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SUSTAINABLE LONG TERM STRATEGIES FOR PHYLLOXERA MANAGEMENT UNDER AUSTRALIAN CONDITIONS



FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **DNR 03/03**

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Research Organisation: **DPI-Rutherglen**

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Sustainable long-term strategies for phylloxera management under Australian conditions

A final report on project DNR 03/03

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Abstract

The overall aim of this research project was to focus on the most appropriate management strategies available to minimise the risk of new phylloxera infestations and where infestations do occur ensure appropriate strategies are utilised to ensure their containment. New developments during the project period included a comparative analysis of screening protocols for phylloxera resistance to rootstocks, phylloxera genotype-specific risk assessments, risk assessments for use of cultural methods in the form of composted green waste and marc formulation. Awareness of industry and the scientific community was enhanced through facilitation and participation in National Phylloxera Management Workshops, facilitation and coordination of an International Grapevine Phylloxera Symposium, oral and poster presentations given at National and International conferences and the project leaders participation (as Technical Adviser) in the National Phylloxera Technical Reference Group meetings. Existing and new research linkages were developed and enhanced with phylloxera research groups in Europe (University of Hohenheim & University of Vienna) and the USA (UC Davis).

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 2003-June 2006. The outputs of the project have resulted in an increased scientific knowledge of grapevine phylloxera, which has been passed on to industry and researchers at all levels. The research activities have allowed the development of improved management practices through a detailed understanding of the population dynamics and dispersal characteristics of phylloxera genetic lineages. This has enabled the identification of risk periods and zones within phylloxera-infested vineyards in the Glenrowan, Buckland Valley, Nagambie and Rutherglen. Phylloxera awareness activities have been significantly enhanced during the course of the projects through:

- A variety of extension activities including workshops, field days, seminars, industry and media articles and the Third International Grapevine Phylloxera Symposium.

- 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.

- Training for growers in the early detection and identification of field symptoms of phylloxera infestations 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.

- Development of closer international collaborative links, particularly with the University of California, Davis, University of Vienna and University of Hohenheim ensured that research and development was of a high international standard with a global perspective and avoided the duplication of research pathways.

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

- Improved protocols for screening of phylloxera performance on rootstocks under controlled environment conditions.

- Monitoring of a geographically restricted phylloxera genetic strain detected outside the existing quarantine zones during the course of the project.

Introduction

Project DNR 03/03 – **Sustainable long-term strategies for phylloxera management under Australian conditions**

Time frame: *July 2003 to June 2006*

This is a report on phylloxera research, development and extension activities carried out from July 2003 to June 2006. The work was funded by the Grape and Wine Research and Development Corporation (GWRDC Project DNR 03/03), 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. Ensure effectiveness of phylloxera quarantine protocols through scientific validation of risk factors and disinfestation techniques.

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:

1. Determining the potential natural dispersal of phylloxera within and between vineyards in five grape-growing regions (Rutherglen, Nagambie, King Valley, Buckland Valley and Glenrowan) located in four distinct phylloxera infestation zones (PIZs; Northeast, Nagambie, King Valley and Alpine). This included studies of (below ground) root and above-ground populations of phylloxera, especially in regard to the number and survival of dispersive stages and genotype interactions during the spring, summer and autumn on both grafted and ungrafted grapevines.

2. Quantifying risks of transfer through harvesting operations and dispatch of diagnostic samples. This involved sampling of phylloxera at several new field sites using emergence traps and techniques developed and optimised by the DPI-Rutherglen Research team in a previous GWRDC-funded research project "Targeted Phylloxera Management Options".

3. Facilitating and providing technical support for the validation, development and implementation of National Phylloxera Management Protocols. Provision of scientific support, as technical adviser to the National Phylloxera Technical Reference Group. In addition, assistance was provided to grower groups to develop protocols appropriate to their perceived degree of risk. In particular outputs from this project contributed to the industry developed 5-Year Phylloxera Research and Development Plan.

4. Providing advice on rootstock management through rigorous evaluation of rootstocks, for phylloxera control, under controlled laboratory, glasshouse and field conditions. To develop sustainable rootstock recommendations a range of phylloxera clonal lineages where screened.

5. Testing new approaches to control of phylloxera on ungrafted vines, so that grape-growers are provided with alternative options for control of phylloxera. This involved testing of effects of composted green waste and composted marc formulations under commercial growing conditions.

6. Gathering data on soil characteristics at known phylloxera-infested sites to assist in development of a risk management approach to phylloxera infestation.

7. Monitoring of new phylloxera outbreaks and provided growers in affected regions with updated management protocols.

Project Aims and Outputs

The project objectives and outputs achieved are summarised as follows:

Objectives	**Outputs**
Knowledge transfer via National Phylloxera	*Completion of four Phylloxera Workshops per year*
Data to validate rootstock performance in established trials as related to phylloxera genetics	*Analysis of data on phylloxera genotype geographic location, diversity and seasonal abundance at 3 field sites completed over 3 consecutive seasons.* *Information on the population dynamics of phylloxera genotypes on grafted and ungrafted grapevines, under field conditions in 3 PIZ regions*
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. A range of new/conventional rootstock hybrids screened against 3 phylloxera genotypes.*
Information on immune rootstock under Australian conditions	*Establishment and field grafting at three field sites in Northeast Victoria to enable long-term comparison of phylloxera genotype behaviour on 'resistant' and 'immune rootstocks under Australian growing conditions.*
Scientific validation of influence of soil characteristics on phylloxera clones	*Soil characterisation at five field sites where know phylloxera lineages occur.*
Information on influence of organic management on phylloxera populations/damage	*Three-year studies on influence of composted marc and composted green waste completed. Impact of mulch applications on risks etc defined. Draft recommendations on use of composted marc and composted green waste as interim management options.*
Published articles on phylloxera management	*One published scientific article and 2 industry articles.*
Development, transfer and adoption of research outputs.	*Thirteen workshops presented and facilitated and 200 growers trained in phylloxera management and identification.* *Provided technical advice at all National Phylloxera Technical Reference Group meetings and contributed to the development of 5-Year Phylloxera R & D plan.* *Facilitated and participated in industry and scientific forums including 3rd International Phylloxera Symposium, Cool Climate Symposium and AWITC.*
Detailed distribution maps of phylloxera clones in selected NE Victoria vineyards	*Distribution maps of clones in PIZ regions under development*
Information on influence of fungal pathogens potentially associated with phylloxera damage	*Survey of two infested vineyards completed and published in industry and scientific journals.*

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 the Buckland Valley in 2003 a field-based study was conducted over one grapevine growing season (2004-2005) to monitor the population dynamics, risk of dispersal and impact on ungrafted *Vitis vinifera* L. cv. Pinot Noir grapevine performance of the root feeding grape phylloxera genotype G20. Phylloxera emergence at the soil surface was relatively low compared with previous field studies of more geographically widespread genotypes. Phylloxera significantly reduced grape yields but not grapevine quality. The implications of these results for phylloxera management on ungrafted *V. vinifera* grapevines in phylloxera-infested quarantine regions is discussed.

Introduction

In December 2003 grapevine phylloxera was detected in a commercial vineyard outside of existing quarantine zones and a temporary 5km quarantine zone was declared in the Buckland Valley region. All vineyards in and around this region were surveyed by Plant Standards – Victoria staff and in January 2004 a second vineyard was found to be infested. This surveyed region was later incorporated into an extension of the North East Phylloxera Infested Zone (PIZ) This was the first phylloxera outbreak outside a PIZ since the establishment of the Upton PIZ in April 2000 (Powell, 2003). The occurrence of the Buckland Valley infestation, provided a unique opportunity to monitor (i) the population dynamics of a phylloxera genotype with limited geographic distribution (ii) the rate of spread of phylloxera in a relatively newly-infested ungrafted vineyard and (iii) estimate the relative risks of phylloxera transfer. The same site was also studied as a component of the CRCV project S2.2.5 *Early detection of phylloxera in grapevines (Vitis vinifera) a test bed for spectro-optical and chemical fingerprinting of pest-induced grapevine stress which has been reported previously* (Powell et al., 2006a).

Methods

Site Details

The study site was located in a commercial vineyard near Porepunkah, located close to the Buckland River within 20 kilometres of the boundaries of the North East and King Valley PIZs. Phylloxera was first detected in the vineyard in December 2003. Shortly after detection a preliminary 5km buffer zone (Fig. 1) was enforced followed declaration of the Alpine Valley quarantine zone (Fig. 2) which later became amalgamated into an expanded North East PIZ (Fig. 3). The phylloxera genotype within the study block was characterised using microsatellite markers (see Chapter 5), as G20 which is one of the least common genotypes found in Australia (Corrie et al., 2003) but is one of the six clonal lineages recommended for further studies (Hoffman, 2003). A soil survey was conducted at the study site (see Chapter 7) and the soil classified as an acidic mesotrophic red dermosol (Isbell, 1996).

Experimental Design

The vineyard block, chosen for the study, consisted of a block of 64 rows of ungrafted *V. vinifera* L. cv. Pinot Noir planted in the 1990s. In each of five parallel rows there were five 'monitor vines', therefore a total of 25 infested vines, in parallel panels, selected to monitor above-ground phylloxera dispersal and below-ground phylloxera population dynamics.

Phylloxera Monitoring

To monitor above ground movement an emergence trap was placed adjacent to the vine trunk of each monitor vine. The methodology for emergence trapping is described in Chapter 4. The seasonal abundance of phylloxera dispersive stages moving from below the ground onto the soil surface was measured, over one growing season commencing October 2004 and finishing in May 2005.

Phylloxera Survey

A systematic root survey across and beyond the perimeter of the whole experimental block was conducted in January 2005, where roots of every second vine in every second row, were visually inspected for presence or absence of root galls and/or phylloxera. A visual assessment of vine vigour was also made classifying vines as high, medium or low canopy vigour (Fig. 4).

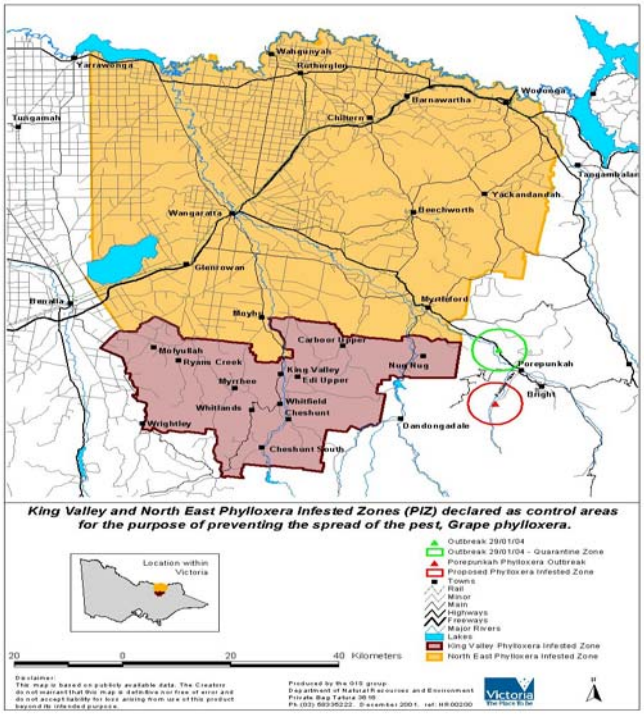


Figure 1. Location of phylloxera outbreaks in Buckland Valley region 5km temporary Phylloxera Infested Zones declared in the Buckland Valley (red circle) and Eurobin (green circle).

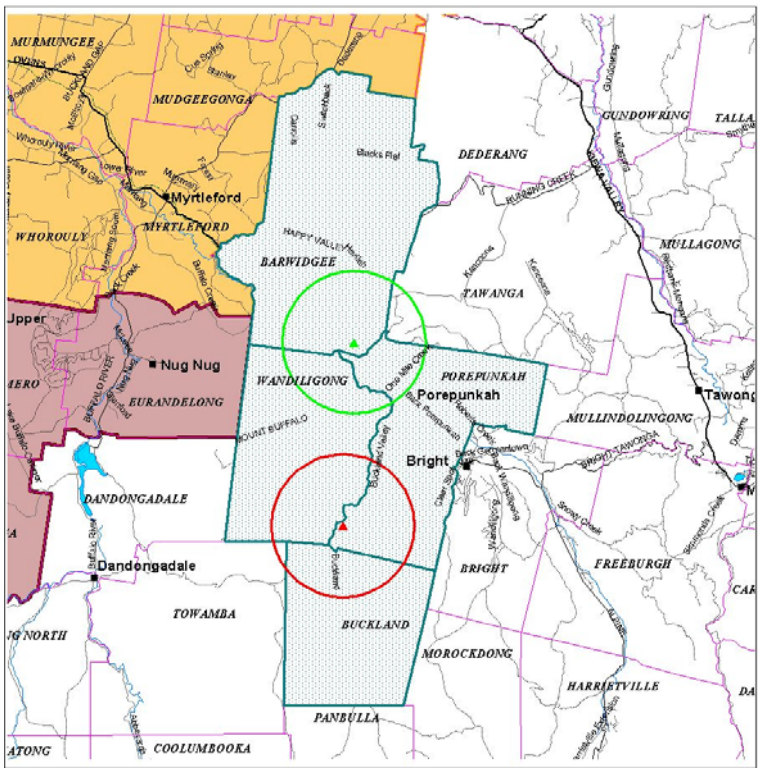


Figure 2. Alpine Valley Phylloxera Infested Zone

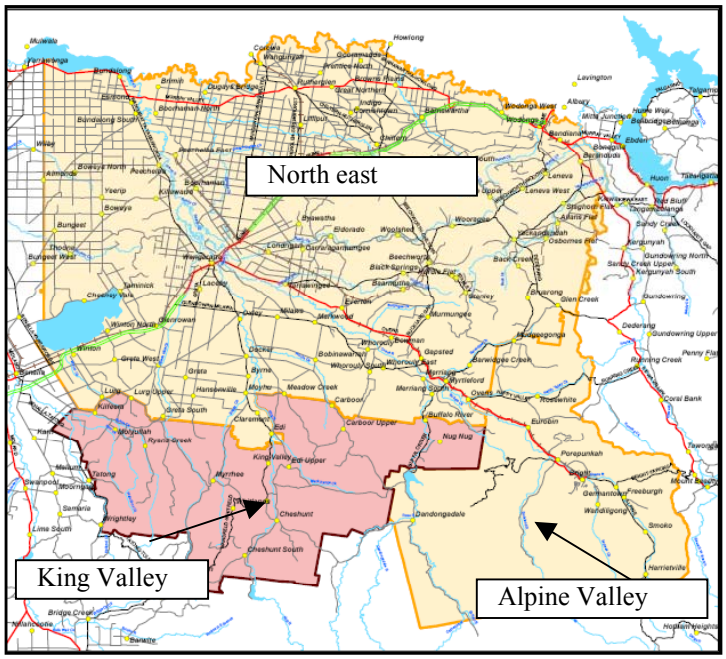


Figure 3. North East Phylloxera Infested Zone, incorporating Alpine Valley PIZ, ajacent to King Valley PIZ



Figure 4. Low and high vigour vines at Buckland Valley field site

Grapevine Performance

Phylloxera causes direct damage to the root system of ungrafted *V. vinifera* grapevines. The damage to the root system eventually results in loss of or reduction in root function and gradual decline in vine health and performance. It may also impact on the quality of the final product (juice or wine). To quantify this impact yield, quality and vigour assessments (as described in Chapter 4) were conducted at this study site at harvest (yield and quality) and post-harvest (pruning) in March and May 2005 respectively. Twenty-five 'monitor' infested vines and twelve uninfested 'control' grapevines were selected for harvest and pruning assessments. All 'monitor' and control vines were hand-harvested on 9th March 2005. The 'trial monitor' vines were spur pruned according to grower specifications and weighed on-site using portable scales on 31st May. Between season monitoring of grapevine performance could not be conducted as the grower removed the infested vines after pruning in winter 2005 and replaced with grafted vines.

Statistical Analysis

All trial data was analysed using GenStat version 8.1 software (Payne et al., 2006) with Analysis of Variance (ANOVA). Least significant differences (LSD) were used to calculate significant differences ($p=0.05$).

Results

Phylloxera Emergence

Phylloxera crawlers were monitored from 20th October 2004 until 9th May 2005 (Fig. 5). Because phylloxera is not active above-ground during the July-September period (Powell et al., 2003) no sampling was conducted during the winter months. During the season, crawlers were first detected in emergence traps in mid-December, peaked in abundance in mid-January with a mean of over 100 crawlers per trap and were still detectable in early May. At harvest (March) although still present above-ground crawler numbers were relatively low (mean of <20 crawlers per trap) but some individual vines still had over 100 crawlers per trap during harvest (data not presented).

Grapevine Performance

The data for the 2005 vintage were collected and analysed as shown in Table 1. For phylloxera-infested grapevines mean yield, mean bunch weight, mean berry weight and pruning weights were significantly lower than uninfested vines. Quality parameters, as assessed by pH and Brix, were not significantly affected.

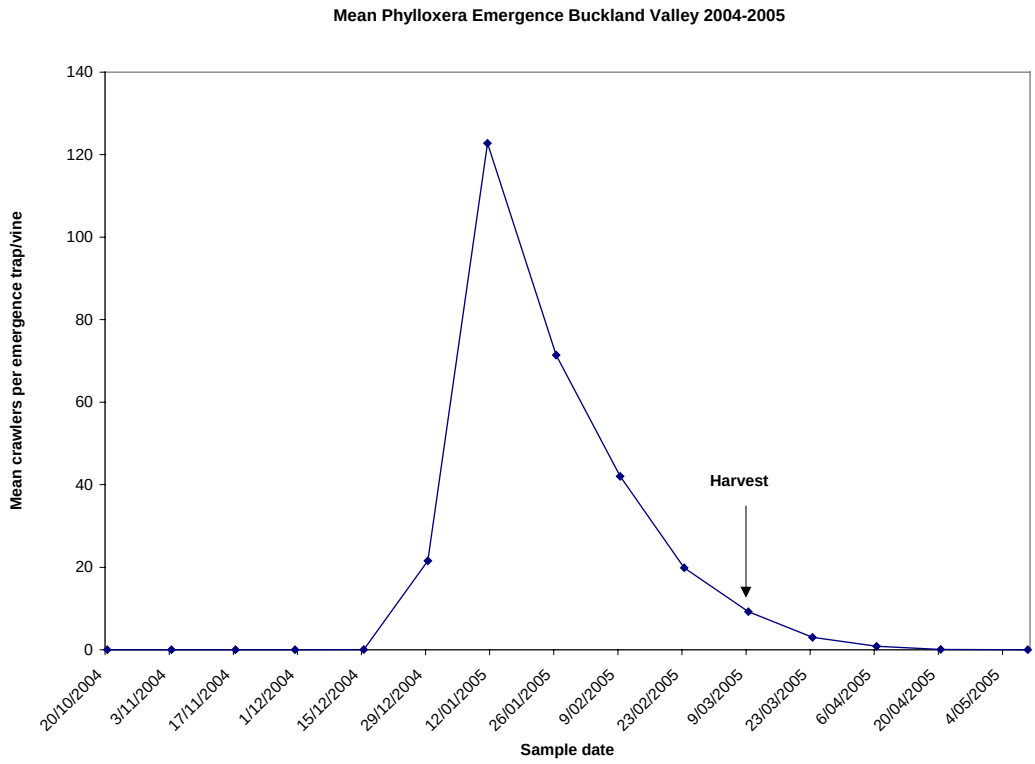


Figure 5. Phylloxera crawlers collected in emergence traps at the base of V. vinifera cv. Pinot Noir grapevines, at the Buckland Valley field site, over the 2004-2005 growing season, where n = 25 vines.

Table 1. A comparison of harvest and pruning data, collected March and May 2005 respectively, from phylloxera-infested and uninfested grapevines in a block of ungrafted *V. vinifera* cv. Pinot Noir at the Buckland Valley field site.

Phylloxera Status	Mean bunch number per vine	Mean yield per vine (kg)	Mean bunch weight (g)	Mean berry weight (g)	Mean Brix (º)	Mean pH	Mean pruning weight (kg)
Infested	106.4	11.93	110.8	1.23	20.55	3.05	1.02
Uninfested	125.5	15.52	124.7	1.32	20.15	3.06	1.74
$LSD_{0.05}$	22.0	2.94	12.93	0.08	0.98	0.05	0.36
Significance	NS	*	*	*	NS	NS	*

Least significant differences (LSD) can be used to compare significant differences between means within the column. When the difference between the means is greater than the LSD the treatments are statistically different (at the 95% confidence level), n=25 (infested) and 12 (uninfested), * = significant difference and NS = non-significance.

Phylloxera Survey

A phylloxera survey conducted on 14th January 2005, one year after the initial survey of the site covering 64 vine rows with up to 169 vines per row. The survey examined every second vine in every second row and as such was more intensive. It showed the main site of infection which was initially thought to be 10 rows had extended to include 24 rows with two satellite infections

appearing to the east and west of the original detection site. The remaining 40 rows of the block were found not to be infested.

Conclusion

This is the first time that the phylloxera strain G20 has been monitored under field conditions on ungrafted *V. vinifera.* A relatively low population abundance, as assessed by above-ground trapping, was recorded, in comparison with earlier studies conducted on more geographically widespread genotypes in the King Valley (Powell et al., 2000, 2006; Herbert et al., 2006a), Nagambie (Powell, 2003) and Upton regions (Powell et al., 2003; Herbert et al., 2006a), which indicates three possible scenarios. Firstly the genotype G20 has relatively low virulence on *V. vinifera* compared with the so called 'superclones' G1 and G4 which are those studied in Nagambie/Upton and the King Valley respectively. Both laboratory and glasshouse, presented in Chapters 2 and 3 respectively, support this hypothesis. Relatively low virulence and low fecundity (egg production), of G20 on *V. vinifera* cv. Shiraz has been recorded in controlled trials compared to G1 and G4 on *V. vinifera.* Earlier studies (Viduka et al., 2003) using excised roots have also shown that G20 has low virulence on *V. vinifera* cv. Sultana compared to G1 and G4. Interestingly the nodosities observed on the infested roots in December 2003 at the Buckland Valley site did appear physically smaller in comparison with those observed in G1 and G4 induced nodosities observed in the field at other sites (K. Powell pers. observation) which also suggests that less root damage occurred. The level of root damage due to G20, in comparison to G1 and G4 induced tuberosities, although not assessed at this site was reduced in controlled glasshouse studies (Chapter 3) which suggests a different level of physiological response by the host plant. This would impact on above ground vine performance parameters such as yield and vigour.

Results from detection studies conducted under field conditions at the Buckland valley site (Powell et al., 2006a) indicated a reduced physiological response from G20 infested vines compared to more virulent phylloxera genetic strains, G1 and G4. Another reason why population abundance may have been low is that phylloxera may have been detected earlier at the Buckland Valley site than in other sites where population studies have been conducted. Alternatively due to lower virulence of this genotype visual symptoms in the canopy may have taken longer to be expressed. Both chemical and spectral studies conducted at the Buckland valley site indicate that differences in spectral and chemical characteristics and photo-protective pigments of the leaves could be detected between infested and uninfested vines (Renzullo et al., 2006; Blanchfield et al., 2006; Tucker et al., 2006). However, it is likely that the detection at the site was 'early' as it was restricted to a relatively small patch within the vineyard block in January 2004. Whereas in the following season after an extensive ground survey was conducted in January 2005 'satellite spots' were evident indicating that the insect had clearly moved beyond the original site of detection. It is difficult to pinpoint when phylloxera was first introduced to the field site.

Finally both soil (Chapter 7) and climate characteristics may also play a role in the level of both infestation and rate of spread at the study site and further research is being conducted at the site to be published at a later date.

Despite the relatively low virulence and abundance of the G20 strain phylloxera has nonetheless affected vine performance causing reduced grape yield, berry weight and pruning weight but not impacted on quality as assessed by Brix and pH. Unfortunately a between- season comparison could not be made as the area was replanted to grafted vines in Autumn 2005. Whilst phylloxera infestation may have contributed to the decline in vine vigour/biomass (as measured by the production of canes) and yield attributes. Seasonal effects may also have played a significant role at the site. In particular drought and local bush fires causing significant smoke taint to the vines at this site in the preceding season which could have impacted on vine performance making the infested vines more stressed and more susceptible to phylloxera attack. Other variables which could impact on phylloxera abundance and grapevine health at the site are site related factors such as soil conditions and climate.

Recommendations

This was is the first field study to show the population dynamics of the phylloxera genotype G20 on ungrafted *V. vinifera* cv. Pinot Noir over a full growing season and is the first ever phylloxera study to be conducted in the cool climate region of the Buckland Valley. It is important to study the impact of less commonly distributed phylloxera clonal lineages like G20 so that growers can modify their outbreak management strategies accordingly for these less virulent genotypes, reduce their economic impact and reduce the risk of transfer both within and outside the infested block. It also important to study the behaviour of this genotype under controlled conditions on resistant rootstocks and this is highlighted further in Chapters 2 and 3. Based on limited data to-date the following preliminary recommendations are made when dealing with the G20 phylloxera genotype:

- Take into account climatic conditions. In this study drought in the season prior to monitoring may have impacted on vine health, which could have also impacted on phylloxera interactions with the vine. The phylloxera was detected in December 2003 and this may have been due to higher stress on the vines in the preceding summer.

- Take into account when peaks of phylloxera abundance occur above-ground and minimise vehicle and personnel movement during this period to avoid transferring phylloxera to uninfested grapevine blocks. 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 representing a significant risk of transfer.

- Monitoring of the phylloxera strain G20 on rootstocks in the field is recommended, as it is apparent from laboratory and glasshouse studies (Chapters 2 and 3) that this clone can survive and reproduce on some rootstocks (in particular Schwarzmann). No field studies on rootstocks infested with G20 have yet been conducted but in a field survey G20 has been detected on Schwarzmann (Corrie et al., 2003).

- Monitor new outbreaks of phylloxera as they occur, so that informed decision-making allows the affected grower to optimise management strategies based on site variables and phylloxera genetics.

Chapter 2. Screening Rootstocks *In Vitro* for Phylloxera Resistance

Objectives:

- *Validation and development of laboratory rootstock screening protocols*
- *Screening of phylloxera performance on common and novel rootstock hybrids*

Summary

An excised root bioassay protocol was used to compare the relative performance of selected phylloxera genetic strains on commercially available and newly developed rootstock hybrids. The technique, which is routinely used for phylloxera screening, showed that the level of virulence and development of different strains was affected by rootstocks. The rootstocks were significantly more resistant to all tested strains of phylloxera compared with ungrafted *Vitis vinifera* vines, as shown by reduced population development on all rootstock's. Phylloxera development was measured on mature lignified roots only. Schwarzmann, 5BB Kober and Börner rootstocks were the most resistant. Some phylloxera strains such as G4 developed to the adult stage on both Ramsey and 110 Richter but still developed at significantly reduced levels compared to *V. vinifera* roots. This protocol allows screening of rootstocks to individual phylloxera genetic strains over a relatively short time period of two to eight weeks. However, quality of root material can influence the results and exclude the whole plant response. It is recommended that whilst excised root bioassays are a useful preliminary screen ultimately whole plant rootstock screening in the form of glasshouse and field trials is recommended.

Introduction

Phylloxera has been present in Australia for over 130 years. In that time, the use of rootstocks with resistance to phylloxera has increased particularly in Phylloxera Infested Zones (PIZ) but only marginally outside PIZs. Still 85% of vines in Australia are still planted to ungrafted highly susceptible European grapevines (*Vitis vinifera* L.). Despite realistically being the only long-term option for phylloxera management there are several major grape-growing regions where rootstock uptake is low including major viticultural areas in South Australia, Western Australia, New South Wales and Victoria.

Most rootstocks used today have been screened for phylloxera resistance overseas, under different conditions to those observed in Australia. Australia has at least 83 different phylloxera genotypes (Herbert et al., 2006b), some of which have different virulence levels on different vine species (Powell 2003, Viduka et al., 2003, Korosi et al., 2006). Therefore commercially available and new rootstock hybrids being developed for use need to be screened against phylloxera genotypes found in Australia.

Access to phylloxera genotypes, the facilities and expertise for rootstock screening under quarantine conditions and the location of the DPI-Rutherglen in the centre of the North East Phylloxera Infested Zone (PIZ), provide the opportunity for rootstock screening to be conducted under controlled environment conditions.

