapping - phylloxera spatial and temporal distribution

Phylloxera populations were monitored with emergence traps as described earlier. A Differential Global Positioning System (DGPS) was used to determine accurate trap locations. Initial field positions were collected with a Trimble GeoXT GPS, followed by post-processing using Land Victoria GPSnet data, for sub-meter accuracy.

Trapped insects were collected every 3-4 weeks from January to May 2008 by washing into plastic vials, with 100% ethanol. Trap samples were transported under quarantine conditions to the DPI-Rutherglen quarantine laboratory and examined under low power binocular microscope. Emergent phylloxera life-stage numbers, consisting of crawler and alate (winged adults) were recorded.

Traps were placed in the six known infested blocks of the property. Trap placement was based on (i) ground survey data and (ii) visual assessment of weak spots by project staff (Fig. 5). In each block up to 15 traps were used. Trap placement commenced on 14th January 2008 and all traps were removed on 7th May 2008. Total trap numbers for the season and trap locations are shown in Fig. 6. Relatively low phylloxera numbers (0-100) were recorded in blocks 1-3 inclusive, whereas higher trap numbers were recorded in blocks 4-6 inclusive. Relatively high trap numbers were detected in blocks 4-6 which are areas in which a visible weak spots was evident when conducting a visual assessment of the canopy vigour (Fig. 5). Phylloxera numbers collected in emergence traps provide an indication of above-ground population movement which also reflect relative below ground phylloxera levels (Herbert et al., 2006).



Fig. 5. Weak spots evident at Site 2 (Block 6) showing low vigour vines in the foreground

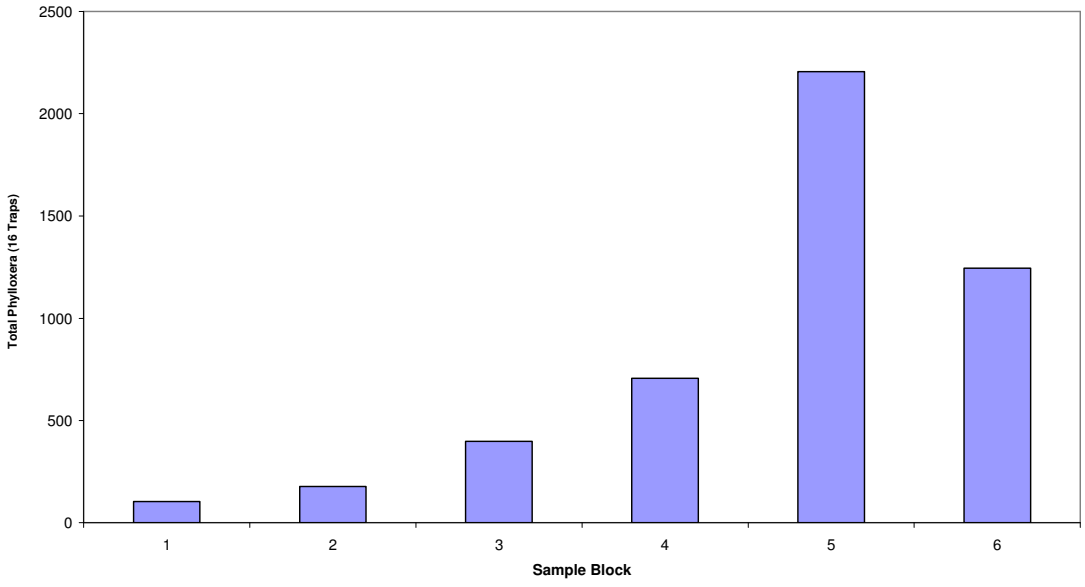


Fig. 6. Relative total abundance of phylloxera detected above ground using emergence traps in six phylloxera-infested blocks of the property

Electromagnetic ground surveys

Electromagnetic (EM) induction sensors measure bulk electrical conductivity which is determined by relative proportions of clay, salinity and moisture in the soil. The EM-38 sensor collects data from 0-1.5 metres reflecting the typical depth of grapevine roots (and coincidentally the known depths at which grapevine phylloxera is found on the root system) and in the vertical dipole position 65% of its response comes from the 25-150 cm soil depth the data from which is collected by two passes. Typically in the vineyard environment EM-38 data is collected by mounting the equipment on a rubber sledge and pulling via an all-terrain vehicle (ATV) through the centre of the vine mid-row. To accurately map a vineyard area a differential global positioning system (DGPS) is used in conjunction.

In May 2008 project staff in collaboration with staff from the University of New England, Armidale conducted an EM38 survey of all vineyard blocks on the property. EM38 surveys are being evaluated by DPI Rutherglen project staff as a research tool to determine to what extent soil properties such as electrical conductivity and soil moisture may assist in detection of 'hot spots' for phylloxera activity in project DPI 08/01. EM38 data can also be used for other grapevine management activities.

Measurement of apparent soil electrical conductivity was completed using a Geonics® EM-38RT unit (Geonics Ontario, Canada) operated in vertical dipole mode. Prior to conducting each set of measurements throughout the course of the experiment, the instrument was switched on and warmed up for a period of 15 minutes, and zeroed following the standard Geonics protocol. Immediately following the zeroing procedure, the unit was placed on the ground at a pre-designated calibration point and the EC_a measured and checked to ensure it was repeatable to within 5%.

Two-dimensional surveys were completed by towing the EM-38 unit behind an ATV on a rubber sled at a speed of 10 km hr^{-1} with particular attention paid to ensuring the sled did not deviate more than 10 cm either side of track, thereby minimizing trellis-induced artefacts in the readings. The EM-38 unit itself was placed in a thermoplastic case with styrofoam

insulation on either side and a cover over the top to reduce case temperature fluctuations. The distance between the unit and the surface of the ground, as dictated by the bottom of the case and thickness of the rubber sled was approximately 15 mm. The continuous output data stream from the EM-38 unit was fed into a Trimble TSCe® datalogger along with the DGPS location information, every second, from a Trimble DGPS (Trimble, Sunnyvale California, USA).

The images obtained during the survey are shown. Figure 7 shows the area of the property in which phylloxera was detected using ground surveys and emergence traps. Trap numbers are relatively higher in Blocks 4-6 and are relatively low in Blocks 1-2. Higher EC values were generally recorded in the Northern area of Blocks 3, 4, 5 and 6a. Figure 8 shows the EM 38 data for Blocks 6b-10. No trapping was conducted in any of these blocks.

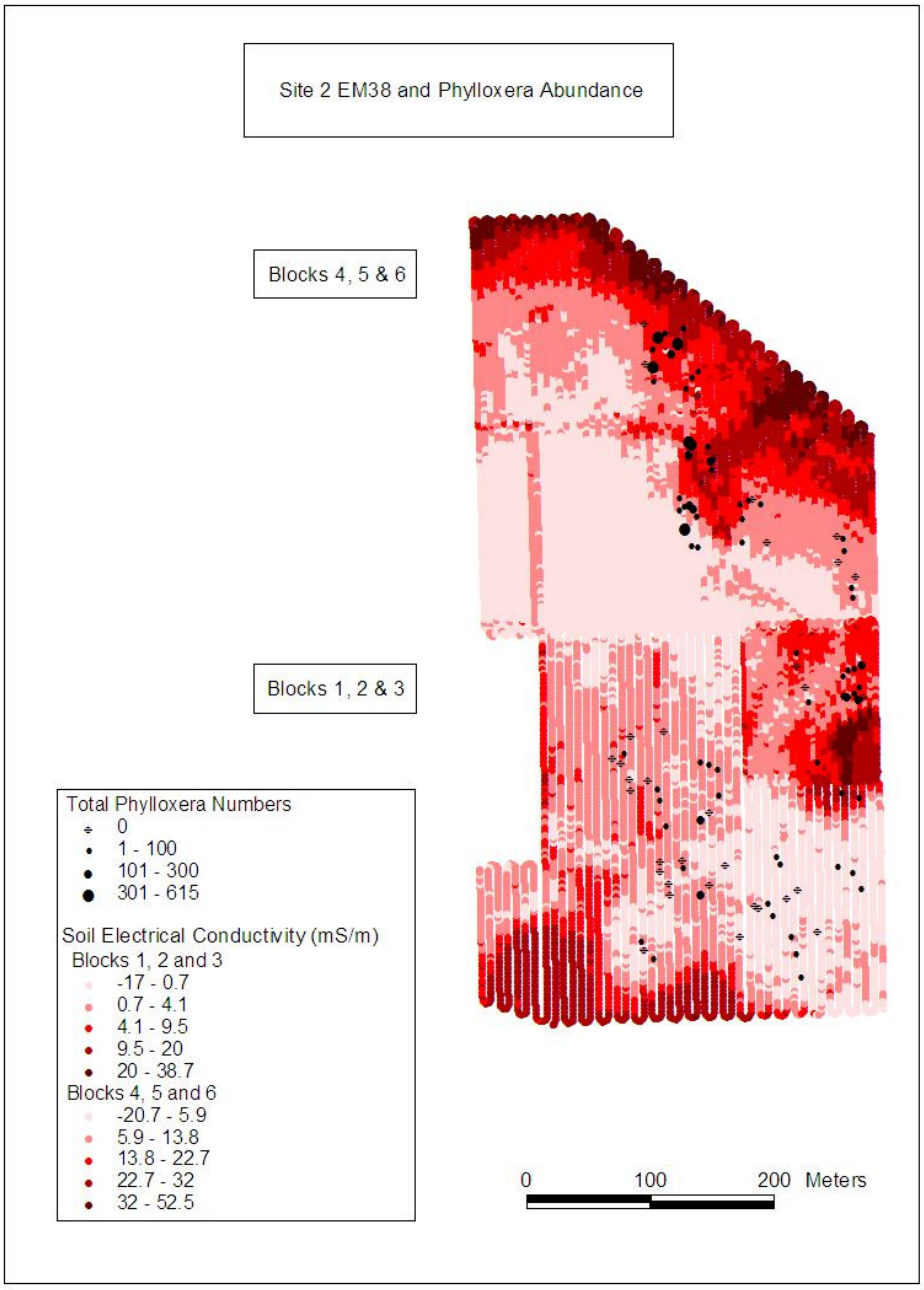


Fig. 7. An electromagnetic induction (EM38) map from survey data collected May 2008, and emergence trap data collected January-May 2008, in six phylloxera-infested *Vitis vinifera* blocks at Site 2



Fig. 8. An electromagnetic induction (EM38) map from survey data collected May 2008, and emergence trap data collected January-May 2008, in *Vitis vinifera* blocks at Site 2 where no phylloxera activity was monitored

Multispectral aerial surveys

Airborne multispectral imagery collected in February 2008 (Figs. 9-11), was supplied by the Phylloxera and Grape Industry Board of South Australia. Imagery was processed by the PGIBSA as true colour, Plant Cell Density (PCD) and False Colour Infra Red. A true colour image is produced by combining three independently recorded waveband images (red, green and blue). A false colour image utilises a NIR red and green camera. Healthy vigorous vines appear red, while bare ground appears blue/green. Three types of images were produced for each of the study sites using aerial sensors:

1) True-colour image (Fig. 9): information from the blue, green and red wavebands is projected into blue, red and green of the screen or printer, generating an image similar to a colour photograph.

2) False-colour image (Fig 10): green, red and near infrared wavebands are projected into the blue, green and red of the screen/printer. Regions of greater photosynthetically active biomass strongly reflect near infrared radiation, which is highlighted in this type of image.

3) Plant Cell Density (PCD; Fig 11) has high values for photosynthetically active biomass (PAB) and low for a low PAB. PCD is calculated from the formula:

$$PCD = \frac{NIR}{RED}$$

Multispectral ground surveys

The side-looking Greenseeker™ reflectance sensor (N Tech Inc., USA) was also trialled at Site 2 to allow comparison with imagery collected using airborne sensors. The system

was mounted on an ATV, and alternate grapevine rows were measured from both sides of the canopy. Light-emitting diodes transmitted infrared and red wavelengths, and the reflected signal was recorded by a pair of photo-detectors. The system measures Normalised Difference Vegetation Index (NDVI). NDVI quantifies the relative difference between the near infrared reflectance peak and the red reflectance trough from the spectral reflectance profile (Proffitt et al., 2006) and is calculated from the formula:

$$NDVI = \frac{NIR - Red}{NIR + Red}$$

Where NIR and Red are the reflectances in each waveband. For highly vegetated targets the NVDI value will be high.

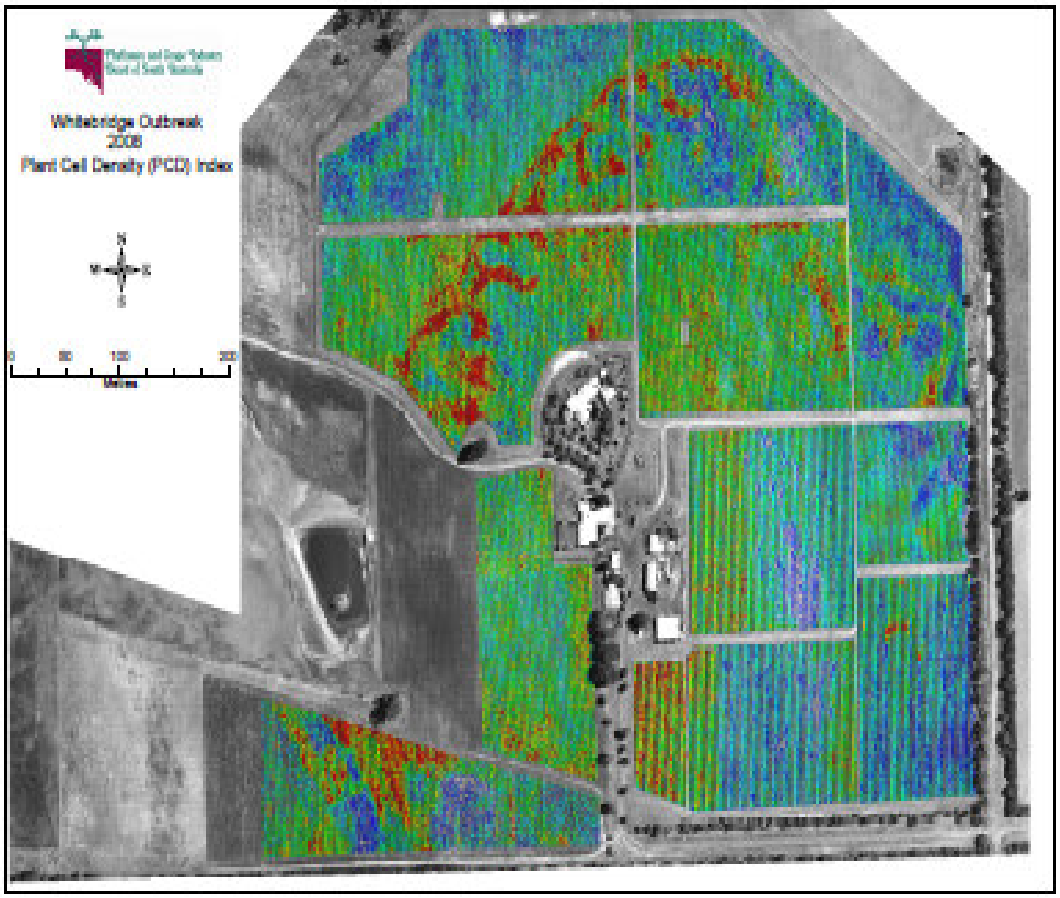


Fig. 11. PCD aerial imagery of Site 2 showing of weak areas in several grapevine blocks



Fig. 9. True colour aerial imagery of Site 2 showing distribution of weak areas in several grapevine blocks

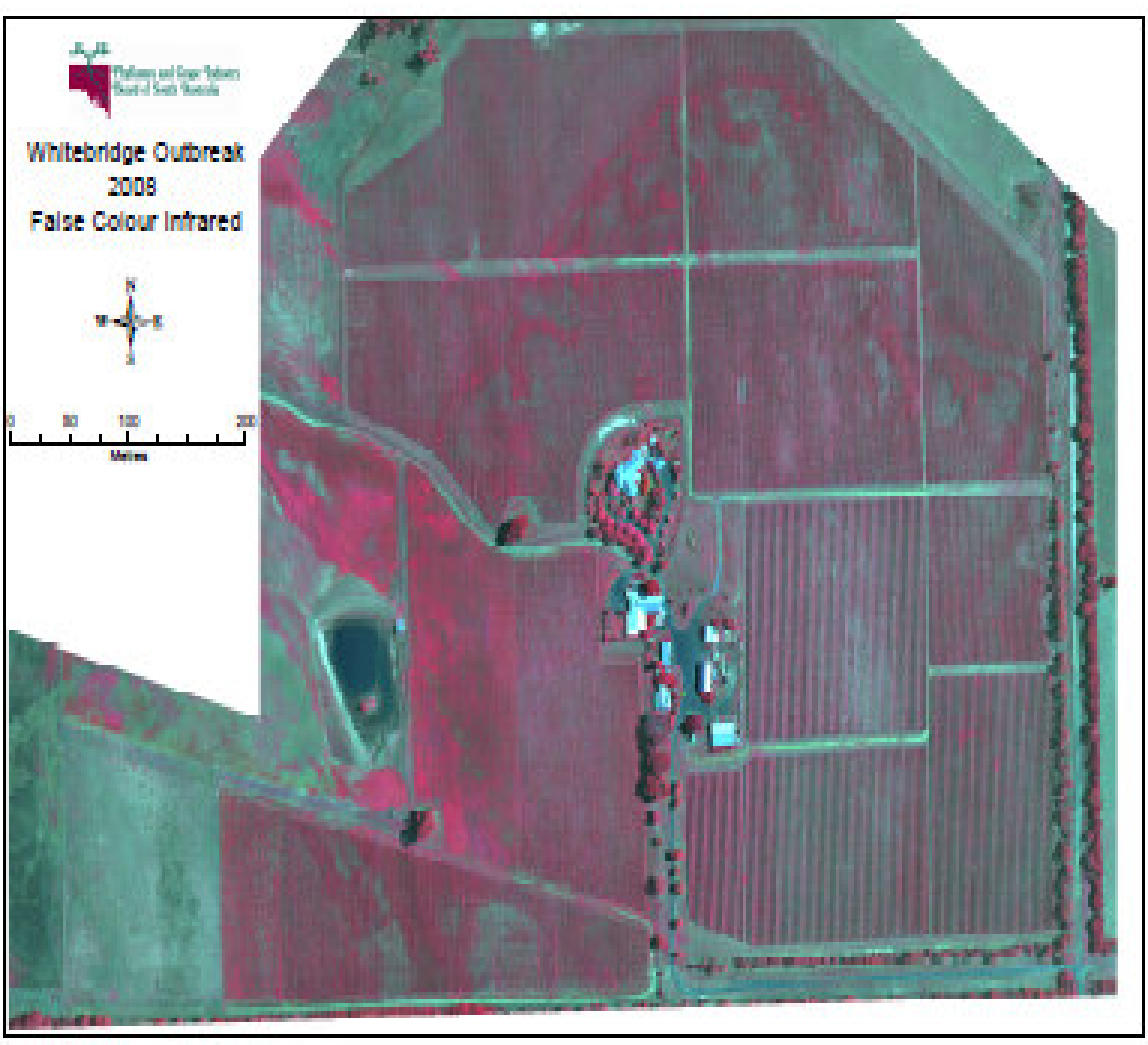


Fig. 10. False colour aerial imagery of Site 2 showing of weak areas in several grapevine blocks

In March 2008 DPI Rutherglen in collaboration with Wine Network conducted a spectral survey of the entire vineyard over a 2-day period using a 'Greenseeker' spectral device attached to an ATV. The imagery obtained provides and indication of relative canopy vigour and may be considered potentially more sensitive than aerial surveys as they can identify relatively small weak spots not visible on aerially conducted surveys. Initially data was analysed for 4 infested blocks of the 13 surveyed. However due to a software corruption issue the remaining data was sent to the USA for analysis. Data from infested Blocks 1 and 6 could not be recovered but imagery from the four other blocks was recovered and an example of such imagery is shown (Fig. 12). The imagery indicated areas of weak canopy which would be useful to monitor over successive seasons. Weak canopy is one of the potential indications of phylloxera presence and can prove useful in targeted phylloxera detection. Although weak spots can also be due to other causes such as frost damage, poor drainage, water stress, nutritional issues, salinity, herbicide drift, underlying soil characteristics and other pests and diseases.

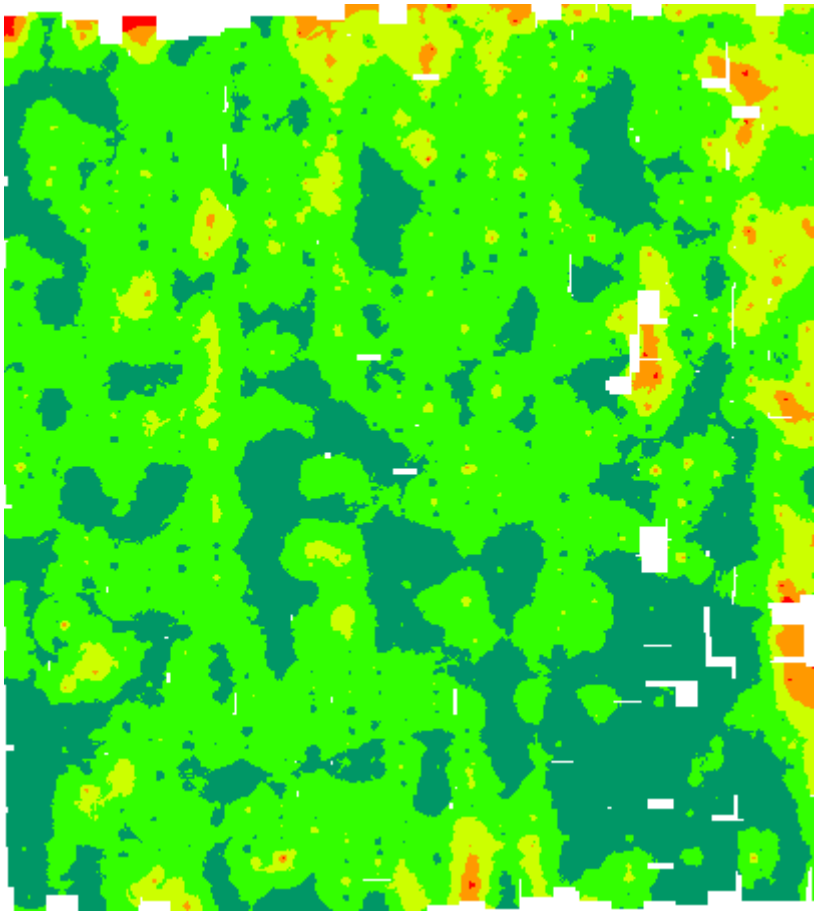


Fig. 12. Greenseeker imagery collected in a phylloxera-infested bock. Orange regions indicate relatively lower vigour vines darker green regions indicate relatively high vigour vines. Weak areas are potential sites for trap placement

Data integration and interpretation

The overall focus of the research activity was collect data to determine if a combination of spectral canopy, soil sensing and trapping data could ultimately identify or improve detection at the field sites. In order to do this data requires complex analysis and integration in collaboration with a number of research providers. Preliminary observations of the ongoing analysis are included in this report. But further analysis will be reported as a component of project DPI 08/01.