A variety of screening techniques have been used to assess resistance to phylloxera including *in vitro* bioassays, such as the use of tissue culture plants (Grzegorczyk & Walker, 1998; Kellow et al., 2002) and excised roots (Granett et al., 1987: Powell, 2003). Before rootstocks can be screened for field resistance to phylloxera they need to be evaluated using different genotypes of phylloxera under controlled conditions in the laboratory. A range of new hybrid rootstocks and conventionally used rootstocks were screened for resistance to up to three phylloxera genotypes using the excised root bioassay system.

Methods

Plant and Insect Material

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

Three phylloxera genotypes designated, G1, G4 and G20 were used in the trials and collected from single source vineyards in the Nagambie, King Valley and Buckland valley regions respectively. Prior to the experiment, populations of each genotype were maintained separately on excised *V. vinifera* cv. Shiraz roots in a dark controlled environment growth room (25±2°C) at DPI-Rutherglen Centre. The phylloxera populations 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) by CESAR, Melbourne University (see Chapter 5).

Excised Root Bioassay Procedure

All bioassays were conducted on excised grapevine root pieces based on a modified method of Granett et al. (1987). Three bioassays carried out in during the project period are reported here. An additional two bioassays were conducted but are not reported as the root quality was either very poor and did not support good control populations or the genetic identity of some rootstocks could not be verified.

A total of seven new rootstock hybrids, supplied by CSIRO Merbein, and five commercially available rootstocks were screened. In each bioassay four to seven rootstock treatments and up to three phylloxera genetic strains were used, depending on availability of root material and phylloxera clonal lineages. All root material, 10-15 mm in diameter, were cut into 60-70 mm long sections. They were washed with sterile distilled water and dipped into 7.3g Ridomil/3L water to deter fungal attack and rinsed three times in ultra pure water to ensure no excess fungicide was left

on the root piece. The proximal end of the blotted dry sections were wrapped with cotton wool, moistened with sterile water and placed in plastic petri-dishes lined with moistened filter paper.

Treatments (ie. rootstocks) and controls were replicated 7-10 times depending on the availability of sufficient root material for each of the three bioassays. One-two root pieces from the same rootstock or control were placed in a 140 x 25mm petri-dish lined with filter paper. The control consisted of either *V. vinifera* cv. Sultana (Bioassay 1 and 2) or *V. vinifera* cv. Shiraz (Bioassay 3), which are highly susceptible to phylloxera and support large populations *in vitro* and demonstrates classic vine decline symptoms *in planta*.

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 $23^{o}C \pm 2^{o}C$. After the phylloxera eggs had been introduced to the root pieces; the rate of phylloxera development was assessed at weekly intervals by counting of individual life-stages using a lower power binocular microscope. For each phylloxera strains initial infestation and screening occurred on three separate consecutive days to prevent cross-contamination.

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 8.1 for Windows software (Payne et al., 2006). 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 1, which was conducted over a six-week period, the performance of the phylloxera strain G4 was compared on three rootstocks, Ramsey, Schwarzmann and Börner and compared to the control *V. vinifera* cv. Sultana (Fig. 6). On the rootstock Börner phylloxera did not develop beyond the crawler stage indicating a highly resistant response. On the rootstock Schwarzmann phylloxera did not develop beyond the intermediate stage and by week four of the trial 100% mortality was achieved indicating a highly tolerant response. On Ramsey G4 developed to the adult stage and began producing eggs by week four. On *V. vinifera* all life-stages were observed on control root pieces, indicating a highly susceptible response, and by week four due to high phylloxera numbers on controls counting was discontinued. All rootstocks screened had significantly lower phylloxera levels ($P<0.05$) than the *V. vinifera* control (Fig. 6), and hence an improved level of tolerance to phylloxera. Significant differences were observed when comparing rootstocks and controls, at different sampling points (data not presented). Overall phylloxera developed relatively poorly on both Schwarzmann and Börner when compared to both Ramsey and the control. Development on Ramsey was delayed and significantly lower abundance (excluding

eggs) was recorded in the first two weeks of the bioassay compared to the control *V. vinifera*. Egg production overall was significantly higher on control roots after four weeks compared to Ramsey and no eggs were produced on Börner or Schwarzmann (Fig. 7).

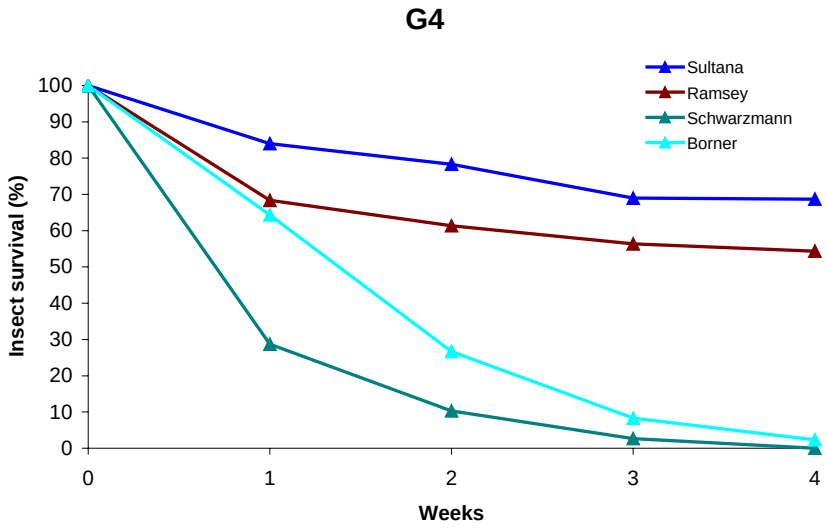


Figure 6. Excised bioassay one: comparison of phylloxera strain G4 survival on rootstocks Ramsey, Schwarzmann and *V. vinifera* cv. Sultana

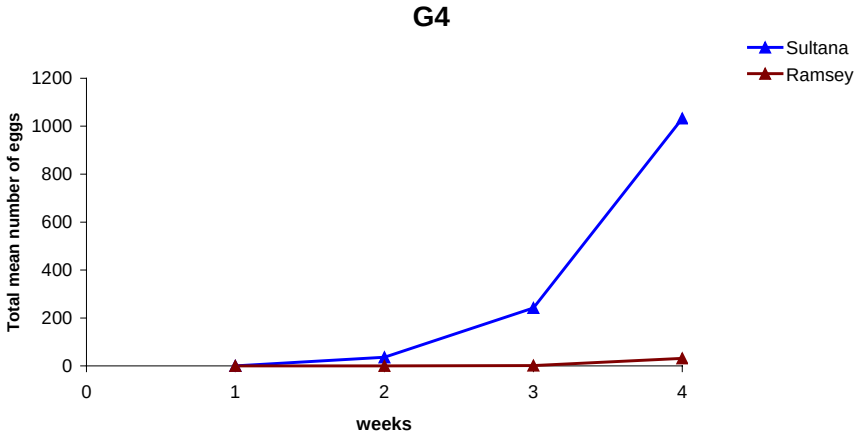


Figure 7. Excised bioassay one: comparison of phylloxera strain G4 egg production on rootstocks Ramsey and *V. vinifera* cv. Sultana

Bioassay Two

Five *Vitis* rootstock hybrids developed by CSIRO Merbein were screened to assess the relative performance of two known virulent phylloxera strains G1 and G4 in comparison with *V. vinifera*. All rootstocks screened had lower phylloxera levels, for both strains tested G4 (Fig. 8) and G1 (Fig. 9), than the *V. vinifera* cv. Sultana and hence an improved level of tolerance to phylloxera. No phylloxera developed beyond the crawler stage on any of the rootstock hybrids. However,

unfortunately the quality of the root material for the control vines meant that development and survival of phylloxera on *V. vinifera* was lower than observed in other trials thus highlighting the importance of excised root quality when conducted such screening methods. All *V. vinifera* root material was sourced from plantings at CSIRO Merbein. Time of collection and management of the vines prior to root excision could have influenced the root quality.

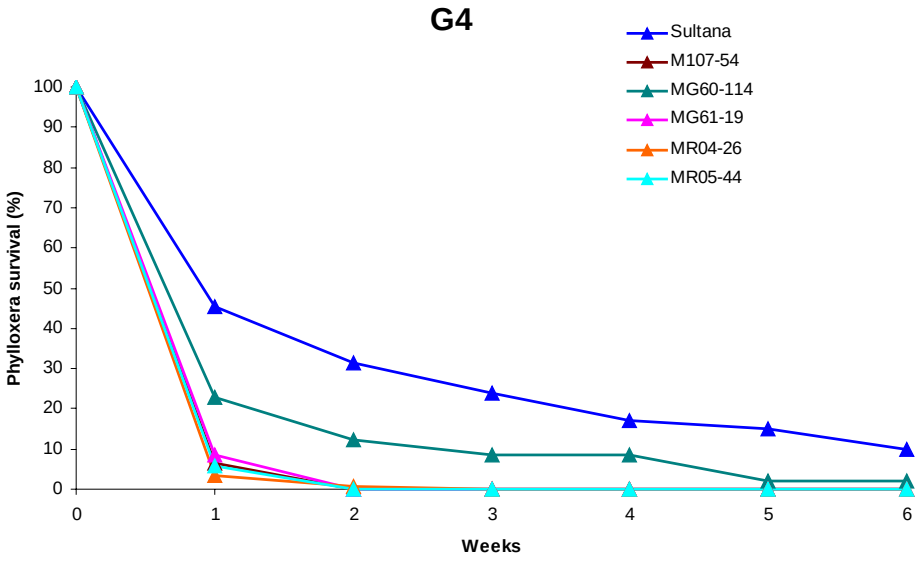


Figure 8. Excised bioassay two: comparison of phylloxera strain G4 survival on five newly developed rootstock hybrids and *V. vinifera* cv. Sultana

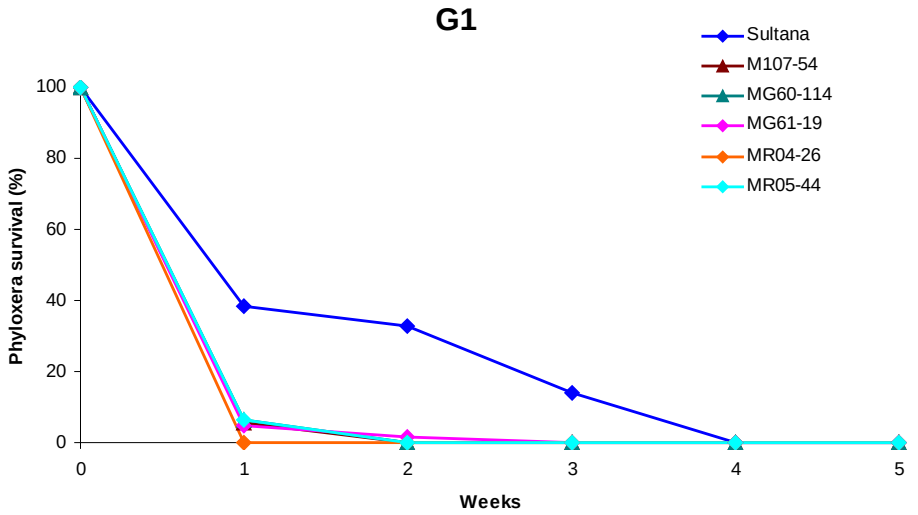


Figure 9. Excised bioassay two: comparison of phylloxera strain G1 survival on five newly developed rootstock hybrids and *V. vinifera* cv. Sultana

Bioassay Three

In bioassay three, due to poor performance of control root material in the previous bioassay, *V. vinifera* cv. Shiraz was used for control roots. In this bioassay survival of all phylloxera genotypes was significantly higher on controls compared to all rootstocks allowing the trial to continue over an eight-week period (Fig. 10). All rootstocks screened had significantly lower phylloxera abundance for all three phylloxera genetic strains than *V. vinifera*. Survival duration and development differed between genetic strains and rootstocks. Crawlers, eggs and adults of strains G4, G1 and G20 developed on *V. vinifera* control suggesting a high level of susceptibility of *V. vinifera* to all three phylloxera strains. Significant differences in phylloxera strain performance on *V. vinifera* were observed with G20 having significantly lower numbers of insects and lower egg production than both G4 and G1. This indicates a lower overall level of virulence for the G20 clone on *V. vinifera.*

G4 survived for up to and including eight weeks on the control but less than 2 weeks on Ramsey and Schwarzmann (10a). Strain G4 did not develop beyond the crawler stage on Börner, Ramsey, Schwarzmann, M920 or 5BB Kober. This genotype only developed to the adult stage on 110 Richter and *V. vinifera*. Egg production was significantly lower on 110 Richter compared to *V. vinifera* (Fig. 11).

G1 survived for up to and including eight weeks on the control and 110 Richter but less than 2 weeks on Börner and Schwarzmann (Fig. 10b). On all other other rootstocks G1 survived between 4-7 weeks with Ramsey surviving for up to 7 weeks. G1 did not develop beyond the crawler stage on Börner, Schwarzmann, M920 or 5BB Kober. This strain only developed to the adult stage on 110 Richter, Ramsey and *V. vinifera*. No eggs were observed on 110 Richter but eggs were produced on Ramsey, albeit in significantly lower numbers than on the *V. vinifera* (Fig. 11).

G20 survived for up to and including 8 weeks on the control only and less than 2 weeks on Ramsey and Schwarzmann (Fig. 10c). On all other other rootstocks G20 survived between 3-5 weeks. G20 did not develop beyond the crawler stage on Börner, Schwarzmann, M920, 110 Richter, Ramsey or 5BB Kober. This genotype only developed to the adult stage on *V. vinifera* and egg production was significantly lower compared to G1 and G4 on the control
(Fig. 11).

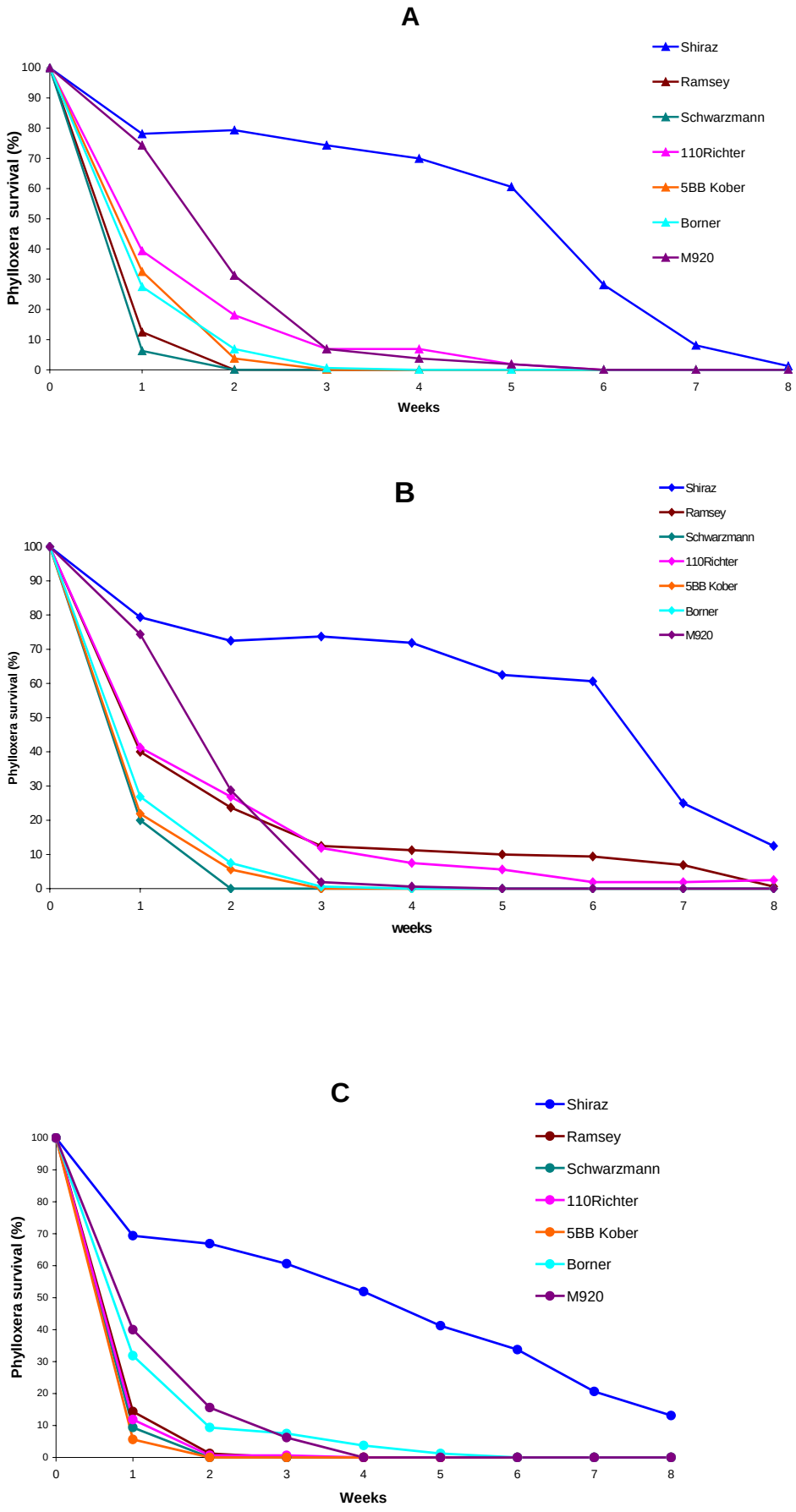


Figure 10. Excised bioassay three: comparison of phylloxera strains (A) G4, (B) G1 and (C) G20, on six rootstock hybrids and *V. vinifera* cv. Shiraz

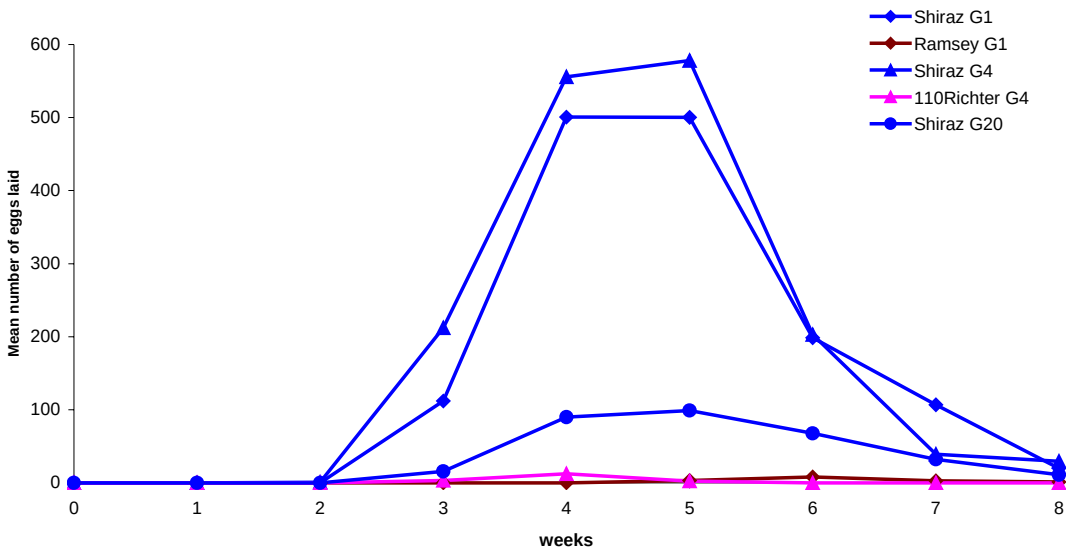


Figure 11. Excised bioassay three: comparison of egg production of three phylloxera strains, G1, G4 and G20 on rootstocks and *V. vinifera* cv. Shiraz

Discussion

Boubals (1966) noted that the presence of phylloxera galls (tuberosities) on lignified (main) roots was the most important indicator of phylloxera damage. The decline of AXR-1 in the Napa Valley, California was attributed to a new biotype of phylloxera, designated Biotype B to distinguish it from phylloxera incapable of killing AXR -1, termed Biotype A (Granett et al., 1996).

The bioassays described here where conducted specifically on excised lignified roots as development of phylloxera and associated root damage on the main roots are likely in the whole plant system to impact on vine performance. The relative level of establishment, development and survival of three selected clonal lineages of phylloxera found in Australia, were assessed on different commercially available and newly developed rootstocks. Screening of all 83 genotypic genotypes would be impractical in terms of resource allocation for the project and six clonal lineages have been recommended for screening (Hoffman, 2003). However, comparing relatively common genotypes such as G1 and G4 (representing 69% distribution) against a more geographically-restricted genotype (such as G20) allows some recommendation on rating of phylloxera resistance to be made. This study therefore focused on screening of rootstocks against three genotypes G1, G4 and G20 isolated from three PIZs within Victoria.

All phylloxera genetic strains tested showed significantly lower levels of performance on all tested rootstocks when compared to *V. vinifera*. However, even on *V. vinifera* clonal performance differed significantly. The G20 strain showed lower levels of development and egg fecundity in comparison

with G1 and G4. This could have important implications in terms of management of ungrafted vines in the field especially if vine decline may take longer due to lower root damage from less virulent phylloxera strains. It could also impact on the relative ability to detect the less virulent strains in the field due to their lower abundance and possibly lesser impact on vine performance. This is an important reason to monitor different phylloxera strains under field conditions on ungrafted vines and is highlighted further in Chapter 1, where G20 was monitored under commercial growing conditions.

Although all genotypes tested showed relatively poor performance on rootstock hybrids there were important differences between their development levels on different commercially available and newly developed rootstocks and some differences between bioassays were also observed. For example genotype G4 in bioassay one was able to develop to the adult stage and produce eggs on Ramsey whereas and G1 followed a similar developmental trend on Ramsey in bioassay three. However, in bioassay three G4 did not develop to adulthood on Ramsey. This indicates that some characteristic of the root material may have influenced phylloxera establishment and subsequent development. Root material for excised bioassays was sourced from field-grafted blocks in Merbein. Time of collection of material and site conditions (eg drought, climate) at time of collection could have impacted on the nutritional quality of this material, which would be critical for phylloxera development. Although environmental conditions in the laboratory are easily controlled for these experiments quality of root material for testing can be variable. This highlights that one of the confounding variables in using the excised bioassay as a routine rootstock screening system is root quality. Another variable is sometimes fungal contamination, which was not a major issue in this study.

Recommendations

On some rootstocks although phylloxera developed beyond the crawler stage the population abundance was significantly lower than on *V. vinifera.* Nevertheless some strains developed to the adult stage and began producing eggs on lignified roots of hybrids. This is not an indication that rootstock breakdown will occur, but it does show that the risk of transfer is higher when this happens. Those rootstocks, on which eggs were produced, included Ramsey (eggs produced by G1 and G4) and 110 Richter (G4).

- Careful selection of rootstocks to minimise quarantine risks would be advisable when the genetic strain of phylloxera in the infested zones have been characterised.

It is important to note that whilst the phylloxera genotypes were verified the rootstock material supplied was not always particularly in the case of the rootstock Börner.

- Future recommendations include the DNA testing of all rootstock material before phylloxera screening is conducted to ensure correct parentage is identified. This has been recommended due to some anomalies in the reaction of some rootstocks that were tested more than once in different trials. For example Ramsey was tested in two trials, however, its reactions to G4 were different in two trials. This could also be due to the time of year in which the root material was collected in the field.

Although the excised root system is rapid and produces result within a relatively short time period (ie 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.

- Due to some phylloxera survival on some rootstocks in all experiments, further evaluation is recommended, using additional genotypes of phylloxera and under pot trial conditions, to determine the impact of phylloxera on the vine decline and phylloxera genotype survival and development of each rootstock under growing conditions.

- A rapid screening procedure in a glasshouse system is recommended as the next phase in rootstock screening as an *in planta* test is closer to the field conditions than the *in vitro* test using the excised root bioassay system. This is discussed further in Chapter 3.

- Ultimately field screening will need to be conducted so that rootstock performance under phylloxera infested conditions can be monitored. This is discussed further in Chapter 4.

Chapter 3. Screening Rootstocks *In Planta* for Phylloxera Resistance

Objective: Optimisation of rootstock screening protocols and testing of selected phylloxera clonal lineages

Summary

A modified glasshouse protocol is described for co-cultivation of phylloxera genetic strains with potted grapevines and to assess phylloxera development and root response under glasshouse conditions. Using this protocol, individual phylloxera strains were contained in root enclosures that allowed quantification of individual life-stages. The new protocol allowed screening of rootstocks to individual phylloxera strains by preventing cross contamination and allowing more detailed quantification of phylloxera development and mature root response. For assessing phylloxera resistance/tolerance, results obtained using the new screening technique are considered to be (i) more realistic than the widely used excised-root bioassay systems, which exclude a whole plant response, and (ii) more rapid and robust than field-based studies where external variables, such as climate, soil properties and vine management, can influence results.

Vitis vinifera cv. Shiraz grapevines, grafted to 5 different rootstocks of American parentage, which are widely used in south-eastern Australia, were screened for resistance to three phylloxera genetic strains known to occur in Victoria: G1, G4 and G20 as typified with microsatellite marker analysis (Chapter 5). All three strains of phylloxera developed on *V. vinifera* vines, as shown by higher population development and necrotic tuberosity development. No significant differences were detected between the G1 and G4 phylloxera genotypes on *V. vinifera* but significantly poorer performance of G20 was observed. On rootstocks however, phylloxera performance was significantly reduced and some strains did not develop beyond the first instar (crawler) stage on some rootstocks. Phylloxera development was measured on both mature lignified and non-lignified roots. Ramsey tolerated phylloxera feeding, allowing development to adulthood and reproduction, but at greatly reduced levels compared to *V. vinifera* roots and with less damage to the grapevine root system. The poorest phylloxera performance was recorded on Börner.

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, 2003). Quarantine regulations have been in place in Australia since the late 1800s (Boehm, 1996). By geographically isolating viticulture regions and adhering quarantine protocols the spread of phylloxera has been minimised. Phylloxera in Australia is currently only found in localised regions, called Phylloxera Infested Zones (PIZ) in New South Wales and Victoria (National Vine Health Steering Committee, 2003). However, recent infestations outside existing quarantine zones were detected in the

Buckland Valley region, Victoria in December 2003 and Murchison, Victoria in July 2006. This is clearly indicative that quarantine breakdowns do occur sporadically. The only available long-term control option is the use of phylloxera resistant rootstocks, which offer sustainable production and a reduction in the risk of phylloxera transfer.

The Australian experience with phylloxera-resistant rootstocks commenced in 1900 with the rootstocks *Vitis riparia* and *V. rupestris* and their hybrids being released in Victoria (Whiting, 2003). Australian vine growers have over 20 different commercially available rootstocks of American *Vitis* parentage to choose from. The most commonly used rootstock varieties are the hybrids of four American *Vitis* species: *V. riparia, V. rupestris, V. campinii* and *V. berlandieri*. Despite the relative success of resistant rootstocks in controlling phylloxera, experiences overseas has shown that tolerance levels to phylloxera depend on the genetic strain of phylloxera attacking the rootstocks. In California the AXR-1 resistant rootstock failed in the 1980s, due the presence of phylloxera 'Biotype B' and there have been reports in Germany of 5C Teleki succumbing to phylloxera attack resulting in reduced performance (Whiting, 2003).

There are 83 known genetic strains of grape phylloxera that have been identified to-date in Australia (Herbert et al, 2006b). 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 (designated G1, G3, G4, G19, G20 and G30) have been recommended for screening against commonly used rootstocks (Corrie et al., 2003; Hoffman, 2003). Testing of these six clonal lineages is a pre-requisite to ensure sustainable rootstock recommendations for the Australian viticulture industry.

For this study we tested the performance of three phylloxera genotypes (G1, G4 and G20). The two most common and widespread genotypes in Australia are G1 and G4 (Corrie at al. 2002). Genotype G20 is less geographically widespread but it was detected at the site of one of the most recent phylloxera outbreaks in the Buckland Valley, Victoria in December 2003. The three phylloxera genotypes were tested against four commonly used rootstocks (Ramsey [*V. candicans* x *V. rupestris*], Schwarzmann [*V. riparia* x *V. rupestris*], 110 Richter [*V. berlandieri* x *V. rupestris*] and 5BB Kober [*V. berlandieri* x *V. riparia*], with self-grafted *V. vinifera* as a control. In addition a reportedly 'immune' rootstock Börner, which is used widely in Europe and has recently been introduced to Australia for breeding programs was also included in the trial. A modified glasshouse screening method, as described by Powell (2003), was used in two trials to examine interactions of the selected rootstocks with the selected phylloxera genotypes. Phylloxera population abundance and development and tuberosity (lignified root swelling) formation on grapevine roots were quantified along with several measures of vine performance.

Methods

Plant Material and Growth Conditions

In Trial 1 one-year old grapevine rootlings were sourced from the Sunraysia and Mid-Murray Phylloxera Exclusion Zone, from Sunraysia Nurseries in Gol Gol New South Wales in November 2003. The rootlings included the two treatment bench-grafted rootstocks: Schwarzmann (clone EXWA NW131), Ramsey (clone A11V2 NW130) and Shiraz (clone PT23 NW90) as control. The scion in each case was Shiraz (clone PT23 NW90), including the self-grafted control to exclude grafting as a confounding factor.

In Trial 2 one-year old grapevine rootlings were sourced from the Boulevard Nurseries in the Sunraysia and Mid-Murray Phylloxera Exclusion Zone, Victoria in May 2005. The rootlings included the five treatment bench grafted rootstocks: Schwarzmann (clone R1A2S10), Ramsey (clone A11V2), Börner (two-year old), 110 Richter (clone A5V19), 5BB Kober (clone R1A1S6) and Shiraz (clone PT23) as control. 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 (ie phylloxera inoculation).

Insect Material

Three phylloxera strains, G1, G4 and G20, were used in each trial. Each strain was single sourced from roots of ungrafted *V. vinifera* vines from commercial vineyards in PIZs n Victoria, Australia (G4-King Valley, G1-Nagambie and G20-Buckland Valley).

Phylloxera strains 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 by staff at the Centre for Environmental Stress and Adoption Research (CESAR), Melbourne (Chapter 5).

Experimental Design - Trial One

A randomised complete plot design was established to examine the interaction between phylloxera genotype and rootstock, using a four by three factorial design. This accommodated the four infestation types (three phylloxera genotypes and a non-infested control) and the three rootstock types. Eight blocks (replicates with twenty four combinations each) were set-up within the glasshouse 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.

Experimental Design - Trial Two

A randomised complete plot design was established to examine the interaction between phylloxera genotype and rootstock, using a four by six factorial design. This accommodates the four infestation types (three phylloxera genotypes and a non-infested control) and six rootstock types. Eight blocks (replicates with twelve combinations each) were set-up within the glasshouse to account for any variations in the environmental conditions. Each phylloxera strain was set-up on consecutive days to prevent cross-contamination of the strains during infestation.

Phylloxera Infestation

For both glasshouse trials infestation of rootstocks with phylloxera strains occurred on three 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" (as described in Powell, 2003; Fig. 12). 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 (Fig. 12). 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 (Fig. 13). Two to four vines were infested at a time, in the laboratory, to minimise stress to the vines and potential changes in host plant-phylloxera interactions.



Figure 12. Enclosure containing attached lignified root section of potted grapevine infested with phylloxera eggs of a single known strain



Figure 13. Pot enclosure to prevent cross contamination of phylloxera strains between rootstock 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 (Fig. 12). The root system was visually checked for insects 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 (Fig. 14). The infested root piece was gently scrubbed with a fine soft-bristled 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.



Figure 14. Collection of phylloxera life-stages from infested root enclosure

Several additional measurements were taken at the end of the second glasshouse trial to assess the impact of phylloxera infestation on individual vines and assess the performance of uninfested rootstocks as compared to uninfested *V. vinifera*. The measures included root dry weight, plant stem dry weight, total leaf area, total leaf number, total leaf dry weight.

Chlorophyll Assessment

Leaf chlorophyll measurements were obtained in trial two with a hand-held SPAD-502 Chlorophyll meter (Minolta™) as described in Powell and Herbert (2005). Readings were taken monthly for each vine with the total readings for three leaves averaged to give a single chlorophyll value per vine.

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 8.1 for Windows software (Payne et al., 2006). 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.

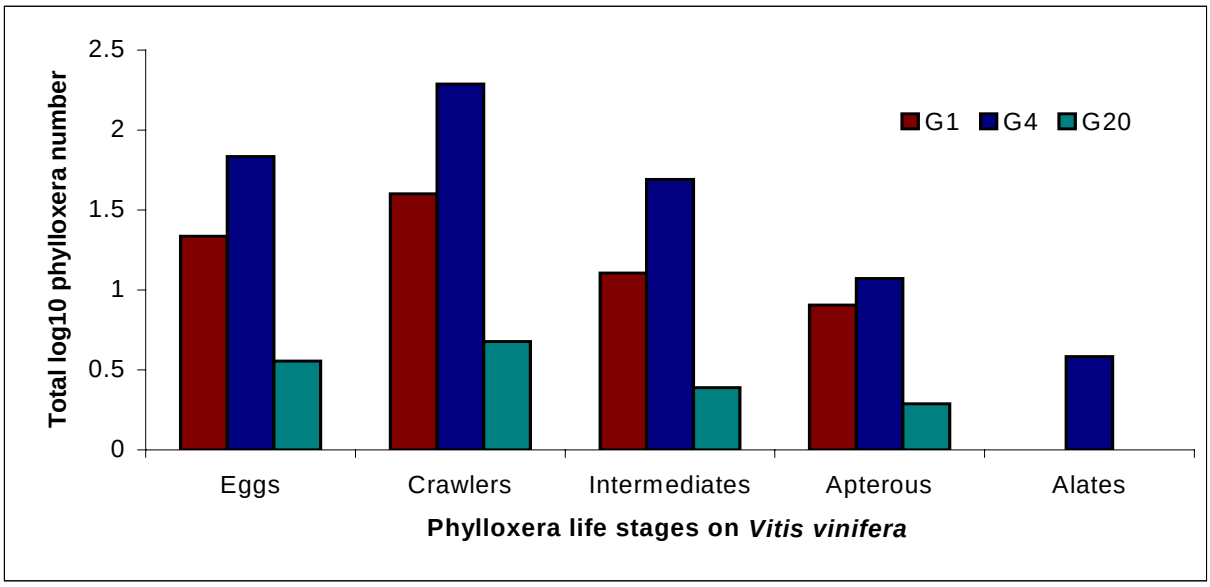
Results

Phylloxera Performance on Vitis vinifera

Trial 1 and 2

The mean total number of individual life-stages present after ten weeks for each phylloxera genotype tested on the control *V. vinifera* cv. Shiraz for both glasshouse trials is shown in Fig. 15. Overall, after ten weeks, the phylloxera strains G1 and G4 produced significantly higher population numbers than the genetic strain G20, in both trials, which indicates that both G1 and G4 were more virulent than G20. This observation has also been noted in field trials (Chapter 1) and laboratory trials (Chapter 2). For all genetic strains the most abundant phylloxera life-stages after ten weeks were eggs and crawlers (first instar). In both trials all life-stages were found for the three genetic strains with the exception of winged adults (alates), which were only observed on G4 infested vine roots.

(A)



(B)

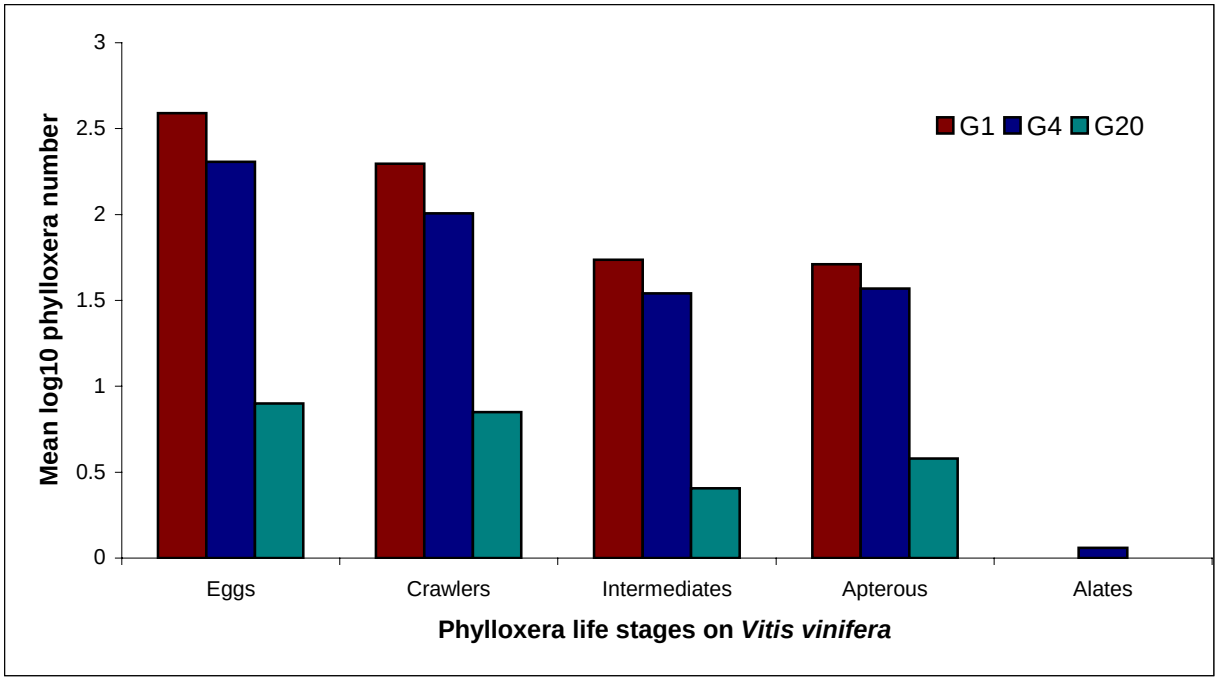


Figure 15. Life-stages of three phylloxera genetic strains, G1, G4 and G20, after ten weeks glasshouse incubation on V. vinifera cv. Shiraz (A) Trial 1 (B) Trial 2.

Phylloxera Performance on Grafted Rootstocks

Trial 1

The mean total number of phylloxera present after ten weeks for each phylloxera genotype tested on rootstocks for glasshouse trial one is shown in Fig. 16. Clear differences between the performance of the three phylloxera genotypes were observed. All three genetic strains G1, G20 and G4 did develop on Ramsey and Schwarzmann but numbers were relatively low compared with the controls. Of all three genetic strains tested G20 was the poorest performer even on the controls.

Trial 2

The mean total number of phylloxera present after ten weeks for each phylloxera genotype tested on rootstocks for the second glasshouse trial is shown in Fig. 17. Differences in performance between the three phylloxera genotypes screened on the five different rootstocks were evident. None of the genotypes tested were able to develop on the rootstock Börner indicating that, at least for the three clonal lineages tested, it appears to show an immune response. The clones G1 and G4 did not develop on either Schwarzmann or 5BB Kober but did develop on 110 Richter and Ramsey. G20 did not develop on Ramsey or Börner but developed on all other rootstocks.

Grapevine Roots Response to Phylloxera Genotype

Tuberosities - Trial 1

Tuberosities (Fig. 18) and nodosities were significantly more abundant on *V. vinifera* than on rootstocks (Fig. 19) for all phylloxera genetic strains tested indicating more root damage to the growing susceptible vine, which would impact on performance. Nodosities were observed for all three phylloxera clones on *V. vinifera* and on a single Schwarzmann vine (infested with G20) and four Ramsey plants (data not shown). Necrotic tuberosities (Fig. 18a) where observed for all phylloxera strains on *V. vinifera*. However, tuberosity numbers on *V. vinifera* roots were only significantly different between G4 and G20 infested vines. No necrotic tuberosities where observed on any rootstock however raised dome tuberosities (Fig. 18b), were observed on Ramsey for all 3 phylloxera strains and on Schwarzmann for the G20 strain only. The number of tuberosities developed on vines infested with any of the three phylloxera strains tested did not differ significantly on either Ramsey or Schwarzmann (Fig. 19).

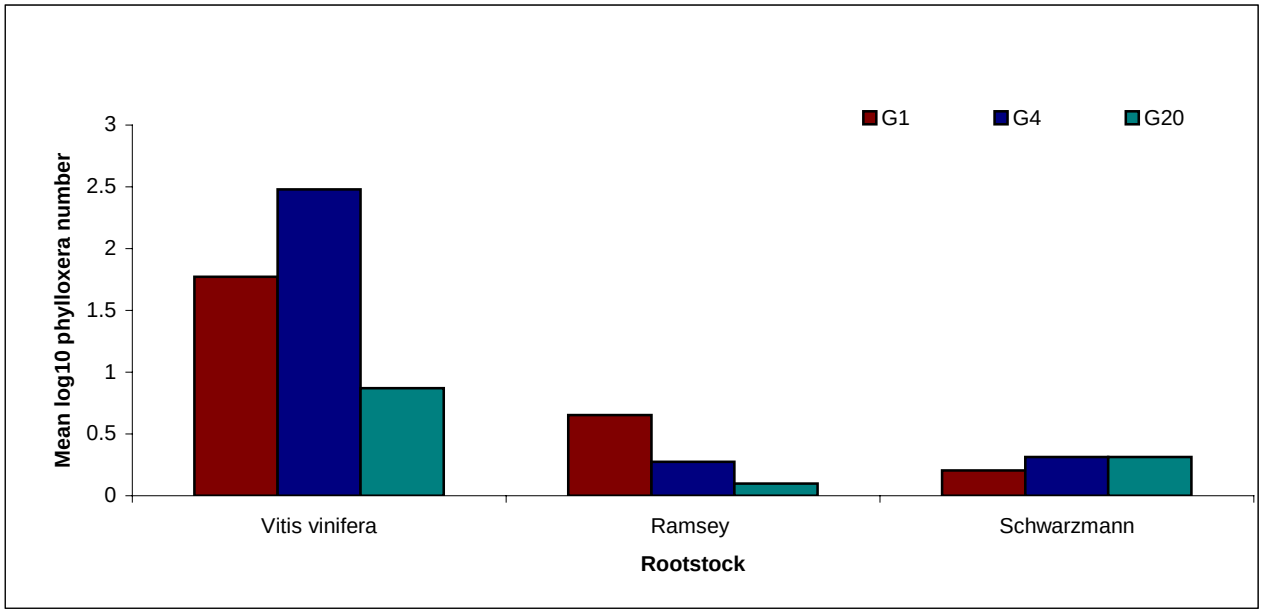


Figure 16. Total abundance of three phylloxera genetic strains, G1, G4 and G20, after ten weeks glasshouse incubation on *V. vinifera* cv. Shiraz, Ramsey and Schwarzmann

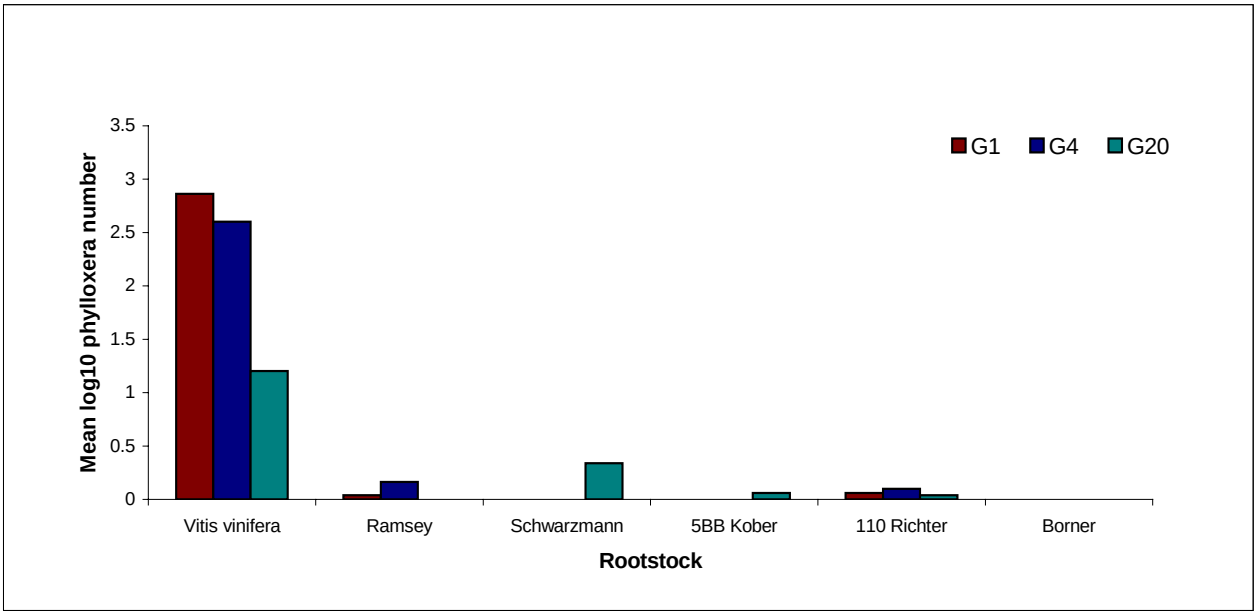


Figure 17. Total abundance of three phylloxera genetic strains, G1, G4 and G20, after ten weeks glasshouse incubation on *V. vinifera* cv. Shiraz, Ramsey, Schwarzmann, 110 Richter, 5BB Kober and Börner.

Tuberosities - Trial 2

Nodosities were observed for all phylloxera strains on *V. vinifera* and on two Schwarzmann plants infested with G20 (data not shown). Necrotic tuberosities where observed on all phylloxera-infested *V. vinifera* regardless of genetic strain (Fig. 17). No necrotic tuberosities where observed on any rootstock. However, raised-dome tuberosities (Fig. 18b) were observed on all rootstocks with the exception of Börner. But the presence of domed tuberosities was dependent on phylloxera strain. For example tuberosities were observed on Ramsey, regardless of the genetic strain present. On 110 Richter tuberosities were only observed on the G4-infested vines. However, for 5BB Kober tuberosities were only observed in the presence of the G1 strain. Overall more tuberosities were observed on Ramsey than any other rootstock with the exception of *V. vinifera* control (Fig. 20).



Figure 18a. Necrotic tuberosities observed on phylloxera-infested *V. vinifera*



Figure 18b. Domed tuberosities observed on phylloxera-infested on Ramsey

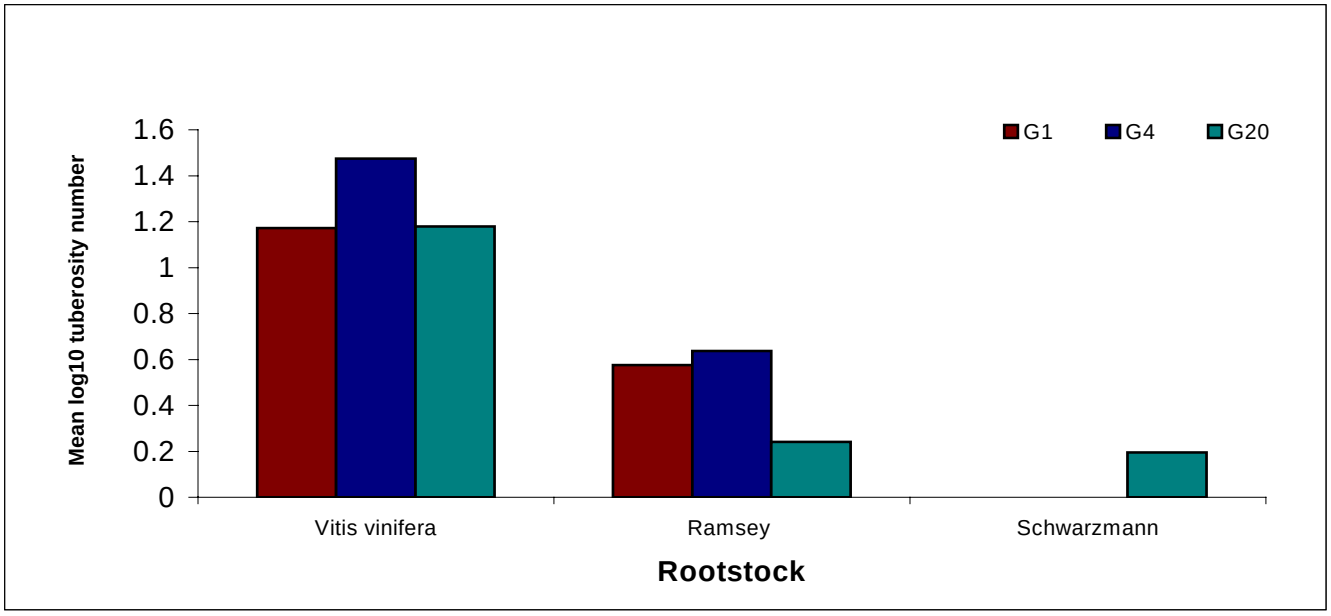


Figure 19. Total abundance of tuberosities developed on *V. vinifera* cv. Shiraz, Ramsey and Schwarzmann after ten weeks glasshouse incubation infested with three phylloxera genetic strains, G1, G4 and G20

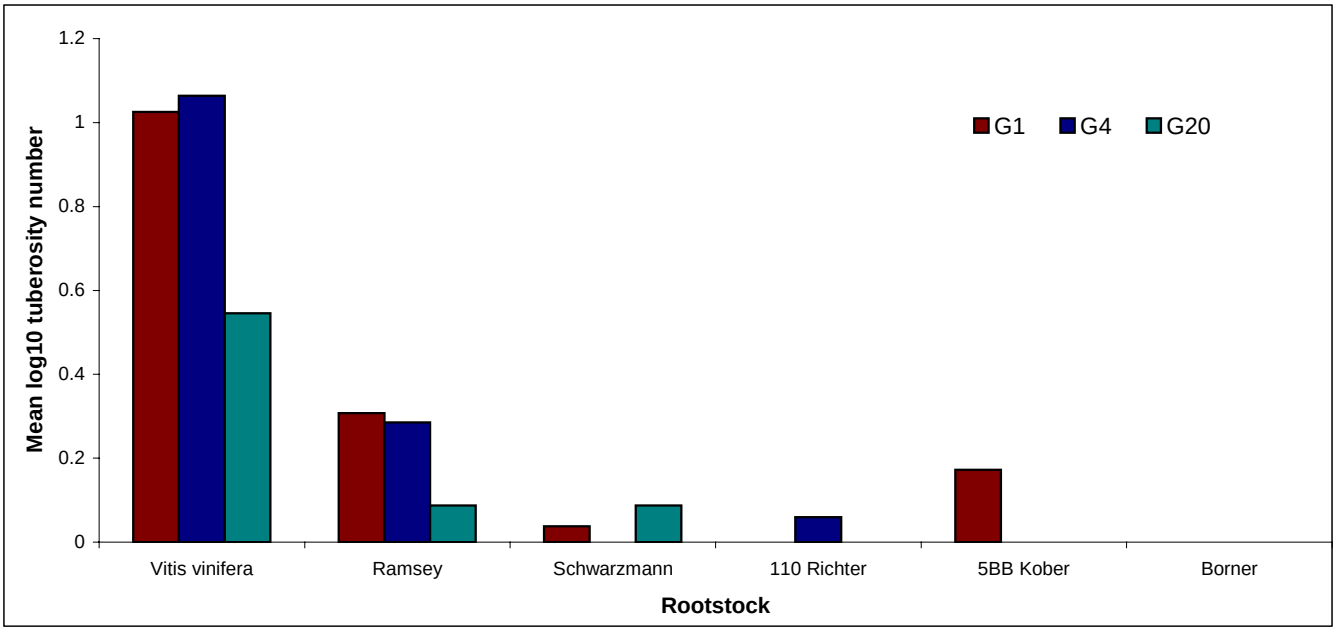


Figure 20. Total abundance of tuberosities developed on *V. vinifera* cv. Shiraz, Ramsey, Schwarzmann 110 Richter, 5BB Kober and Börner after ten weeks glasshouse incubation infested with three phylloxera genetic strains, G1, G4 and G20

Grapevine performance in response to phylloxera genotype

Rooting development.

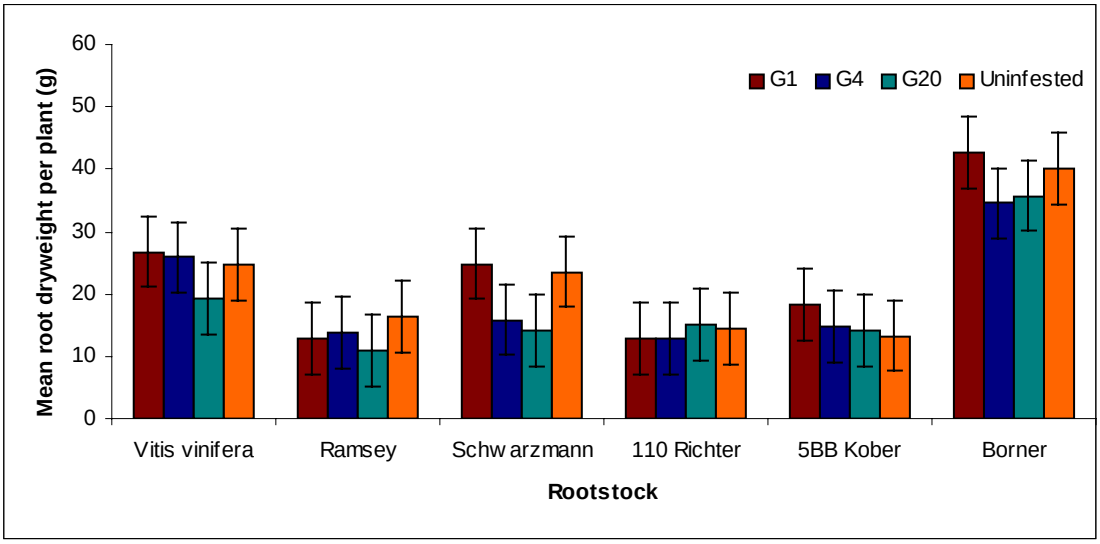
After ten weeks infestation no significant difference in total root dry weight was observed between phylloxera-infested or uninfested vines regardless of rootstock treatment. Overall Börner had the highest root mass compared with all rootstocks and *V. vinifera* (Fig. 21a). Schwarzmann and *V. vinifera* had similar root mass whilst 110 Richter, 5BB Kober and Schwarzmann had the lowest root mass. The negligible impact of phylloxera on overall root mass, despite production of necrotic and non-necrotic tuberosities on lignified roots, may be due to the fact that low numbers of phylloxera were contained in a relatively small proportion of the root mass.

Leaf and stem development

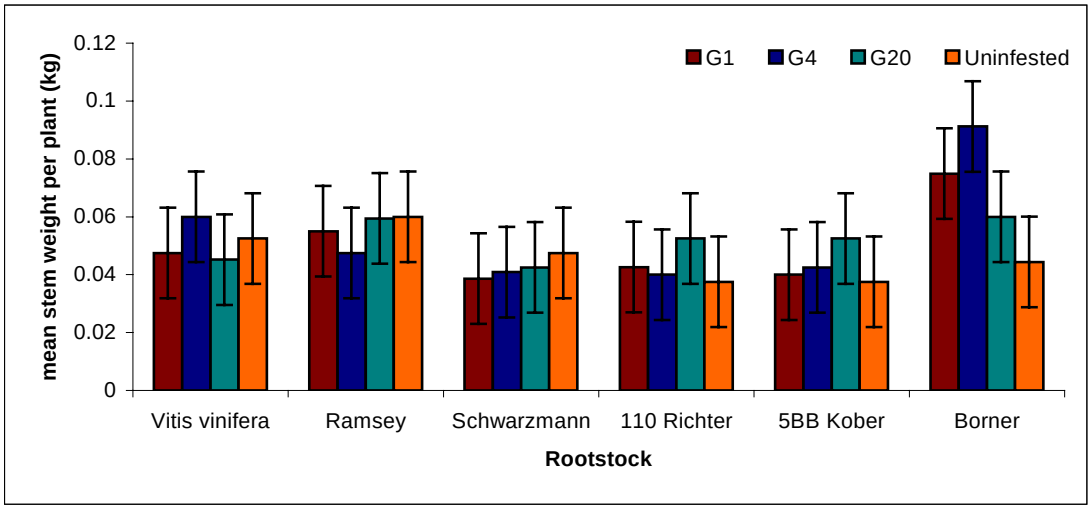
After ten weeks infestation no significant difference was observed in stem weight, leaf area, leaf number, leaf dry weight (Figs. 21b-21e) between phylloxera-infested or uninfested vines regardless of rootstock treatment. Overall Börner had the highest leaf dry weight and stem weight compared with all rootstocks and *V. vinifera* (Fig. 21b-21e). The negligible impact of phylloxera on overall canopy, despite production of necrotic and non-necrotic tuberosities on lignified roots, may be due to the fact that low numbers of phylloxera were contained in a relatively small proportion of the root mass.