Preliminary conclusions

This research trial has provided preliminary background information to allow for :

- Further examination of the relationship between soil factors and phylloxera.
- Support the hypothesis that phylloxera may prefer to establish in higher EC_a areas and may move to lower EC_a as population pressure on declining vines increases. (as indicated as described by Bruce et al., 2009)
- Areas of higher EC_a may facilitate the earlier expression of classical stress symptoms like reduced vine vigour.
- High EC_a areas could potentially be used as focus areas for areas to monitoring for further phylloxera spread in infested vineyards.

At the time that the experimental work was completed (May 2008) grapevine phylloxera was detected within at least six blocks of the property predominantly on the Eastern side as indicated by ground survey and trapping data collected. In some blocks relatively high population levels (i.e. > 100 phylloxera per trap) where detected and represent a high risk of transfer to uninfested vines on a range of vectors such as machinery, grapevine material and clothing/footwear as described in the National Phylloxera Management Protocols (2008). Aerial and ground collect imagery highlighted weak spots due to phylloxera in six blocks on the Eastern side of the property. The property was predominantly planted to ungrafted *Vitis vinifera* varieties which are highly susceptible to grapevine phylloxera therefore decline and further spread in the vineyard blocks is likely over coming seasons. This risk can be reduced by adhering to quarantine and disinfestation protocols (refer to National Phylloxera Management Protocols, 2008) but ultimately the use of phylloxera-resistant rootstocks is recommended and there are a number of reference source where further information on choice of rootstocks can be obtained (Whiting, 2003; Dry, 2008).

CHAPTER 2

IN VITRO SCREENING FOR PHYLLOXERA RESISTANCE

Objective: Information on phylloxera clones on selected rootstocks – through laboratory screening

Summary

In a number of experiments the performance of selected phylloxera genotypes on *Vitis vinifera and Vitis* rootstocks was assessed in laboratory trials. Excised root bioassay protocols were used to compare the relative performance of selected grape phylloxera genotypes on both commercially available and newly developed rootstock hybrids. The excised root bioassay technique has been used worldwide routinely for phylloxera screening for more than a decade to compare the level of virulence and development of phylloxera 'populations' on *Vitis* rootstocks. However, more recently this method has been developed to compare specific clonal lineages of grapevine phylloxera (Powell et al., 2003). This study compared six selected grape phylloxera genotypes on up to seven rootstock types. Grape phylloxera genotypes differed in fecundity (egg production) and egg to adult survivorship on both *Vitis vinifera* and rootstock hybrids. Therefore the results of this work have important implications for the future selection of rootstocks for phylloxera management.

All rootstocks were generally more tolerant to all tested strains of phylloxera compared with ungrafted *Vitis vinifera*, as shown by reduced population development on all rootstock's, but the level of tolerance did differ between phylloxera strains. However caution is advisable when basing a resistance rating solely on *in vitro* studies. It is recommended that whilst excised root bioassays are a useful preliminary and relatively rapid screening system, they may produce potentially higher population abundance than when using whole plant systems for screening. Whole plant rootstock screening, in the form of glasshouse and field trials, although relatively time consuming is recommended as the second and third tier screening

approach, as this not only examines the interactions at a whole plant level, but also allows an assessment of relative damage to the root systems of both lignified and non-lignified root types and can also allow an assessment of the impact of phylloxera populations on plant vigour, yield and quality.

Overall the results indicate that only some phylloxera genotypes, notably G1 and G4, are highly virulent on *V. vinifera*, which may help explain relative patterns of field damage and continued survival and production of *V. vinifera* vines in some infested areas. Pre-screening of rootstocks against selected phylloxera genotypes will enable more strategic protection of areas where particular genotypes may potentially establish.

Introduction

Although phylloxera was first detected in Australia in 1877 since then the use of phylloxera resistant rootstocks has increased notably in PIZs but only marginally outside PIZs. However 85% of vines in Australia (Powell, 2008) are still planted to ungrafted highly susceptible European grapevines (*Vitis vinifera* L.). In most major PEZ grape-growing regions rootstock uptake is still remarkably low including South Australia, New South Wales and Western Victoria.

In 2003 systematic screening commenced in Australia to selected clonal lineages of phylloxera known to occur in the country (Powell et al, 2006). Eighty three different phylloxera genotypes (Umina et al., 2007), some of which have different virulence levels on different vine species (Powell 2003, Korosi et al., 2007) have been identified to-date and commercially available and new rootstock hybrids being developed for use are now being screened against selected phylloxera genotypes. A systematic screening process therefore commenced in a previously funded GWRDC project Sustainable Phylloxera Management Phase 1 (DNR 03/03) using three phylloxera genotypes (G1, G4 and G20) and has continued in this project with an additional 3 phylloxera strains (G7, G19, and G30) and additional rootstocks.

Methods

Plant and insect material

Lignified roots of all rootstocks and controls, either *V. vinifera* cv. Sultana or *V. vinifera* cv. Shiraz source material, depending on availability and root quality, were collected from a field collection maintained at CSIRO Merbein located in the Western Phylloxera Exclusion Zone (PEZ) of Victoria.

Six phylloxera genotypes designated, G1, G4, G7, G19, G20 and G30 were used in the trials all collected from single source commercial vineyards in the Nagambie (G1), King Valley (G4) and North East (G7, G19, G20 and G30) Victorian region respectively. Prior to each experiment, populations of each genotype were maintained separately on excised *V. vinifera* cv. Shiraz or cv. Sultana roots (Kingston et al., 2007) in a dark controlled environment growth room (25±2°C) at the DPI-Rutherglen Centre under quarantine conditions. The phylloxera lines were genotyped both prior to trial commencement (to ensure single phylloxera strains were utilised) and after cessation of the trial (to ensure no cross-contamination of genotypes had arisen during the course of the trial) as described in Powell (2006).

Excised root bioassay procedure

All bioassays were conducted on excised grapevine root pieces based on a modified method of Kingston et al. (2007). Treatments (i.e. rootstocks) and controls (i.e. *Vitis vinifera*) were replicated 7-10 times depending on availability of source root material. Two root pieces from the same rootstock or control were placed in a 140 x 25mm Petri-dish lined with filter paper. Twenty, two-to-five day old eggs of known phylloxera strains from pure stock cultures, of single sourced populations, were placed on each root piece. The Petri-dish edges were sealed with Vitifilm™ to avoid cross contamination of genotypes and stored in the dark in plastic containers at 23^{o}C ± 2^{o}C. After the phylloxera eggs had been introduced to the root pieces, the rate of phylloxera development was assessed weekly by counting of individual life-stages using a lower power binocular microscope. For each phylloxera strain initial

infestation and screening occurred on separate consecutive days to prevent cross-contamination. All bioassays carried out in during the project period are reported here. A total of five new rootstock hybrids, supplied by CSIRO Merbein were also screened.

Statistical Analysis

For these experiments the total number of each phylloxera life-stage surviving up to eight weeks was used for statistical purposes. Statistical analyses were conducted using GenStat 10 for Windows software (Payne et al., 2007). Life-stage data were analysed using non-parametric methods at each week of the trial. A Mann-Whitney U (Wilcoxon rank-sum) paired test was used to compare the results between any two rootstocks and genotypes.

Results

Bioassay One

In Bioassay one, which was conducted over a eight-week period, the performance of the phylloxera strains G7, G19 and G30 were compared on four rootstocks: 1103 Paulsen, 110 Richter, Börner and *V. vinifera* cv. Sultana (Figs. 13-18).

Vitis vinifera

All three genotypes G7, G19 and G30 developed to adulthood and produced eggs on *V. vinifera* (Figs. 13-18) The level of egg production did however differ between genotypes with higher egg production recorded from G30 than either G7 or G19.

1103 Paulsen

On the rootstock 1103 Paulsen G7 phylloxera did not develop beyond the crawler stage indicating a highly resistant interaction (Fig. 13) and therefore could not develop to the adult stage to produce eggs (Fig. 14). In contrast G19 developed to the adult stage on 1103 Paulsen (Fig. 15) and produced relatively high egg numbers per adult when compared with

the *V. vinifera* control (Figure 16). G30 phylloxera was not tested against this rootstock as this has been reported previously (Powell, 2003).

110 Richter [*V. berlandieri* x *V. rupestris*]

On 110 Richter all three genotypes G7, G19 and G30 developed to adulthood (Figs. 13, 15 &17) and produced eggs (Figs. 14, 16 & 18) indicating a tolerant reaction. G7 and G30 produced more eggs per adult than on *V. vinifera*.

Börner [*V. riparia* x *V. cinerea*],

On the rootstock Börner, which is reputedly the only 'truly' phylloxera resistant rootstock (Blank et al., 2009) G19 phylloxera did not develop beyond the crawler stage (Fig. 15) and indicating a highly resistant response. However, G7 and G30 phylloxera developed beyond the crawler stage on this rootstock (Figs. 13 & 17) and produced eggs (Figs. 14 & 18) at similar levels to *V. Vinifera.* This is the first reported incidence of phylloxera being able to develop on the rootstock Börner under controlled conditions.

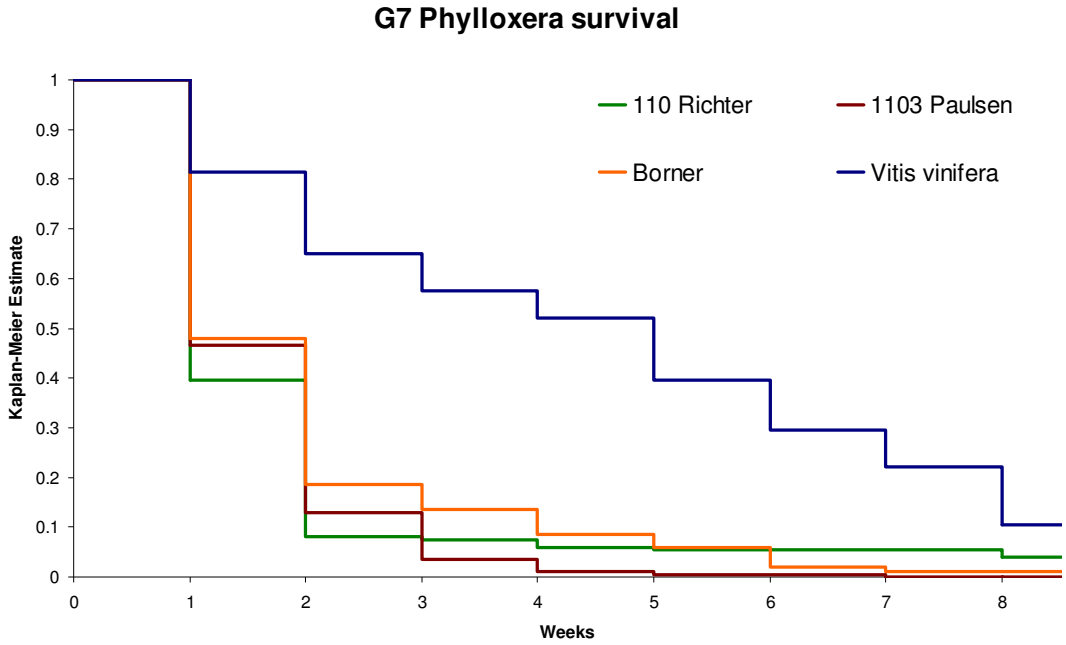


Fig. 13. Comparison of phylloxera strain G7 survival on rootstocks 110 Richter, Börner, 1103 Paulsen and *V. vinifera* cv. Sultana

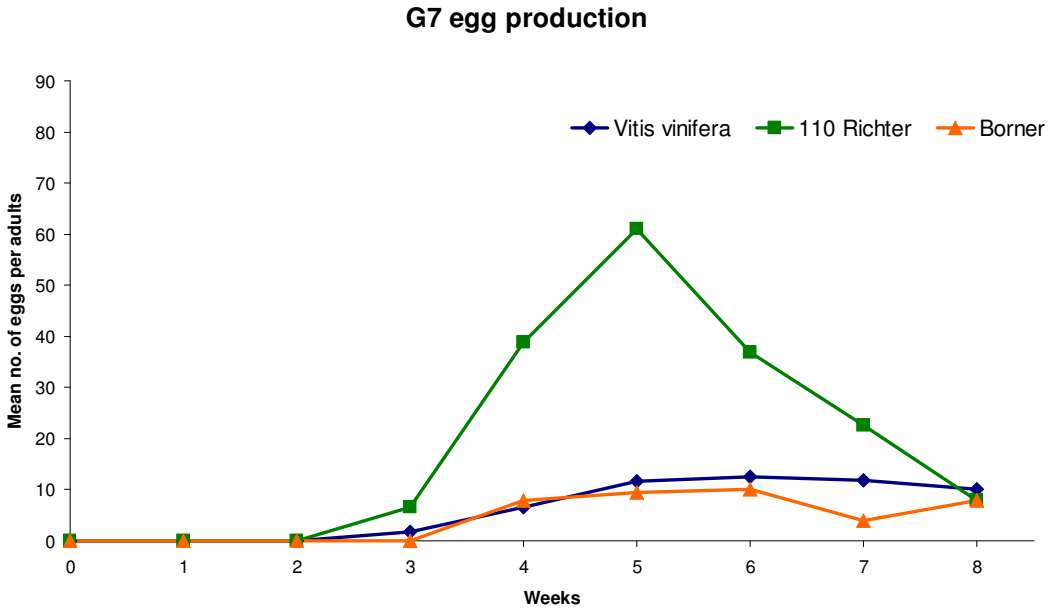


Fig. 14. Comparison of phylloxera strain G7 egg production on rootstocks 110 Richter, Börner, 1103 Paulsen* and *V. vinifera* cv. Sultana where * signifies zero egg production on 1103 Paulsen

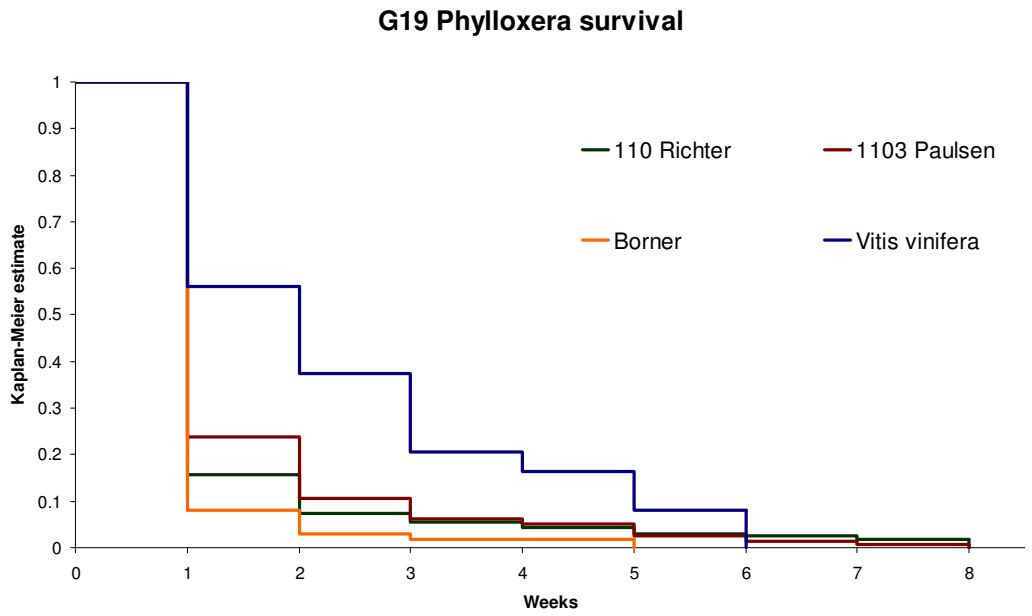


Fig. 15. Comparison of phylloxera strain G19 survival on rootstocks 110 Richter, Börner, 1103 Paulsen and *V. vinifera* cv. Sultana

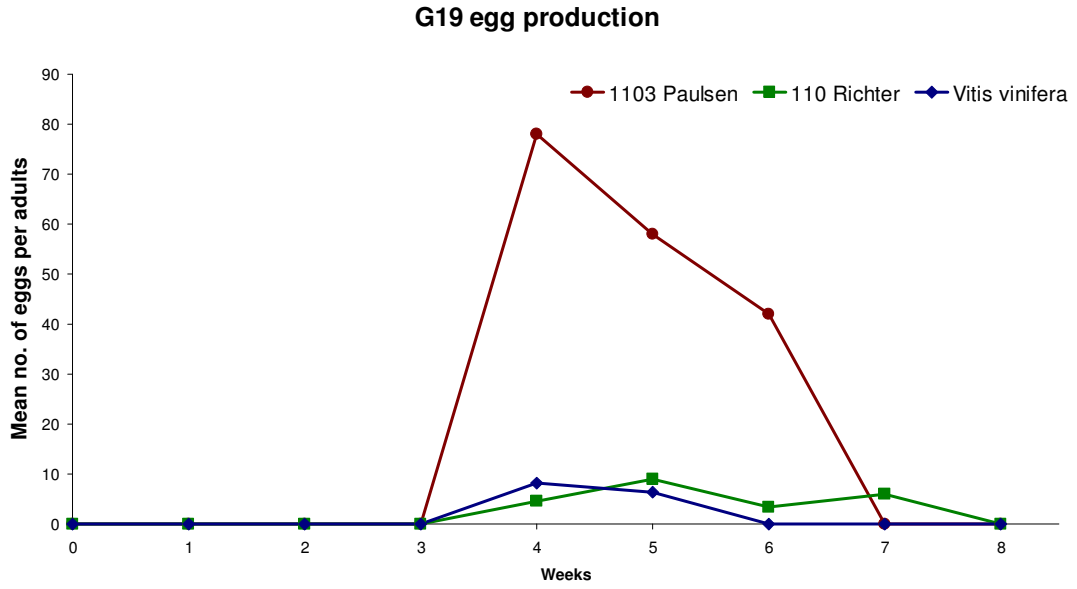


Fig. 16. Comparison of phylloxera strain G19 egg production on rootstocks 110 Richter, Börner*, 1103 Paulsen and *V. vinifera* cv. Sultana, where * signifies zero egg production on Börner

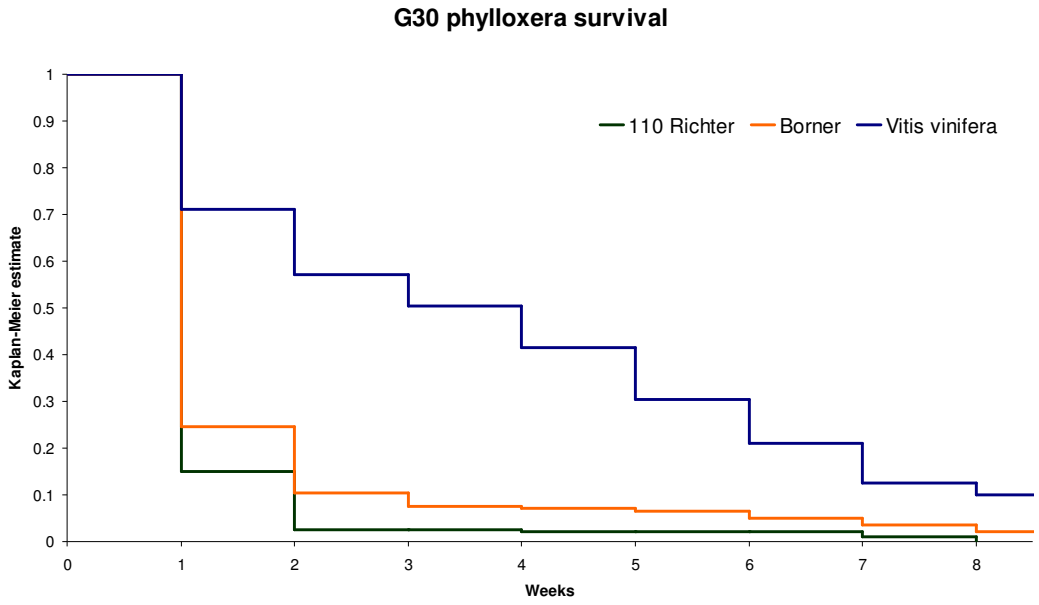


Fig. 17. Comparison of phylloxera strain G30 survival on rootstocks 110 Richter, Börner and *V. vinifera* cv. Sultana

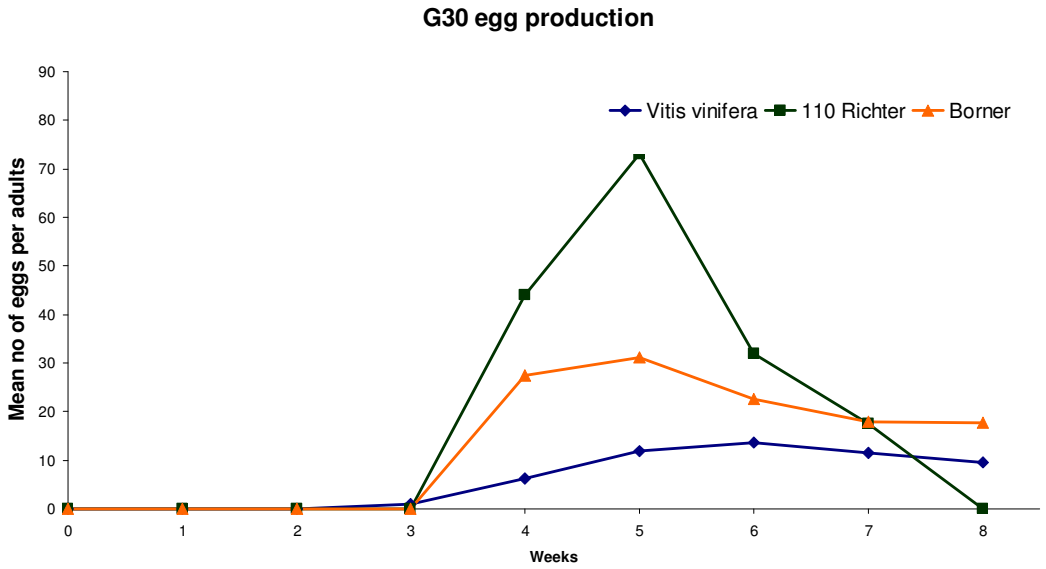


Fig. 18. Comparison of phylloxera strain G30 egg production on rootstocks 110 Richter, Börner and *V. vinifera* cv. Sultana

Bioassay Two

In Bioassay two, which was conducted over a eight-week period, the performance of the phylloxera strain G20 was assessed on four rootstocks, Schwarzmann, 1103 Paulsen, 140 Ruggeri and *V. vinifera* cv. Sultana (Figs. 19-20). On rootstocks 1103 Paulsen and 140 Ruggeri G20 performed poorly and did not develop beyond the crawler stage (Fig. 19) indicating a highly resistant response. However, G20 phylloxera developed beyond the crawler stage on Schwarzmann and produced eggs (Fig. 20).

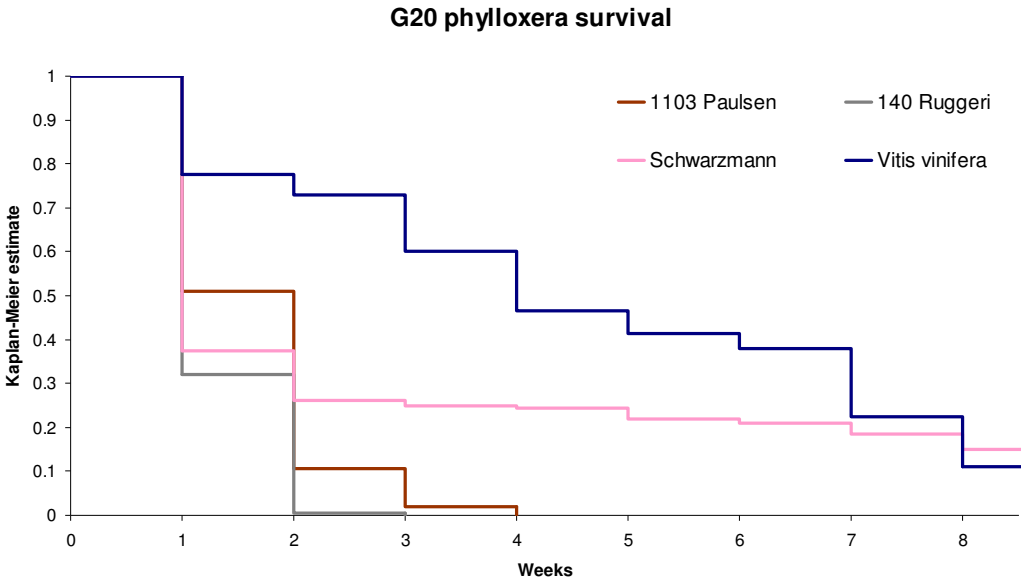


Fig. 19. Excised bioassay two: comparison of phylloxera strain G20 survival on rootstocks Schwarzmann, 140 Ruggeri, 1103 Paulsen and *V. vinifera* cv. Sultana

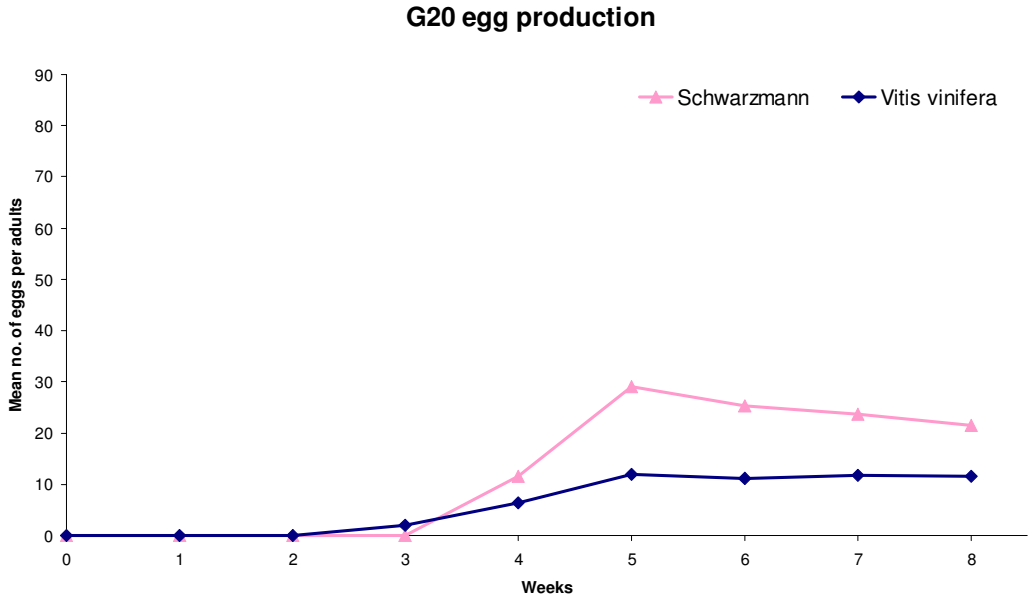


Fig. 20. Excised bioassay two: comparison of phylloxera strain G20 egg production on rootstocks Schwarzmann, 140 Ruggeri*, 1103 Paulsen* and *V. vinifera* cv. Sultana, where * signifies zero egg production on 1103 Paulsen or 140 Ruggeri

Bioassay Three

In bioassay three, DPI-Rutherglen, in collaboration with CSIRO Merbein, conducted laboratory screening of five hybrid rootstocks, MG54-83, MG60-61, MG60-113, MR 6-31 and MR8-23, for resistance to two clonal lineages of phylloxera G1 and G4. All plant root material was sourced from CSIRO Merbein and was assumed to be true type and was not DNA typed prior to screening. The results when comparing the rootstocks with *Vitis vinifera* cv. Shiraz indicated a high level resistance to phylloxera genotypes G1 and G4, the two most geographically widespread genotypes known to occur in Australia.

The two phylloxera genotypes, G1 and G4, used in this trial were sourced from single source vineyards in the Yarra and King Valleys' respectively and DNA typed pre- and post-trial. Populations of each genotype were maintained on *Vitis vinifera* cv. Shiraz root pieces under controlled growth room conditions (23°C ± 2°C).

Phylloxera development and survival for both genotypes tested was relatively poor on rootstocks MG 60-113 and MG 60-61; neither G1 nor G4 phylloxera developed beyond first instar (crawler) life-stage (Figs. 21 & 22) indicating a high level of phylloxera resistance. G1 phylloxera crawlers survived on MG60-113 for up to four weeks and on MG60-61 for five weeks (Fig. 21). On the same two rootstocks G4 crawlers survived for five and four weeks respectively (Fig. 22). Both phylloxera genotypes on MG54-83 developed to adulthood although in significantly lower ($P<0.05$) numbers than on *V. vinifera* cv. Shiraz and lived on past the length of the trial (Figs. 21 & 22). G1 phylloxera development on rootstock MG54-83 was significantly different ($P<0.05$) to that on MG60-113 for the first four weeks of the trial. From the second to the third week of the trial there was a significant difference ($P<0.05$) in G1 phylloxera numbers on MG60-61 and MG54-83. For the eight-week trial period the number of G4 phylloxera which developed on *V. vinifera* cv. Shiraz was significantly higher ($P<0.05$) than that on all the rootstocks tested. There was no significant difference ($P>0.05$) in G4 phylloxera numbers between any of the three rootstocks tested.

Neither of the two phylloxera genotypes tested developed to adulthood on rootstocks MG60-113 and MG60-61, therefore no eggs were produced on these two rootstocks (Figs. 23 & 24).

On the control, *V. vinifera* cv. Shiraz, both genotypes started producing eggs by week three and reached peak egg numbers by week five. On rootstock MG54-83 G1 phylloxera started producing eggs in week six (Fig. 23) an indication of delayed development compared to the control and the egg number (relative fecundity) was significantly lower than on the control. G4 phylloxera followed a similar trend on MG54-83 rootstock with total egg numbers being significantly lower than on the control roots however, this genotype produced eggs earlier in the trial, in week four (Fig. 24). When M8-23 and M6-31 are compared (Figs. 25-28) survival of both G1 and G4 was relatively poor compared to V. Vinifera. Egg production occurred for both genotypes on *V. vinifera* but only for G4 on M8-23.

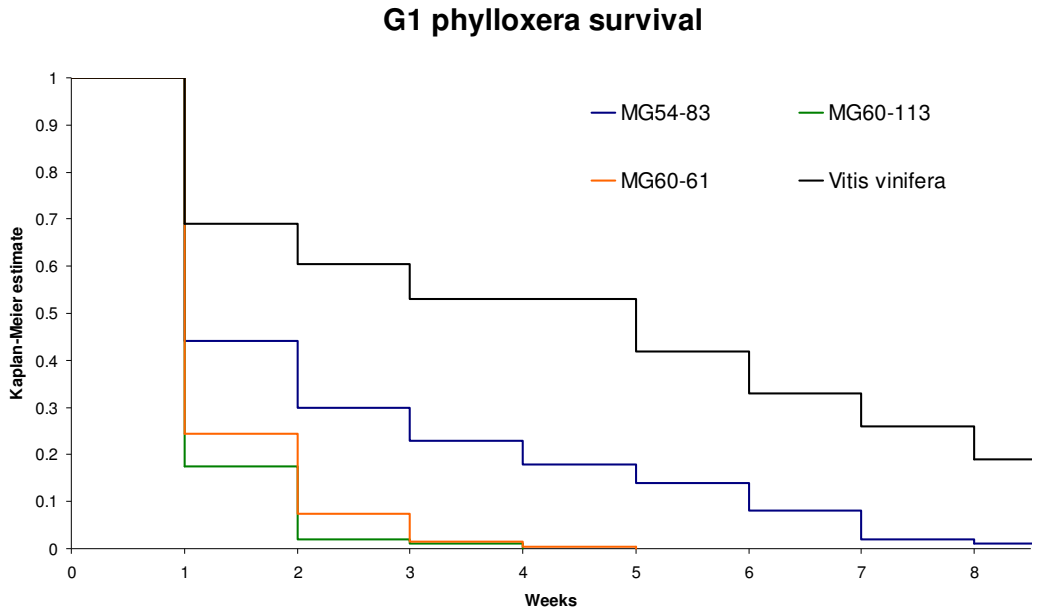


Fig. 21. Phylloxera genotype G1 survival on rootstocks MG54-83, MG60-113, MG60-61 and *Vitis vinifera* cv. Shiraz

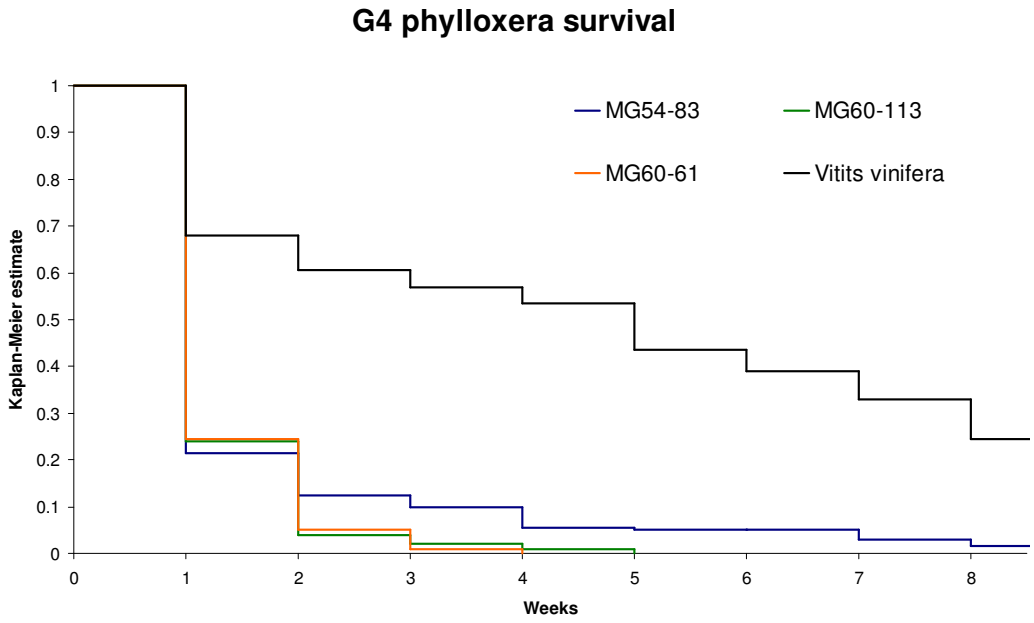


Fig. 22. Phylloxera genotype G4 survival on rootstocks MG54-83, MG60-113, MG60-61 and *Vitis vinifera* cv. Shiraz

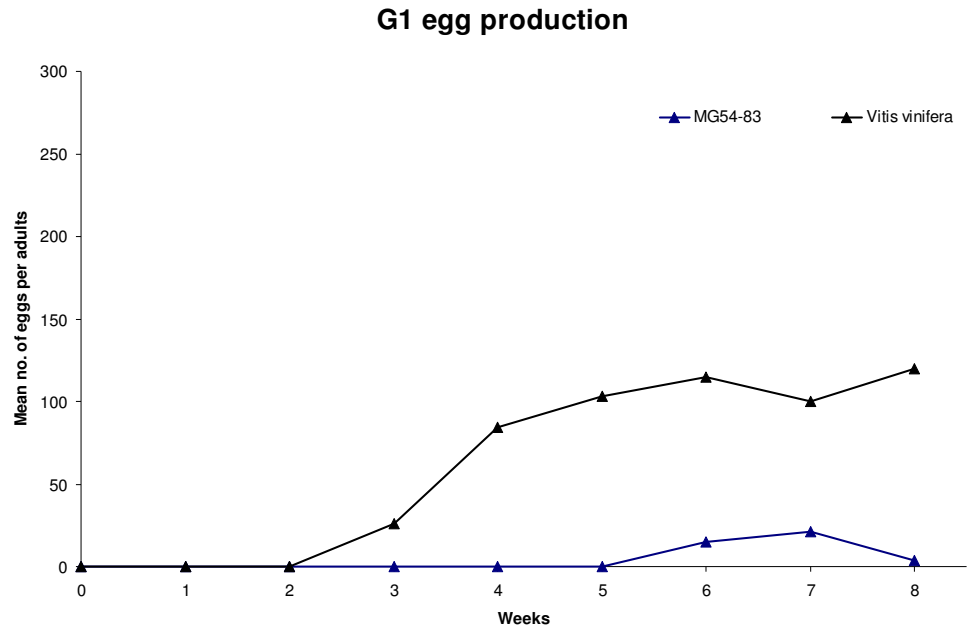


Fig. 23. Phylloxera genotype G1 egg production on rootstocks MG54-83 and *Vitis vinifera* cv. Shiraz

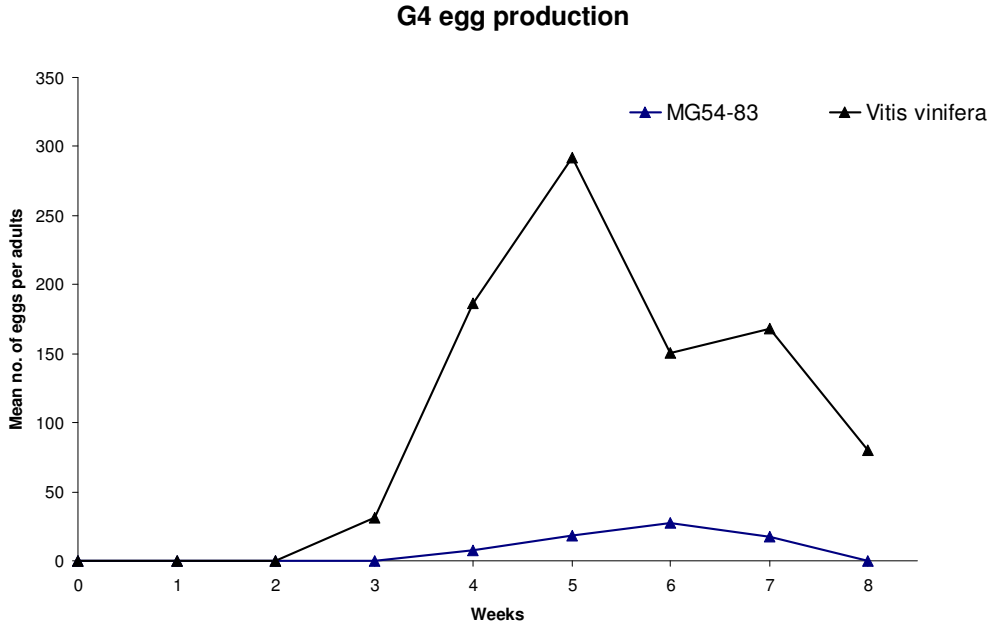


Fig. 24. Phylloxera genotype G4 egg production on rootstocks MG54-83 and *Vitis vinifera* cv. Shiraz

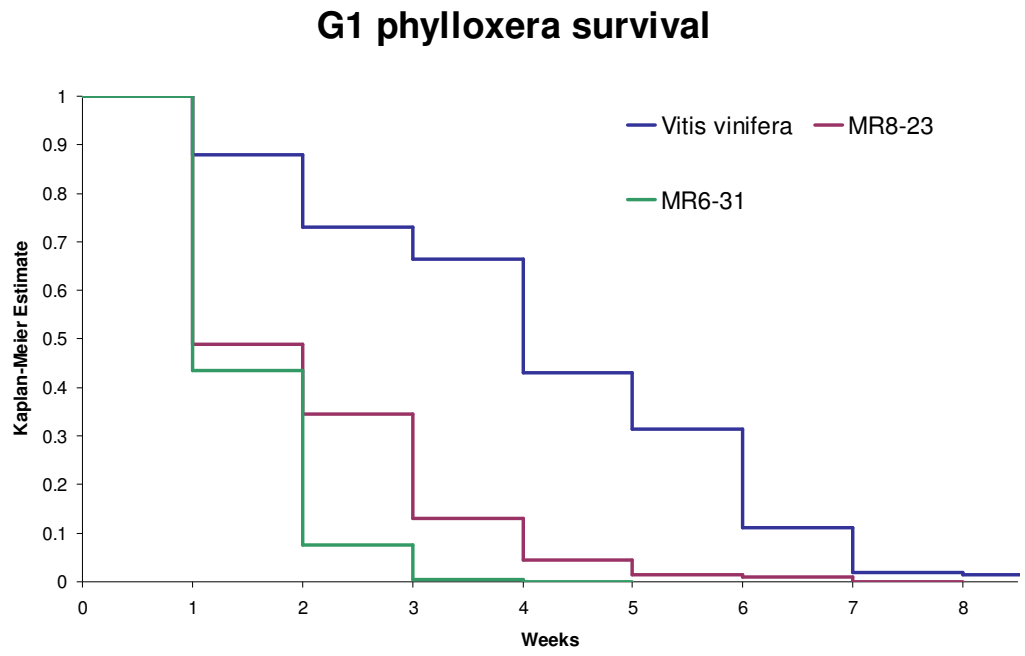


Fig. 25. Phylloxera genotype G1 survival on rootstocks MR8-23, MR6-31 and *Vitis vinifera* cv. Shiraz

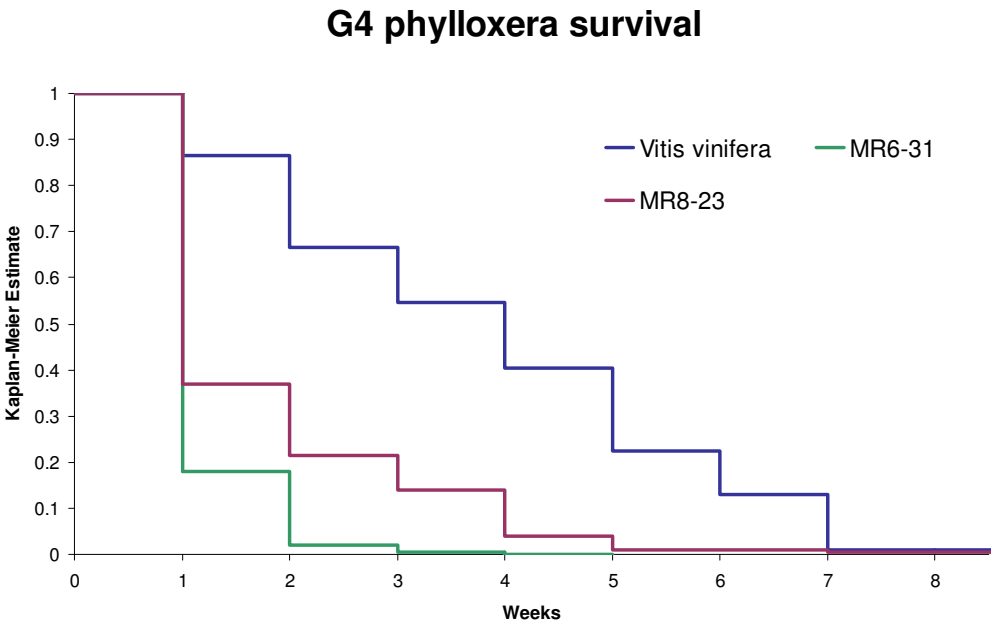


Fig. 26. Phylloxera genotype G4 survival on rootstocks MR8-23, MR6-31 and *Vitis vinifera* cv. Shiraz