Plant pigment response

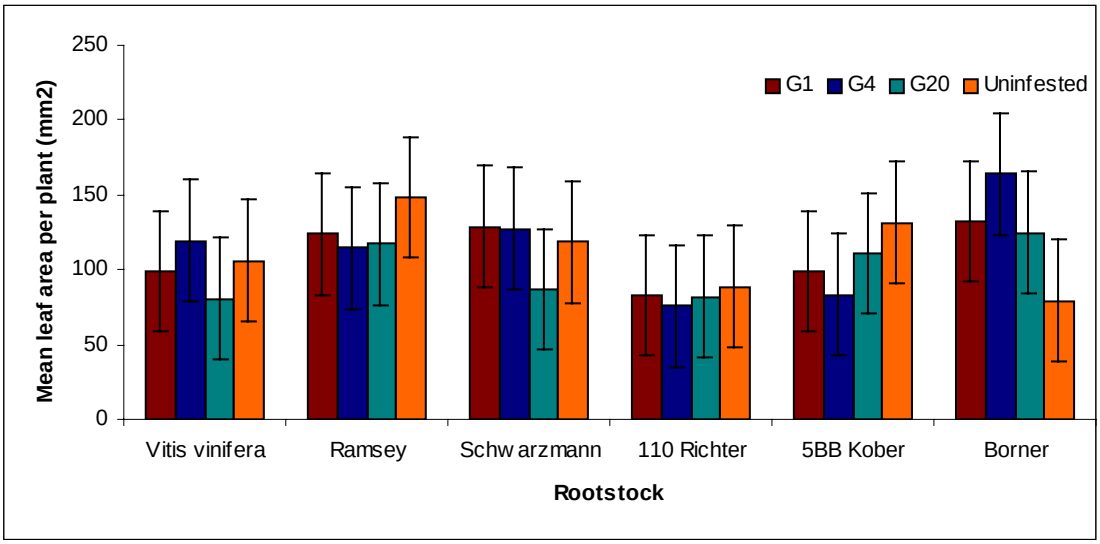
After 10 weeks infestation no significant difference was observed in chlorophyll measurements for phylloxera infestation (data not presented).


Mean root dryweight per plant (g)
60
50
40
30
20
10
0
G1
G4
G20
Uninfested
Vitis vinifera
Ramsey
Schw arzmann
110 Richter
5BB Kober
Borner
Rootstock

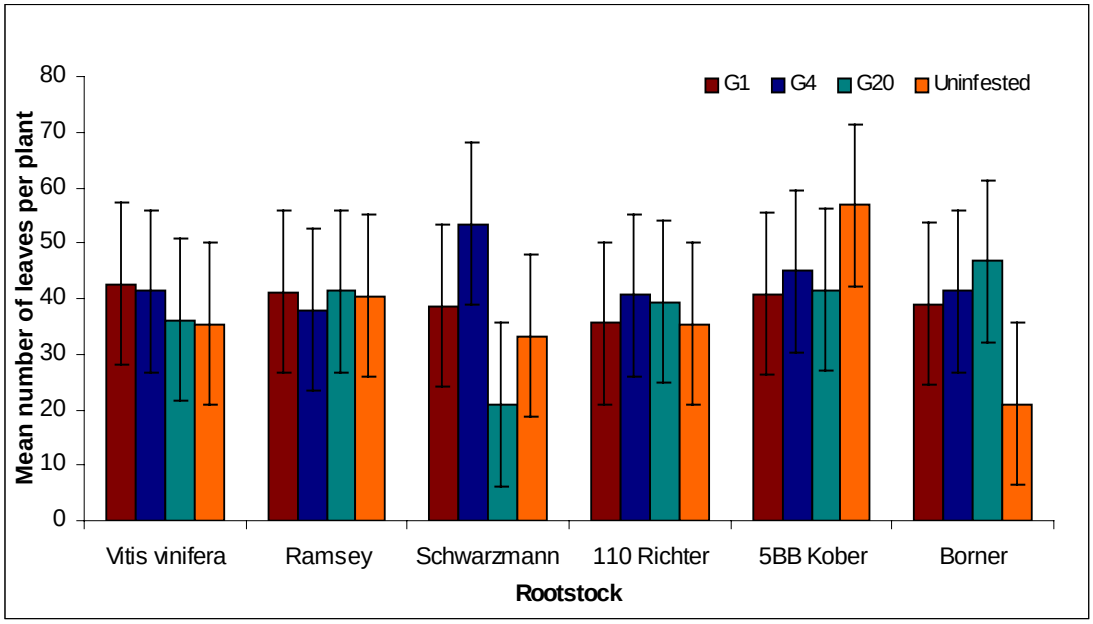
A


mean stem weight per plant (kg)
0.12
0.1
0.08
0.06
0.04
0.02
0
G1
G4
G20
Uninfested
Vitis vinifera
Ramsey
Schwarzmann
110 Richter
5BB Kober
Borner
Rootstock

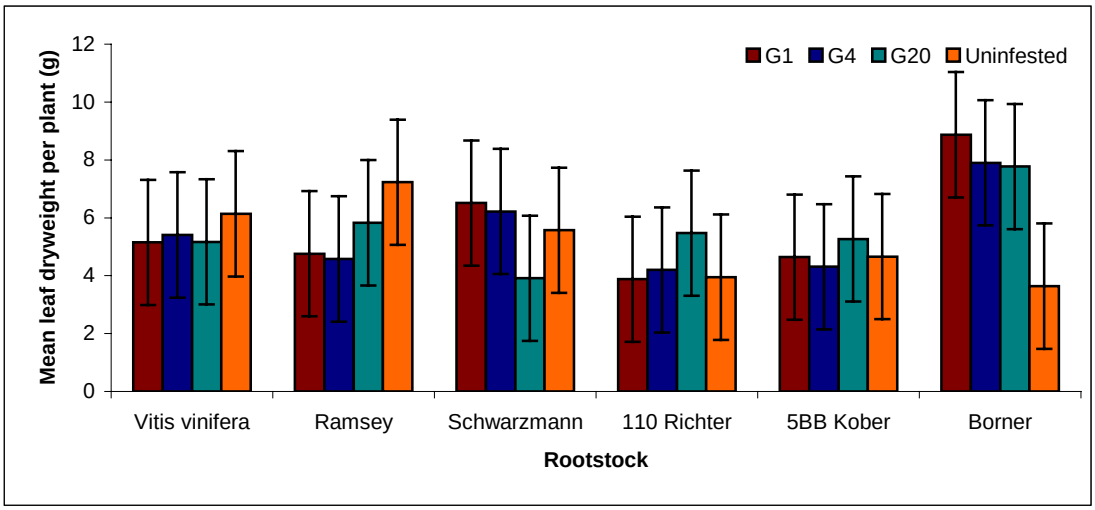
B


Mean leaf area per plant (mm2)
250
200
150
100
50
0
G1
G4
G20
Uninfested
Vitis vinifera
Ramsey
Schw arzmann
110 Richter
5BB Kober
Borner
Rootstock

C



D



E

Figure 21. Mean (A) root mass, (B) stem weight (C) leaf area (D) leaf number and (E) leaf weight for the four different phylloxera infestation treatments on six rootstocks

Discussion

The options available for screening phylloxera genotype-rootstock genotype reactions include laboratory (refer to Chapter 2), glasshouse (current Chapter) and field trials (refer to Chapter 4). Laboratory screening, using excised roots, is a rapid simple procedure but results may be variable and misleading, as they are dependent on the viability of excised root material and the time of its collection. In addition 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. Glasshouse screening on the other hand moves a step closer to field screening as it allows a whole plant response to be assessed on lignified and non-lignified roots and some measures of vine performance (and hence resistance characteristics) to be assessed.

Field screening is although essential is labour intensive, affected by environmental conditions and is a long-term proposition as it takes several years before results become available. Field screening is also reliant on planting in known infested areas and this may preclude screening of rootstocks against a range of phylloxera genotypes, making decisions on resistance ratings difficult.

From the six clonal lineages that have been recommended for testing against commonly available American rootstocks, three (G1, G4 and G20) were tested in the two glasshouse trials. *Vitis vinifera* showed least resistance to all three phylloxera genotypes with higher phylloxera abundance and more necrotic tuberosities formed on the roots. This result is consistent with previous glasshouse studies when G1 and G4 phylloxera clones were tested against Ramsey and Schwarzmann (Powell, 2003). On *V. vinifera* the strain G20 clearly showed both lower abundance and root damage than the G1 and G4 strains indicating lower virulence. This support observations from both laboratory and field studies (Chapters 2 and 4).

In laboratory trials where excised roots have been tested, G1 and G4 clones outperformed G20 on *V. vinifera* ((Chapter 2), Viduka et al 2003). In the field G4 and G1 produce higher population levels than G20 on ungrafted *V. vinifera* (Chapter 4). In the field G20 has to-date only been detected on Schwarzmann (Corrie at al, 2002) and *V. vinifera* (Powell et al., 2006a). In our glasshouse study G20 developed to the adult stage, layed eggs and produced non-necrotic tuberosities on Schwarzmann but in significantly lower numbers than on *V. vinifera* control. This however, does not indicate that there is a risk of breakdown in resistance of Schwarzmann vines, but there would be a risk of transfer. Whilst the number of insects developing on rootstocks was significantly lower in both trials compared to *V. vinifera* some rootstocks, for example Börner, showed high levels of resistance. No insects could develop to the adult stage on Börner and no tuberosities were evident on these vines when infested with any of the three phylloxera strains. On Ramsey all there phylloxera strains could develop to the adult stage and although egg production levels were low compared to *V. vinifera* Ramsey does appear to present a significant risk of transfer compared to the other rootstocks tested. This supports previous findings that these rootstocks have good phylloxera tolerance, but the level of tolerance varies (Powell, 2003). Assessment of

tuberosity formation may provide a useful indicator of the level of host plant response to phylloxera infestation and provide a preliminary indicator of rootstock resistance. Tuberosity morphology varied between treatments with *V. vinifera* showing necrotic tuberosities, which may provide a site for secondary fungal invasion. Other rootstocks (eg. Ramsey) developed domed non-necrotic tuberosities indicating a different level of host plant defence response to phylloxera feeding. On some rootstocks (eg. Börner), tuberosities did not develop at all and insects did not survive on these rootstocks.

Our observations are consistent with that of Kellow (2000). Kellow used micro-propagated Ramsey and Schwarzmann to test VWL-1 phylloxera genotype collected from the King Valley. VWL-1 phylloxera is equivelant to G4. Kellow et al (2002) did not observed root damage on Schwarzmann but on Ramsey root swellings that were morphologically different to *V. vinifera* were described. However, Kellow's observations were restricted to non-lignified roots of younger plant material. In conclusion, these glasshouse studies have proven that the virulence levels of phylloxera strains differs on distinct rootstocks and rootstocks respond to phylloxera feeding differently.

Four widely used rootstocks, 5BB Kober, 110 Richter, Schwarzmann and Ramsey, were screened under glasshouse conditions to assess the performance of three clonal linaeges of phylloxera found in Australia. In addition Börner supplied by Geisenheim Plant Breeding Station Germany was screened for the first time in Australia under glasshouse conditions. All the rootstocks tested represented three groups of hybrid crosses: *Vitis riparia* x *V. rupestris*, *V. champinii* and *V. berlandieri* x *V. riparia.* All rootstocks had significantly lower phylloxera populations present on the main root system than the control ungrafted *V. vinifera* which suggests that the rootstocks are tolerant whereas *V. vinifera* is clearly highly susceptible.

Overall the trial supports the assumptions that the five rootstocks tested have high to good phylloxera tolerance, although clearly the levels of tolerance do vary. The protocol developed provided the opportunity to screen rootstocks in a relatively short time period to a range of phylloxera genotypes under controlled growth conditions and prevented cross-contamination of phylloxera which has been observed in other glasshouse trials (Buchanan, 1990).

Recommendations

Rootstock Recommendations

- All rootstocks tested in this trial showed high levels of tolerance to G1, G4, and G20 phylloxera strains. However, further screening should be conducted using additional selected genotypes and rootstocks to ensure that sustainable rootstock recommendations can be made.

- Further rootstock and phylloxera genotype screening is required in order to give Australian growers comprehensive advice on rootstock selection.

Modified Screening Protocols

- The use of this rapid screening procedure in a glasshouse system is recommended for further rootstock screening as an *in planta* test is closer to the field and vine physiological conditions than the more widely used excised root bioassay system. The procedure could be further modified to take sub-samples during the ten-week trial period to develop genotype-specific phylloxera life-tables and model phylloxera development over time on various vine types. In addition the trial period could be extended or protocol modified to be able to further assess the effects of phylloxera feeding on vine performance. The protocol could also be further modified to examine interactions between phylloxera genotype x rootstock x soil environment.

Further Screening

- The results presented here highlight the variability of susceptibility of *V. vinifera* to phylloxera genotypes. Future bioassays would need to incorporate additional genotypes of phylloxera under glasshouse pot trial conditions, to determine the impact of phylloxera on the vine decline and phylloxera genotype survival and development of *V. vinifera* and rootstocks under optimal growing conditions. Examining the genotype-host plant susceptibility would assist in identifying the mechanism behind phylloxera resistance and susceptibility.

Chapter 4. Phylloxera Genetic Diversity and Population Dynamics Under Field Conditions

Objective: To examine interactions between phylloxera genetic strain and grafted rootstocks under field conditions

Summary

Field-based studies were conducted, over three consecutive grapevine growing seasons in three commercial vineyards, to monitor the population dynamics, risk of dispersal and impact on grapevine rootstock performance of several phylloxera genetic strains. Phylloxera emergence at the soil surface was range form relatively low to relatively high and this was dependent on genetic strain and site-related variables. Phylloxera significantly reduced grape yields and quality at some but not all sites. The implications of these results for phylloxera management on ungrafted *Vitis vinifera* grapevines in phylloxera-infested quarantine regions is discussed.

Introduction

Rootstocks of American *Vitis* parentage have long been identified as the main control option for grapevine phylloxera. To-date rootstock recommendations, for the Australian viticulture industry were based mainly on (i) overseas field and controlled environment studies and (ii) limited field studies in Australia where phylloxera genetics has not been taken into account as a variable. More recently the potential impact of phylloxera genetic diversity and its implications for sustainable rootstock recommendations has been considered in limited studies under controlled conditions (Powell 2003; Viduka et al., 2003; Korosi et al., 2006). Advances in the use of microsatellite markers have increased our available knowledge on phylloxera genetics (Corri et al., 2003) and a detailed comprehensive genetic assessment of phylloxera in Australia has been conducted (Hoffman, 2003). Eighty-three clonal strains have been identified to-date (Herbert et al., 2006b) in Australia and six clonal lineages recommended for screening (Hoffman, 2003). We selected three study sites to examine the interactions of some of these clonal lineages with a range of commonly used rootstocks and map the geographic diversity of some of the lesser described genotypes. The study sites had been established in the mid-1980s (Whiting, 2003) as part of a wider study to assess the suitability of a range of rootstocks for use in Victorian vineyards.

Methods

Site Details and Experimental Design

Site 1 - Rutherglen

The study site was located in a commercial vineyard in Rutherglen, within the North-East Phylloxera Infested Zone (PIZ; Fig. 22). Phylloxera was first detected in the vineyard at the turn of the twentieth century but was not detected in the study block until 1985. After which time yields of ungrafted vines at the site dropped significantly (John Whiting, pers.comm.). The vineyard had previously been surveyed (Hoffman, 2003) and predominant phylloxera had been characterised as genotype G7. The G7 genotype has not been found to-date in the Nagambie or King Valley PIZs. A soil survey was conducted in August 1999 (as described in Powell, 2003). Soil was described as duplex in nature with a red loam topsoil and a yellow subsoil (Whiting pers. comm).

The vineyard block, chosen for the study, consisted of a block of six rows of ungrafted *V. vinifera* L. cv. Muscadelle (clone IV622055) planted in 1978. The row spacing was 3.66 metres and vine spacing was 2.13 metres. Three alternate rows there were used to monitor vines which were set in a randomised complete block design for above-ground phylloxera dispersal and below-ground phylloxera genotype presence. On the monitor vines emergence traps were used to monitor above-ground movement. A total of five replicates for each rootstock were monitored in season one and this replicate number was increased to ten per rootstock in seasons two and three. Rootstock treatments examined were Richter 110, ARG-1, Schwarzmann and 5BB Kober. Ungrafted *V. vinifera* cv. Muscadelle was used as the control treatment.

Site 2 - Glenrowan

The study site was located in a commercial vineyard in Glenrowan, within the North-East Phylloxera Infested Zone (PIZ) (Fig. 22). Phylloxera was first detected in the Glenrowan region in 1909 and vines were planted in a block adjacent to a known phylloxera-infested block. The vineyard had previously been surveyed (Corrie et al., 2003) and the predominant phylloxera had been characterised as genotype G1. Soil at the site is described as a sandy duplex soil (M. Reid pers. comm).

The vineyard block, chosen for the study, consisted of a block of four rows of grafted and ungrafted *V. vinifera* L. cv. Brown Muscat planted in 1976. The row spacing was 3 metres and vine spacing was 2 metres. Three rows there were used to monitor vines which were set in a randomised complete block design for above-ground phylloxera dispersal and below-ground phylloxera genotype presence. On the monitor vines emergence traps were used to monitor above-ground movement. All insects collected in emergence traps were genotyped (Chapter 5). Root samples and insects from root samples were collected in November 2003, February 2005, 2006 to confirm rootstock and phylloxera genotype (Chapter 5). A total of five replicates for each rootstock were monitored in season one and, due to low detectable numbers throughout the growing season, the

replicate number was increased to nine per rootstock in seasons two and three. Rootstock treatments examined were 110 Richter, Ramsey, Schwarzmann and Rupestris du Lot. Ungrafted *V. vinifera* cv. Brown Muscat was used as the control treatment.

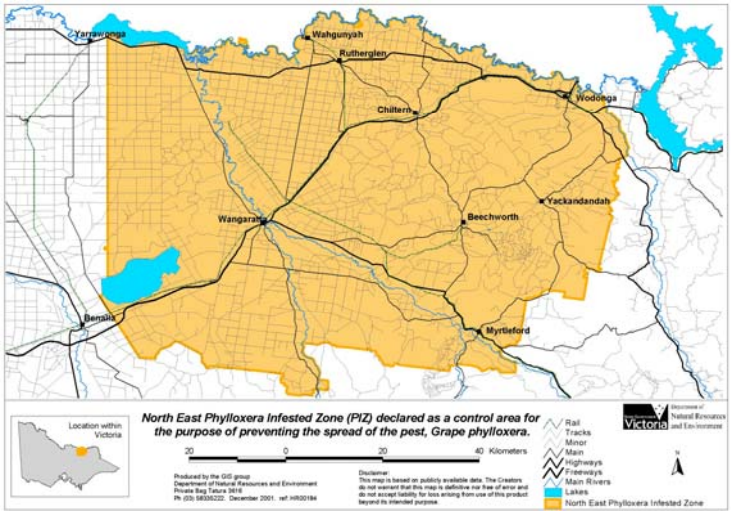


Figure 22. North East phylloxera infested zone (PIZ) at the time of rootstock monitoring

Site 3 - Nagambie

The study site was located in a commercial vineyard in Nagambie, within the Nagambie PIZ (Fig. 23). Phylloxera was first detected in the vineyard block in 1978 three years after initial planting at the site (Buchanan 1990). The vineyard had previously been surveyed (Corrie et al., 2003) and the phylloxera at the site had been characterised as genotype G1. This G1 genotype is relatively widespread and has been found to-date in the Nagambie, Upton, North East and Orchard Hills PIZs but not in the King Valley PIZ. To-date it is the only genotype known in the Upton, Orchard Hills and Nagambie regions.

The vineyard block, chosen for the study, consisted a block of grafted and ungrafted *V. vinifera* L. cv. Cabernet Sauvignon (clone planted IV692217) planted in 1984. The row spacing was 3.35 metres and vine spacing was 1.83 metres. Ten adjacent rows there were used to monitor vines which were set in a randomised complete block design of 5 replicate blocks for above-ground phylloxera genotype dispersal and below-ground phylloxera genotype presence. On the monitor vines a single emergence trap was placed adjacent to the vine trunk (Powell et al., 2000). A total of five replicates for each rootstock were monitored over all three seasons. Rootstock treatments examined during the trial were 110 Richter, ARG-1, Schwarzmann, Ramsey, 5BB Kober, 140 Ruggeri, Rupestris du Lot and 5C Teleki. Ungrafted *V. vinifera* cv. Cabernet Sauvignon was used as the control treatment. In some instances where phylloxera could not be found in either emergence traps or on surveyed roots of some rootstocks sampling was discontinued after a single season.

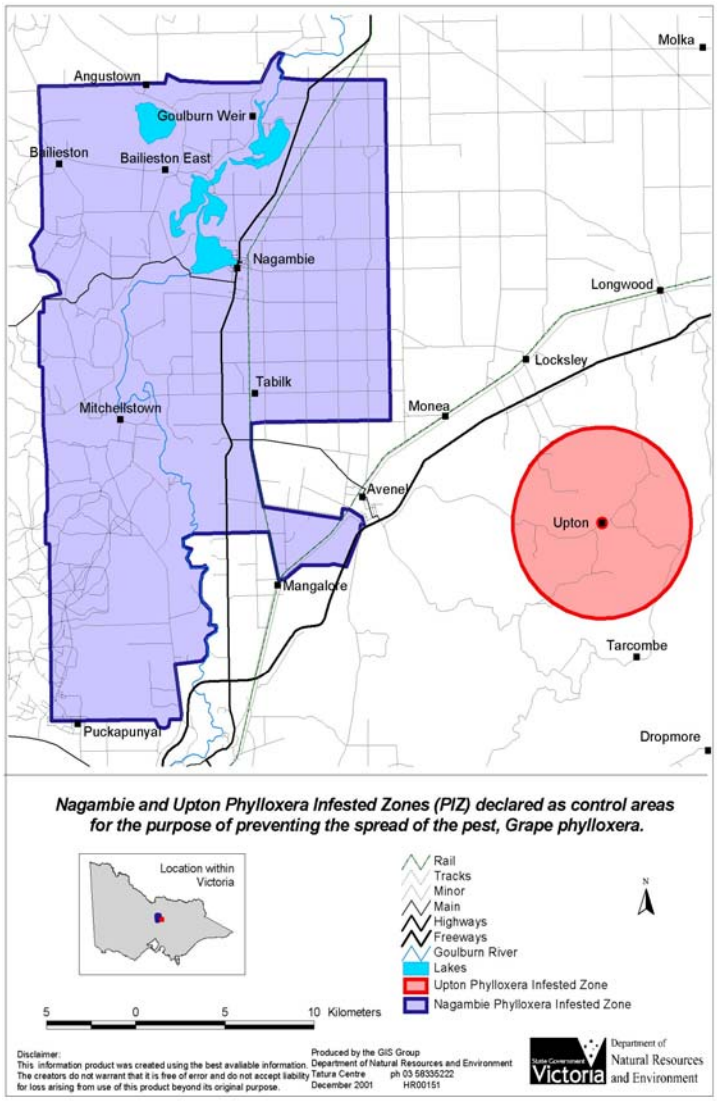


Figure 23. Nagambie and Upton Phylloxera Infested Zones

Phylloxera Monitoring

At all three field sites to assess phylloxera population movement above-ground a non-destructive emergence trap (Powell et al., 2000), were used for each monitor vine and statistical analysis covered a period of eight to eleven fortnights for each season. The seasonal abundance of phylloxera dispersive stages, (namely first instars (crawlers) and adult winged forms (alates)) emerging from below-ground onto the soil surface was measured over three successive seasons commencing December 2003 and finishing in April 2006 using 'emergence traps' placed adjacent to each monitor vine. Emergence traps (Fig. 24) consisted of translucent plastic containers (4 litre Décor™), 22 cm diameter x 13 cm depth, open at one end, rinsed with tap water and inverted onto the soil surface at a distance of 10 cm from the sample vine trunk. Traps were fixed flush with the soil surface using metal tent pegs. On emergence from the soil phylloxera were trapped in condensation on the sides of the trap. At fortnightly intervals insects were removed by washing the trap with 70% methanol and collected in plastic containers. Traps were then rinsed with tap water

and re-positioned. Collected phylloxera life- stages were identified and quantified using a low power binocular microscope.



Figure 24. Emergence trap positioned adjacent to infested monitor grapevine to quantify phylloxera emergence from the soil and sticky traps located on the vine trunk to monitor phylloxera movement within the canopy

Grapevine Performance

To quantify the impact of phylloxera presence on yield, quality and vigour assessments were conducted at this study site at harvest (yield and quality) and post-harvest over three successive seasons. All 'monitor' infested vines were selected for harvest and pruning assessments. All 'monitor' vines were hand-harvested. Grape bunches were counted and weighed in the field using a portable balance. Samples of fifty berries were collected from each vine and weighed to determine mean berry weight. The berry samples where then transferred to the DPI-Rutherglen quarantine laboratory where they were crushed and the juice tested for sugar level by refractometer and pH by electrode. Canes of the ' trial monitor' vines were cane pruned or spur pruned dependent on growers recommendations at each site and weighed on-site using portable scales. Harvest and pruning dates at all sites were determined by the vineyard manager to closely align with commercial practices in the regions.

At all phylloxera population monitoring sites yield and quality assessments were taken at harvest.

Statistical Analyses

All trial data was analysed using GenStat Version 8.1 software (Payne et al., 2006). Repeated Measures Analysis of Variance (ANOVA) was used for data collected over time (ie emergence trap data). Standard ANOVA was used for one off events (ie. harvest and pruning data). Where necessary data was transformed according to Log_{10} and least significant differences (LSD) were used to calculate significant differences ($p=0.05$). In season 3 at the Nagambie trial site pruning

data only was transformed (square root) in order to meet the assumptions in ANOVA, which states that all observations must be independent, have constant variance and are normally distributed.

Results

Site 1 - Rutherglen

Phylloxera Monitoring

Phylloxera emergence was monitored from early December 2003 to April 2006. The peak of phylloxera crawler abundance above-ground was consistently low over all three seasons (Table 2). Numbers were too low, even on ungrafted control vines, to conduct comparative statistical analysis of phylloxera abundance either within or between seasons. Nevertheless some interesting observations on abundance levels can be made (Table 2).

Surprisingly in some seasons ungrafted *V. vinifera* did not record the highest phylloxera abundance (Table 2). The low numbers on *V. vinifera* could not be attributed to previous root damage as the ungrafted vines at the site showed no obvious symptoms of vine decline or any classical symptoms of reduced vigour due to phylloxera-infested root damage. Phylloxera was found all rootstock types monitored. In the first season numbers were very low for all treatments and controls and for that reason the number of replicates was doubled in subsequent seasons. Richter 110 had the lowest total number of phylloxera over all seasons combined. No phylloxera were detected on ARG-1 during season 1. The highest total number of phylloxera, over all seasons combined, were recorded on Schwarzmann and own roots. The abundance of phylloxera overall differed between seasons. The highest phylloxera numbers were recorded on all rootstocks and the control in season 2.

Table 2. Relative abundance of phylloxera crawlers caught over three successive seasons on four rootstock types and ungrafted *V. vinifera* cv. Muscadelle over three successive seasons at Rutherglen field site

Rootstock	Season 2003-04[1]	Season 2004-05[2]	Season 2005-06[2]	Combined Total
V. vinifera	8	34	9	51
ARG-1	0	29	1	30
5BB Kober	4	17	3	24
110 Richter	6	9	2	17
Schwarzmann	5	26	23	54
Mean per trap	0.92	2.55	1.52	

1= sum of 5 replicates per rootstock 2 = sum of 9 replicates per rootstock treatment

Genotype Interactions

Numbers were too low, even on ungrafted control vines, to conduct comparative statistical analysis of phylloxera genotype abundance or rootstock interactions either within or between seasons.

Nevertheless some interesting observations can be made on the association between phylloxera clones and rootstock genotype and seasonal detection capability using emergence traps (Table 3). The predominant genotype found at the site was G7, which was found on all rootstocks and control. However, it was not found on every rootstock type in each season. In the 2003-2004 season G7 was only detected on 110 Richter yet was found in the following season on all rootstocks and control. This limited detection in this first season could be an artifact of having a low number of trap replicates it may also be due to some climatic effect reducing phylloxera emergence. In season 1 the genotypes G91 and G90, which had not previously been detected in Australia, were detected on 5BB Kober and own roots respectively but where not detected at all in the following two seasons. Confirmation of this genotype identity will be made in further studies. This suggests an extremely low level of virulence for these two genotypes. Genotype G30 was only detected on the rootstock ARG-1 and only during the 2004-2005 season. The genotypes G1 and G4 were only detected on own rooted vines and Schwarzmann and were less prevalent than genotype G7 and detection was seasonally dependent.