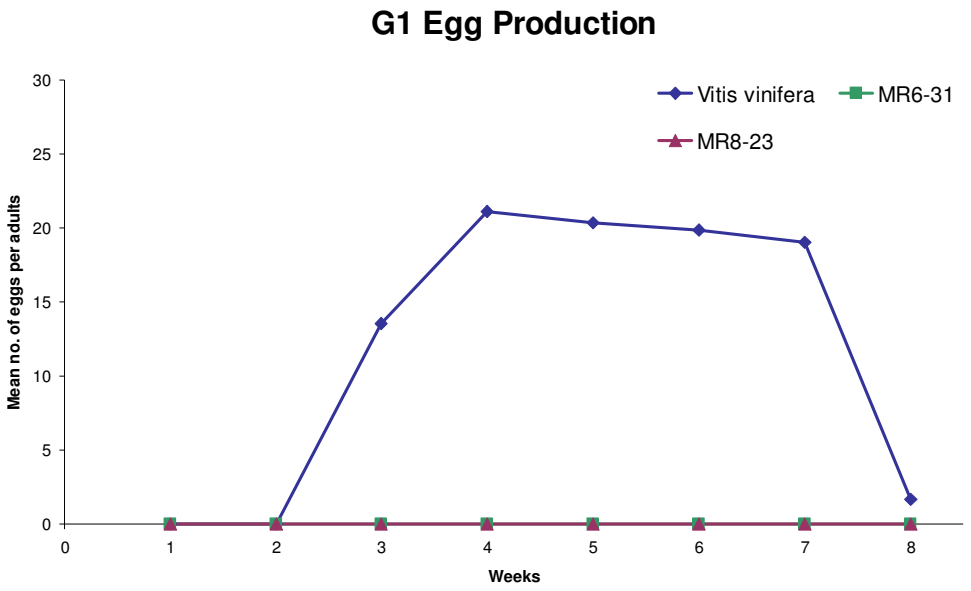


Fig. 27. Phylloxera genotype G1 egg production on rootstocks MR8-23, MR6-31 and *Vitis vinifera* cv. Shiraz

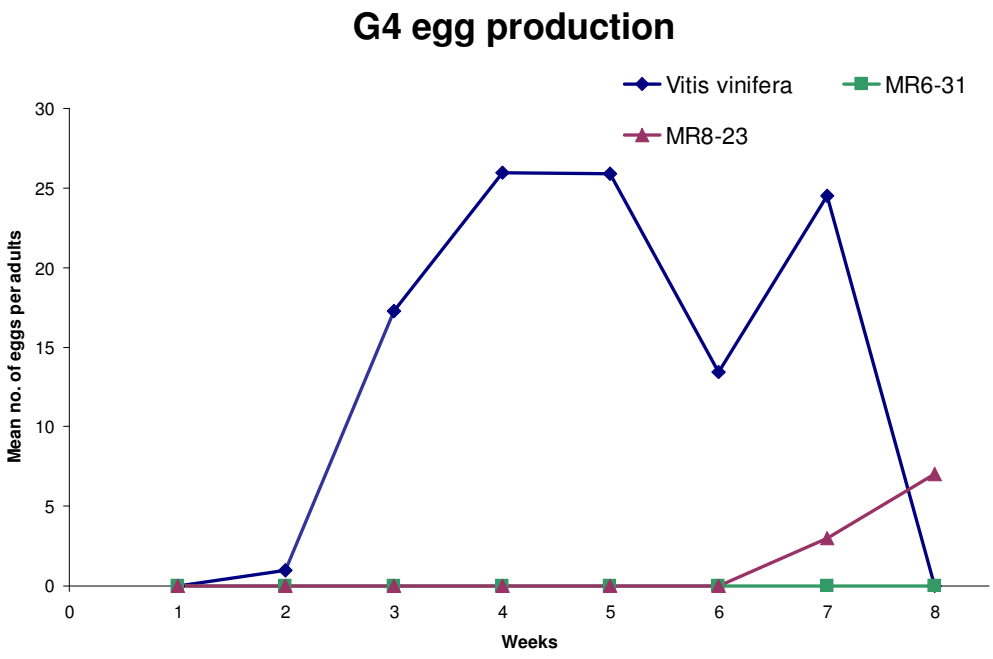


Fig. 28. Phylloxera genotype G4 egg production on rootstocks MR8-23, MR6-31 and *Vitis vinifera* cv. Shiraz

Discussion

There are in Australia at least 83 known phylloxera genotypes of which six clonal lineages have been recommended for testing. During the course of this project and the previous related project we have gone some way to screening them against a wide range of rootstocks with different *Vitis* parentage and scored preliminary resistance ratings albeit limited to *in vitro* screening (Table 2). Recent studies have shown that caution is advised when interpreting results from excised root bioassays as this type of screening protocol (i) excludes a whole plant reaction (ii) is reliant on obtaining healthy root tissue for initial screening (iii) is reliant on timing of collection of root material (i.e. vine physiology at the time of collection may influence insect interactions) and (iv) does not consider the impact that other abiotic and biotic variables may have on the insect-host plant interactions. Therefore further screening studies are advisable under glasshouse (Chapter 3) and field conditions (Project DPI 08/01) to ensure rootstock recommendations are resilient in the long-term.

When five newly bred hybrids were tested they showed good levels of resistance to G1 and G4 phylloxera. However it would be advisable, prior to commercial release, to screen the rootstocks MG60-113, MG60-61, MG54-83, MR8-23 and MR6-31 and any further new hybrids against all six recommended phylloxera clonal lineages.

Most previous studies have focused on the phylloxera genotypes G1 and G4 as these are the two most widely distributed in phylloxera infested regions of Australia (Powell, 2006). We have here presented data on not only the same phylloxera genotypes but also an additional 4 genotypes.

All phylloxera strains tested showed relatively lower levels of performance (in terms of survival on all tested rootstocks compared to *V. vinifera*. However, even on *V. vinifera* clonal performance differed significantly. The G7, G19, G20 and G30 strains showed lower levels of development and egg fecundity in comparison with G1 and G4. This has important implications for our ability to detect phylloxera on ungrafted *Vitis vinifera* in the field as vine decline could take significantly longer to be expressed under lower phylloxera population

pressure. In addition the timing of replanting of a vineyard to resistant rootstocks would be delayed when genotypes of lower virulence are present. Although all genotypes tested here showed relatively poor performance on rootstock hybrids there were important differences between their development levels and ability to produce eggs and hence maintain populations on different commercially available rootstocks signifies this would impact on detection efficiency in grafted vineyards and would also influence the relative risk of transfer.

Recommendations

On some rootstocks although phylloxera developed beyond the crawler stage the population abundance was generally lower than on *V. vinifera.* Some strains nevertheless developed to the adult stage and began producing eggs on lignified roots of hybrids. This is not necessarily an indication that rootstock breakdown will occur but it does show that the risk of transfer is higher when this happens. For example three rootstocks on which eggs were produced included 1103 Paulsen (eggs produced by G19) and 110 Richter (G7, G19, and G30), Börner (G7, G30) and Schwarzmann (G20). Based on these observations careful selection of rootstocks to minimise quarantine risks would be advisable when the genetic strain of phylloxera in the infested zones have been characterised.

However caution is advised when interpreting laboratory results as although the excised root system is rapid and produces result within a relatively short time period (i.e. weeks compared to years in field trials). The *in vitro* system is not ideal when wanting to examine phylloxera-host-plant interactions, but it is useful for preliminary comparative screening of a range of rootstocks. It also allows weekly observations of phylloxera development. A whole plant system under controlled conditions is physiologically closer to field conditions and other factors can be modified such as nutrition, water and soil conditions which cannot be altered in the excised root system. A rapid screening procedure in a glasshouse system is recommended as the next phase in rootstock screening as they are *in planta* tests closer to the field

conditions than the in vitro test using the excised root bioassay system. This is discussed further in Chapter 3.

Table 2. Provisional rankings of rootstock resistance, tolerance and susceptibility based solely on tier one *in vitro* excised root screening under controlled conditions.

Genotype/Rootstock	G1	G4	G7	G19	G20	G30
Vitis vinifera	✓S	✓S	✓S	✓S	✓S	✓S
Ramsey	✓T	✓T	✓T	✓T	✓T	✓T
Schwarzmann	✓R	✓R	✓T	✓T	✓T	✓T
Börner	✓R	✓R	✓T	✓R	✓R	✓T
110 Richter	✓T	✓T	✓T	✓T	✓T	✓T
1103 Paulsen	✓T	✓T	✓R	✓T	✓R	✓R
140 Ruggeri	✓T	✓T	✓R	✓R	✓R	✓R
5BB Kober	✓R	✓R	✓T	✓T	✓T	✓T

* where ✓=- tested, x = not yet tested, S= susceptible, T = tolerant and R= resistant

CHAPTER 3

IN PLANTA SCREENING FOR PHYLLOXERA RESISTANCE

Objective: Information on phylloxera clones on selected rootstocks – through glasshouse screening

Summary

Using the glasshouse rootstock screening protocol developed in project DNR 03/3 (Powell, 2006) for co-cultivation of phylloxera genetic strains with potted grapevines an assessment of phylloxera development and root response under glasshouse conditions was made using five selected grape phylloxera genotypes. Using this protocol phylloxera genotypes were contained in root enclosures which allowed quantification of individual life-stages. The protocol allowed screening of rootstocks to individual phylloxera genotypes by preventing cross contamination and allowing more detailed quantification of phylloxera development and mature root response. For assessing phylloxera resistance/tolerance, results enabled a comparison of five phylloxera genotypes against up to seven rootstocks. *Vitis vinifera* cv. Shiraz grapevines, grafted to seven rootstocks of American *Vitis* parentage, which are widely used in Australia, were screened for resistance to up to five phylloxera genetic strains known to occur in Australia. As also observed *in vitro* bioassays (Chapter 2) grape phylloxera genotypes differed in egg production and population development on both *Vitis vinifera* and rootstock hybrids and in addition root damage levels differed between phylloxera genotypes and rootstocks.

All tested strains of phylloxera developed on *V. vinifera* vines, as shown by population development and necrotic tuberosity development. On *V. vinifera* relatively low phylloxera levels were detected when examining genotypes G7, G19 and G30 when compared with

previous data using G1, G4 and G20 populations (Powell et al., 2006). On rootstocks however, G7, G19 and G30 phylloxera performance was relatively poor on some rootstocks particularly Börner, 140 Ruggeri and 110 Richter where insects did not survive the ten-week trial period. G7, G19 and G30 phylloxera development was measured on mature lignified roots and Ramsey, Schwarzmann and 5BB Kober tolerated phylloxera establishment, allowing development to adulthood and reproduction of all three genotypes tested, and at similar levels compared to *V. vinifera* roots but less damage to the grapevine root system. G1 and G4 was tested against 1103 Paulsen and 140 Ruggeri. Whilst 1103 Paulsen tolerated both phylloxera genotypes. 140 Ruggeri only tolerated G4 and not G1. However on these two rootstocks G1 and G4 abundance was significantly lower than on *V. vinifera* and root damage was very low in comparison.

Introduction

Currently there are only two recommended long-term options for the management of grape phylloxera in Australia; use of resistant rootstocks and quarantine (Powell, 2008). The use of resistant rootstocks can enable vines to be productive in phylloxera infested regions but if rootstocks can sustain even low populations of phylloxera they still pose a significant quarantine risk. Selection of rootstocks based on their interaction with known phylloxera genotypes may minimise this risk. For example if some genotypes cannot feed, establish and develop on some rootstocks at all then the risk is negligible. Whereas if some genotypes establish and are as abundant on rootstocks as *V. vinifera* then the quarantine risk of transfer is high.

There are 83 known genetic strains of grape phylloxera that have been identified to-date in Australia (Umina et al., 2007). In the North East Victorian PIZ all 83 strains are present, whereas only single phylloxera genetic strains have been identified in all other Victorian PIZs. Six clonal lineages have been recommended for screening against commonly used rootstocks (Hoffman, 2003). Testing of these six clonal lineages is a pre-requisite to ensure sustainable rootstock recommendations for the Australian viticulture industry and systematic

glasshouse screening initially commenced using G1, G4 and G20 (Powell et al. 2006). Screening on the remaining three selected phylloxera genotypes is a component of this project.

A preliminary laboratory screening, using excised roots (Chapter 2), is the first tier in screening for phylloxera resistance or tolerance being a relatively rapid simple procedure but results may be variable due to root quality degradation of the trial period which may potential influence phylloxera development. Granett et al (2001) has shown that when comparing phylloxera population levels on excised and attached grapevine roots, excised roots support a higher phylloxera survival than attached roots. This suggests that excised root bioassays can lead to overestimates of phylloxera virulence and hence under estimates of rootstock tolerance in the whole plant situation. Glasshouse screening is the second-tier screening phase offering a whole plant response and an assessment of root damage albeit under controlled environmental conditions.

For this study we tested the performance of five phylloxera genotypes (G1, G4, G7, G19 and G30). The two most common and widespread genotypes in Australia are G1 and G4 (Corrie at al. 2002) whilst G7, G19 and G30 have been detected in the North east PIZ on V. Vinifera and some rootstocks. The rootstocks tested were Ramsey [*V. candicans* x *V. rupestris*], Schwarzmann [*V. riparia* x *V. rupestris*], 110 Richter [*V. berlandieri* x *V. rupestris*], 140 Ruggeri [*V. berlandieri x V. rupestris*], 1103 Paulsen [*V. berlandieri x V. rupestris*], 5BB Kober [*V. berlandieri* x *V. riparia*], Börner [*V. riparia* x *V. cinerea*], with self-grafted *V. vinifera* as a control. Phylloxera population abundance and development and tuberosity (lignified root swelling) formation on grapevine roots were quantified ten weeks post-infestation.

Methods

Plant material and growth conditions

Two trials were conducted using one-year old grapevine rootlings which were sourced from within the Western Phylloxera Exclusion Zone (PEZ), from Boulevard

Nurseries in Mildura Victoria in December 2006. The rootlings included seven treatment bench grafted rootstocks: Schwarzmann (clone R1A2S10), Ramsey (clone A11V2 NW130), 140 Ruggeri (clone Q45-3A), 5BB Kober (clone R1A2S10), Börner (clone EX VAM), 110 Richter (clone A5V19), and 1103 Paulsen (200HT EX VAM). The scion in each case was Shiraz (clone PT23), including the self-grafted control to exclude grafting as a confounding factor.

The rootlings were potted at the Department of Primary Industries - Rutherglen Centre into 20cm diameter pots with potting media (80% sterile potting mix: 20% Perlite) then kept in a shadehouse, under drip irrigation, to enable good root development before transferring them to a controlled-temperature glasshouse six months prior to treatment application (i.e. phylloxera inoculation).

Insect material

Five phylloxera genotypes, G1, G4, G7, G19 and G30, were used. Each genotype was single sourced from roots of ungrafted *V. vinifera* vines from commercial vineyards in PIZ's in North East Victoria, Australia. Phylloxera strains from a single source were maintained on *V. vinifera* cv. Shiraz excised root pieces under controlled conditions in growth cabinets (25±3°C; 12 hours L:D) using a modified method after Granett et al. (1987). Prior to commencing the experiment and at trial completion phylloxera were genotyped using microsatellite markers (see Powell et al., 2006).

Experimental design

A randomised complete plot design was established to examine the interaction between phylloxera genotype and rootstock, using a factorial design. Were factors were infestation type (phylloxera genotype and a non-infested control) and rootstock type). Eight replicates were used per treatment in a block design to account for any variations in the environmental conditions. Each phylloxera genotype was set up on consecutive days to prevent cross-contamination of the genotypes during infestation.

Phylloxera infestation

For all glasshouse trials infestation of rootstocks with phylloxera strains occurred on separate consecutive days to prevent cross-contamination. To infest, each vine was taken out of its pot and the roots were cleaned of potting mix to locate a single, lignified root piece. Twenty phylloxera eggs were placed on moistened filter paper strips. The roots of the vines were exposed, a root piece was selected (2.75±1.15cm diameter) and the filter paper was wrapped around the root in a tube formation. A small amount of sterile soil mixture (80% potting mix, 20% Perlite) was enclosed around the root and tube, in a mesh rectangle (8x10cm; 60µm pore) to form a "phylloxera enclosure" (Powell, 2003). The enclosure was secured at both ends and sealed with plastic cable ties and Tanglefoot™ to prevent phylloxera escaping to the rest of the root system. The vine was placed in a sterile 20cm diameter pot with and addition of fresh sterile soil mixture added where required (80% potting mix, 20% Perlite) and enclosed in a mesh bag (60µm pore size) sealed with Tanglefoot™ and cable ties to prevent cross-contamination of genotypes between vines. Two to four vines were infested at a time, in the laboratory, to minimise stress to the vines.

Post infestation growth conditions

At commencement of both trials the vines were fertilised with 3.5g Osmocote™ and 500ml Thrive™ (8g of Thrive™ mixed in 4.5L water) per potted vine. Each vine was drip irrigated for two minutes daily. To prevent the vines from going to dormancy, artificial growth lights were turned on each day - 6am-8pm and 1am-2am - during the trial. Gemini Tinytag Ultra™ dataloggers monitored the temperature and relative humidity in the glasshouse. The temperature settings were minimum 18°C and maximum 26°C 8am-9pm, min 18°C max 22°C 9pm-8am. Relative humidity was maintained at 50% using a Flipperdry 400 de-humidifier.

Experimental harvest

After ten weeks, sequential harvesting took place based on period since infestation. Vines were removed from their pots and the potting mix was carefully removed from the roots to locate the root enclosure. The root system was visually checked for phylloxera and nodosities to determine if any phylloxera had 'escaped' form the enclosure. The enclosure was removed by cutting the encapsulated root at either end of the mesh. The enclosure was opened, rinsed with water and all material was captured in a 60µm sieve. The infested root piece was gently scrubbed with a fine toothbrush to remove any remaining phylloxera. The washed material was then collected in tap water in a plastic vial and examined under a dissecting microscope. All phylloxera life-stages were recorded and the excised root was examined for the presence of nodosities (galls on non-lignified roots), tuberosities (swellings on lignified roots) and phylloxera life-stages.

Statistical analyses

For these experiments the total number of phylloxera life-stages surviving after ten weeks and the root damage at this date was used for statistical purposes. Analyses were conducted using GenStat 10 for Windows software (Payne et al., 2007). Since the data was not normally distributed life-stage and tuberosity data were analysed using non-parametric methods. Kruskal-Wallis one-way analysis of variance was used to determine the difference in rootstock resistance to each phylloxera genotype. When Kruskal-Wallis test results showed differences between the rootstocks or the genotypes then a Mann-Whitney U (Wilcoxon rank-sum) paired test was used to compare the results between any two rootstocks.

Results

Trial 1- Phylloxera performance on Vitis vinifera and grafted rootstocks

Phylloxera abundance

The mean total number of phylloxera present after ten weeks for each phylloxera clone tested on rootstocks for glasshouse trial one (Fig. 29). Clear differences between the performance of the three phylloxera clones G7, G19 and G30 were observed. All three genetic strains developed on Ramsey, Schwarzmann and 5BB Kober with G30 having marginally higher abundance on all three rootstocks when compared with G7 and G19. Interestingly abundance of all three genotypes on *V. vinifera* was not significantly higher ($P>0.05$) than on the grafted rootstocks Ramsey, Schwarzmann and 5BB Kober. After 10 weeks G7, G19 and G30 did not survive on Börner or 140 Ruggeri.

Grapevine root response to phylloxera genotype

Tuberosities were significantly ($P<0.05$) more abundant on *V. vinifera* than on all rootstocks tested (Fig. 30) for all phylloxera genetic strains tested with the exception of G7 ($P>0.001$) on Ramsey ($P=0.267$). Indicating generally more root damage to the growing susceptible vine, which would ultimately impact on vine performance over time. Tuberosities were significantly more abundant on G30 ($P<0.001$) infested *V. vinifera* roots than either G7 or G19 infested *V. vinifera* roots. Necrotic tuberosities where observed for all phylloxera genotypes on *V. vinifera.* No necrotic tuberosities where observed on any grafted rootstock but slightly however raised dome shaped tuberosities were observed on some rootstock genotypes combinations particularly Ramsey. All three phylloxera genotypes were able to initiate dome-shaped tuberosities on Ramsey as also described in Powell (2006). But similar root reaction on Schwarzmann and 5BB Kober was dependant on phylloxera genetic strain. No tuberosities were evident on Börner or 140 Ruggeri irrespective of phylloxera clonal lineage treatment.