Table 3. Phylloxera genotype associated with rootstocks and ungrafted *V. vinifera* over three successive seasons at Rutherglen field site

Rootstock	Season 2003-04[1]	Season 2004-05[2]	Season 2005-06[2]	Total genotypes (3 seasons combined)
V. vinifera	G90	G7, G1	G1, G4	4
ARG-1	nd	G7, G30	nd	2
5BB Kober	G91	G7	G7	2
110 Richter	G7	G7	G7	1
Schwarzmann	G1	G7	G7, G4	3

1= sum of 5 replicates per rootstock 2 = sum of 10 replicates per rootstock

Vine Performance

The harvest data for all three seasons is shown in Table 4. In season 1 the mean bunch weight of Schwarzmann was significantly lower than both 5BB Kober and own-rooted *V. vinifera* (Table 4a) There were no other significant effects observed between rootstocks for all other harvest parameters during this first season.

In the second season total bunch weight per vine, mean bunch weight per vine and mean berry weight was significantly lower for own rooted vines than both 5BB Kober and 110 Richter (Table 4b). The mean bunch weight per vine and mean berry weight were also significantly lower for ARG-1 compared to 5BB Kober and 110 Richter. The juice pH was significantly more acidic for own roots and 110 Richter, compared to Schwarzmann, 5BB Kober and ARG-1.

In the third and final season there were no significant differences between treatments for mean total bunch number, mean total bunch weight or mean bunch weight per vine per vine (Table 4c). There were however significant treatment differences in mean berry weight – significantly lower mean berry weight was recorded on own rooted vines compared with all rootstock treatments. Own

rooted vines also had significantly higher acid pH than all rootstock treatments. Brix levels were significantly lower for own rooted vines compared to all rootstocks with the exception of Schwarzmann.

Unfortunately pruning data was only collected at the site on one occasion (July 2002) therefore no vigour assessment could be made between seasons. However, a visible assessment of vigour conducted at harvest time in each season indicated no obvious signs of vigour decline.

Table 4. Harvest data from a phylloxera-infested rootstock trial at Rutherglen trial site. Summary results from ANOVA

(a) Season 2003-2004

Rootstock	Mean total bunch number per vine	Mean total bunch weight per vine (kg)	Mean bunch weight per vine (g)	Mean berry weight per vine (g)	Mean °Brix	Mean pH
V. vinifera	68	12.24	186.2	1.49	25.1	4.32
ARG-1	83	15.01	180.6	1.38	25.8	4.35
5BB Kober	77	15.53	198.5	1.53	25.6	4.32
110 Richter	90	15.83	174.5	1.44	25.9	4.34
Schwarzmann	80	11.65	147.1	1.52	26.3	4.46
LSD(5%)	27	4.92	38.0	0.22	1.6	0.17
Significance	NS	NS	*	NS	NS	NS

(b) Season 2004-2005

Rootstock	Mean total bunch number per vine	Mean total bunch weight per vine (kg)	Mean bunch weight per vine (g)	Mean berry weight per vine (g)	Mean °Brix	Mean pH
V. vinifera	108	11.43	105.8	1.31	25.75	4.15
ARG-1	104	12.08	115.6	1.28	26.65	4.23
5BB Kober	108	14.54	133.5	1.51	26.90	4.24
110 Richter	107	14.61	139.0	1.54	26.55	4.15
Schwarzmann	101	11.97	121.6	1.40	27.00	4.28
LSD(5%)	18	2.78	17.6	0.16	1.50	0.06
Significance	NS	*	*	*	NS	*

(c) Season 2005-2006

Rootstock	Mean total bunch number per vine	Mean total bunch weight per vine (kg)	Mean bunch weight per vine (g)	Mean berry weight per vine (g)	Mean °Brix	Mean pH
V. vinifera	77	15.16	197.3	1.45	24.58	4.03
ARG-1	84	14.75	206.6	1.67	25.64	4.21
5BB Kober	74	15.58	213.9	1.90	25.74	4.25
110 Richter	80	16.38	207.5	1.77	25.90	4.16
Schwarzmann	83	15.73	193.3	1.62	25.22	4.26
LSD(5%)	13	3.50	40.4	0.16	0.91	0.10
Significance	NS	NS	NS	*	*	*

Vines were hand harvested April of each season. Least significant differences (LSD) can be used to compare significant differences between means within the column. When the difference between the means is greater than the LSD the treatments are statistically different (at the 95% confidence level), n=5 (season 1) n=9 (seasons 2 and 3), * =significant difference, NS =not significant.

Site 2 - Nagambie

Phylloxera Monitoring

Phylloxera emergence was monitored from late December 2003 to early April 2006. The peak of phylloxera crawler abundance above-ground was consistently low or non existent on all rootstocks over all three seasons with the exception of ARG-1 and the ungrafted control *V. vinifera* cv. Cabernet Sauvignon (Table 5). Winged alates were detected on both control and ARG-1 vines but at relatively low levels (data not presented). Peaks in emergence of alates followed similar trends for crawler emergence except in Season 3 when alates peaked in April. In season one phylloxera was only detected on ARG-1, *V. vinifera* and 5BB Kober. Phylloxera abundance was highest over an eight-week period from the end of December until late February.

Table 5. Relative abundance of phylloxera crawlers caught in emergence traps, over three successive seasons on four rootstock types and ungrafted *V. vinifera* cv. Cabernet Sauvignon, at Nagambie field site

Rootstock	Season 2003-04	Season 2004-05	Season 2005-06	Combined Total
V.vinifera	541	1262	530	2333
ARG1	1675	4108	4877	10660
5BB Kober	1	1	0	2
5C Teleki	-	-	0	0
110 Richter	0	0	0	0
140 Ruggeri	0	-	-	0
Ramsey	0	0	0	0
Rupestris du Lot	-	2	0	2
Schwarzmann	0	-	1	1

Where sample number =5 traps per vine per season

In the second season of monitoring peak numbers were recorded for ARG-1 and *V. vinifera* in late December a smaller secondary peak occurred on ARG-1 in early March. Phylloxera was also detected in relatively low numbers on 5BB Kober and Rupestris du Lot during the season. In the final season phylloxera were detected in emergence traps on ARG-1 and *V. vinifera* from mid-November through to early April (when monitoring was discontinued). Peak crawler numbers occurred in mid-December on ARG-1 and *V. vinifera* with a secondary peak on ARG-1 in mid February. A single phylloxera was also detected on a single Schwarzmann vine in late November.

Surprisingly overall three seasons ungrafted *V. vinifera* did not record the highest phylloxera abundance (Fig. 25) and this is attributed to the considerable decline in vigour of the own rooted vines and reduced root systems form decades of root damage induced by phylloxera feeding. Phylloxera was consistently found in higher abundance on ARG-1 and peaks in emergence followed similar trends to the own rooted vines with the exception of a later secondary peak on

ARG-1 only. Phylloxera was also detected albeit in very low numbers on 5BB Kober, Rupestris du Lot and Schwarzmann. Throughout the trial no phylloxera was detected on 110 Richter, Ramsey, 140 Ruggeri and 5C Teleki. In the first season numbers were relatively low for ARG and own rooted vines all treatments and controls but increased markedly in season 2 and 3.

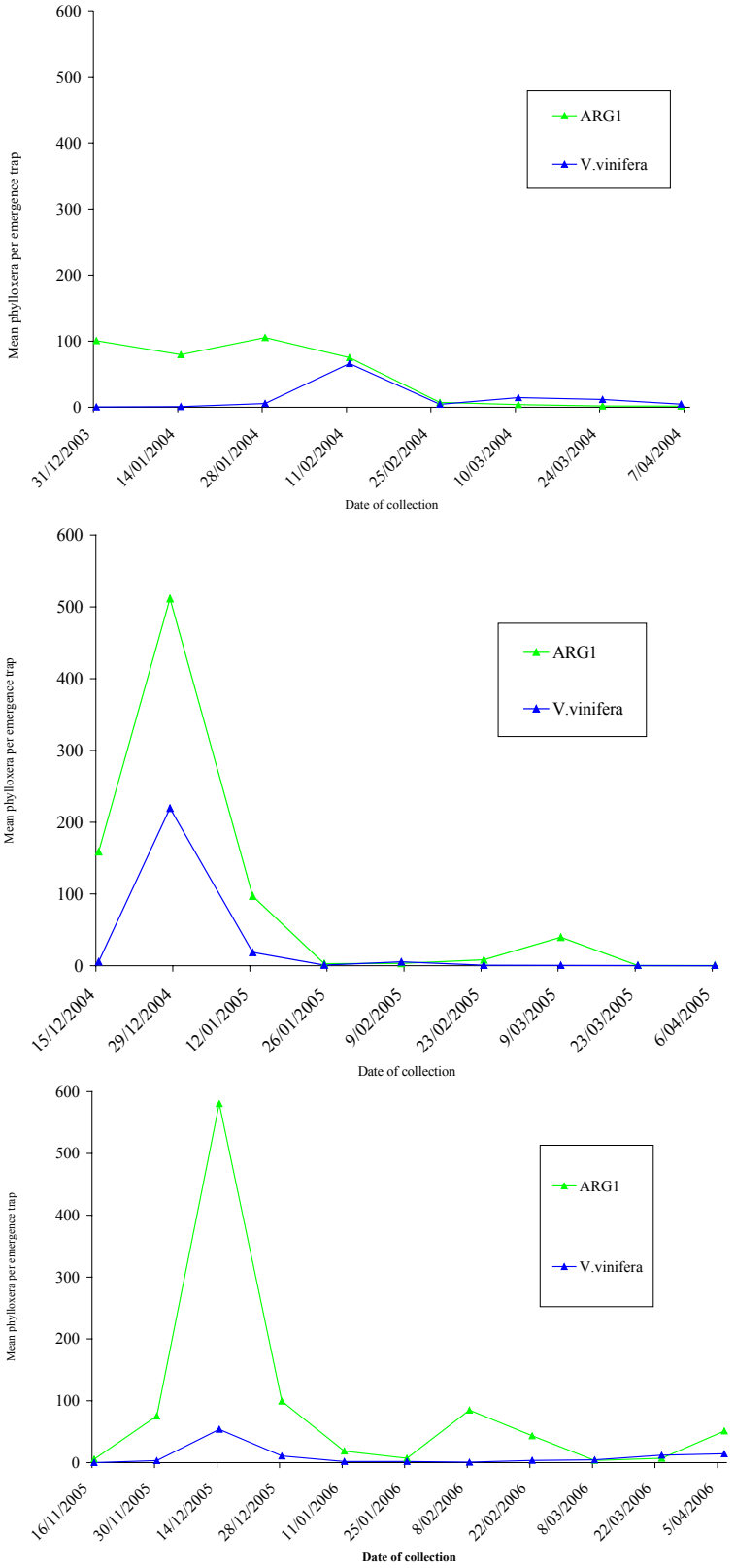


Figure 25. Relative phylloxera abundance at the Nagambie site over three seasons

Genotype Interactions

The only genotype found at the site was G1, which was found in all seasons predominantly on ARG-1 and ungrafted *V. vinifera*. It was also found in very low numbers on 5BB Kober, Rupestris du Lot and Schwarzmann but not for all seasons (Table 6).

Table 6. Phylloxera genotype associated with rootstocks and ungrafted *V. vinifera* over three successive seasons at Nagambie field site

Rootstock	Season 2003-04	Season 2004-05	Season 2005-06	Total genotypes (3 seasons combined)
V. vinifera	G1	G1	G1	1
ARG1	G1	G1	G1	1
5BB Kober	G1	G1	nd	1
5C Teleki	-	-	nd	0
110 Richter	nd	nd	nd	0
140 Ruggeri	nd	-	-	0
Ramsey	nd	nd	nd	0
Rupestris du Lot	-	G1	nd	1
Schwarzmann	nd	-	G1	1

Grapevine Performance

The rootstock harvest data for all three seasons is shown in Table 7. In all three seasons *V. vinifera* had significantly lower yield and pruning weights compared with all rootstock treatments. ARG-1 was the only rootstock that showed significantly reduced grape yields and pruning weights when compared with all other rootstocks and these differences were significant in the first and second seasons. Harvest data for seasons 1 and 2 showed similar overall trends but the overall yield reduction in season 3 for all treatments is attributed to significant hail damage, which occurred at the site in early 2005. Significant differences in pH and Brix levels were observed when comparing *V. vinifera* and against all rootstocks in seasons 1 and 2.

Table 7. Harvest data from a phylloxera-infested rootstock trial at the Nagambie trial site. Summary results from ANOVA.

(a) Season 1 (2003-2004) Harvest date: April 14 2004

	Mean total bunch number per vine	Mean total bunch weight per vine (kg)	Mean bunch weight per vine (g)	Mean berry weight per vine (g)	Mean Brix (°)	Mean pH	√Pruning [1]
V. vinifera	9.8	0.39	0.013	0.39	28.6	3.84	8.46
ARG-1	70.8	4.80	0.068	0.88	22.9	3.52	19.76
5BB Kober	79.4	7.90	0.102	0.88	24.6	3.65	34.92
5C Teleki	-	-	-	-	-	-	-
110 Richter	86.2	7.75	0.088	0.89	24.6	3.52	33.06
140 Ruggeri	112.4	10.40	0.094	0.85	24.7	3.67	42.22
Ramsey	80.8	7.78	0.098	0.90	23.1	3.68	34.57
Rupestris du Lot	-	-	-	-	-	-	-
Schwarzmann	93.0	8.10	0.088	0.82	24.6	3.72	31.34
LSD(5%)	20.0	2.14	0.0218	0.23	1.6	0.15	6.52
Significance	*	*	*	*	*	*	*

(b) Season 2 (2004-2005) Harvest date: March 31, 2005

	Mean total bunch number per vine	Mean total bunch weight per vine (kg)	Mean bunch weight per vine (g)	Mean berry weight per vine (g)	Mean Brix (°)	Mean pH	√Pruning [1]
V. vinifera	6.6	0.10	0.008	0.29	27.4	3.61	8.11
ARG-1	50.2	1.98	0.038	0.58	24.8	3.43	18.58
5BB Kober	64.2	4.78	0.077	0.77	24.9	3.50	32.96
5C Teleki	-	-	-	-	-	-	-
110 Richter	62.2	4.38	0.068	0.77	25.3	3.45	31.70
140 Ruggeri	-	-	-	-	-	-	-
Ramsey	71.0	5.67	0.080	0.74	24.2	3.50	33.20
Rupestris du Lot	74.8	5.21	0.072	0.77	25.2	3.60	35.95
Schwarzmann	-	-	-	-	-	-	-
LSD(5%)	16.1	1.69	0.022	0.14	2.1	0.13	5.13
Significance	*	*	*	*	*	*	*

(c) Season 3 (2005-2006) Harvest date: March 15, 2006

	Mean total bunch number per vine	Mean total bunch weight per vine (kg)	Mean bunch weight per vine (g)	Mean berry weight per vine (g)	Mean Brix (°)	Mean pH	√Pruning[1]
V. vinifera	17.6	0.56	0.022	0.52	26.6	3.75	9.42
ARG-1	41.4	1.76	0.043	0.75	24.7	3.47	18.95
5BB Kober	60.6	5.80	0.097	0.90	24.9	3.43	36.23
5C Teleki	60.4	5.10	0.085	0.93	25.6	3.38	34.59
110 Richter	62.0	4.96	0.079	0.89	25.0	3.36	32.13
140 Ruggeri	-	-	-	-	-	-	-
Ramsey	69.4	5.83	0.083	0.92	24.9	3.46	33.25
Rupestris du Lot	58.8	5.51	0.090	0.89	24.9	3.51	33.36
Schwarzmann	60.2	4.12	0.069	0.87	25.2	3.46	28.91
LSD(5%)	12.6	1.72	0.025	0.14	1.7	0.17	6.08
Significance	*	*	*	*		*	*

[1]Data was transformed (square root) in order to meet the assumptions in ANOVA, which states that all observations must be independent, have constant variance and are normally distributed.

Site 3 – Glenrowan

Phylloxera Monitoring

Phylloxera emergence was monitored from mid-November 2003 until early April 2006. The peak of phylloxera crawler abundance above-ground was relatively low on all rootstocks over all three seasons (Table 8). Over all seasons combined ungrafted *V. vinifera* although recording the highest phylloxera abundance had relatively low numbers compared to those described in the Nagambie trial despite being infested with the same phylloxera genotype G1. This may be partially attributed to older age of the vines and the considerable decline in vigour and reduced root systems of the own rooted vines from decades of root damage induced by phylloxera feeding. In season 1, phylloxera was only detected on Schwarzmann and *V. vinifera*. Phylloxera abundance on *V. vinifera* peaked in mid-February whereas abundance peaked in late January on Schwarzmann (Figure 26).

In the second season of monitoring peak numbers were recorded for 110 Richter, Schwarzmann, Ramsey, Rupestris du Lot and *V. vinifera*. Peak abundance was recorded on *V. vinifera* in mid-January and again slightly earlier on Schwarzmann with a smaller secondary peak occurred on Schwarzmann in early March. Phylloxera numbers on 110 Richter, Ramsey and Rupestris du Lot were relatively low compared to both *V. vinifera* and Schwarzmann.

In the final season phylloxera were detected in emergence traps on all rootstocks and *V. vinifera* from late-November through to early April (when monitoring was discontinued). Peak crawler numbers occurred in mid-December on most rootstocks with the exception of Schwarzmann with peak abundance in late December and *V. vinifera* showed a secondary peak in late January.

Table 8. Relative abundance of phylloxera crawlers caught over three successive seasons on four rootstock types and ungrafted *V. vinifera* cv. Muscat over three successive seasons at Glenrowan field site

Rootstock	Season 2003-04[1]	Season 2004-05[2]	Season 2005-06[2]	Combined Total
V. vinifera	109	164	118	391
110 Richter	0	0	25	25
Ramsey	0	0	4	4
Rupestris du Lot	0	2	161	163
Schwarzmann	38	72	107	217
total/season	147	238	415	800
mean per trap	7.35	5.67	9.88	

1= sum of 5 replicates per rootstock 2 = sum of 9 replicates per rootstock treatment

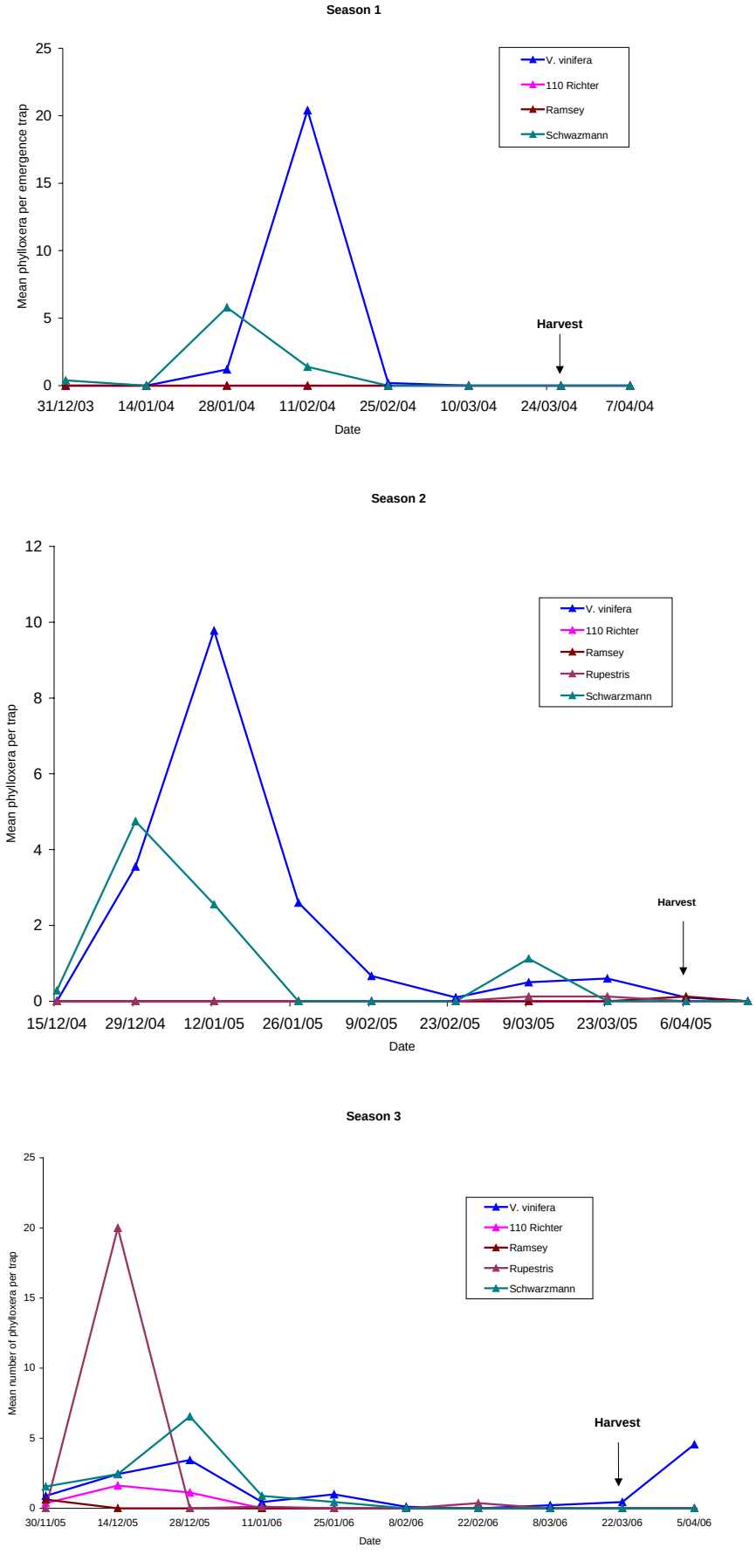


Figure 26. Relative abundance of phylloxera at the Glenrowan site over three seasons

Genotype Interactions

Some interesting observations can be made on the association between phylloxera clones and rootstock genotype and seasonal detection capability (Table 9). Six genotypes were detected at the site. The predominant genotype found at the site was G1, which was only found on 110 Richter, *V. vinifera* and Schwarzmann. G1 was only found on Schwarzmann in one season (season 3). The remaining five genotypes G3, G4, G35, G37 and G59 were all found on Schwarzmann and in addition G4 was also detected on Rupestris du Lot in season 3. However, genotypes were not always found in every season. For example, in the 2003-2004 season G35 and G37 were only detected on Schwarzmann and not detected on any other season. Whereas G59 was found in all three seasons on Schwarzmann.

Table 9. Phylloxera genotype associated with rootstocks and ungrafted *V. vinifera* over three successive seasons at Glenrowan field site

Rootstock	Season 2003-04[1]	Season 2004-05[2]	Season 2005-06[2]	Total genotypes (3 seasons combined)
V. vinifera	G1	G1	G1	1
110 Richter	nd	nd	G1	1
Ramsey	nd	nd	nd	0
Rupestris	nd	nd	G4	1
Schwarzmann	G35, G37, G59	G4, G59	G1, G3, G59	6

1= sum of 5 replicates per rootstock 2 = sum of 9 replicates per rootstock

Vine Performance

The harvest data for all three seasons is shown in Table 10. In season 1 the yield of *V. vinifera* was significantly lower than all rootstocks and this was reflected in overall grape yield, berry weight, pruning weight and bunch number. There were no other significant effects observed between rootstocks for all other harvest parameters during this first season.

In season 2 *V. vinifera* grape yield was again significantly lower than all rootstocks and this was particularly apparent in total bunch weight per vine, mean bunch weight per vine, mean berry weight and pruning weights. Grape quality as assessed by pH and Brix were significantly affected when comparing some rootstocks. In the third and final season *V. vinifera* was significantly lower than all rootstocks. There were some significant differences between rootstock treatments also.

Table 10. Harvest data from a phylloxera-infested rootstock trial at Glenrowan trial site. Summary results from ANOVA.

(a) Season 2003-2004

Rootstock	Mean total bunch number per vine	Mean total bunch weight per vine (kg)	Mean bunch weight per vine (g)	Mean berry weight per vine (g)	Mean ºBrix	Mean pH	Pruning weight (kg)
V.vinifera	2.7	0.41	32.00	0.52	30.78	3.89	0.15
110 Richter	41.6	3.85	87.90	1.34	28.80	3.83	0.86
Ramsey	47.6	4.17	84.70	1.29	29.29	3.86	0.86
Rupestris du Lot	-	-	-	-	-	-	-
Schwarzmann	33.8	3.22	90.40	1.40	27.90	3.85	0.56
LSD (5%)	11.82	1.85	48.87	0.52	3.10	0.15	0.16
Significance	*	*	*	*	NS	NS	*

(b) Season 2004-2005

Rootstock	Mean total bunch number per vine	Mean total bunch weight per vine (kg)	Mean bunch weight per vine (g)	Mean berry weight per vine (g)	Mean ºBrix	Mean pH	Pruning weight (kg)
V.vinifera	35	2.005	62.2	1.21	27.56	4.08	0.18
110 Richter	50.9	4.819	94.9	1.25	28.22	3.97	0.33
Ramsey	74.2	6.749	93.6	1.41	27.11	3.99	0.57
Rupestris du Lot	45.3	3.913	85.2	1.22	27.56	4.20	0.58
Schwarzmann	42.3	3.95	79.9	1.25	25.78	4.10	0.50
LSD (5%)	20.7	1.399	17.26	0.11	2.54	0.12	0.18
Significance	*	*	*	*	NS	*	*

(c) Season 2005-2006

Rootstock	Mean total bunch number per vine	Mean total bunch weight per vine (kg)	Mean bunch weight per vine (g)	Mean berry weight per vine (g)	Mean ºBrix	Mean pH	Pruning weight (kg)
V.vinifera	21.9	0.67	27.7	1.15	32.38	4.16	0.32
110 Richter	45.4	2.04	45.2	1.00	30.00	3.98	0.44
Ramsey	56.2	2.09	38	0.91	32.13	4.22	0.87
Rupestris du Lot	33.7	1.26	37.2	0.73	32.11	4.30	0.83
Schwarzmann	39.2	1.37	33.3	0.84	32.07	4.31	0.80
LSD (5%)	15.59	0.71	11.7	0.16	0.96	0.16	0.23
Significance	*	*	*	*	*	*	*

Table 11. Relative abundance of phylloxera crawlers (total) caught over three successive seasons on rootstocks and ungrafted *V. vinifera* at three field sites

Rootstock	Site 1 – Rutherglen	Site 2 - Nagambie	Site 3 - Glenrowan
V. vinifera	51	2333	391
ARG-1	30	10660	NT
5BB Kober	24	2	NT
5C Teleki	NT	0	NT
110 Richter	17	0	25
140 Ruggeri	NT	0	NT
Ramsey	NT	0	4
Rupestris du Lot	NT	2	163
Schwarzmann	54	1	217

Table 12. Phylloxera genotype associated with rootstocks and ungrafted *V. vinifera* over three successive seasons at three field sites

Rootstock	Site 1 - Rutherglen	Site 2 - Nagambie	Site 3 - Glenrowan	Total genotypes
V. vinifera	G1, G4, G7, G90	G1	G1	4
ARG-1	G7, G30	G1	-	3
5BB Kober	G7, G91	G1	-	3
5C Teleki	-	ND	-	0
110 Richter	G7	ND	G1	2
140 Ruggeri	-	ND	-	0
Ramsey	-	ND	-	0
Rupestris du Lot	-	G1	G4	2
Schwarzmann	G1, G4, G7	G1	G1, G3, G4, G35, G37, G59	7

Between Site Comparisons

At the Rutherglen site, despite phylloxera being present at the time of planting (ie the mid-1980s) no appreciable decline in grapevine health of either rootstocks or ungrafted *V. vinifera* was observed over 3 successive seasons. Phylloxera numbers caught in emergence traps were relatively low, compared to those recorded in the Nagambie and Glenrowan sites, over all 3 seasons (Table 11). A preliminary less detailed survey conducted by Corrie et al. (2002) confirmed the presence of genotype G7 in the Rutherglen vineyard. In our study G7 was the predominant genotype being found in all three seasons and also had a wider host range than other genotypes detected at the site,

as it was detected on *V. vinifera*, 5BB Kober, Schwarzmann, ARG-1 and 110 Richter (Table 12). This finding has important implications for quarantine risk as this genotype can survive, albeit at relatively low levels, on these commonly used rootstocks. In contrast other genotypes at the Rutherglen site where less prevalent in both their temporal and spatial abundance. For example the genotype G30 was only detected on ARG-1 in one of three seasons and not found on any of the other rootstocks therefore in comparison with G7 there is a lesser risk of transfer. Interestingly the genotype G1 which is known to occur in several other phylloxera-infested regions where it has been shown to be highly virulent causing severe vine damage over several seasons (eg: Nagambie, Upton, Glenrowan, Orchard Hills) was detected at the Rutherglen site but only in low numbers on both Schwarzmann and *V. vinifera* vines. This is in stark contrast to the relatively high abundance levels of G1 on *V. vinifera* found at the Nagambie site (Tables 11 and 12) in this study and at other sites in Nagambie and Upton where significant damage to ungrafted vines occurs (Powell 2000, 20003). This suggests that site reflected factors may play a role in the low occurrence of G1 at the Rutherglen site.