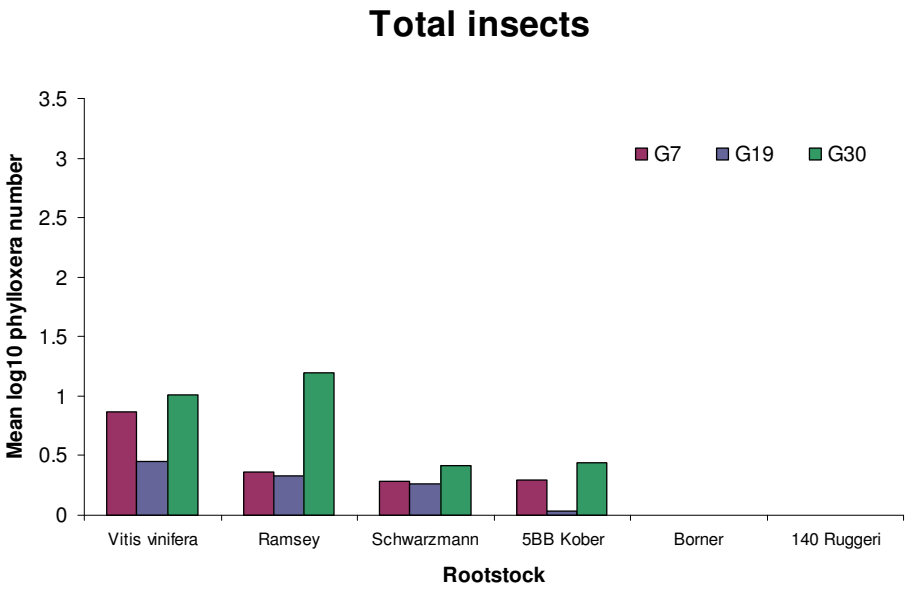


Fig. 29. Total abundance of three phylloxera genetic strains, G7, G19 and G30, after ten weeks glasshouse incubation on *V. vinifera* cv. Shiraz, Ramsey, Schwarzmann, 5BB Kober, Börner and 140 Ruggeri

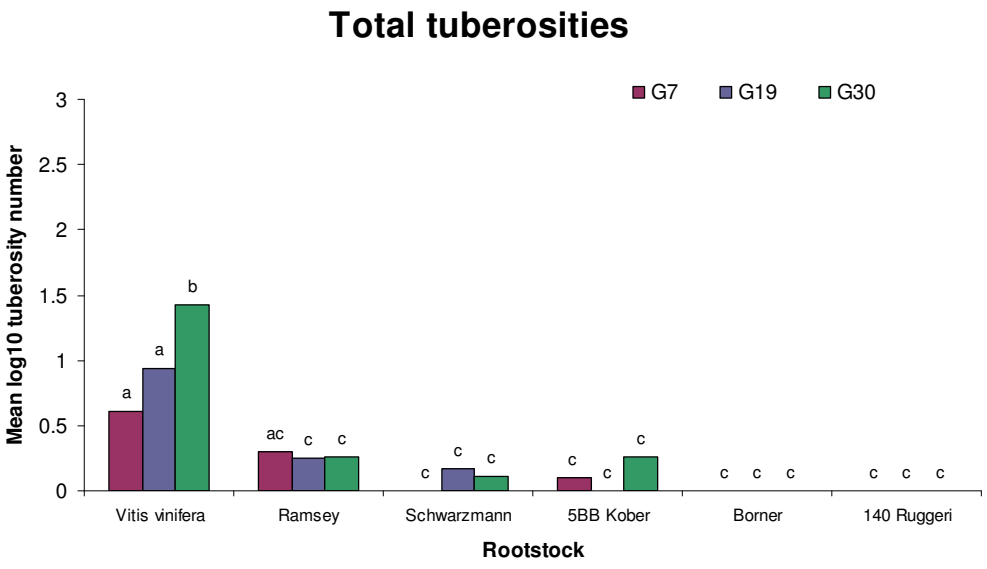


Fig. 30. Total abundance of three phylloxera genetic strains, G7, G19 and G30, after ten weeks glasshouse incubation on *V. vinifera* cv. Shiraz, Ramsey, Schwarzmann, 5BB Kober, Börner and 140 Ruggeri

Trial 2 - Phylloxera performance on Vitis vinifera and grafted rootstocks

1103 Paulsen

The mean total number of phylloxera present after ten weeks for each phylloxera genotype tested on 1103 Paulsen in glasshouse trial two (Fig. 31). Clear differences between the performance of the four phylloxera genotypes, G1, G4, G19 and G30 were observed. All four genetic strains developed on *V. vinifera* at significantly higher levels ($P<0.05$) than on rootstocks. After 10 weeks G1, G4 and G19 were able to establish on 1103 Paulsen albeit at significantly lower levels than the control *V. vinifera.* G30 phylloxera did not survive after 10 weeks on 110 Paulsen. G1, G4, G19 and G30 phylloxera genotypes initiated low levels of non-necrotic tuberosities on 1103 Paulsen compared to high levels of necrotic tuberosities on *V. vinifera* (Fig. 32).

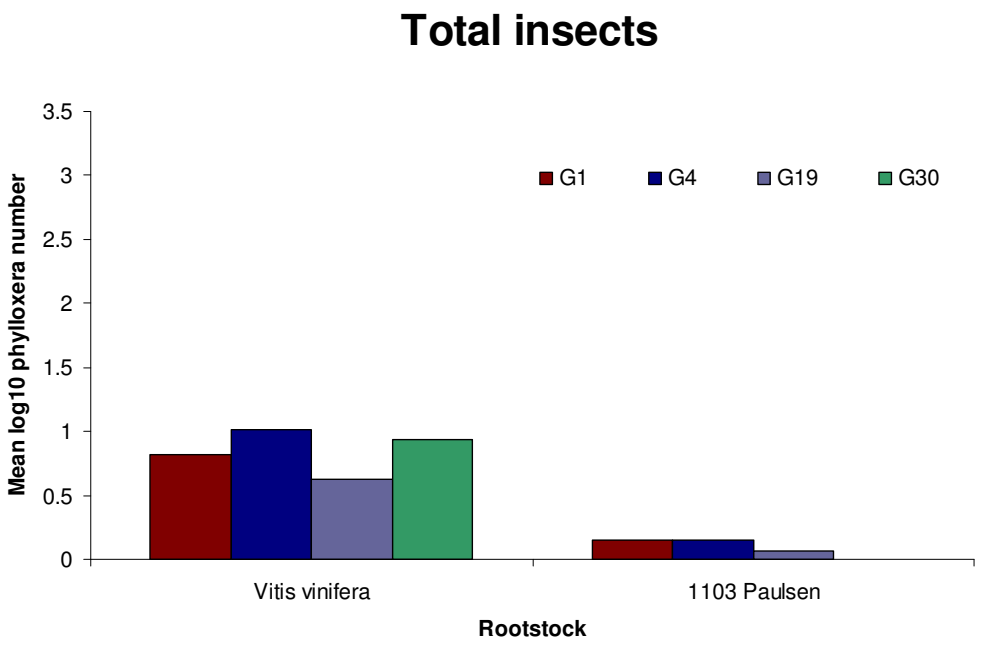


Fig.31. Total abundance of four phylloxera genetic strains G1, G4, G19 and G30, after ten weeks glasshouse incubation on *V. vinifera* cv. Shiraz and 1103 Paulsen

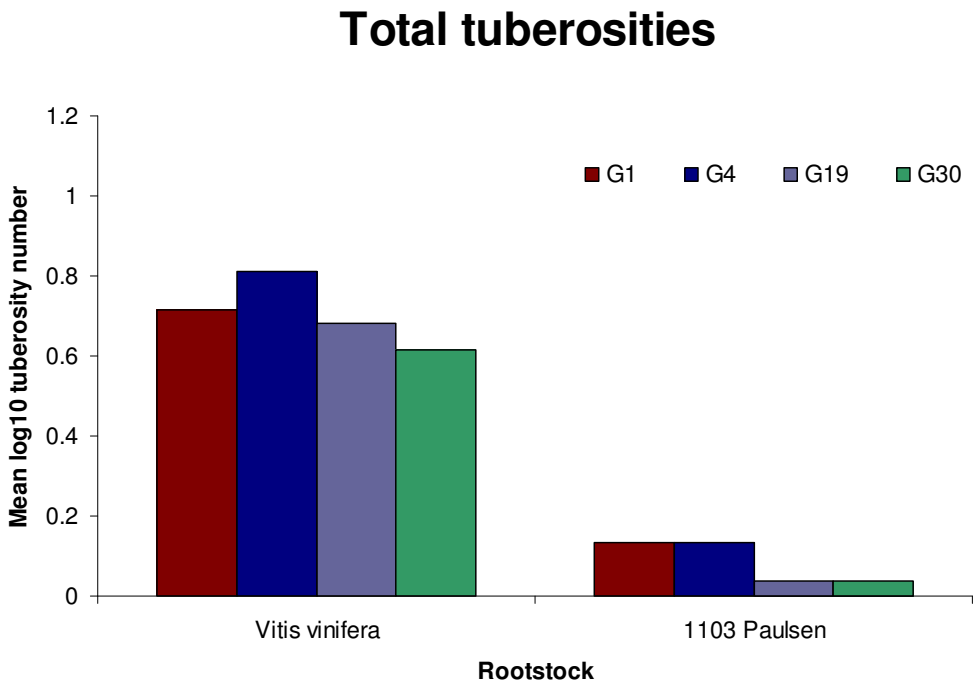


Fig. 32. Total abundance of tuberosities initiated by four phylloxera genetic strains G1, G4, G19 and G30, after ten weeks glasshouse incubation on *V. vinifera* cv. Shiraz and 1103 Paulsen

110 Richter

The mean total number of phylloxera present after ten weeks for each phylloxera genotype tested on 110 Richter in glasshouse trial two (Fig. 33). Clear differences between the performance of the three phylloxera genotypes, G7, G19 and G30 were observed. All three genetic strains did develop on *V. vinifera* after 10 weeks but G7, G19 and G30 did not survive after 10 weeks on 110 Richter. Necrotic tuberosites were present on *V. vinifera* in the presence of G7, G19 and G30. On 110 Richter only G19 phylloxera initiated low levels of non-necrotic tuberosities as compared to high levels of necrotic tuberosities on *V. vinifera* (Fig. 34).

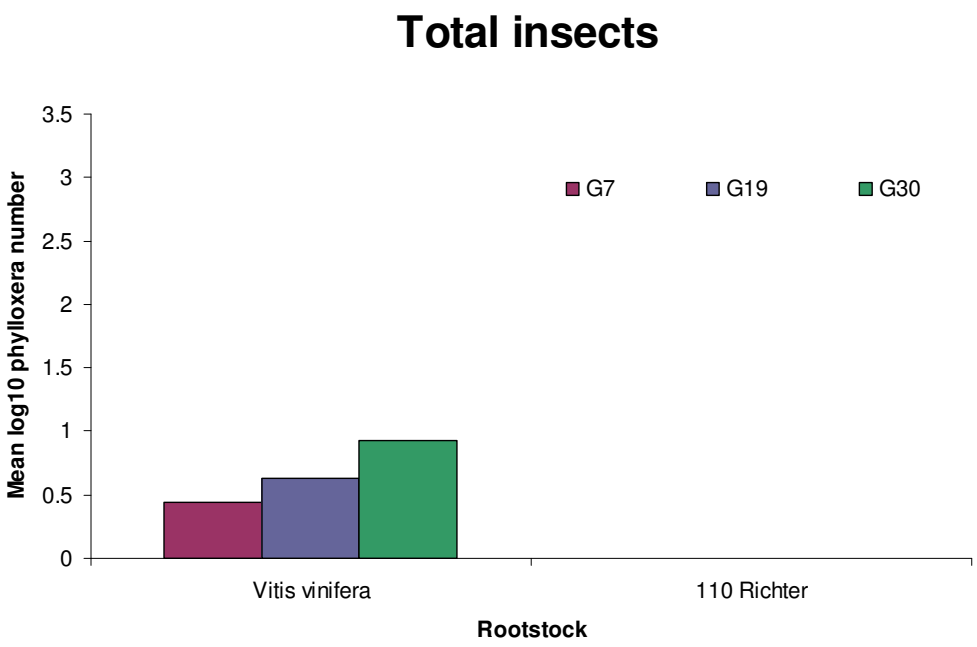


Fig. 33. Total abundance of three phylloxera genetic strains G7, G19 and G30, after ten weeks glasshouse incubation on *V. vinifera* cv. Shiraz and 110 Richter

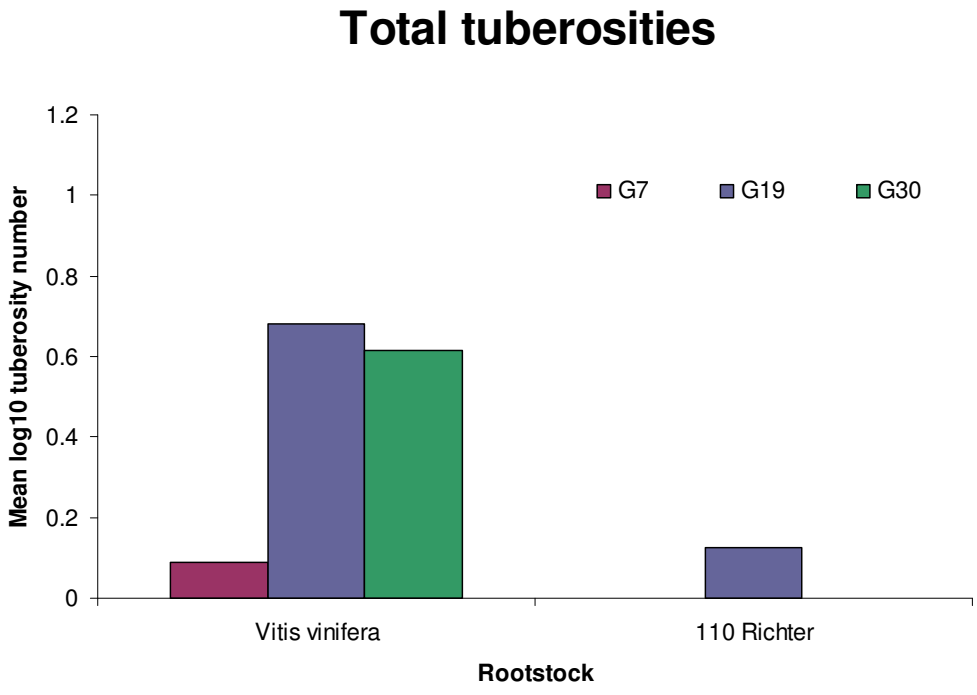


Fig. 34. Total abundance of tuberosities initiated by three phylloxera genetic strains G7, G19 and G30, after ten weeks glasshouse incubation on *V. vinifera* cv. Shiraz and 110 Richter

140 Ruggeri

The mean total number of phylloxera present after ten weeks for each phylloxera genotype tested on 140 Ruggeri in glasshouse trial two (Fig. 35). Clear differences between the performance of the two phylloxera genotypes, G1 and G4 were observed. Both genetic strains developed on *V. vinifera* after 10 weeks and there was not significant difference in abundance levels. On 140 Ruggeri significantly lower abundance of G4 was recorded compared to the control and no G1 was detected on this rootstock after the end of the trial period. There was minimal root damage to 140 Ruggeri (Fig. 36).

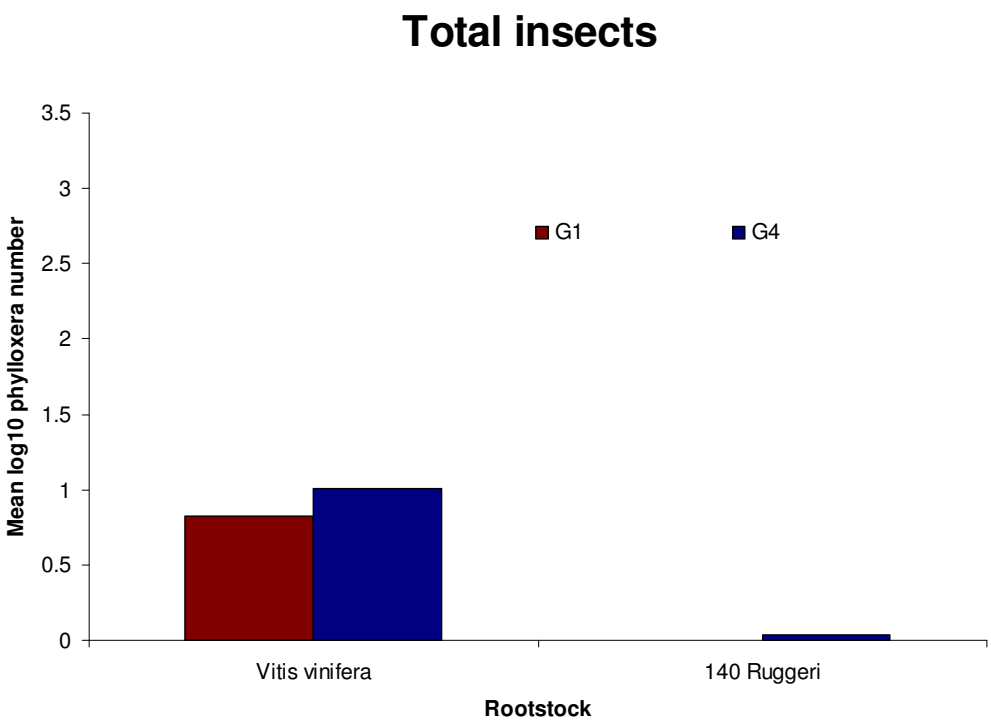


Fig. 35. Total abundance of two phylloxera genetic strains G1 and G4, after ten weeks glasshouse incubation on *V. vinifera* cv. Shiraz and 140 Ruggeri

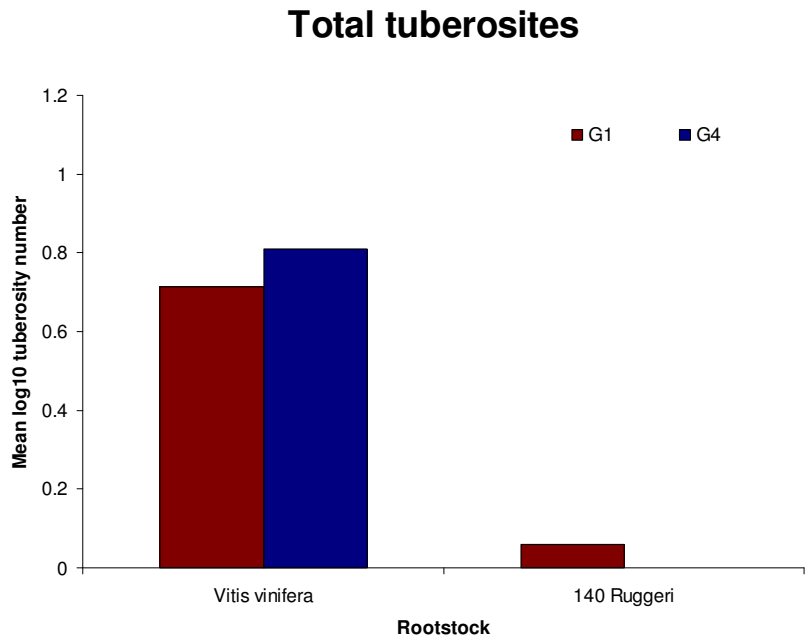


Fig. 36. Total abundance of two phylloxera genetic strains G1 and G4, after ten weeks glasshouse incubation on *V. vinifera* cv. Shiraz and 140 Ruggeri

Discussion

From the six phylloxera clonal lineages that have been recommended for testing against commonly available American rootstocks, five (G1, G4, G7, G19 and G30) were tested in the three glasshouse trials presented here. *Vitis vinifera* showed susceptibility to all five phylloxera genotypes with generally higher phylloxera abundance and more necrotic tuberosities formed on the roots than for any of the rootstocks where only non-necrotic tuberosities developed. On *V. vinifera* G7, G19 and G30 phylloxera showed relatively lower abundance than previous trials conducted using G1, G4 and G20. This finding may also help to explain why G7, G19 and G30 are less commonly distributed geographically and generally found in lower abundance under field conditions in Australia. Their lower abundance on *V. vinifera* could also inadvertently make these genotypes more difficult to detect using conventional ground-truthing techniques, thereby highlighting the need for more sensitive detection methods. In laboratory trials, where excised roots have been tested (Chapter 2), G7, G19 and G30 clones also performed relatively poorly on *V. vinifera* when compared with previous trials using G1, G4 and G20 (Powell et al., 2006).

These results combined with previous glasshouse studies using G1, G4 and G20 (Powell, 2006) moves us a step closer to developing rootstocks recommendations for specific phylloxera genotypes. The third, and final, tier in rootstock screening is field validation which is being conducted as part of the GWRDC funded project "*The 3 'R's Rootstock, Resistance and Resilience*" which commenced in October 2008.

A table of rootstocks tested to date (combining results from this chapter and the corresponding chapter in Powell et al., 2006) summarise the findings to date (Table 3). These results indicate that selection of rootstocks with high resistance (i.e. no establishment of populations after 10 weeks under glasshouse conditions) is highly dependant on phylloxera genotype. For instance Börner appears to have a broad resistance to all six genotypes tested here. In contrast Ramsey appears to have broad 'tolerance' to phylloxera, which means the insect can develop to the adult stage and produce eggs and the rootstock can support

populations of six different phylloxera genotypes. This however, does not necessarily indicate that there is a risk of breakdown in resistance of Ramsey vines, but there may be a potential risk of transfer if Ramsey were planted into a known phylloxera-infested vineyard. Other rootstocks such as Schwarzmann and 5BB Kober are highly resistant to G1 and G4 phylloxera but relatively tolerant to G7, G19, G20 and G30.

Table 3. Provisional rankings of rootstock resistance, tolerance and susceptibility based on whole plant screening under controlled environment conditions.

Genotype/Rootstock	G1	G4	G7	G19	G20	G30
Vitis vinifera	✓S	✓S	✓S	✓S	✓S	✓S
Ramsey	✓T	✓T	✓T	✓T	✓T	✓T
Schwarzmann	✓R	✓R	✓T	✓T	✓T	✓T
Börner	✓R	✓R	✓R	✓R	✓R	✓R
110 Richter	✓T	✓T	✓R	✓R	✓T	✓R
1103 Paulsen	✓T	✓T	x	✓T	x	✓T
140 Ruggeri	✓T	✓T	✓R	✓R	x	✓R
5BB Kober	✓R	✓R	✓T	✓T	✓T	✓T

* where ✓=- tested, x = not yet tested, S= susceptible, T = tolerant and R= resistant

As indicated in previous glasshouse trials (Powell et al., 2006) an assessment of tuberosity formation may provide a useful indicator of the level of host plant response to phylloxera infestation and may also provide a preliminary indicator of how resilient rootstock resistance may be. Tuberosity morphology varied between treatments with *V. vinifera* showing necrotic tuberosities, which may provide a site for secondary fungal invasion. Other rootstocks (e.g. Ramsey) developed domed non-necrotic tuberosities indicating a different level of host plant defense response to phylloxera feeding. On some rootstocks (e.g. Börner) tuberosities did not develop at all and insects did not survive on these rootstocks.

Recommendations

Field screening

Glasshouse and laboratory studies presented in Chapters 2 and 3 of this report do provide preliminary indications of relative resistance/tolerance of selected rootstocks against selected phylloxera genotypes. However ultimately further rootstock and phylloxera genotype screening particularly under field, and consequently different environmental, conditions is required in order to give Australian growers comprehensive advice on rootstock selection in particular Phylloxera Infested Zones or to protect uninfested regions.

Rapid rootstock screening

Glasshouse and laboratory screening have proven that phylloxera genetic strains develop differently on different rootstocks and this indicates that rootstocks may modify the feeding behaviour of different phylloxera genetic strains. One method of testing phylloxera feeding behaviour is the EPG system (Kingston et al., 2007) which has to-date only been tested extensively using a single genotype on *Vitis vinifera* in order to characterise feeding patterns produced when different phylloxera life- stages feed on susceptible vine roots. This method if developed further could potentially proved a more rapid method of rootstock screening as currently replicates of up to eight treatment plants can be assessed over several hours rather than several months (or in the case of field trials several seasons).

Rootstock Recommendations

All rootstocks tested in these trials showed high levels of tolerance to G1, G4, G7, G19 and G30 phylloxera strains. However, further screening should be conducted using additional selected genotypes and rootstocks to ensure that sustainable rootstock recommendations can be made. Overall the data collected supports the assumptions that the seven rootstocks tested to-date have high to good phylloxera tolerance, although clearly the levels of tolerance do vary (Table 3) and there are some differences in ratings compared to *in vitro* studies (Table 2).

Further screening options

The results presented here highlight the variability of susceptibility of *V. vinifera* and resistant or tolerance of rootstocks to individual phylloxera genotypes. Future bioassays would need to incorporate mixed genotype populations of phylloxera under pot trial conditions, to determine if there is competition between genotypes. Additionally the impact of soil type on phylloxera genotype establishment has rarely been considered (Powell, 2007). This potential interaction could be assessed in either glasshouse or field trials.

CHAPTER 4

IMPROVED QUARANTINE PROTOCOLS

Objective: Scientific validation of National Phylloxera Management Protocols - Improved heat treatment recommendations

Summary

Grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), is a significant insect pest of grapevines and can cause mild to severe economic damage to ungrafted European grapevine *Vitis vinifera* L. depending on the insects clonal type (Chapters 1-3). Dispersive stages of phylloxera can be inadvertently transferred from infested vineyards on several potential 'vectors' including viticultural machinery and equipment (Korosi et al., 2009). Dry heat treatment of machinery, such as tractors and harvesters, has been used in Australia as a disinfestation procedure to restrict the spread of phylloxera to uninfested vineyards, but this method does not consider the potential efficacy of the treatment on phylloxera clonal lineage, life-stage or the combined effects of heat and humidity. Over the 83 phylloxera genotypes identified in Australia two dominant and widespread genotypic classes, G1 and G4 occur, and are those considered to have relatively higher potential risk of transfer and subsequent establishment potential. In this study we tested these two grape phylloxera strains under controlled environment conditions at two relative humidity levels (30% and 100%) and four temperature (30°C, 35°C, 40°C and 45°C) treatment combinations to find the optimal combination to achieve 100% phylloxera mortality over the shortest-time period. One hundred per cent mortality of both genetic strains was achieved at 45ºC for 75 minutes irrespective of relative humidity treatment. However some differences in clonal susceptibility to heat and humidity were observed. At 40ºC, 100% phylloxera mortality was achieved at 30% RH after 90 minutes and 105 minutes for G1 and G4 genetic strains respectively. Phylloxera survival was observed at all other heat, humidity and time treatment combinations.

The results of this work have now been endorsed by the National Phylloxera Technical Reference Group and have been incorporated into the modified National Phylloxera Management Protocols (NVHSC, 2008).

Introduction

Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) (Hemiptera: Phylloxeridae), is a significant economic insect pest that feeds on the root and leaves of *Vitis* species (Granett et al. 2001). European grapevines (*Vitis vinifera* L.) are most susceptible to grape phylloxera infestation, potentially killing the plants within 5-10 years depending on insect genotype virulence and relative abundance (Herbert et al., 2006; 2009). There is no reported effective method to eradicate this insect, however there are two widely used management options; the use of phylloxera-resistant rootstocks of American *Vitis* parentage to minimise the impact of phylloxera on infested grapevines and implementation of quarantine protocols to reduce the risk of phylloxera transfer to non-infested areas (Powell, 2008).

Phylloxera population dynamic studies conducted worldwide in infested vineyards (King and Buchanan 1986; Porten & Huber 2003; Powell et al., 2003) have identified that the most dispersive life-stage of phylloxera is the first instar nymph commonly referred to as the 'crawler'. Crawlers are present in the vine canopy, on both foliage and fruit, during the growing season (King & Buchanan 1986; Powell et al. 2000). This highlights the risk period of accidental phylloxera transfer, particularly on viticultural machinery, during the summer months and harvest, when most grapevine management activities occur. Phylloxera first instars have been detected on mechanical harvesters (King & Buchanan, 1986) and they can subsequently survive some post-harvest grape handling processes, such as transport in grape bins, crushing, destemming and pressing (Deretic et al., 2003) and on winery waste (Korosi et al., 2009). This indicates the possibility of transferring live dispersive stages of phylloxera on machinery and grape products from infested vineyards.

In Australia grape phylloxera is predominantly managed through quarantine zones. Phylloxera Infested Zones (PIZ's) are areas known to be infested with phylloxera. To prevent

the spread from PIZ's to Phylloxera Exclusion Zones (PEZs) the National Phylloxera Management Protocols have been developed (NVHSC 2008). These protocols contain movement procedures, minimum hygiene requirements and quarantine treatments for a range of phylloxera risk vectors from a PIZ.

Prior to conducting this experimental work the recommendation for dry heat treatment of viticultural machinery (NVHSC, 2008) in a temperature controlled heatroom, was to keep machinery at 45°C for 120 minutes. Dry heat is also commonly used in treating stored products and fresh fruit commodities for insect pests and to disinfest machinery of weeds and fungal pathogens (Tang et al., 2007).

Temperature impacts on phylloxera survival and development in the presence of the host plant food source, grapevine roots. However, laboratory life-table analyses on grape phylloxera (Granett & Timper 1987; Turley et al., 1996; Fisher & Albrecht 2003; Makee 2003) have focused more on the optimum conditions for feeding site establishment, development rate, survival, reproduction of grape phylloxera of different geographical origin rather than its potential use as a disinfestation procedure. There is limited data on phylloxera response to elevated temperatures above 30°C and this has focused only either on a geographic 'population' of undetermined genetic lineage (Buchanan 1990) or a single-sourced genetic variant of grapevine phylloxera (Korosi et al., 2008). In Australia 83 genetic strains of phylloxera have been characterised (Umina et al., 2007) from which genotype G1 is found to be the most geographically widespread genotype and G4 appears to be more virulent in regions where it has established such as the King Valley (Powell et al., 2003; Herbert et al., 2006). This observation would make these two genotypes the most likely source for further phylloxera spread within Australia. First instar nymphs are the most active dispersive life-stage of phylloxera (Powell et al., 2003) making it the most significant quarantine risk; therefore this life-stage was the focus of the study.

This study had two components. Firstly, determining the temperature and humidity tolerance of phylloxera first instars was tested based on simulated 'heat shed' conditions. Dry heat treatment is used in Australia as a disinfestation treatment for viticultural machinery

(NVHSC, 2008). In addition phylloxera first instar survival and egg viability in water was tested at two temperatures as a pilot study to determine the effect of cooler temperatures and water immersion on phylloxera

The overall aim of this study was to determine the minimum time limits for phylloxera mortality under a range of humidity and temperature combinations and to assess the impact of such treatments on two genetically diverse phylloxera strains.

Materials and methods

Insect maintenance

G1 and G4 phylloxera genetic strains were single sourced from infested vineyards in the Northeast Victoria PIZ, then established as stock cultures using an excised grapevine root system (Kingston et al., 2007) and genotyped using microsatellite markers (Umina et al., 2007). All phylloxera life-stages were carefully manipulated using a fine sable hair paintbrush to prevent damage. Prior to treatment freshly oviposited phylloxera eggs were carefully removed from the excised *V. vinifera* cv. Sultana root pieces. They were then placed in damp filter paper lined Petri dishes, sealed with Parafilm and covered with aluminium foil, to exclude light. The eggs were then incubated at 25±2°C for 5-7 days in a constant temperature room. On hatching newly emerged phylloxera first instars were removed and used in all disinfestation trials.

Dry heat disinfestation

The impact of temperature and humidity on phylloxera first instars was assessed using 'disinfestation' chambers enclosed with desiccators. Round plastic, screw-top desiccators (13cm[h] x9.5cm[d]) were used to maintain constant relative humidity. 150ml of saturated salt solutions were added to maintain relative humidity in each chamber. For 100% RH distilled water was used and for 30% RH saturated magnesium-chloride solution (Buchanan 1990). A cylindrical (5.5cm[h] x 4.5cm[d]) plastic container was placed in the centre of each desiccator and partially filled with humidifier solution to restrict buoyancy. Plastic mesh

(9.5cm [d]) was placed on top of this container as a platform, to keep the vials containing phylloxera above the humidifier solution level and prevent drowning (Fig. 37).

Twenty newly-emerged phylloxera first instars were placed on a 1cm^2 moist filter paper strip. Each strip was placed into a modified plastic vial (5.5cm [h]; 2.5cm [d]). The top and base of each vial was cut out and replaced with a 25µm pore stainless steel mesh that prevented phylloxera escape while still allowing airflow.

Disinfestation vials containing the moist filter paper and phylloxera first instars were placed onto the plastic platform in the desiccator chamber. The chamber lid was screwed on and placed in a constant temperature incubator (Linder & May Pty. Ltd., Brisbane, Australia). Temperatures ranged from 30-45±2ºC at 5ºC increments while humidity level was maintained at either 30% or 100%. Phylloxera first instar survival at 15 minute intervals was assessed in the range of 30-120 minutes,

Separate desiccators were used for each treatment combination (temperature/humidity /time) with two replicate disinfestation vials in each desiccator; each replicate containing twenty first instar phylloxera. TinyTag™ data loggers placed in a separate desiccator recorded the temperature and humidity throughout the trial period. Survival was assessed using a low powered binocular microscope.

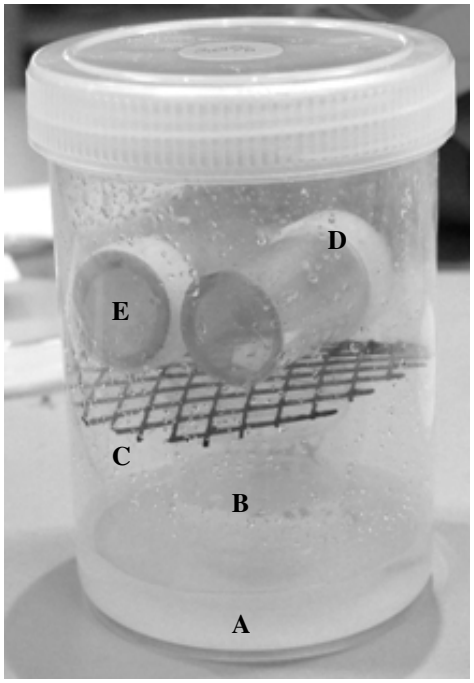


Fig. 37. Desiccator chamber with: A – water or magnesium chloride solution; B – small plastic container with water or salt solution; C – plastic mesh; D – modified plastic vials with phylloxera; E – wire mesh to retain phylloxera and allow air flow

Water and cold temperature treatment

To test the affect of water and temperature on phylloxera egg and first instar survival. Laboratory trials were conducted under quarantine conditions at the DPI – Rutherglen Centre. Single sourced G4 phylloxera was laboratory reared on excised roots (as described earlier) was used in this trial. Phylloxera first instar and egg survival in water was assessed at two temperatures 5ºC and 25ºC (which is considered and optimal control temperature). To maintain temperature experiments were conducted in controlled temperature rooms.

Quadrant™ 5.1 litre plastic containers were filled with 4 litres of autoclaved tap water. In each container plastic vials (5.5cm length; 2.5cm diameter) containing phylloxera were carefully submerged under water. The top and base end of each vial were cut out and replaced with 25 μm pore size stainless steel mesh that prevented insect escape while still allowing water to pass through and ensure no air bubbles were present.

Separate water containers were used for each phylloxera life-stage tested, with nine replicate vials in each desiccator; each replicate contained either five first instars or five eggs.

Phylloxera first instar survival and egg hatching was assessed daily using a low powered binocular microscope.

Statistical analysis

All data were analysed using GenStat for Windows 10th Edition software (Payne et al., 2007). Phylloxera survival between time points was assessed using Kaplan-Meier survival analysis, adopting non-parametric tests for survival curve comparisons. A log-rank test was used to test the null hypothesis that there is no difference between the treatments in the probability of an event (i.e. death) at any time point. The analysis is based on the times of events (i.e. deaths). For each such time the observed number of deaths was calculated in each group and the number expected if there were no differences between the groups.

Results

Dry heat disinfestation

Phylloxera mortality under different temperature and humidity regimes are shown in Figs 38 and 39. The upper thermal limit for 100% first instar mortality was achieved at 45ºC for both phylloxera genetic strains regardless of relative humidity treatment. G1 phylloxera exposed to 45ºC for 75 minutes reached 100% mortality at both 30% and 100% RH whereas for G4 phylloxera 75 minutes duration was required at 30% RH but only 45 minutes at 100% RH to reach 100% mortality.

At 40ºC 100% mortality was only achieved at 30% RH for both genetic strains at 90 minutes and 105 minutes for G1 and G4 respectively. At 100% RH a relatively small number of both phylloxera strains survived the 40ºC treatment.

None of the other genetic strain, humidity and temperature combination treatments tested achieved 100% phylloxera mortality. Differences in mortality between phylloxera genetic strains occurred at sub-lethal temperatures. At 30% RH up to 80% and 45% G1 phylloxera survival was recorded at 30°C and 35°C respectively. Whereas G4 phylloxera, at 30% RH, survival was around 60% at both temperatures. Pairwise comparison of G4 survival curves at

each temperature between the two relative humidities showed no significant difference at 30, 35 and 40ºC ($P>0.05$) but a significant difference at 45ºC ($P<0.001$). The results were similar with G1 except at all temperatures tested, the survival curves were not significantly different ($P>0.05$). The general trend for both genotypes was for better survival at the higher relative humidity.

Water and cold temperature

Over an 11-day trial period no phylloxera eggs hatched when submerged in water at 5ºC (Fig. 40). However by day 11, at 25ºC, 67% of phylloxera eggs had hatched underwater into first instars (Fig. 40) but in the absence of a food source only survived for a maximum of two days underwater. Phylloxera first instar nymphs survived for a maximum of five and seven days at 25ºC and 5ºC respectively (Fig. 41).

Discussion

Dry heat disinfestation

In this study we validated the upper time limits for first instar survival of two phylloxera genetic strains, G1 and G4, at combination of two relative humidity treatments (30% and 100% RH) and four temperature (30ºC, 35ºC, 40ºC and 45ºC) treatments.

All first instar larvae of both genetic strains died at 45ºC when exposed for 75 minutes regardless of relative humidity, although G4 first instars died earlier at 45 minutes at 100% RH. At 40ºC, 100% mortality was only achieved at 90 minutes and 105 minutes for G1 and G4 genetic strains respectively but only at 30% RH. At 40ºC and 100% RH there was some survival after two hours treatment. However 100% relative humidity is an unlikely scenario under heat shed conditions as when equipment is heated up to 45ºC relatively humidity drops to around 30 % relative humidity (pers. comm. Richard Hamilton).

Temperatures of 30°C and 35°C could not be considered as viable disinfestation treatments as survival was 45-60% depending on relative humidity. Mean summer air temperatures in Northeast Victoria, where phylloxera predominates regularly exceed 35ºC, with A horizon

soil temperatures around 30-32ºC (K. Powell, unpublished data). The Northeast Victorian PIZ has been infested by phylloxera for over a century and both G1 and G4 are still prevalent in the zone, but G4 is primarily restricted to the cooler climate region of this PIZ. Therefore G1 seems may be better adapted to warmer temperatures of 30-35ºC and lower humidities than G4 which is found in the cooler climate regions like the King Valley.

Previous grape phylloxera life-table analyses and heat-mortality studies, which did not consider phylloxera genotype, determined the upper thermal limit for grape phylloxera life-stages to be between 36°C and 40°C (Granett & Timper 1987; Buchanan 1990). Previous grape phylloxera time-mortality studies indicated that phylloxera dies within one hour of exposure to temperatures exceeding 40°C between 13-95% RH (Buchanan, 1990). However, in Buchanan's' study all phylloxera life-stages of undetermined genetic strain were used and they were allowed access to grapevine root material and the influence of humidity and temperature on root physiology was not considered.

In contrast in our study only the first instar life-stage of known genetic background in the absence of food source was used, as these are the most likely conditions in which phylloxera will be found on machinery prior to disinfestation. We found that both grape phylloxera genotypes when tested at 40°C survived for 75 minutes at 30% RH, and for over two hours at 100% RH.

In a typical dry heat shed, depending on seasonal conditions and time of heat treatment, relative humidity is within a range of 30-50% (pers. comm. Richard Hamilton, Southcorp Wines, Australia), but 30% RH is typically measured on grape harvesters during a 45ºC heat treatment (pers. comm. Richard Hamilton). For our study we also chose 100% RH as a control to simulate the conditions in the heat room when the entering machinery is wet, but as temperature increases in the heat room, relative humidity would decrease well below this level.

Water and cold temperature

Phylloxera active stages can survive in the absence of a food source for a maximum of 11 days under optimal temperature and ambient conditions (Kingston et al., 2009). When submerged in water in our trial maximum survival of crawlers was 7 days at 25ºC. When temperature was reduced to 5ºC maximum survival was 8 days. Previous studies (Buchanan et al., 1997) have examined phylloxera first instar survival and egg viability in water at 25ºC only and phylloxera tested was in the presence of a food source (i.e. root galls). In their study up to 88% eggs hatched over a 48 hour period and all first instar nymphs died after 5 days in water at 25ºC. Our results indicate that in the absence of a food source survival of phylloxera life-stages in water signifies a possible risk of transfer in waste water or irrigation water. The results also indicate that cold storage at 5ºC would be an ineffective method of disinfestation for phylloxera.

Recommendations

Dry heat disinfestation

Based on our study machinery heated to 45°C should be kept at that temperature for the minimum of 75 minutes to achieve 100% phylloxera crawler mortality, irrespective of phylloxera genotype. Whereas if the machinery is heated to only 40°C this time of heat treatment should be extended to a minimum of 105 minutes. Phylloxera disinfestation protocols have now been modified to recommend a minimum of 90 minutes at 45°C or 120 minutes at 40°C (NVHSC, 2008).

There have been 83 different genetic strains identified in Australia (Umina et al., 2007) and in our study we only tested two of these. We found that there are some survival differences between these two genotypes in different temperature-humidity combinations. Ultimately further studies should be conducted to determine thermal and humidity limits to further validate heat treatment protocols for disinfestation.

In addition laboratory based excised root grape phylloxera studies (Granett & Timper 1987; Fisher & Albrecht 2003; Makee 2003) focusing on the optimum conditions for speed of

development; survival and reproduction of grape phylloxera of different geographical origins do not take into account the importance of relative humidity. In this study we found that the combination of temperature and humidity influences the survival of different phylloxera genotypes, therefore further laboratory based excised root phylloxera studies should consider these tritrophic interactions particularly when using this method as a screening technique for rootstock resistance.