At the Rutherglen site crawlers were more abundant on the rootstock Schwarzmann which had slightly more phylloxera present than ungrafted *V. vinifera* and three phylloxera genotypes were detected on Schwarzmann. The detection of G1 in the field on Schwarzmann at the Rutherglen site is in contrast to other field observations. At the Glenrowan site although G1 was present on *V. vinifera* it could not be detected on Schwarzmann. Although crawlers were only found in low numbers during the course of the trial they still represent a significant risk of transfer, albeit relatively low, compared to sites monitored elsewhere (and reported in other sections of this report). The observation that phylloxera did occur on all rootstocks tested only highlights the importance of maintaining effective quarantine protocols even when planted on rootstocks.

Discussion

As there were no significant differences between phylloxera population levels on the vines over three successive seasons the significant differences observed in vine performance characters are unlikely to be due to the level of phylloxera infestation. It is more likely that other site factors such as weather or management practices have influenced these changes. This observation maybe due to the relatively low level of phylloxera populations at this site, and apparently low level of virulence, observed on the roots of the vines monitored. This is an interesting observation as other sites monitored during the course of the project in the King Valley, Nagambie and Upton showed significant yield decline between the two seasons.

Recommendations

Monitoring at this particular site has highlighted the fact that phylloxera population dynamics and in particular abundance on ungrafted vines can be extremely low. Phylloxera genotype virulence

and its interaction with edaphic characteristics may be a key factor in the low population levels observed.

- Further studies need to be undertaken to determine what other soil or climatic factors maybe influencing the host-insect interactions. This could be done by studies where abiotic and biotic variables can be manipulated and controlled under laboratory and glasshouse conditions.

These trials have highlighted that early detection of phylloxera using ground or aerial based systems may not be very satisfactory in some instances such as this where phylloxera populations are extremely low and visual symptoms of vine decline above-ground are not apparent.

- Future early detection methods will need to be able to detect phylloxera under such conditions to reduce the risk of this particular type of low virulence strain from being inadvertently moved to uninfested.

Chapter 5. Phylloxera and *Vitis* DNA Typing

Summary

When conducting trials to determine the performance of different phylloxera strains to ungrafted and grafted grapevines under field and controlled conditions it is essential to confirm the genetic identity of both the pest and the host plant. In this chapter the methods used to determine the genetic identity of the phylloxera strain and rootstocks used in rootstock trials (Chapters 2 and 3) and those collected from field trials (Chapters 1 and 4).

Methods

Phylloxera DNA Typing

Over 1000 insects were DNA typed during the course of the project for six microsatellite loci. For phylloxera population studies insects were collected from field sites located in the Buckland Valley, King Valley, Rutherglen, Glenrowan and Nagambie from either root samples or emergence trap samples (as described in Chapters 1 and 4). For glasshouse and laboratory based rootstock trials (Chapter 2 and 3) phylloxera were typed prior to inoculation of rootstock vine root material and also at trial completion to confirm no genotype cross-contamination had occurred. All insect samples collected for DNA typing were stored in 100% ethanol at -20°C and transferred to the CESAR laboratories under quarantine conditions for analysis.

DNA Extraction Method

The first stage in phylloxera DNA typing is to extract genomic DNA from the insect samples using a modified protocol (Herbert et al., 2006b). Single insects were placed in a sterile 0.5ml Eppendorf tube and tubes were then centrifuged briefly to deposit insects at the tube base. Then 50µl of a 5% Chelex® 100 (BioRad) solution, heated to 90°C, was added to each tube. Insects were ground in the Chelex solution using a sterile plastic grinding pestle and incubated in a water bath at 65°C for 1 hour. The tubes were then further incubated in a water bath at 90°C for 10 minutes and centrifuged at 13,000 rpm for 2 minutes. The bulk of the supernatant (containing insect DNA) was transferred from the tubes to a DNA stock plate for use with PCR and DNA stock plates (ie DNA extract) were stored at – 20°C.

PCR Amplification Method

Amplification of the microsatellite loci DVIT1-6 were performed as follows described in Corrie et al. (2002, 2003). PCR amplification was performed in a 10µl volume containing:

4.4µM forward and reverse untagged primers (Dvit1, Dvit2, Dvit3, Dvit4, Dvit5 and Dvit6) for each primer pair;
0.02µM Licor (fluorescent tagged) forward primer (Dvit1F, Dvit2F, Dvit3F, Dvit4, Dvit5F and Dvit6F) for each primer pair;
1X Platinum PCR Reaction Buffer;
1.5mM magnesium chloride;
0.2mM of the four dNTP's;
0.001mg BSA (Taq stabilizer);
0.5 Units Platinum Taq and autoclaved ultrapure water to a final volume of 10µl.

PCR (polymerise chain reaction) consisted of an initial denaturation step at 94 °C for 2 minutes; followed by 30-35 cycles (Table 13) of 94ºC denaturation for 30 seconds, X annealment (Table 13) for 30 seconds and 72°C extension for 30 seconds; followed by a final 72°C extension step for 4 minutes. Electrophoresis of the PCR products was performed and scored using a Licor IR2 DNA Analyser. Allele sizes were scored manually using standards for comparison.

Table 13. Definition of the six microsatellite loci used to determine the genotypes of grape phylloxera samples. Further details of PCR profiles for DVIT1-6 can be found in Corrie et al., 2002, 2003

Locus name	Repeat motif[a]	GenBank Accession no	Flanking primer sequences (5`→ 3`)	Allele sizes (bp)
DVIT1	$(CA)_9(CG)_4$	AYO56815	F: CGTTCGTTCTGGTATCGTTATT R: TAACGACCCGACTGAAATGTAG	128, 132, 134, 136, 138
DVIT2	$(CT)_{14}(TA)_{16}$	AYO56816	F: GCTTAATTTTGTGTCTCAAGTTA R: TAATGCTTCGTTTTCTAAGTGC	257, 259, 261, 289
DVIT3	$(AT)_9(GT)_{11}$	AYO56817	F: CCAAAACAACCAAGATTT TCT CC R: GATCCAAACTATGACAAACACCC	171, 175, 190
DVIT4	$(AAT)_{11}$	AYO56818	F: TCTTCAAAAATGTTACATGAT R: TATACAATGAATGGTATCAATTC	154, 156, 159, 162, 164, 168
DVIT5	$(A)_{13}$	AY371193	F: GAAATCCGTTCGGTGAGAGC R: TATGGTCAATGGTCAATCCGTC	129, 131, 132, 133, 140, 141, 142
DVIT6[β]	$(AAT)_9$	-	F: TGGACGATGGTTTTCATAGC R: TTGATTGTCATTGGTTTTGC	199, 205, 208, 211

[a]*Annealment temperatures and number of cycles for the six microsatellite loci*
Dvit 1: 50°C, 30 cycles
Dvit 2: 47°C, 35 cycles
Dvit 3: 47°C, 35 cycles
Dvit 4: 48°C, 30 cycles
Dvit 5: Touchdown = 55°C -45°C with 1°C /cycle reduction for 10 cycles followed by 20 cycles at 45°C.
Dvit 6: 50°C, 30 cycles.

[β] Primer details and PCR amplification protocol available from Astrid Forneck (University of Vienna, Austria)

***Vitis* DNA Typing**

Over 100 grapevine root samples were DNA typed during the course of the project for five microsatellite loci. For phylloxera population studies roots material was collected from vines which were monitored for phylloxera interactions and vine performance at rootstock trial field sites located in the Rutherglen, Glenrowan and Nagambie (as described in Chapter 4). For glasshouse and laboratory based rootstock trials (Chapters 2 and 3) selected root material were typed post inoculation to confirm rootstock parentage. All root samples collected for DNA typing were stored at –80°C and transferred in liquid nitrogen to the CESAR laboratories under quarantine conditions for analysis.

DNA Extraction Method

The first stage in *Vitis* DNA typing is to extract genomic DNA from the root samples. A 0.1g sample of root material was ground with liquid nitrogen and transferred to a sterile 1.5ml Eppendorf tube. Then 300µl of Plant DNAzol (Invitrogen) was added to each tube and mixed by inverting 10 times. This was followed by addition of 300µl pure chloroform and gentle shaking at room temperature for 5 minutes. Tubes were then centrifuged at 13,000 rpm for 10 minutes. The bulk of the supernatant (containing plant DNA) was transferred from the tubes to a fresh sterile 1.5ml Eppendorf tubes and 250µl of ice-cold 100% ethanol was added to each tube. Samples were stored at room temperature for 5 minutes and then centrifuged at 5000rpm for 5 minutes. Ethanol was gently pipetted from each tube and 300µl of 1 part DNAzol: 0.75 part 100% Ethanol was added. Tubes were gently vortexed, stored at room temperature for 5 minutes and then centrifuged at 5000rpm for 5 minutes. The DNAzol: Ethanol mixture was gently pipetted from each tube and 300µl of 70% ethanol added. Tubes were again gently vortexed followed by centrifugation at 5000rpm for 5 minutes. The ethanol was gently pipetted from each tube and tubes were air-dried for 30 minutes. The resultant DNA was resuspended in 35-100µl of 1XTE (pH 8.0) dependent upon DNE pellet size and resuspended overnight at room temperature. All DNA extractions were stored at – 20°C.

PCR Amplification Method

PCR amplification was performed in a 10 µl volume containing:

0.1 µM of forward primer (VVS1F, VVS2F, VMD5F, VMD7F and VMD28F) for each primer pair;

0.04µM of reverse and forward primers (VVS1, VVS2, VMD5, VMD7 and VMD28) for each primer pair;

0.04µM forward primer (VVS1F, VVS2F, VMD5F, VMD7F and VMD28F) end-labelled with [α^{33}P]-dATP (10 mCi/ml; General Electric) for each primer pair;

1X Platinum PCR reaction buffer;

2.0mM magnesium chloride;

0.175mM of the four dNTP's;

0.001mg BSA (Taq stabilizer);

0.5 Units Platinum Taq and autoclaved ultrapure water to a final volume of 10µl.

PCR consisted of an initial denaturation step at 94°C for 2 minutes; followed by 30-35 cycles (Table 14) of 94ºC denaturation for 30 seconds, X annealment (Table 14) for 30 seconds and 72°C extension for 30 seconds, followed by a final 72°C extension step for 4 minutes.

Electrophoresis of the PCR products was performed using standard techniques on 5% polyacrylamide gels with 1x TBE buffer. After electrophoresis the gel was dried and exposed to X-ray film (Kodak). Allele sizes were visually scored against standards.

Table 14. Primer sequences of the five microsatellite loci used to determine the genotypes of the *Vitis* rootstock samples.

Locus name	Flanking primer sequences (5`→ 3`)
VVS1	F: ACA ATT GGA AAC CGC GTG GAG
	R: CTT CTC AAT GAT ATC TAA AAC CAT G
VVS2	F: CAG CCC GTA AAT GTA TCC ATC
	R: AAA TTC AAA ATT CTA ATT CAA CTG G
VMD5	F: CTA GAG CTA CGC CAA TCC AA
	R: TAT ACC AAA AAT CAT ATT CCT AAA
VMD7	F: AGA GTT GCG GAG AAC AGG AT
	R: CGA ACC TTC ACA CGC TTG AT
VMD28	F: AAC AAT TCA ATG AAA AGA GAG AGA GAG A TCA
	R: TCA ATT TCG TAT CTC TAT TTG CTG

Annealment temperatures and number of cycles for the five microsatellite loci

VVS1: 56°C, 30 cycles
VVS2: 50°C, 30 cycles
VMD5: 56°C, 35 cycles
VMD7: 52°C, 35 cycles
VMD28: 56°C, 30 cycles

Chapter 6. Establishment of Rootstock Performance Trials Under Phylloxera-Infested Conditions

Objective: To establish field trials to compare the performance of the rootstock Börner with commercially available rootstocks and monitor the impacts of phylloxera on rootstock performance.

Background

In the late 1970s-mid 1980s several trials were established around Victoria to compare the performance of a range of rootstocks (Whiting 2003). Some of those trials were established in phylloxera-infested regions. Since the establishment of these trials some of the rootstocks used are no longer widely used (eg ARG1 and Harmony) and new rootstocks such as Börner, which is reportedly immune to phylloxera, have become available through breeding programs overseas. In addition our knowledge on the complexity of phylloxera genetics has increased and it is clear from controlled environment studies that phylloxera genotypes have different levels of virulence on rootstocks. Most of the existing rootstock trials in phylloxera-infested vineyards are unsuitable for phylloxera studies due to the decline in the susceptible *Vitis vinifera* and the lack of Börner. Therefore three new trials were established to compare the performance of Börner with widely used rootstocks and to monitor the activity and performance of different phylloxera genetic strains on these rootstocks.

Methods

Three study sites were chosen for establishment of the rootstock trials. At each study site phylloxera had previously been monitored for a number of years on ungrafted vines and phylloxera diversity at each site described (Powell, 2003). Trial plots were planted either in existing known infested soil or adjacent to vine blocks known to be infested to allow natural dispersal of phylloxera to the trial blocks. At the Rutherglen (Fig. 27) and Nagambie (Fig. 28) sites a randomised complete block design was established comprising 16 blocks with eight plots in each block, were planted on 14th and 22nd October 2003 respectively. At the King Valley site (Fig. 29) a randomised complete block design was established comprising 18 blocks with eight plots in each block representing single vine treatments were planted on 10th October 2003. The following single vine treatments at each site were used for each plot:

1. 140 Ruggeri (*Vitis berlandieri x V. rupestris*)
2. 1103 Paulsen (*V. berlandieri x V. rupestris*)
3. SO4 (*V. berlandieri x V. riparia*)
4. Teleki C (*V. berlandieri x V. riparia*)
5. Börner
6. 99 Richter (*V. berlandieri x V. rupestris*)
7. 5C Teleki (*V. berlandieri x V. riparia*)
8. Ungrafted *V. vinifera* L. (control)

Results

All vines were monitored for successful establishment and field grafting. Monitoring for vine performance and phylloxera performance was originally due to commence in July 2006 as part of a new research program Sustainable Phylloxera Management Phase 2. However, due to funding limitations this work will not be continued.

Recommendations

- Having three new rootstock trials in three different phylloxera-infested regions offers the unique opportunity for a comparative field screening of Börner performance against commercially available rootstocks under different soil conditions and to known phylloxera genetic strains.



Figure 27. Rutherglen rootstock field site



Figure 28. Nagambie rootstock field site



Figure 29. King Valley rootstock field site

Chapter 7. Scientific Validation of Influence of Soil Characteristics on Phylloxera Clones

Objectives:

- *Determine the influence of soil type on phylloxera distribution and development*
- *To identify potential phylloxera risk areas in major wine-grape producing areas of Australia*

Summary

Detailed soil characterisation was undertaken in phylloxera-infested vineyards in five Phylloxera Infested Zones in Victoria to assist in risk analysis based on soil properties.

Introduction

The life-cycle of grapevine phylloxera in Australia is dominated by the asexual root galling (radicicoliae) form of the insect. As the radicicoliae spends most of its life-cycle in close association with the host plants root system and soil environment, it is fundamentally important to understand how the soil environment impacts on phylloxera population, dispersal and level of root damage induced. Phylloxera has long been associated with soils having predominantly clay characteristics with a suggestion that it is the physical cracking properties of the soil that influence the insects' establishment and dispersal. This theory is now being questioned (J. Granett, pers. comm). The association of clay/sand content with phylloxera risk is based primarily on anecdotal evidence. There has been limited characterisation of soil types in phylloxera-infested vineyards known to be infested with specific phylloxera genotypes to draw any association between genotype distribution and soil characteristics. This fundamental knowledge is required to understand the spatial and temporal distribution of phylloxera and to attempt to classify 'risk regions' based on soil characteristics and likelihood of phylloxera establishment. It is also extremely important to understand the impact of soil properties on phylloxera interactions with vine roots when developing robust early detection systems. The level of sensitivity of both canopy-based and soil-based detection systems can be dependent on phylloxera's interaction with the host plant under different soil conditions. This is being further explored in a GWRDC-funded project *Optimising detection and management methods for soil borne pest incursions* to be published at a later date.

There are only limited studies on the impact of soil characteristics on phylloxera. Davidson and Nougaret (1921) suggest that phylloxera "live longer in rich deep well drained soils" and phylloxera-infested vines "die more rapidly in poorly drained" soils. One of the oft-quoted key references to the influence on soil type on phylloxera is from a Californian study conducted last century (Nougaret and Lapham, 1928). In their paper the authors, following a five-year soil survey of Californian vineyards, concluding that grapevines grown in soils with 60% or less sand content

are more susceptible to phylloxera damage. Buchanan (1990) concluded that phylloxera caused significant damage to young vines in the presence of dense clay subsoils. In recent years the association between phylloxera and soil characteristics has been examined in more detail in Europe (Reisenzein et al., 2006) and the USA (Fisher et al., 20003). But only limited studies have been conducted in Australia (Powell et al., 2003) and these have not taken into account the relative virulence of the phylloxera genetic strain at each site. To improve our understanding of soil impacts on phylloxera distribution a survey was conducted at known phylloxera-infested sites in North east Victoria to characterise soils were phylloxera has established.

In this study we conducted a baseline survey of 5 phylloxera-infested vineyards from 5 Phylloxera Infested Zones in North-East Victoria, to define common characteristics of the soils which may influence phylloxera abundance and hence highlight high risk areas.

Method

Soil Surveys

Soil surveys were conducted in December 2004 at five commercial vineyard sites. Soil pits where dug to a depth of 1.5 metres with a backhoe. Pits were dug in a T-shape (Fig. 30) or a rectangle (Fig. 31) with a straight end face and a sloping entrance. The pits were a minimum of 1 metre wide. Soil was characterised on-site and further classified on the basis of soil texture and chemistry. Soil pH classification was based on that reported by Cass et al (2002): less than 5.5 acid; 5.5-6.5 slightly acid; 6.5-8.0 neutral; 8.0-8.6 calcareous and greater than 8.6 alkaline. Soil were considered sodic if they had > 6% sodium, calcium deficient if < 50% calcium, and magnesium deficient if <25% magnesium present. If total nitrogen was <0.2% and total potassium was <80%, soils were considered deficient in these nutrients.



Figure 30. T-shaped soil pit located adjacent to a phylloxera-infested grapevine block at the Buckland Valley field site.



Figure 31. Rectangular shaped soil pit, with sloping entrance, located between phylloxera-infested grapevine rows at the Upton field site.

Results

Soils at all sites were classified on the basis of soil texture and chemistry (Isbell, 1996) and the classifications are outlined in Table 15. Further details for each of the five sites are described.

Table 15. General soil and phylloxera genotype description at each field site

Location/Site number	Phylloxera genotypes present*	Soil classification
Rutherglen	Several	Sodic mesotrophic brown dermosol
King Valley	G4	Bleached acidic red dermosol
Buckland Valley	G20	Acidic, mesotrophic, red dermosol
Nagambie	G1	Bleached, eutrophic, red dermosol
Upton	G1	Reticulate, magnesic, yellow chromosol

*as reported in Powell 2003; Powell et al 2006a

Site 1 - Rutherglen

In the Rutherglen region a vineyard block in which one-year old rootstock field trial (see Chapter 6) had been established, was chosen adjacent to a block known to be lightly infested with a range of phylloxera genetic strains (Corrie et al., 2003). The population dynamics at this site have previously been monitored over three successive seasons and been shown to have relatively low abundance in comparison with those monitored in other PIZs (Powell et al., 2003). These genotypes to-date have caused minimal vine damage despite phylloxera being first recorded in the vineyard at the turn of the 19th century.

The soil was classified as a sodic mesotrophic brown dermosol. The site geology and landscape is one of Quaternary alluvium sediments on a level plain. There was a gradual increase in texture (clay content) with depth down the soil profile (Fig. 32). Soil texture at the site generally demonstrated a duplex nature with sandy clay loam A horizons over medium to heavy clay subsoils. Top soil pH was slightly acidic and sub-soil pH ranged from slightly acid to alkaline (Table 16). Soil was deficient in potassium (Table 17). Sodium and potassium levels were relatively stable moving down the profile. In general, in the A horizon, a relatively high fine sand content (50%) and clay content increased to 48% in the B horizon (Fig. 32). Concentrations of exchangeable aluminium were generally low (<10mg/kg) throughout the soil profile. Exchangeable calcium levels were not deficient. Magnesium and sodium levels increased with increasing depth Roots were common down to 60cm depth. The deeper subsoil (from 70 cm depth) becomes strongly sodic and dispersive. This results in restricted root and water movement at this depth.

Soil Profile Morphology- Rutherglen (Site 1)

Surface Soil

Ap 0-15 cm Brown (7.5YR4/3); *fine sandy clay loam*; weak coarse blocky structure; hard-setting surface condition; firm to very firm consistency (dry); many macropores present; pH 5.4; clear change to:



Subsoil

B1 15-30 cm Strong brown (7.5YR4/6); *light clay (fine sandy);* weak to moderate sub-angular blocky structure; many fine to medium macropores; firm consistency (slightly moist); pH 6.0; gradual change to:

B21 30-50 cm Strong brown (7.5YR5/6) with few (5%) dark red (2.5YR4/6) mottles; *light medium clay (fine sandy)*; moderate – strong, medium to fine polyhedral structure; weak consistency (moist); few (5%) medium sized (5 mm) ferromanganiferous nodules; roots common to 60 cm; pH 6.1; gradual change to:

B22 50-70 cm Brownish yellow (10YR6/6) with many (40%) strong brown (7.5YR5/6) mottles; *medium clay*; moderate - strong, medium to fine sub-angular blocky structure; weak consistency (moist); few (5%) medium (5-10 mm) ferromanganiferous and clay nodules; and few (5%) manganese stains; pH 6.6; gradual change to:

B23 70-95 cm Light yellowish brown (2.5Y6/4) with many (30%) strong brown (7.5YR4/6) mottles; *medium heavy clay*; moderate – strong, medium to fine sub-angular blocky structure; firm consistency (moist); manganese staining common; few (5%) ferromanganiferous and clay nodules; roots to 80 cm; pH 7.6; gradual change to:

B24 95-110+ cm Light olive brown (2.5Y5/4) with many (30%) reddish yellow (7.5YR6/6) mottles; strong – medium to fine sub-angular blocky; firm consistency (moist); few (2.5%) fine manganese flecks; pH 8.9.

Table 16. Key Profile Characteristics – Rutherglen (Site 1)

	pH	Salinity Rating	Sodicity	Dispersion
Surface (Ap horizon)	Strongly Acid	Very Low	Non-Sodic	None
Subsoil (B21 horizon)	Slightly Acid	Very Low	Non-Sodic	None
Deeper subsoil (at 95-110+ cm)	Strongly Alkaline	Very Low	Strongly Sodic	Strong

Table 17. Key Chemical Characteristics – Rutherglen (Site 1)

Horizon	Horizon Depth	pH (water)	pH $CaCl_2$	EC dS/m	Exchangeable Cations Ca	Mg	K	Na meq/100g	Ex Al mg/kg	Ex Ac meq/100g	Field pF2.5	Wilting Point pF4.2	Coarse Sand (0.2-2.0mm)	Fine Sand (0.02-0.2mm)	Silt (0.002-0.02mm)	Clay (<0.002mm)
Ap	0-15	5.4	4.7	0.08	3.2	0.92	0.82	0.13	<10	6.0	24	6.3	10	50	18	16
B1	15-30	6.0	5.2	0.06	3.1	1.7	0.37	0.26	<10	4.6	23.3	8.4	7	43	16	31
B21	30-50	6.1	5.1	<0.05	2.3	2.2	0.28	0.38	<10	3.6	24.7	9.1	7	42	14	35
B22	50-70	6.6	5.2	0.07	2.0	4.4	0.43	1.4		5.1	25.8	12.4	7	34	15	41
B23	70-95	7.6	6.2	0.09	2.6	6.4	0.59	2.8			29.1	16.3	5	31	17	48
B24	95-110+	8.9	7.9	0.14	3.0	7.3	0.62	3.5			30.4	15.4				

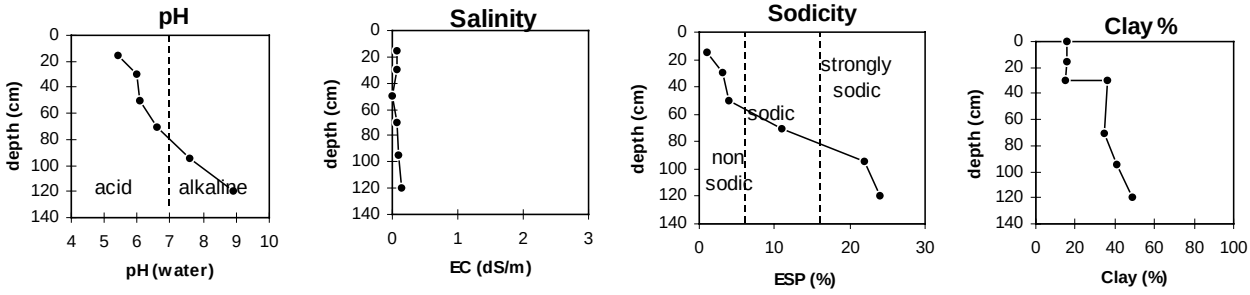


Figure 32. Influence of soil depth on soil chemistry – Rutherglen (Site 1)

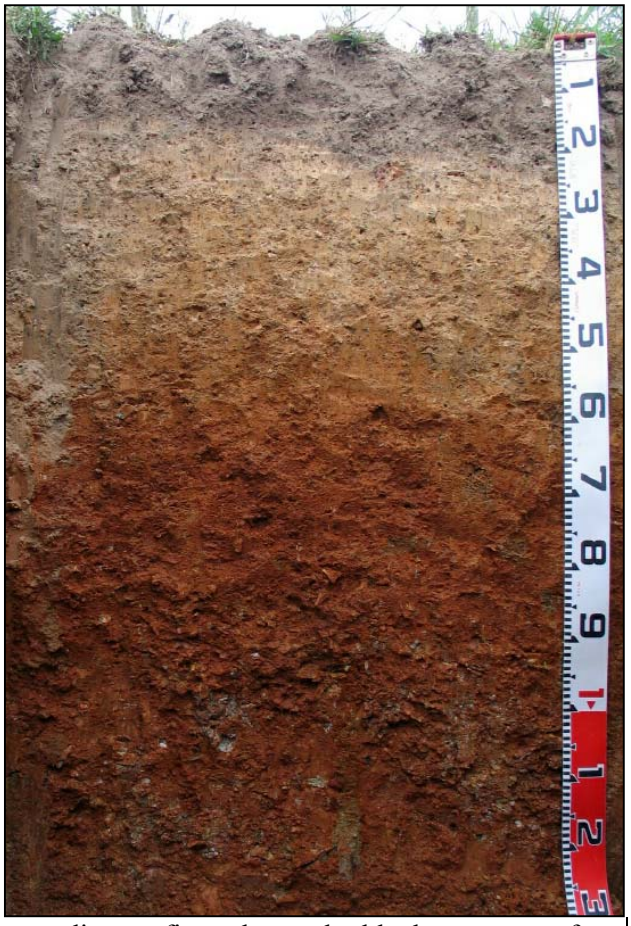
Site 2 – King Valley

In the King Valley region a vineyard block was selected in which a one-year old rootstock field trial (see Chapter 6) had been established, adjacent to a block known to be heavily infested with phylloxera genotype G4. This phylloxera strain at the site has caused considerable vine damage to the vineyards since it was first detected in 1997 and has been monitored for three successive seasons (Powell et al., 2006b) and been shown to have high population abundance and high risk of transfer. The soil was classified as a bleached-acidic mesotrophic red dermosol. There is a gradual increase in texture (clay content) with depth down the soil profile. Soil texture at the site demonstrated a duplex nature with light sandy clay loam A horizons overlying light to medium clay subsoils. Top soil pH was generally slightly acid with sub-soil more acidic than corresponding top soil (Table 18). The soil was deficient in potassium (Table 19). High clay content (up to 54%) was observed which increased marginally moving down the profile (Fig. 33). Concentrations of exchangeable aluminium were toxic (up to 860mg/kg) throughout the soil profile with the lowest level being in the A horizon. Exchangeable calcium was acceptable and generally decreased with increasing soil depth. Magnesium levels were not deficient and increased with increasing soil depth. Sodium levels were acceptable.