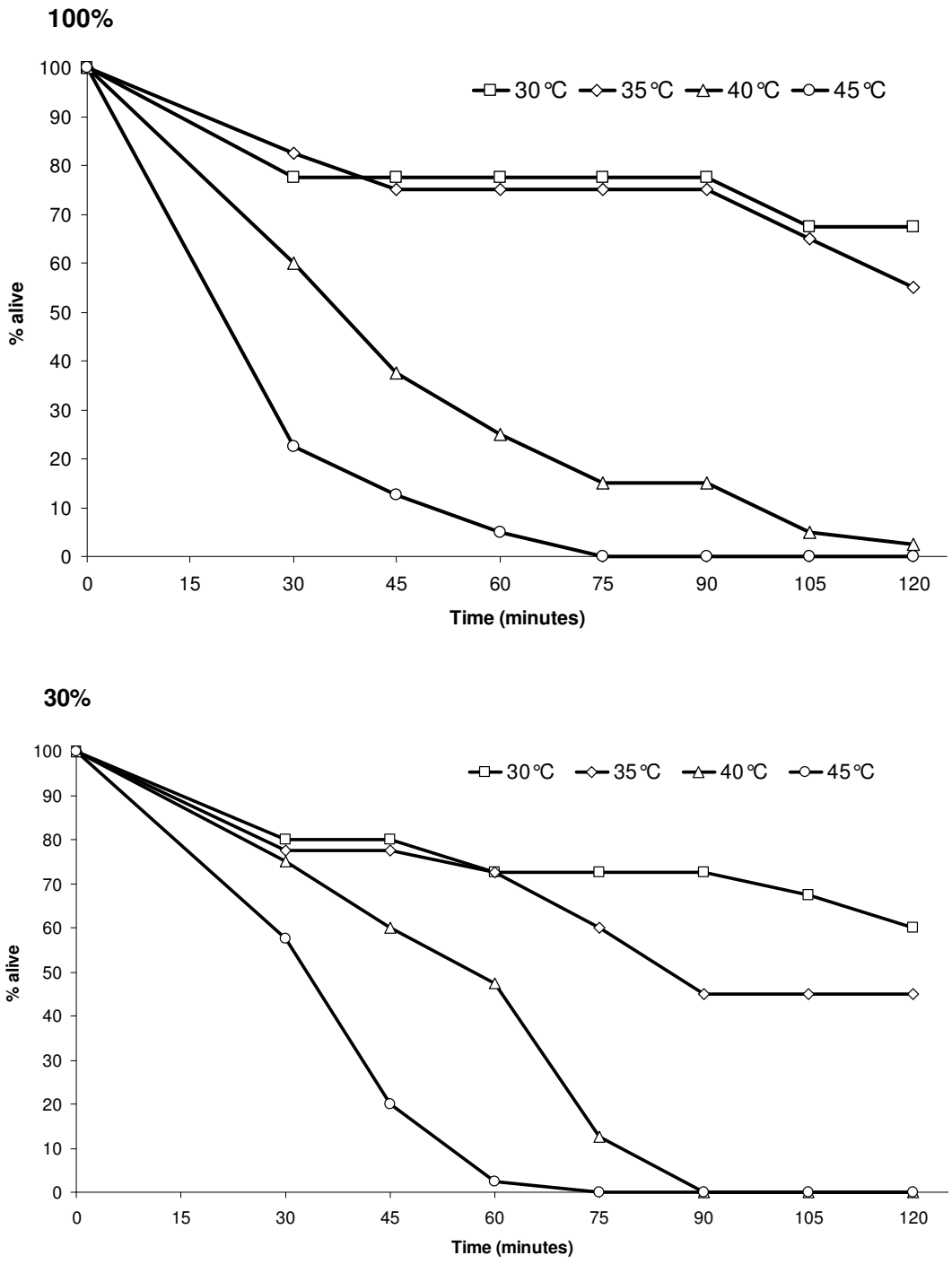


Fig. 38. First instar G1 phylloxera survival at 30% and 100% relative humidity and four temperatures

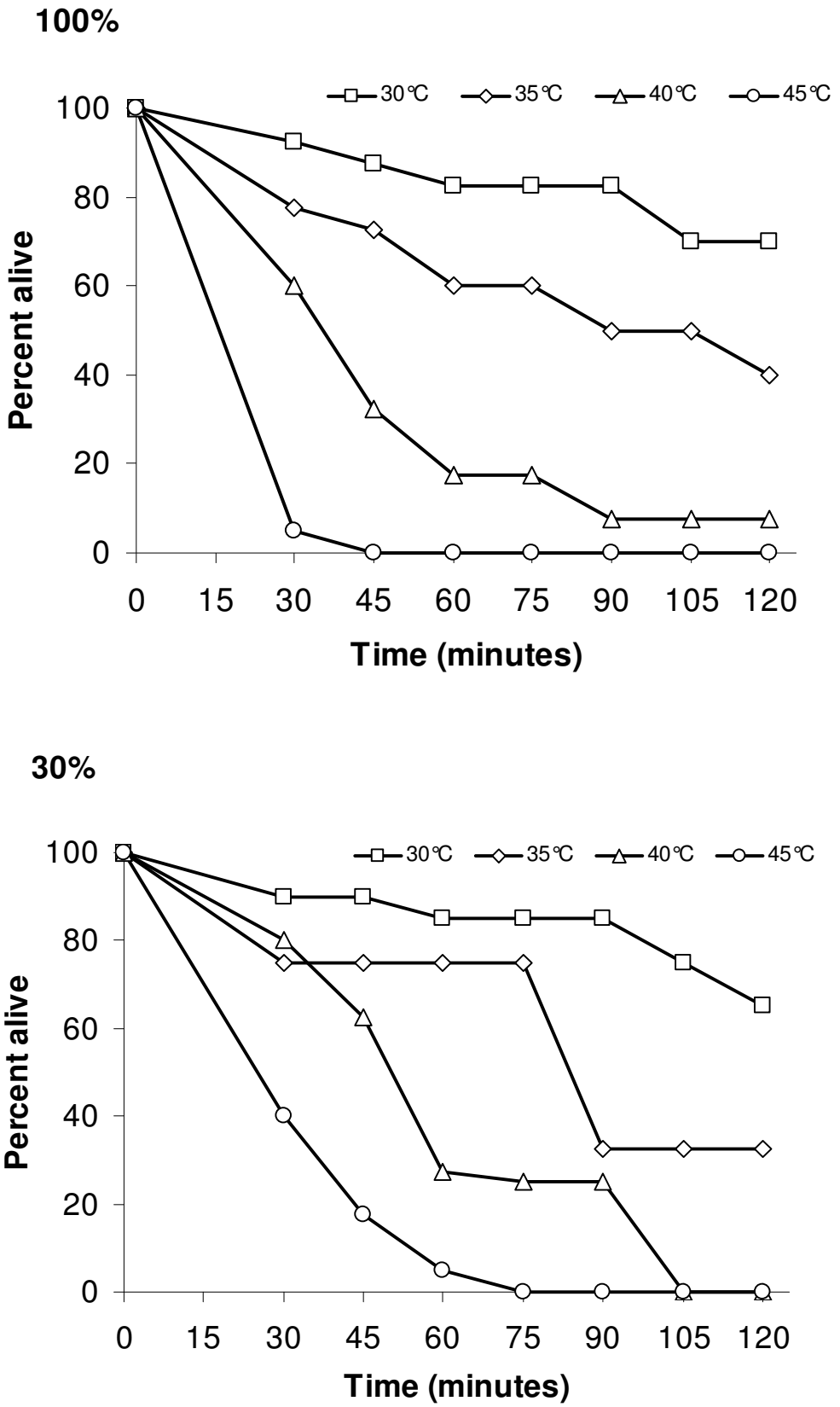


Fig. 39. First instar G4 phylloxera survival at (a) 30% and (b) 100% relative humidity and four temperatures

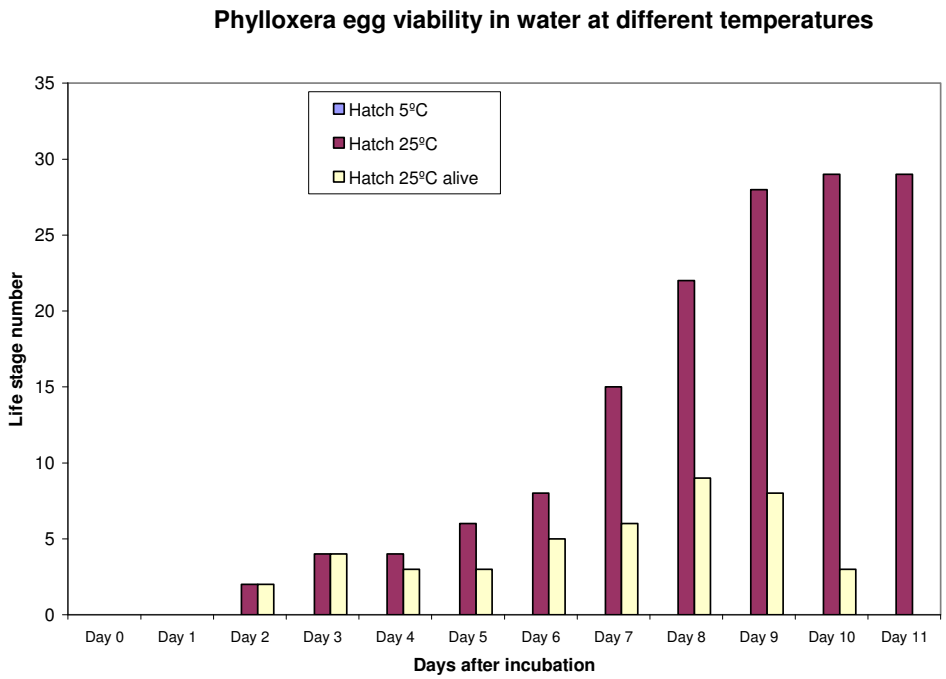


Fig. 40. Phylloxera egg survival and hatchability when submerged in water at two temperatures

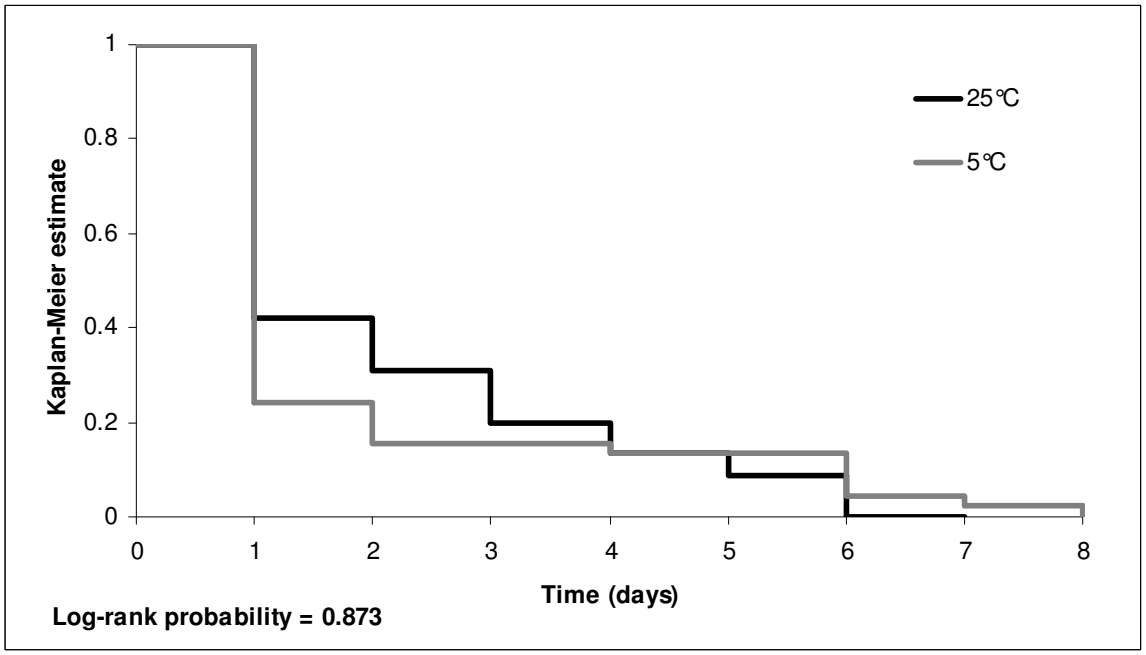


Fig. 41. Kaplan-Meier estimate of phylloxera first instar survival when submerged in water at two temperatures

CHAPTER 6

PHYLLOXERA EDUCATION AND AWARENESS ACTIVITIES

Objective: To facilitate and deliver Annual National Phylloxera Management Workshops and related extension and awareness activities

Summary

Phylloxera Identification and Management workshops were an essential component of the three-year Sustainable Phylloxera Management Phase II project (Powell, 2006). Between July 2006 and June 2009 a total of 17 workshops were delivered to over 400 participants from four states. There were three types of workshops depending on the target audience.

Workshop evaluation for the first time was changed from a paper based questionnaire to include KeePad™ technology an interactive, computer-based data collecting system. This is the first time this technology was used for phylloxera workshop evaluation. The technology gave us the opportunity to objectively evaluate in finer detail than previously the level of knowledge retained by the workshop participants during the presentations.

Introduction

Education and awareness are essential components of phylloxera management. The Phylloxera Identification and Management Workshops were designed to deliver information to the viticulture and related industry personnel about phylloxera management, including rootstock selection, disinfestation protocols, detection methods and quarantine to prevent the spread of phylloxera.

Although these workshops were originally delivered to members of the viticulture and oenology industry, we felt the need to diversify and to reach a broader audience to enhance

awareness of the risks. During the three-year period we organised several one- and two-day workshops but also participated in other activities where a half-day phylloxera awareness activity could be incorporated. These were mostly for visitors to the DPI Rutherglen Centre from different professional organisations including The Alpine Valleys Leadership Group, DPI and DSE cadet program, DPI and DSE Young Professionals Forum and students from local high schools and universities.

Method

The audience and level of background knowledge for the Phylloxera Management Workshops is so diverse that there was a need to deliver the message tailored to the audience using three different formats:

Comprehensive workshop format

This is the 'classical workshop' we have been delivering since 1994, with minor modifications to the viticulture industry. In this format participants are given comprehensive information on phylloxera during a one- or two-day workshop covering the following topics:

a. **Phylloxera biology, geography and history** – explaining about the origins of phylloxera, its habitat, life-cycle and geographical distribution in the world and Australia.

b. **Phylloxera genotypes** – introducing the participants to the concept and relevance of genetic variability within grapevine phylloxera and the potential consequences for management and detection.

c. **Population dynamics and distribution** – providing information on the seasonal phylloxera distribution in the vineyard and highlighting risk periods.

d. **Quarantine and risk management** – giving an overview of the National Phylloxera Management Protocols, including Phylloxera Management Zones and disinfestation protocols as well as phylloxera management methods including the use of 'resistant' rootstocks.

e. **Detection methods** – traditional vineyard survey methods for growers and survey teams (ground surveys) and highlighting new detection methods coming on-stream in the future e.g. DNA probe, chemical fingerprinting, aerial surveys etc.

f. **Disinfestation facilities** – excursion taken to a local winery within a PIZ that deals with viticultural machinery and equipment coming in and going out to infested and uninfested vineyards. Participants are shown facilities used in disinfestation including heat shed, wash-down bay, bin dipping.

g. **Field survey and grower's talk** – participants are taken to a phylloxera infested vineyard to see above ground symptoms of phylloxera; using shovels and hand lenses then examined phylloxera on the root system. Participants also meet with a grower from a phylloxera infested vineyard who explains their post outbreak management processes.

h. **Phylloxera in the laboratory** – during this session the participants are taken into the laboratory to look at live phylloxera life-stages under the microscope as well as emergence trap samples containing abundant phylloxera crawlers and winged adults.

i. **Hypotheticals** – in this session the participants are given different scenarios of a phylloxera outbreak to discus what they would do if the scenarios were 'real'.

j. **Research update** – a comprehensive up to date session on new phylloxera research conducted at the DPI Rutherglen Centre including new information on genotype and rootstock interactions, early detection methods and risk analysis.

The one-day format of this workshop was advertised nationally through viticulture industry journals (Australian and New Zealand Grapegrower and Winemaker, National Grapegrower and Australian Viticulture). Participants were usually people involved in the viticulture industry. The two-day workshop was organised in conjunction with the Phylloxera and Grape Industry Board of South Australia, participants were mostly from South Australia or were employees of companies based in South Australia.

Evaluation was only done at the end of these workshops. In 2007 the classical paper based evaluation forms were given to the participants at the end of the day. From 2008 we introduced a new, electronic evaluation called KeePad™ Technology. It is a PowerPoint based questionnaire with response devices. Each slide asks a question and provides a number of possible answers. Participants are given a response device each and they have to press the numbered button that they think corresponds to the correct answer. The same set of questions was asked at the commencement and closure of the workshop and the answers were compared to see how much the participants had learned.

For the workshop, each participant was given a comprehensive booklet called Phylloxera Workshop Notes to enable them to find all the information covered and other resources for further reading or viewing.

Vineyard survey workshop format

Participants for this workshop were contractors to the Victorian and Queensland Governments who were engaged to conduct phylloxera surveys for the new Phylloxera Exclusion Zones (PEZ).

This was a shortened version of the Comprehensive workshop, mostly focusing on the vineyard survey technique and risk of transfer. There was less time spent in a classroom

situation and more time hands on with practical training. The classroom component was similar to the one day workshop (topics a-f and e) with more time spent on explaining surveys and population dynamics. The practical side of the workshop (topic g) was held in an infested vineyard where the participants conducted a phylloxera survey. Every participant had to be able to identify phylloxera symptoms in the vineyard (e.g. reduced canopy, weak spot, undervine weeds) and find the insects themselves. Participants were also given a handout on survey techniques showing phylloxera life-stages and symptoms.

Visiting Professionals format

DPI-Rutherglen Centre attracts various professional groups that are interested in science. To these groups a 30-60 minute overview was delivered. A practical component included showing phylloxera under the microscope and a theoretical component explained the risks involved that most vineyard/winery/cellar door visitors need to be aware of and a short update on current research.

These professionals included the following groups:

1. Alpine Valleys Community Leadership Group – a leadership program for community development.
2. DPI and DSE Cadet program – mostly DSE with a few DPI cadets ranging from first to fourth year university students.
3. DPI and DSE Young Professionals Forum – meeting, networking and learning opportunity for young professionals from DPI and DSE.
4. Educational institutions – high school students from Rutherglen High School and viticulture students from Swinburne University.

Results

Over the three-year duration of the project 17 workshops were conducted and over 400 people educated on phylloxera to different levels (Table 4). Workshop locations were chosen where there was either an infested vineyard so the participants gained hands on experience of phylloxera or at DPI Rutherglen Centre where phylloxera populations are kept under controlled quarantine conditions.

Table 4. Participation levels and locations of Phylloxera Workshops in the three year project period (July 2006-June 2009).

	2006 July-Dec	*2007 Jan-Dec*	*2008 Jan-Dec*	*2009 Jan-June*	*TOTAL*
No. of workshops	3	7	5	2	17
Workshop locations	Milawa, Murchison	Porepunkah, Rutherglen, Yarra Valley	King Valley, Porepunkah, Rutherglen	Porepunkah, Rutherglen	
No of attendees	83	173	89	61	406

Visiting professional's workshops were the most numerous (Table 5) with over 200 participants over the three-year period. Followed by the Classical Workshops with just over 150 and the least numerous ones were the Survey team workshops.

Table 5. Participation levels for different types of Phylloxera Workshops in the three year project period (July 2006-June 2009).

WORKSHOP TYPE	*2006 July-Dec*	*2007 Jan-Dec*	*2008 Jan-Dec*	*2009 Jan-June*	*TOTAL*
Classical workshops	23	66	45	17	151
Survey teams	20	22	12	0	54
Visiting professionals	40	85	32	45	202
TOTAL	83	173	89	61	406

A demographic breakdown ratio (Table 6) of workshop participants over the three year period shows an approximate 20:80 split between people involved in the day-to-day-vineyard operations and transient vineyard operations. This is due to the large number of people attending the visiting professional workshops.

Table 6. Workshop participants by occupation (% of total for each year) at all workshops in the three year project period (July 2006-June 2009).

	2006 July-Dec	*2007 Jan-Dec*	*2008 Jan-Dec*	*2009 Jan-June*
Grapegrower	1.2	3.5	3.4	3.3
Viticulturist	0	4	0	0
Vineyard worker	6.2	5.8	8.5	1.7
Vineyard manager	10	5.2	10.1	6.6
Day to day vineyard operations total	**17.4**	**18.5**	**22**	**11.6**
Consultant	1.2	14	5.1	3.4
Contractor	26	11.6	13.6	0
Nursery staff	0	0.5	1.7	0
Winemaker	0	0.5	0	0
Student	49.4	8.7	20.3	0
Other	6	46.2	37.3	85
Transient vineyard involvement	**82.6**	**81.5**	**78**	**88.4**

Occupational status of participants (Table 7) shows a spread between company, self- and government-employed participants. Company employed participants dominated the workshops for all years except for 2007. The percent of self-employed increased during the three-year period. Government employed people dominated the workshops in 2007.

Table 7. Occupational status of all workshop participants (% of total for each year) in the three year project period (July 2006-June 2009).

	2006 second half	*2007*	*2008*	*2009 first half*
Company employed	80	28.5	80.8	46.1
Self employed	5	6.4	11.5	23.1
Government employed	15	55.1	7.7	30.8

Evaluation process

At the end of each Classical Workshop an evaluation was conducted. Originally it was a survey form that the participants fill in. This is a very subjective way of determining

how much a person has learned from each session. We used this method to evaluate the workshop in 2006 and early 2007. In the second half of 2007 we changed our evaluation method to a more objective and quantifiable one. KeePad™ Technology is an interactive, computer based data-collecting system. This is the first time this was used in phylloxera evaluation. In the first year of introducing this technology the response from the participants was overwhelmingly in favour for the new technology.

In the second half of 2008 and early 2009 we perfected the types of questions asked from the participants to be able to objectively evaluate how much they learned during the workshops. Participants were asked a couple of demographic questions (e.g. their employment status and occupation) followed by 21 multiple choice questions on phylloxera (Fig. 42). Each participant had a response device assigned to them where they pressed the number that corresponded to the answers. Participants were asked to the best of their knowledge answer the questions at the beginning of the workshop and the same set of questions at the end. This gave us information on the participant's pre workshop background on phylloxera and how much they learned during the one- or two-day workshop. A sample selection of data generated from the information colleted during the workshops held in December 2008 and January 2009 is described in Figs 43-47.

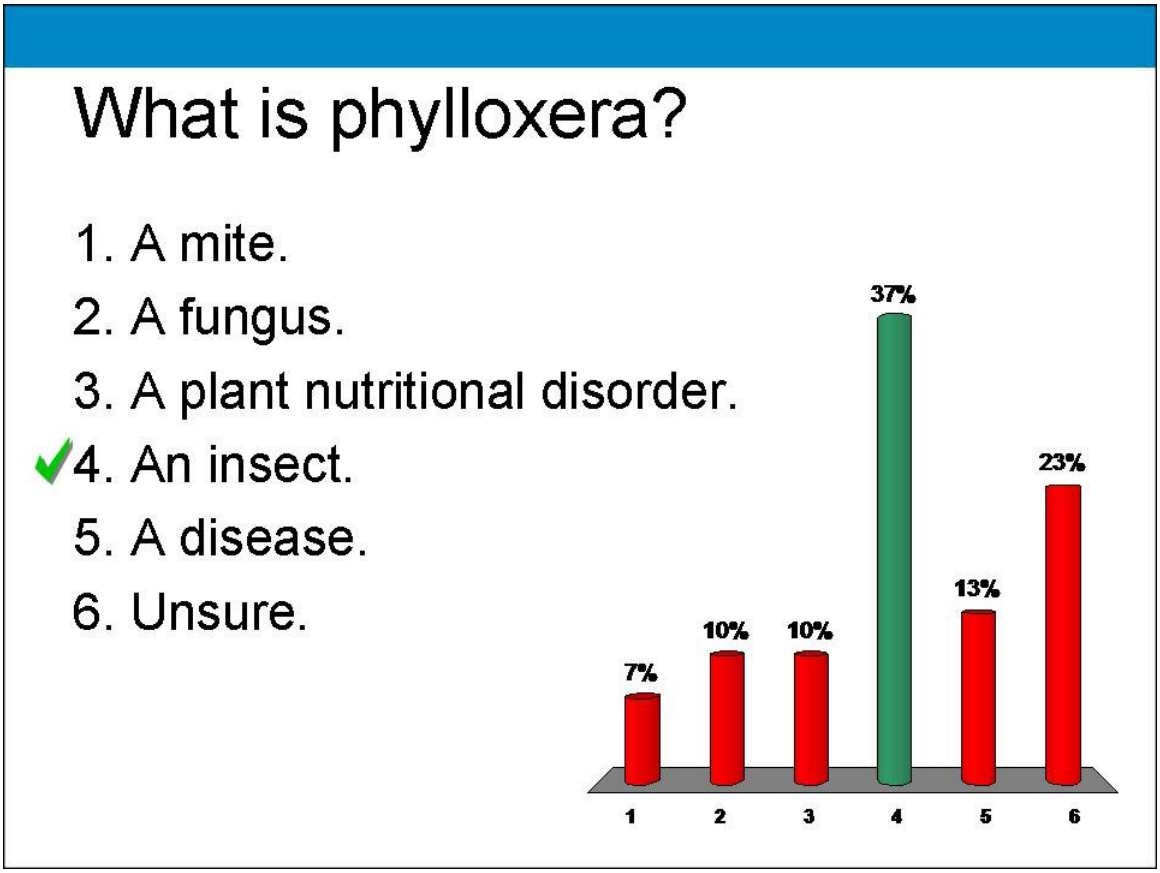


Fig. 42. Example of a sample test question asked at the start of a phylloxera workshop. The correct answer is indicated by a green tick next to the number and the percentage of correct (green bar) and incorrect (red bars) answers given

Over 60% of the participants were company employed (Fig. 43) followed by self employed and government employed.

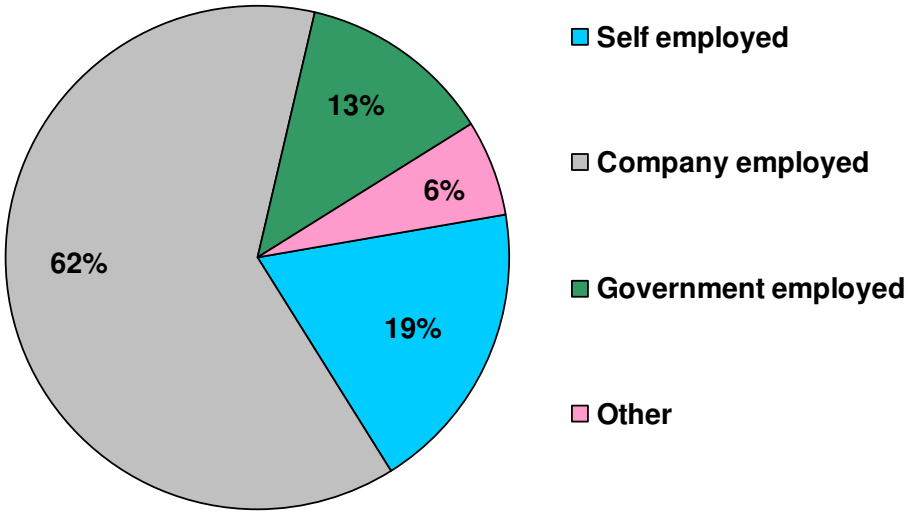


Fig. 43. Classical workshop attendees based on employer type (% of total) in 2008/09

Over two thirds of all participants in 2008/09 were vineyard managers, vineyard workers, consultants and grape growers (Fig. 44). The most numerous group was vineyard managers (31%) followed by vineyard workers (18%), consultants (15%), grape growers (12%) and finally nursery workers (3%). There was a large number of participants (21%) belonging to occupations not specified.

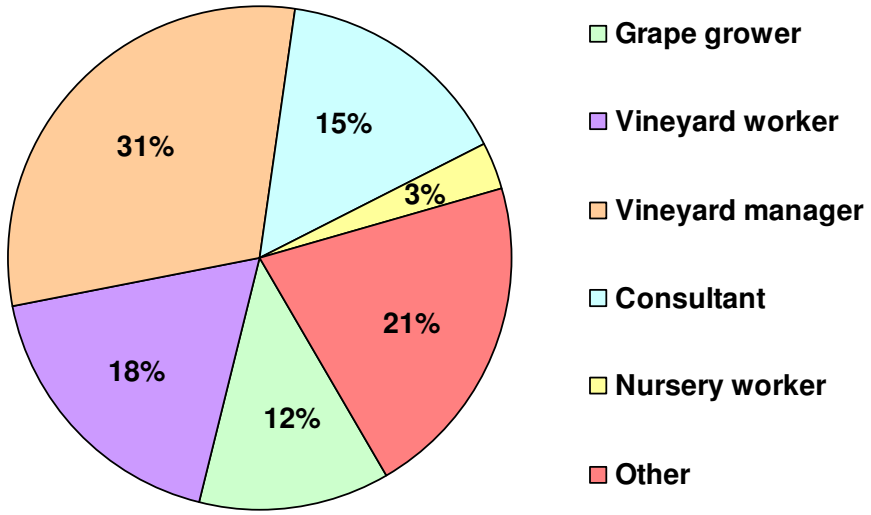


Fig. 44. Occupation type (% of total) of classical workshop participants in 2008/09

At the start of the workshop less than half of the answers given by participants were correct (Fig. 45a) indicating only a cursory knowledge of grapevine phylloxera. Whilst at the end nearly 80% of the answers provided were correct (Fig. 45b). Company employed people were responsible for 77% of all the answers (Fig. 45) and at the start only 28% of these were correct (Fig. 45a) by the end the knowledge gained has almost doubled (Fig. 45b). Self employed people also nearly doubled their correct answers by the end of the workshop. The most improved group was the government employed participants.

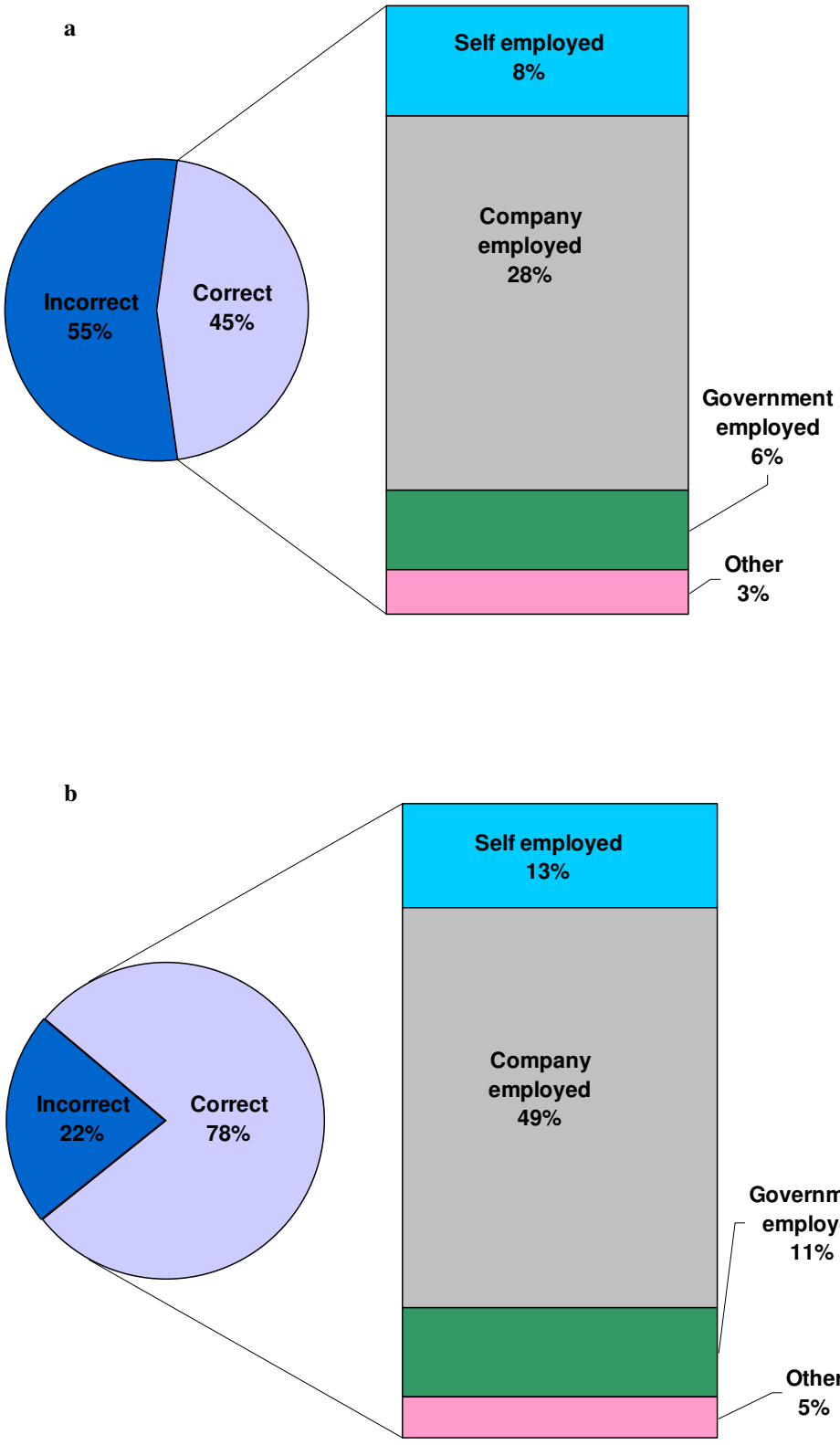


Fig. 45. Percentage of all correct and incorrect answers given by the participants at the start (a) and at the end (b) of the workshops

A further breakdown of individual professions showed similar trends with all participating professions gaining improved awareness (Fig. 46).

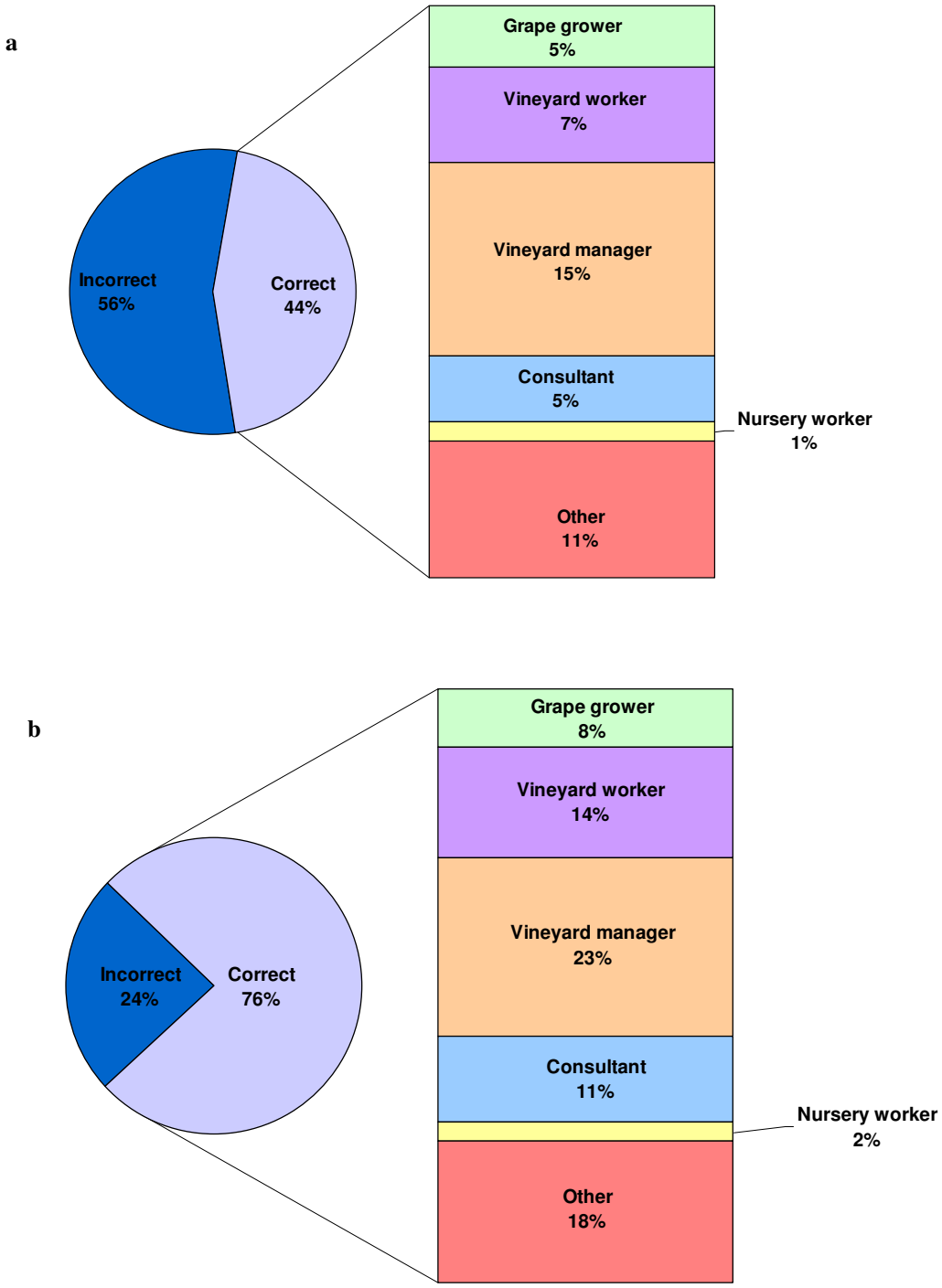


Fig. 46. Percentage of all correct and incorrect answers given by the participants (based on their employment status) at the start (a) and at the end (b) of the workshops

At the end of the workshop participants were asked if they would do anything differently after what they seen and heard when they go back to their own operations. Here are a few quotes from the individual sessions conducted:

Disinfestation and protocols:

"Revise phylloxera quarantine protocols and better implement the appropriate ones."

"Initiate cleaning protocols and some level of quarantine on our vineyards."

"Have tighter protocols in the vineyard."

"Change shoes between vineyards."

"Be more vigilant with quarantine protocols: already worry about boots and vehicles but didn't realise clothing is a risk."

"Have been operating in coordination with protocols already. More awareness is always good."

"Will pay more attention to the concentration of bleach solution and time spent in it. Make sure the solution is changed frequently."

"Tighter protocols re disinfestation of clothing and machinery and water collection."

"Have a second pair of boots with me."

"Purchase a footbath kit for myself when moving between vineyards."

"Clean boots more often and spend more time in the bleach bath."

"Better record keeping and stricter entry points in the vineyard."

"Enforce and encourage vineyard hygiene."

"Review and revise current site protocols."

Machinery and people:

"Update and increase preventive and treatment of visitors, workers and contractors."

"Be more diligent in people entering and machinery being used in the vineyard."

"Restrict people driving cars/machinery on the vineyard."

"Stricter entry protocols for winery staff and contractors."

"More phylloxera signage, examine all machinery entering the property."

"Sign in/out of contractors and better controls."

"I'll be more vigilant on any interstate people and winery staff, e.g. grape samples."

Detection:

"Be more aware of the signs of early effects of phylloxera in the vineyard."

"Inspect higher number of vines in the vineyard."

"Look for phylloxera in suspect spots within the vineyard."

"More vigilant in looking for phylloxera signs in the vineyard."

Relaying learned information:

"Provide more uptake information with respect to biology, control etc."

"Convey knowledge to fellow workers."

"More vigilant in advising people of potential risk."

"Talk to contract crew, try to educate them, give them more information during induction."

"Improve awareness of staff and contractors through training."

"Talk to management about any upcoming planting."

"Educate others in our area, try to influence my neighbours."

"Encourage other viti staff to attend in the future."

"More education/awareness for employees, contractors."

Miscellaneous quotes:

"Remove vines near buildings with public access.'

"Look at rootstocks for planting decisions."

"Improve records/procedures for contractors."

"Improve our sign in facilities to include a question about where the visitor has been."

"When planting vines in the future, explore the need to use rootstocks."

"Improve signage and implement sign in book."

National and International Presentations and Information Distribution:

Part of the education and awareness activities was to present poster and/or oral presentations at national and international conferences to scientific and industry audiences.

Presentations were made at the following conferences:

1. The 13th Australian Wine Industry Technical Conference, Adelaide, South Australia, July-August 2007.
2. The 4th International Phylloxera Symposium, Keszthely, Hungary, September 2007.
3. Australian Entomological Society 38th Annual General Meeting and Scientific Conference, Beechworth, Victoria, September 2007.
4. Symposium on Plant Interactions With Aphids. Wageningen University, Netherlands, 2008.
5. Australian Entomological Society 39th Annual General Meeting and Scientific Conference, Orange, New South Wales, September 2008.

Awards and recognition

The following awards where presented to the Phylloxera research team during the course of the project:

2006 - Team Finalist for the Daniel McAlpine Outstanding Achievement Award at the DPI Science Awards, Melbourne 2006.

2007 - Team Finalist and Winners of the Daniel McAlpine Outstanding Achievement Award at the DPI Science Awards, Melbourne 2007.



Conclusions

The workshop evaluation surveys demonstrate that phylloxera workshops are an effective way of increasing phylloxera awareness. Participants are ready to make changes to their practices in a way of changing their protocols, improve detection techniques or educate others.

Phylloxera extension and awareness activities are very important part of phylloxera management. During the three years a higher number of participants than previous years came to these activities, representing over 400 participants. In recent years the number of phylloxera outbreaks within Victoria has increased and this shows the need to educate more viticulture and oenology industry people on phylloxera management. The challenge is to reach more and more people each year, not only people within the industry but those with linkages to it.

Annual phylloxera workshops, industry and media articles all highlighted phylloxera awareness and latest research outputs to a range of stakeholders in the Australian viticulture

industry. Conference presentations, participation in and organisation of the 4th International Phylloxera Symposium in Hungary and the International Congress of Entomology in South Africa complimented both research and extension activities of the project. The extension activities played a key role in the project and increased industry and researchers' awareness of phylloxera research highlights, conducted in Australia, both nationally and internationally. The effectiveness of national phylloxera extension activities was assessed at a workshop level through workshop evaluation clearly demonstrated an improved awareness of phylloxera management issues.

Recommendations

The steady number of participants to the phylloxera education and awareness activities shows that there is the need to continue with these activities. Recent phylloxera outbreaks in the Yarra valley, Lancefield and Porepunkah highlighted the fact that more needs to be done in phylloxera awareness. One of the options is to deliver more phylloxera education activities such as the phylloxera workshops. Flexibility in workshop delivery is a key to effective education and awareness. Instead of asking the participants to come to a phylloxera infested area, there is a possibility to change the format to a "roadshow" which can be taken closer to the target audience but this makes delivery of the detection component more challenging. Tailor made workshops in not infested regions would not have the hands on component but could take into account region specific issues.

APPENDIX 1: COMMUNICATIONS

Oral presentations conducted during the course of the project included:

1. Triphasic screening of cultivated grapevines *Vitis* species for resistance to grapevine phylloxera *Daktulosphaira vitifoliae.* KS Powell, GA Korosi, CJ Trethowan & AA Hoffmann 13th International Congress of Entomology. 06-12 July 2008, Durban (South Africa).
2. Development of sustainable phylloxera resistant rootstock recommendations for Australian conditions based on triphasic screening using genetically distinct clonal lineages of *Daktulosphaira vitifoliae.* K.S. Powell, G.A. Korosi & C.J. Trethowan. Second National Rootstock Workshop Merbein, May 2008.
3. Comparative life-stage feeding in the Phylloxeridae family. K. Kingston, K Powell & P. Cooper. Symposium on Plant Interactions With Aphids. Wageningen University, Netherlands 18-21 August, 2008.

Selected poster presentations conducted during the course of the project included:

4. Are we winning the battle with Phylloxera? CJ Trethowan, G Korosi, PR Clingeleffer & KS Powell. Australian Entomological Society 39th Annual General Meeting & Scientific Conference. 28 Sept.-01 Oct. 2008, Orange (NSW).
5. Glasshouse rootstock screening for grape phylloxera resistance. Korosi, GA., CJ. Trethowan & KS. Powell Australian Entomological Society 39th Annual General Meeting & Scientific Conference. 28 Sept.-01 Oct. 2008, Orange (NSW).

Involvement with Working groups/Reference groups:

1. Technical Adviser to National Phylloxera Technical Reference group).

2. Entomology Adviser to Plant Health Australia on Industry Biosecurity Group reviewing and revising the National Viticulture Industry Biosecurity Plan.

Referred publications

Herbert, K.S., A.A. Hoffman, Powell., K.S. et al. 2006. "Changes in grape phylloxera abundance in ungrafted vineyards." J Econ Ento **99**(5): 1774-1783.

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APPENDIX 2: INTELECTUAL PROPERTY

APPENDIX 3:BIBLIOGRAPHY

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APPENDIX 4: STAFF

Project Activities

The projects core activities were carried out by DPI-Rutherglen staff. Phylloxera and *Vitis* genotyping was conduct by staff at CESAR under the supervision of Professor Ary Hoffman. The project activities were carried out in close collaboration with local growers in North east Victoria, the Phylloxera and Grape Industry Board of South Australia, the National Phylloxera Technical Reference Group and CSIRO Merbein.

Project Staff

DPI-Rutherglen staff involved in the project activities included Dr Kevin Powell (Project Leader), Ginger Korosi, and Carolyn Trethowan.
CESAR staff involved in genotyping work included Vanessa White.

Adherence to Quarantine Protocols

All fieldwork was carried out under permits that specified procedures and disinfestation protocols designed to prevent the spread of phylloxera by project staff. Field visits were arranged in consultation with vineyard managers. All field equipment, clothing and footwear was cleaned and disinfested after visiting infested vineyards. Field collected samples were stored and transported in sealed containers under permit from the vineyard to laboratory facilities at DPI Rutherglen Centre. All field collected samples were processed in laboratory facilities at DPI Rutherglen Centre which is located within the North-East Victoria Phylloxera Infested Zone (PIZ). Insect and plant materials which required genetic characterisation were transferred under quarantine regulations under permit issued by Plant Standards-Victoria.

Acknowledgments

The phylloxera research and extension team would like to express their gratitude to all growers who have allowed trials to be conducted on their properties.

APPENDIX 5: BUDGET RECONCILIATION