Soil Profile Morphology- King Valley (Site 2)

Surface Soil

A1 0-15 cm Very dark greyish brown (10YR3/2); *light very fine sandy clay loam*; weak medium, parting to moderate fine to very fine sub-angular blocky structure; firm consistency (dry); medium to coarse (2-20 mm) angular quartz fragments common (15%); pH 6.4; clear change to:



A21 15-20 cm Very dark greyish brown (10YR3/2); sporadically bleached; *light very fine sandy clay loam*; firm consistency (dry); medium (2-10 mm) quartz fragments common (15%); pH 5.5; clear change to:

A22 20-35 cm Brown (7.5YR5/3) with many (20%) reddish yellow (7.5YR6/6) mottles; conspicuously bleached; *very fine sandy clay loam*; firm consistency (dry); few (5-10%) fine to coarse (2-20 mm) quartz fragments; pH 5.1; gradual change to:

A3 35-45 cm Brown (7.5YR5/4); *very fine sandy clay loam*; moderate medium sub-angular blocky structure; weak to firm consistency (moist); few (5-10%) fine to coarse (2-15 mm) quartz fragments; pH 5.2; gradual change to:

Subsoil

B21 45-60 cm Yellowish red (5YR5/6) with many (40%) pale brown (10YR6/3) mottles; *light clay*; moderate – strong, medium to fine sub-angular blocky structure; few macropores; weak to firm consistency (moist); few (5%) quartz nodules; pH 5.1; gradual change to:

B22 60-85 cm Dark red (2.5YR4/6) with few (10%) yellowish brown (10YR6/6) mottles; *medium clay*; moderate - strong, medium to fine sub-angular blocky structure; many (50%) shale fragments (10-30 mm) weak consistency (moist); pH 4.9; gradual change to:

B23 85-120 cm Dark red (2.5YR4/6) with brownish yellow (10YR6/8) mottles; *medium clay*; pH 4.8:

B24 120+ cm Dark red (2.5YR4/6) with few (10%) light yellowish brown (10YR6/4) and grey (10YR6/1) mottles in patches; *sandy clay loam*.

Table 18. Key Profile Characteristics – King Valley (Site 2)

	pH	Salinity Rating	Sodicity	Dispersion
Surface (A1 horizon)	Slightly Acid	Very Low	Non-Sodic	None
Subsoil (B21 horizon)	Very Strongly Acid	Very Low	Non-Sodic	None
Deeper subsoil (at 85-120 cm)	Very Strongly Acid	Very Low	Non-Sodic	None

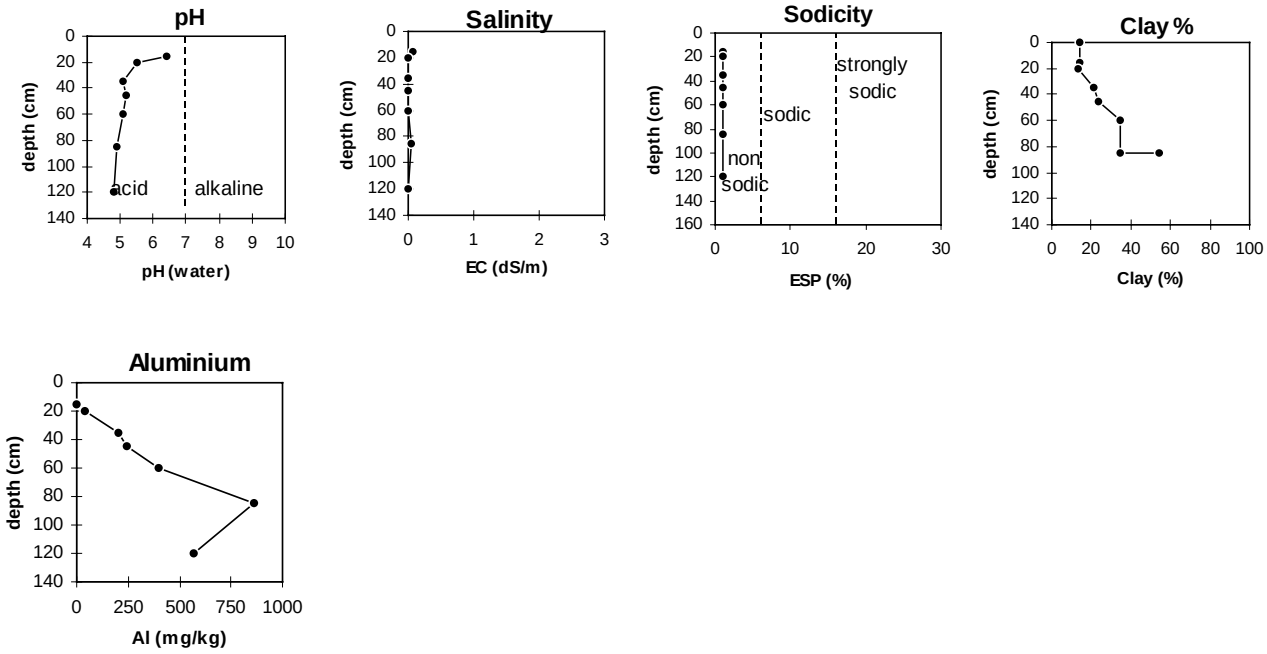


Figure 33. Influence of soil depth on soil chemistry – King Valley (Site 2)

Table 19. Key Chemical Characteristics – King Valley (Site 2)

Horizon	Horizon Depth	pH (water)	pH $CaCl_2$	EC dS/m	Exchangeable Cations meq/100g Ca	Mg	K	Na	Ex Al mg/kg	Ex Ac meq/100g	Field pF2.5	Wilting Point pF4.2	Coarse Sand (0.2-2.0mm)	Fine Sand (0.02-0.2mm)	Silt (0.002-0.02mm)	Clay (<0.002mm)
A1	0-15	6.4	5.8	0.07	7.9	1.6	0.52	0.13	<10	6.6	35.9	8.7	14	28	38	14
A21	15-20	5.5	4.7	<0.05	2.8	0.73	0.23	0.09	43	8.2	28.7	6.6	18	29	37	13
A22	20-35	5.1	4.3	<0.05	1.1	0.44	0.31	0.09	200	6.7	26.0	7.9	13	20	45	21
A3	35-45	5.2	4.4	<0.05	0.95	0.55	0.38	0.08	240	6.5	25.2	9.4	13	22	40	24
B21	45-60	5.1	4.2	<0.05	1.3	1.2	0.60	0.13	400	8.3	27.1	12.7	12	18	32	35
B22	60-85	4.9	4.1	0.05	0.61	1.8	0.49	0.09	860	15	32.6	19.4	12	13	21	54
B23	85-120	4.8	4.1	<0.05	0.16	0.68	0.24	0.08	570	7.8						

Site 3 – Buckland Valley

In the Buckland Valley site (Fig. 34) phylloxera was first detected in December 2003 and is characterised as genotype G20 with low phylloxera abundance (Chapter 1) and relatively low virulence (Chapters 2 and 3). The site has a gentle rise and is on the second terrace level of the Buckland River. The soil was classified as an acidic mesotrophic red dermosol with a general increase in texture (clay content) with depth down the profile.

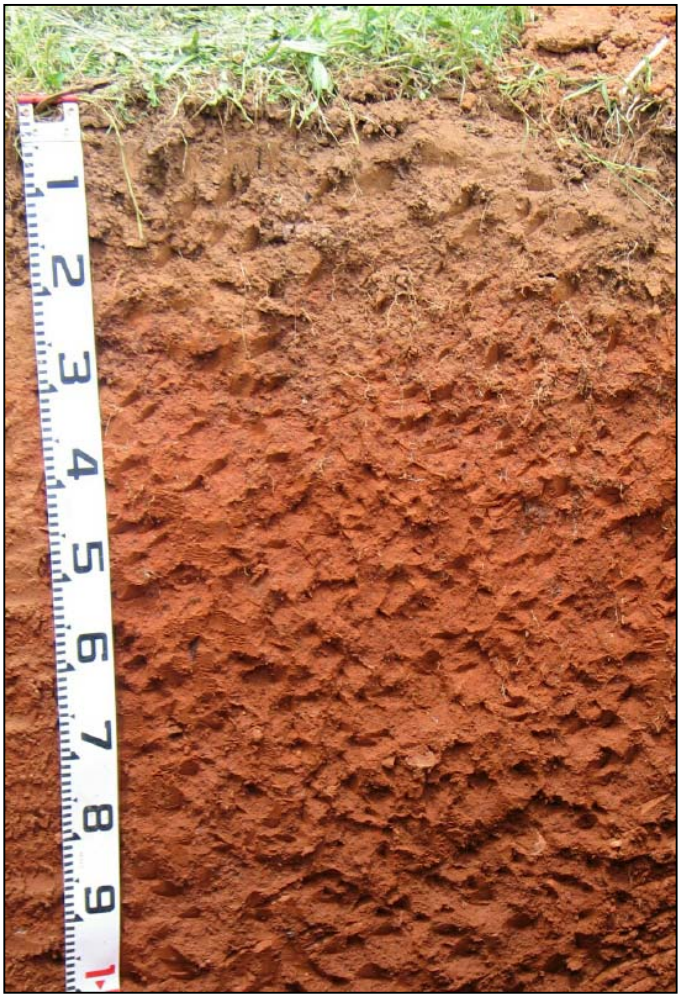
The soil texture at this demonstrated a duplex nature with fine sandy clay loam A horizons over medium clay subsoils (Table 20). All soils were deficient in potassium and magnesium (Table 21). A high clay content was observed in the sub soil (up to 52%) and relatively low sand content (<20%). Soils were not sodic (Fig. 35). Concentrations of exchangeable aluminium reached toxic levels of up to 420mg/kg in the B horizon. Calcium and magnesium levels decreased with increasing soil depth. Top soil was moderately acidic and subsoil pH was strongly acidic.



Figure 34. Buckland Valley field site showing rows of infested grapevines (background) and location of soil pit (foreground)

Soil Profile Morphology - Buckland Valley (Site 3)

Surface Soil
A11 0-12 cm Dark brown (7.5YR3/3); *fine sandy clay loam*; moderate - strong medium to fine sub-angular blocky structure; very firm consistency (dry); pH 5.8; clear change to:



A12 12-20 cm Dark Brown (7.5YR3/4) and light brown (7.5YR6/4 dry); *fine sandy clay loam*; strong medium to fine sub-angular blocky structure; firm consistency (slightly moist); many large macropores present; some (5%) fine dark manganese staining; pH 5.4; clear change to:

Subsoil
B21 20-40 cm Dark red (2.5YR4/6); *light medium clay*; strong coarse to medium sub-angular blocky, parting to moderate to strong fine polyhedral structure; firm consistency (moist); many macropores present; pH 5.2; gradual change to":

B22 40-70 cm Dark red (2.5YR4/8); *medium clay*; moderate to strong coarse medium sub-angular blocky structure; weak to firm consistency (moist); few (5%) rounded quartz pebbles; many macropores present; pH 5.0; gradual change to:

B3 70-100 cm Dusky red (2.5YR3/4); *fine sandy clay loam*; moderate – strong, medium to fine sub-angular blocky structure; weak to firm consistency (moist); pH 5.1.

Table 20. Key Profile Characteristics – Buckland Valley (Site 3)

	pH	Salinity Rating	Sodicity	Dispersion
Surface (A11 horizon)	Moderately Acid	Very Low	Non-Sodic	None
Subsoil (B21 horizon)	Strongly Acid	Very Low	Non-Sodic	None
Deeper subsoil (at 70-100 cm)	Strongly Acid	Very Low	Non-Sodic	None

Table 21. Key Chemical Characteristics – Buckland Valley (Site 3)

Horizon	Horizon Depth	pH (water)	pH $CaCl_2$	EC dS/m	Exchangeable Cations Ca	Mg	K	Na meq/100g	Ex Al mg/kg	Ex Ac meq/100g	Field pF2.5	Wilting Point pF4.2	Coarse Sand (0.2-2.0mm)	Fine Sand (0.02-0.2mm)	Silt (0.002-0.02mm)	Clay (<0.002mm)
A11	0-12	5.8	5.2	0.09	7.6	2.1	1.9	0.09	<10	11	34.3	13.3	10	28	30	26
A12	12-20	5.4	4.7	0.06	5.0	1.0	1.3	0.09	59	11	32.6	12.7	8	22	35	30
B21	20-40	5.2	4.3	0.05	3.7	1.1	1.4	0.07	260	10	33.6	15.9	2	17	34	46
B22	40-70	5.0	4.2	0.06	2.0	0.67	1.6	0.10	420	9.6	34.9	16.6	2	20	28	46
B3	70-100	5.1	4.3	0.06	3.0	0.55	1.9	0.08	420	12	34.5	18.6	3	20	23	52

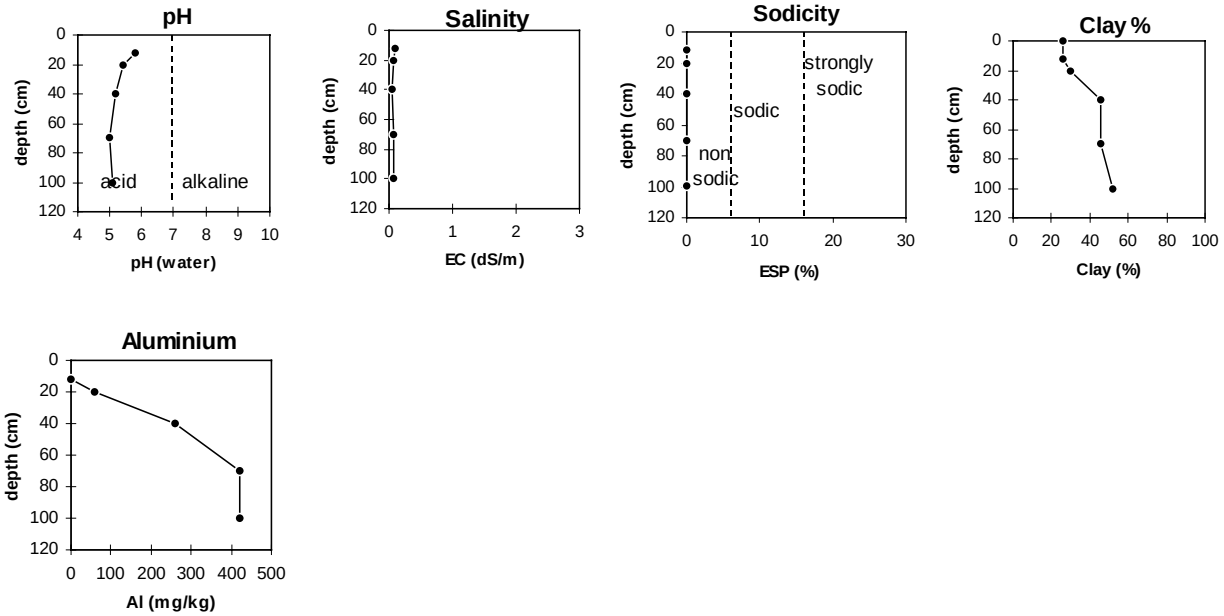


Figure 35. Influence of soil depth on soil chemistry – Buckland Valley (Site 3)

Site 4 - Nagambie

At the Nagambie site a vineyard block in which one-year old rootstock field trial (see Chapter 6) had been established was selected, adjacent to a block known to be heavily infested with a relatively virulent genotype phylloxera genotype G1. G1 has caused considerable vine damage at the selected vineyard since it was first detected in 1969. The vineyard has been monitored for three successive seasons and has been shown to have high population abundance (Chapter 9; Powell et al., 2006c). The soil was classified as a bleached eutrophic red dermosol with medium to thick horizons. The A horizon (with the exception of A1) was more acidic than the sub-soil (Table 22). The soil has a strong texture contrast between surface (A) horizons and upper subsoil (B21) horizon. Soil texture at the site demonstrated a duplex nature with fine sandy loam A horizons over fine sandy clay loam subsoils (Table 23). In the A horizon a relatively low clay content (<10%) was observed but this increased moving down the profile to reach up to 23 % in the B horizon (Fig. 36). Sand content reached up to 68% in the A horizon. All soils were deficient in potassium throughout the soil profile. Exchangeable calcium generally decreased with increasing soil depth,

whereas magnesium levels generally increased with increasing depth. Sodium and potassium levels were relatively stable moving down the profile.

Soil Profile Morphology- Nagambie (Site 4)

Surface Soil
A1 0-10 cm Brown (7.5YR4/3); *fine sandy loam;* weak coarse blocky structure; firm to very firm consistency (dry); pH 7.0; clear change to:



A21 10-20 cm Brown (7.5YR5/4); conspicuously bleached (10YR7/3 dry) with many (5-10%) yellowish red (5YR5/6) mottles; *fine sandy loam*; apedal; firm to very firm consistency (dry); pH 4.9; clear change to:

A22 20-30 cm Yellowish red (5YR5/6); conspicuously bleached (7.5YR7/4); *heavy fine sandy loam*; apedal; very firm consistency (dry); many very fine to fine macropores present; pH4.9; clear change to:

Subsoil
B21 30-45 cm Dark red (2.5YR4/6); *fine sandy clay*; moderate coarse blocky, parting to moderate medium polyhedral structure; strong consistency (dry); many (20%) very fine to fine macropores present; pH 5.7; gradual change to:

B22 45-70 cm Dark yellowish brown (10YR4/4) with many (20%) faint reddish yellow (7.5YR6/8) mottles; *fine sandy clay*; moderate to strong blocky structure; strong consistency (dry); many very fine to fine macropores present; pH 6.7; gradual change to:

B23 70-90 cm Dark yellowish brown (10YR4/4) with many faint reddish yellow (7.5YR6/8) mottles; *fine sandy clay loam*; moderate medium polyhedral structure; very firm to strong consistency (dry); many very fine to fine macropores present; pH 6.7; abrupt change to:

B24 90-100 cm Strong brown (7.5YR5/6); *fine clayey sand*; apedal; weak consistency (slightly moist); abrupt change to:

B25 100+ cm Dusky red (2.5YR4/4); *fine sandy clay loam*; moderate coarse prismatic, parting to moderate coarse blocky structure; pH 6.6.

Table 22. Key Profile Characteristics – Nagambie (Site 4)

	pH	Salinity Rating	Sodicity	Dispersion
Surface (A1 horizon)	Neutral	Low	Non-Sodic	None
Subsoil (B21 horizon)	Moderately Acid	Very Low	Non-Sodic	None
Deeper subsoil (at 100+ cm)	Slightly Acid	Very Low	Non-Sodic	None

Table 23. Key Chemical Characteristics – Nagambie (Site 4)

Horizon	Horizon Depth	pH (water)	pH $CaCl_2$	EC dS/m	Exchangeable Cations Meq/100g Ca	Mg	K	Na	Ex Al mg/kg	Ex Ac meq/100g	Field pF2.5	Wilting Point pF4.2	Coarse Sand (0.2-2.0mm)	Fine Sand (0.02-0.2mm)	Silt (0.002-0.02mm)	Clay (<0.002mm)
A1	0-10	7.0	6.6	0.19	6.1	0.80	0.61	0.12			22.7	5.5	4	65	19	8
A21	10-20	4.9	4.5	0.10	1.2	0.46	0.19	0.11		3	19.4	3.9	2	68	25	6
A22	20-30	4.9	4.5	0.10	1.1	0.48	0.15	0.13		2.3	18.1	3.7	2	66	20	9
B21	30-45	5.7	5.1	0.11	3.0	1.1	0.19	0.25		2.3	19.6	6.9	1	58	16	22
B22	45-70	6.7	5.9	0.07	3.7	1.5	0.16	0.29		2.5	22.3	8.2	1	57	17	23
B23	70-90	6.7	6.0	0.07	3.3	1.7	0.17	0.32		1.8	21.8	7.0	1	66	7	23
B24	90-100	6.4	5.7	<0.05	1.7	1.2	0.08	0.2								
B25	100+	6.6	5.8	0.05	2.8	2.1	0.13	0.21								

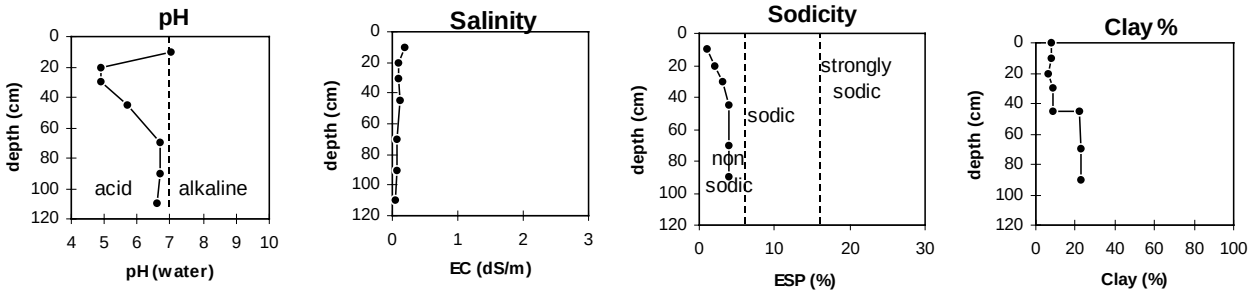


Figure 36. Influence of soil depth on soil chemistry – Nagambie (Site 4)

Site 5 - Upton

As only one vineyard is known infested in the Upton region, where phylloxera was first detected in 2000, samples were collected from two blocks in the same vineyard (Fig. 37) where phylloxera population studies on ungrafted vines have been conducted over three successive seasons (Powell 2003). Phylloxera population abundance at this site has been monitored previously and is known to be moderately abundant (Powell et al., 2003, Herbert et al., 2006a) but has been slow to disperse since it was first detected (K. Powell, pers. observ.). Phylloxera at this site has been characterised as genotype G1 a relatively virulent genotype (Chapters 2 and 3). The site has a granitic geology with low sloping landscape. The soils were classified as reticulate, magnesic, yellow chromosol (Isbell, 1996). Top soil was moderately acidic and sub-soil strongly acidic (Table 24).

The soil has a strong texture contrast between surface (A) horizons and upper subsoil (B21) horizon. Soil texture at the Upton sites generally demonstrated a duplex nature with sandy clay loam A horizons over medium clay loam subsoils (Table 25). Reticulate mottling ("tiger mottles") was observed in deep subsoil (Fig. 38). In the A horizon a relatively low clay and silt content (<10%) was observed but this increased moving down the profile to reach up to 63% in the B

horizon. Sand content reached up to 46% in the A horizon. All soils were deficient in potassium throughout the soil profile. Concentrations of exchangeable aluminium increased moving down the profile reaching toxic levels of up to 410 in the B horizon (Fig. 39). Exchangeable calcium decreased with increasing soil depth, whereas magnesium levels generally increased with depth. Sodium and potassium levels were relatively stable moving down the profile (Table 24).



Figure 37. Upton field site showing phylloxera-infested grapevines

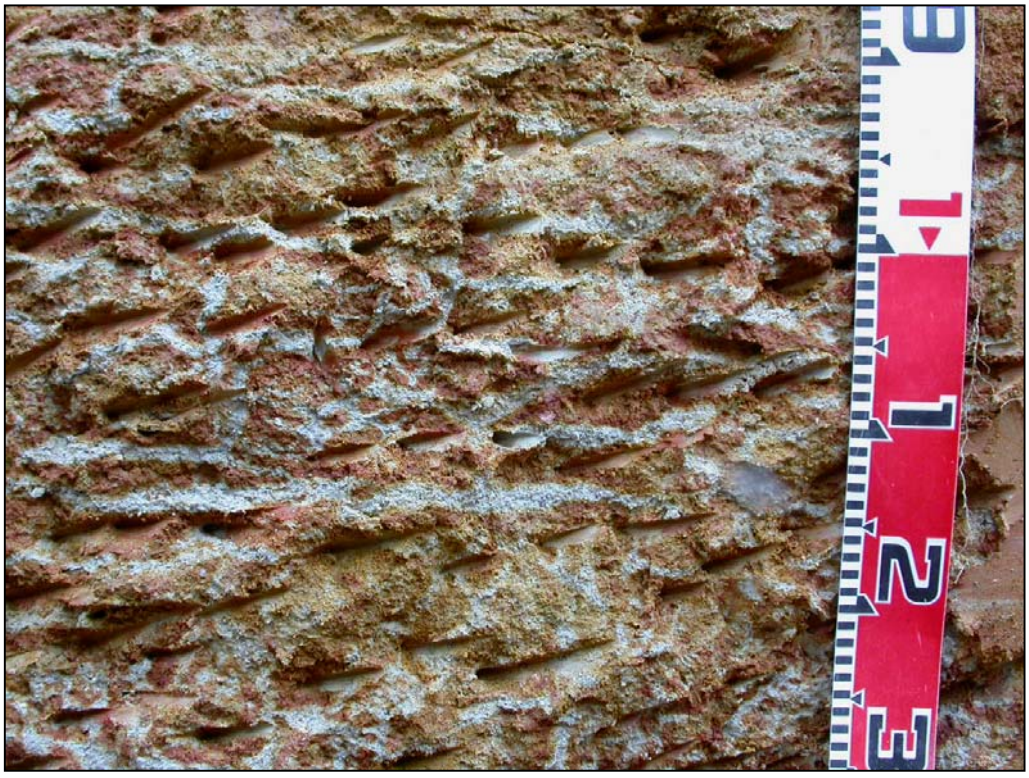


Figure 38. Reticulate "tiger" mottling observed at >1 metre depth at Upton field site

Soil Profile Morphology- Upton (Site 5)

Surface Soil

A1 0-13 cm Very dark greyish brown (10YR3/2); *loam*; weak strong blocky structure; moderate fine to very fine granular structure near surface; weak to firm consistency (dry); few (5%) medium (2-5 mm) quartz fragments; pH 5.9; clear change to:



A21 13-25 cm Yellowish brown (10YR5/4); conspicuously bleached (10YR7/2 dry); *sandy clay loam*; apedal; firm consistency (moist); few (5%) medium (2-8 mm) sub-angular quartz fragments; fine to medium macropores common; pH 5.8; clear change to:

A22 25-45 cm Light yellowish brown (10YR6/4); conspicuously bleached; *heavy sandy clay loam;* apedal; weak to firm consistency (moist); few (5%) medium (2-6 mm) sub-angular quartz fragments; fine to medium macropores common; pH 5.7; abrupt change to:

Subsoil

B21 45-65 cm Brownish yellow (10YR6/6); *medium clay*; moderate medium sub-angular, parting to strong fine sub-angular blocky structure; weak to firm consistency (moist); few (2%) ferruginous and clay nodules; few (5%) quartz fragments; pH 5.6; clear change to:

B22 65-90 cm Brownish yellow (10YR6/6) with many (20%) faint strong brown (7.5YR5/6) mottles; *light medium clay*; strong coarse sub-angular blocky, parting to strong medium to fine polyhedral structure; firm consistency (moist); few (5-10%) medium sized (5-15 mm) ferruginous and clay nodules; quartz fragments; pH 4.9; clear change to:

B3 90-120 cm Light grey (5R7/1) with many (50%) dark red (2.5YR4/6) and strong brown (7.5YR5/6) mottles ("tiger mottling"); *clay loam (gritty);* strong medium to fine sub-angular blocky structure; firm to very firm consistency (moist); few (5%) medium (4-8 mm) ferruginous and clay nodules; medium (4-8 mm) quartz fragments common 10-15%); pH 4.8; gradual change to:

120-140 cm undefined

Table 24. Key Profile Characteristics – Upton (Site 5)

	pH	Salinity Rating	Sodicity	Dispersion
Surface (A1 horizon)	Moderately Acid	Very Low	Non-Sodic	None
Subsoil (B21 horizon)	Moderately Acid	Very Low	Non-Sodic	None
Deeper subsoil (at 90-120 cm)	Very Strongly Acid	Very Low	Non-Sodic	None

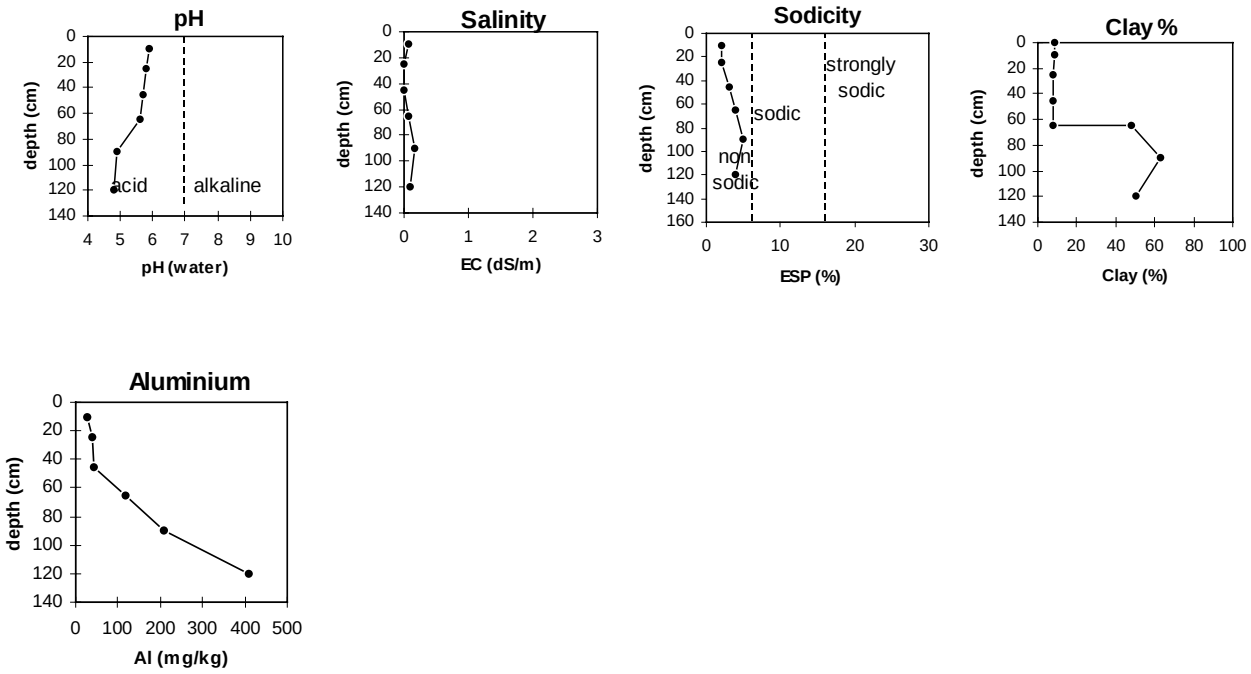


Figure 39. Influence of soil depth on soil chemistry – Upton (Site 5)

Table 25. Key Chemical Characteristics – Upton (Site 5)

Horizon	Horizon Depth	pH (water)	pH $CaCl_2$	EC dS/m	Exchangeable Cations meq/100g Ca	Mg	K	Na	Ex Al mg/kg	Ex Ac meq/100g	Field pF2.5	Wilting Point pF4.2	Coarse Sand (0.2-2.0mm)	Fine Sand (0.02-0.2mm)	Silt (0.002-0.02mm)	Clay (<0.002mm)
A1	0-10	5.9	4.9	0.06	2.6	1.0	0.47	0.24	26	9.2	57.7	7.0	46	25	15	9
A21	10-25	5.8	4.8	<0.05	0.56	0.26	0.14	0.10	38	3.2	14.8	2.9	46	31	10	8
A22	25-45	5.7	4.7	<0.05	0.53	0.25	0.11	0.11	43	3.1	13.8	3.7	46	29	12	8
B21	45-65	5.6	4.5	0.06	1.7	2.0	0.26	0.43	120	7.2	27.2	14.9	25	16	10	48
B22	65-90	4.9	4.5	0.17	0.15	4.7	0.67	0.78	210	11	36.6	22.8	10	14	12	63
B3	90-120	4.8	4.3	0.10	<0.05	2.5	0.36	0.44	410	6.7	32.6	17.9	19	17	12	50

Conclusion

There are a number of factors which can influence phylloxera population abundance under field conditions and it is important to identify these, so that management practices can be adjusted accordingly where possible, to reduce the risk of both inter- and intra-vineyard phylloxera dispersal. Interactions between the susceptible host plant and phylloxera, phylloxera genetics and site-related factors, such as soil type, soil moisture and soil temperature are all likely to influence the population dynamics of the insect. However, there are few published studies, which examine the effect of soil chemical and physical properties on phylloxera populations and those which are published focus primarily on clay/sand ratios. A clearer understanding and in-depth analysis needs

to be undertaken to identify which key soil properties (chemical and/or physical) influence root physiology and the ability of phylloxera to interact with the root system and subsequently establish, develop and disperse.

In Australia there are around 65 known phylloxera-infested vineyards. A soil survey was conducted in 14 vineyards in North-East Victoria (ie 25% of known infested vineyards) as a qualitative analysis of phylloxera-infested soils in an earlier study by Powell (2003). Our study has provided data for an additional five vineyard sites. In effect the two studies combined cover almost 30% of known infested sites. This provides essential baseline information to investigate what factors may influence phylloxera's ability to establish, develop and disperse and subsequently this data will allow us to improve our risk assessments based on soil type which currently relies on one broad category ie sand/clay percentage.

From the studies conducted in Australia to-date it is clear that in general infested soils shared common characteristics including a duplex nature with loams overlaying clay-based subsoil, acid to slightly alkaline pH, relatively high calcium content, nitrogen and potassium deficient soils. One soil examined with <45% clay and silt content had relatively low phylloxera populations but another with < 20% clay has high populations. Buchanan (1990) collected some preliminary soil data at rootstock trial sites in Wagunyah/Rutherglen, Milawa and Nagambie and observed that phylloxera levels were lower in soils with >79% sand content. However, his observations were based on only six rootstock trial blocks, whereas our study combined with the previous study (Powell 2003) now covers 33 commercial blocks in seven grape-growing areas. The percentage of sand was also not always a good indicator of phylloxera levels. Indeed the soil with the highest sand content (58-65%) in our study (Site 4-Nagambie) has relatively high phylloxera abundance.

Interestingly nitrogen and potassium deficiency was evident in all soils tested. Riley (1878) recommended the use of potassium and nitrogen fertilisation to promote vine growth and reduce phylloxera densities. Foliar nutrient applications are widely used by grapegrowers in the USA in phylloxera-infested vineyards (Fisher and Hellman, 2000) and Kopf (2000) has reported that the addition of nitrogen to vines leads to lower production of phylloxera nodosities.

One very interesting feature of some sites where phylloxera levels are particularly abundant (eg: King Valley) is that exchangeable aluminium capacity is at toxic levels. High exchangeable aluminium capacity can reduce plant vigour through reduced root elongation and removal of root hairs, which could in turn weaken or stress the vines making them more susceptible to phylloxera damage. However, at other sites (eg: Nagambie) moderately high phylloxera populations were also observed on soils with low exchangeable aluminium capacity. However, at these sites the vines are older and have sustained phylloxera populations for longer periods of time than the King Valley sites. Other factors influencing phylloxera abundance at different sites could include the phylloxera genotype and its interaction with the vine hosts (this is discussed further in Chapters 2-4).

Discussion

In terms of assessing risks based purely on phylloxera presence in given soil orders only generalisations can be made at this stage until further studies have been conducted. Over the course of this study phylloxera was found in soils in tow orders dermosols and chromosols and in addition to this the previous study (Powell, 2003) detected phylloxera in six orders: chromosols, kandosols, kurosols, sodosols and rudosols. Chromosols are widespread in Eastern Australia and some parts of South west Western Australia and South Australia. Chromosols occur over a wide rainfall range from 300-1200mm (Isbell *et al*., 1999). The kandosols are widespread in most Australian states, with the exception of Victoria and Tasmania, and cover a wide rainfall range (200-4000mm). The kurosols have a clear textural B horizon which are acidic and appear in regions with rainfall range of 600-1200mm. They are not widely distributed nationally but are found in eastern Australia and restricted parts of South west Western Australia, Victoria, South Australia and Tasmania. The rudosols are the third most widespread soils in Australia, with the largest areas being in arid-central and North-west Australia. However, the rainfall ranges are <200mm to over 1000mm. Their distribution in major grape-growing areas is relatively limited. Sodosols cover 13% of Australia and are relatively widespread in Western Australia, Tasmania, Victoria, New South Wales and South Australia.

Recommendations

The link between soil characteristics and phylloxera establishment still requires further analysis. However, key characteristics of infested soils suggest soil acidity, low salinity and high Aluminium exchange capacity could be key factors in phylloxera's ability to establish, develop and disperse.

- Before risk maps and revised detection protocols can be produced, based on soil characteristics, controlled environment and field studies need to be conducted using a selected group of phylloxera clonal lineages. A more comprehensive soil profile data set is also required from all known phylloxera-infested sites. This would also be useful in infested sites to allow site-specific rootstock recommendations to be made. It would also enhance the effectiveness of early detection systems.

Chapter 8. Influence of Composted Green Waste on Phylloxera Population Dynamics

Objective: To determine the effect of vineyard management practices on phylloxera development and virulence and grapevine performance - composted green waste study

Summary

A field study was conducted, over three grapevine growing seasons, to assess the effect of composted green waste on the population dynamics and risk of dispersal of root-feeding grapevine phylloxera and performance of phylloxera-infested ungrafted *Vitis vinifera* cv. Sauvignon Blanc grapevines. An assessment was also made to determine the effect of compost application on grapevine vigour, grape yield and quality. Phylloxera crawler (first instar) emergence above-ground was significantly increased through the annual application of composted green waste. The implications of these results for management of ungrafted phylloxera-infested grapevines are discussed.

Introduction

Composted green waste consists of a mixture of garden waste such as prunings, weeds and lawn clippings. Following composting it has the potential to be used in a number of horticultural situations including as mulch applications in vineyards. In trials conducted by DPI-Knoxfield in the Yarra Valley and Mornington Peninsula composted green waste had beneficial effects including weed suppression and soil moisture increases (Hood, 1999). In California in a recent survey, when comparing differences in phylloxera-related grapevine damage in conventionally managed and organically managed vineyards, Lotter et al. (1999) showed that there was significantly less root damage in the organically managed vineyards.

To-date no studies have been conducted to determine the influence of composted green waste on grapevine phylloxera population abundance, risk of transfer and related vine performance. The 3-year study reported here was conducted in collaboration with the University of California-Davis. The trial was designed to assess the impact of composted green waste mulch in a heavily phylloxera-infested vineyard in the cool climate King Valley Phylloxera Infested Zone of North East Victoria.

Methods

Site Details

The experimental site was in a commercial vineyard located 5 km east of Cheshunt within the King Valley Phylloxera Infested Zone (PIZ, Fig. 40), North-East Victoria, Australia. Phylloxera was first detected in the vineyard in May 1997. The phylloxera at the site was characterised as genotype G4 (Corrie et al., 2003). The experiments were conducted between October 2001 and June 2004 over three consecutive grapevine growing seasons. The commercial vineyard block chosen for the study consisted of 15-year old ungrafted *V. vinifera* cv. Sauvignon Blanc vines. The row spacing was 3 m and vine spacing was 1.8 m and the experimental block consisted of ten experimental rows with a single buffer row between paired experimental rows. A soil survey was conducted in the experimental block, as described in Powell (2003) and was classified as a dystrophic brown kurosol (Isbell, 1996) which demonstrated a duplex nature with a sandy clay loam A horizon overlying the higher clay content B horizons.

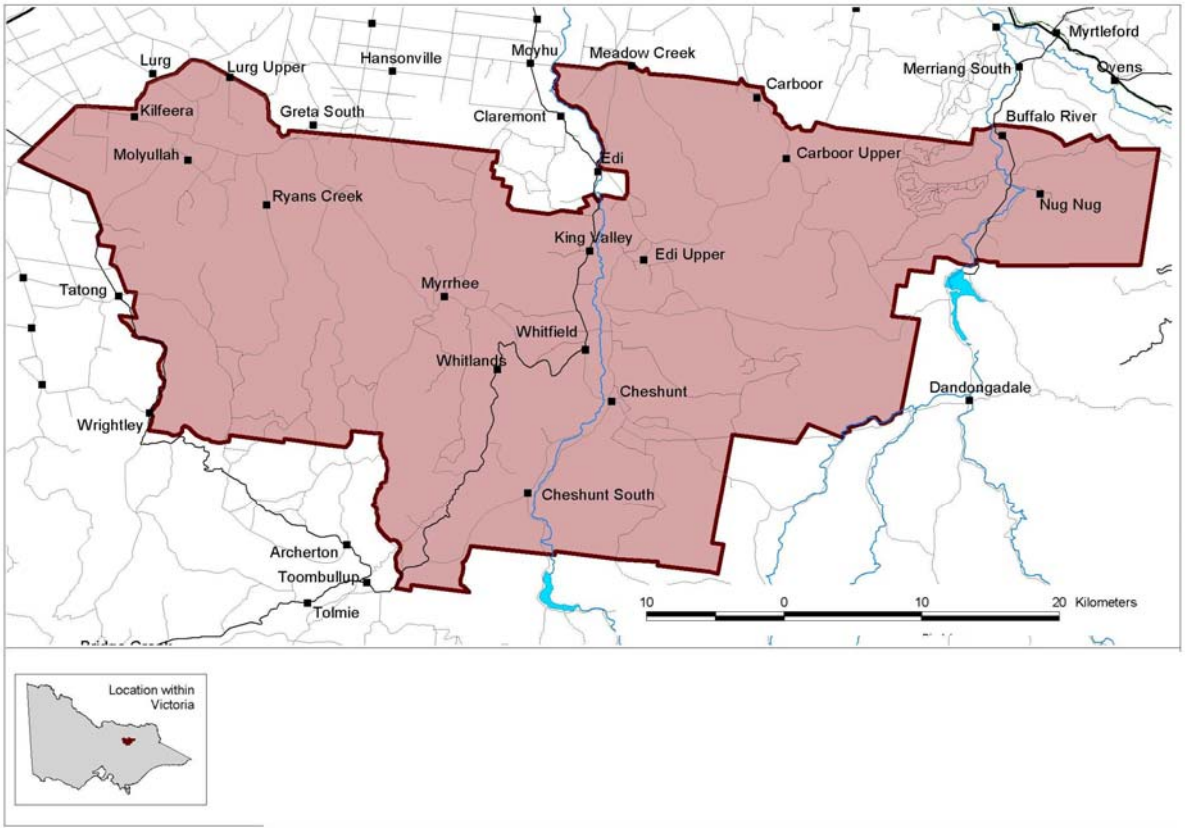


Figure 40. King Valley Phylloxera Infested Zone showing location of composted green waste field trial

Rainfall and air temperature records were obtained from the Moyhu weather station located 15 km from the experimental site. Mean monthly temperatures during the 2001 and 2003 vegetative season (October-May) were 18.5°C and were slightly higher at 19.5°C during the 2002 season. The

mean total rainfall during the vegetative season was 17 mm but varied from 15.6-18.9 mm. Mature composted green waste was prepared commercially through a windrowing process by Greenchip Recycling, Barunduda, Victoria, following Australian composting standards (Anon. 2000) and a chemical analysis conducted prior to application (Table 26).

Table 26. Comparative chemical analysis of vineyard soil and composted green waste.

Description of sample/analysis	Soil	Pre-application composted green waste
Exchangeable aluminium (mg/kg)	<10	12
E.C. (dS/m)	0.1	NA
% w/w Organic matter	5.9	34
% w/w Total C	3.1	18
% w/w Total N	0.19	1.5
Olsen P (mg/kg)	22	690
Exchangeable Ca (meq/100g)	13	15
Exchangeable Mg (meq/100g)	1.6	12
Exchangeable Na (meq/100g)	<0.05	3.3
Exchangeable K (meq/100g)	0.58	14
4 Cations+ (meq/100g)	44	15
Ca:Mg	8.2	1.3
% Calcium	86	34
% Magnesium	11	27
% Sodium	1	8
% Potassium	4	32
PH-H_2O	7.3	NA
PH-$CaCl_2$	6.6	NA

Where NA = not assessed

Trial design

The experiment was laid out in a randomised complete block with five replicates. Within each replicate (block) there were two plots, one plot for the treatment (compost) and one for the control (no compost). Plots were randomly assigned within each block. In each plot ten adjacent vines, with five vines in two adjacent rows, were selected for the treatment and ten for the control. Compost was applied to the undervine soil surface to a depth of 5 cm in October of 2001, 2002 and 2003 of each growing season prior to budburst. The control plots were set up similarly to the treatment plots but compost was not applied to the soil surface. Single uncomposted buffer vine rows were placed between experimental plots and blocks.

Phylloxera Sampling and Monitoring – Above Ground

In each plot two central grapevines were used as 'population monitor vines', to assess above-ground abundance of phylloxera life-stages. To assess phylloxera population movement above-ground two non-destructive trap types, trunk and emergence (Powell et al., 2000), were used for each monitor vine and statistical analysis covered a period of eight fortnights for each season. The seasonal abundance of phylloxera dispersive stages, (namely first instars (crawlers) and adult winged forms (alates)) emerging from below-ground onto the soil surface was measured over three successive seasons using 'emergence traps' placed adjacent to each monitor vine (as described in Chapter 4). At fortnightly intervals insects were removed by washing the trap with 70% methanol and collected in plastic containers. Traps were then rinsed with tap water and re-positioned. Collected insects were identified and quantified using a low power binocular microscope.

Phylloxera movement up and down grapevine trunks was assessed, over three successive seasons, by collecting dispersive life-stages in sticky traps wrapped around the vine trunk (Powell et al., 2000). A 10 cm strip of grey duct tape was wrapped around the vine trunk (sticky side inward to adhere to the trunk) 20 cm above the soil surface and sealed at the top and bottom with a liquid sealant to prevent phylloxera moving into cracks in the vine bark. The duct tape formed a smooth surface on which the adhesive trunk traps could be placed. Trunk traps consisted of two bands of white electrical tape wrapped around the trunks of monitor vines at a distance of 25 cm above the soil surface and 20 mm apart. Tanglefoot™ was applied evenly to the centre of the tape, at a width of 1 cm, using a paintbrush. The lower band was used to collect insects moving up the vine trunk from the soil surface, while the upper band was used to collect insects moving down the trunk from the vine canopy. Trunk traps were removed and replaced every two weeks. On removal, traps were placed on A4 paper, covered with plastic Gladwrap™ to prevent sample contamination and examined under a low power binocular microscope for phylloxera presence. All phylloxera life-stages were identified and quantified.

In season 2001-2002 the above-ground phylloxera counts were analysed from the 13th of December 2001 to the 25th of March 2002. In season 2002-2003 the phylloxera counts were analysed from the 19th of December 2002 to the 26th March 2003. In season 2003-2004, phylloxera counts were analysed from the 17th of December 2003 to the 24th of March 2004.

Phylloxera Sampling and Monitoring – Below Ground

To assess population numbers below-ground a destructive root sampling technique was used twice per growing season in winter (October) and summer (February/March). Root populations of grapevine phylloxera were monitored at six-monthly intervals, over three successive seasons, in October and February-March. Root samples (2-8g dry weight) were taken from a single vine adjacent to the monitor trap vine in one row of the coupled/paired design (n=5). The roots were transported under quarantine conditions to a quarantine laboratory, where they were washed and examined, under a dissecting microscope, to record the number of each phylloxera life-stage. The

roots were weighed to calculate wet weight, then oven-dried at 50°C for 48 h. and reweighed to determine dry weight.

Grapevine Performance

The grapevine response to treatment application and phylloxera presence was assessed twice per season once as a winter pruning and again during the autumn at harvest. Vines were spur pruned by hand (Tassie and Freeman, 1992), in June (winter) of three consecutive seasons (2001-2003), for each treatment and control vine and prunings were weighed on a portable field balance. Grape yield and quality assessments were taken at harvest in March (autumn) for all treatment and control vines (n=50). Total grape bunches were counted per vine and individual bunches weighed in the field using a portable balance. Random samples of 50 berries were collected from each vine and weighed to determine mean berry weight. The berry samples were then transferred in a plastic clip seal bag to a quarantine laboratory where they were manually crushed and the juice tested for sugar level (ºBrix) by a hand-held Atago™ refractometer and for pH by electrode.

Statistical Analyses

A non-parametric analysis, using the Kruskal-Wallis test, was conducted for root population data in StatXact (Cytel Software Corporation, 2003). This test assesses the differences among the average ranks to determine whether there were any significant differences between the compost and the control in the number of root population life-stages.

All other trial data was analysed using Genstat 8.1 (Payne et al., 2006). Data that were collected as a one-off event (harvest data, pruning data) were analysed using standard ANOVA, whereas data that were collected over time (emergence traps, trunk traps) were analysed using repeated measures ANOVA. Repeated measures ANOVA was considered more appropriate for these repeated measures data because there were no evidence of a systematic time correlation. Data on insect abundances from trunk and emergence trapping were $\log_{10}$ transformed and least significant differences (LSD) were used to calculate significant differences at the 95% confidence limits. Post-ANOVA residuals-based diagnostic plots in GenStat showed that, for each characteristic, the data reasonably met the ANOVA assumptions.

Results

Phylloxera Sampling and Monitoring Below-Ground

In October (winter) of each season, relatively low numbers of phylloxera life-stages were generally recorded on grapevine roots (Table 27). Crawlers represented the predominant life-cycle stage. Eggs, intermediate instars and wingless adults were observed, but no winged adults. Due to low numbers of some life-stages statistical analysis was conducted for crawlers, eggs and pooled data for all life-stages. There was no significant difference in crawler, egg or total life-stage abundance

on the roots of compost treated and control vines for any season. There was also no significant treatment by time interaction for each season.

Later in the season (summer) phylloxera life-stages were more abundant on the roots and eggs and crawlers were the most abundant life-stages. There were relatively few intermediate instars and wingless adults, but more than in the winter samples. Despite being generally higher, for each season, for the compost treated vines this difference in crawler, egg or total life-stage abundance was not significant for any season.

Between the two sampling periods, summer and winter for all three seasons there was a significant difference in phylloxera abundance for all life-stages and crawlers alone only in season three ($P<0.01$), with higher abundance in the summer. However, this was not due to a treatment effect and is more likely due to relatively high summer soil temperatures, which are known to affect phylloxera development (Granett and Timper, 1987).

Table 27. Seasonal phylloxera life-stage abundance on grapevine roots (g/dry weight root), over three seasons, with and without composted green waste application

	Eggs		Crawlers		All life-stages	
Treatment/Season	***Winter***	***Summer***	***Winter***	***Summer***	***Winter***	***Summer***
Compost						
Season 1	0.02	20.50	0.64	22.06	0.78	53.71
Season 2	0	13.42	0.95	8.08	0.95	38.47
Season 3	0	10.36	0.22	10.50	0.22	24.42
Control						
Season 1	0.23	17.51	0.31	13.67	0.61	48.62
Season 2	0.19	10.53	1.28	4.00	1.42	23.63
Season 3	0.16	2.86	0.49	8.94	0.99	27.96

Phylloxera Sampling and Monitoring Above-Ground

Phylloxera emergence

Phylloxera crawlers were collected in emergence traps from October to May for the three experimental seasons and peaked in abundance in January (Fig. 41). Data were only analysed from December to March to avoid large number of zero counts in both the control and the compost, in other time periods. In the first season, over the entire sampling period, significantly higher ($P<0.01$) numbers of crawlers were recorded from the compost vines compared with the control vines (Fig. 41a). On three consecutive sampling dates, 9th January, 25th January and 6th February, the difference was significant ($P<0.05$). Overall there was no significant interaction between treatment and time, that is, for both the compost and the control; the trend was similar across the sampling periods.

In the second season crawlers were less abundant overall (Fig. 41b) than the first season (Fig. 41a). Significantly more crawlers were collected in the traps of compost treated vines than control vines ($P<0.01$) over the whole sampling period. On four consecutive sampling dates, between thee 2nd January and 14th February, significant differences were recorded ($P<0.01$). Similar to the first season, there was no significant treatment by time interaction.

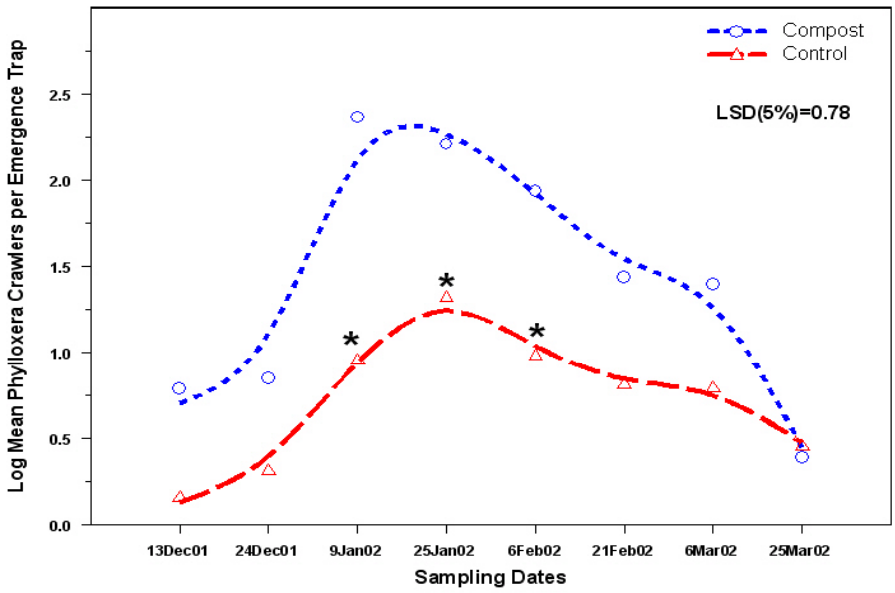
In the third experimental season overall the abundance of crawlers was higher than the two previous seasons. However, there was no significant effect of treatment on crawler number (data not presented). Similar to the first and second season, there was no significant treatment by time interaction and similar trends were observed with higher insect numbers in the summer months.

Phylloxera movement on grapevine trunks

The potential risk of phylloxera transfer on the vine canopy was assessed by the use of trunk traps (Powell et al., 2000). Phylloxera crawlers were collected on the bottom and top trunk traps from October to May for all experimental seasons (data from December to March presented) and peaked in January (Fig. 42). Only bottom trunk trap phylloxera counts were considered in the analysis as this represents movement of phylloxera from the undervine surface of treated vines. The top trunk traps were utilised to avoid overestimates of phylloxera movement, as these traps excluded downward movement of dispersive stages from the 'monitor vine' canopy and neighbouring vines.

In the first season overall, more crawlers were collected from compost treated vines (Fig. 42a). Significantly more crawlers were collected from compost-treated vines on only one sampling date ($P<0.05$). In the second experimental season over the entire sampling period there was no significant difference between compost-treated and uncomposted control vines (data not presented). In the third experimental season phylloxera crawlers caught in bottom trunk traps showed a peak in January and also an increase in numbers in March for both compost-treated and control vines (Fig. 42b). Over the entire sampling period, there was no significant difference between compost-treated and uncomposted control vines. There was a significant difference between the control and the compost on the 24th of March (Fig. 42b).

A



B

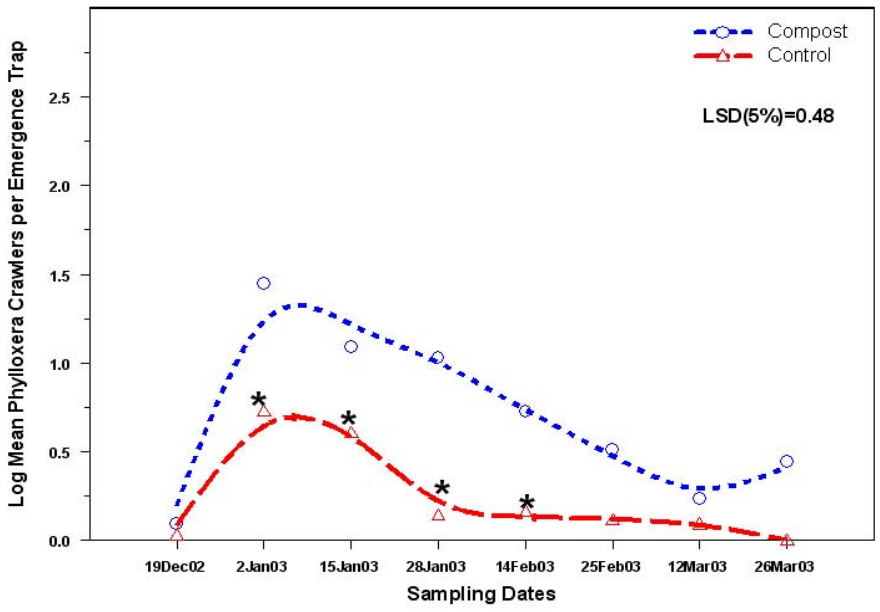
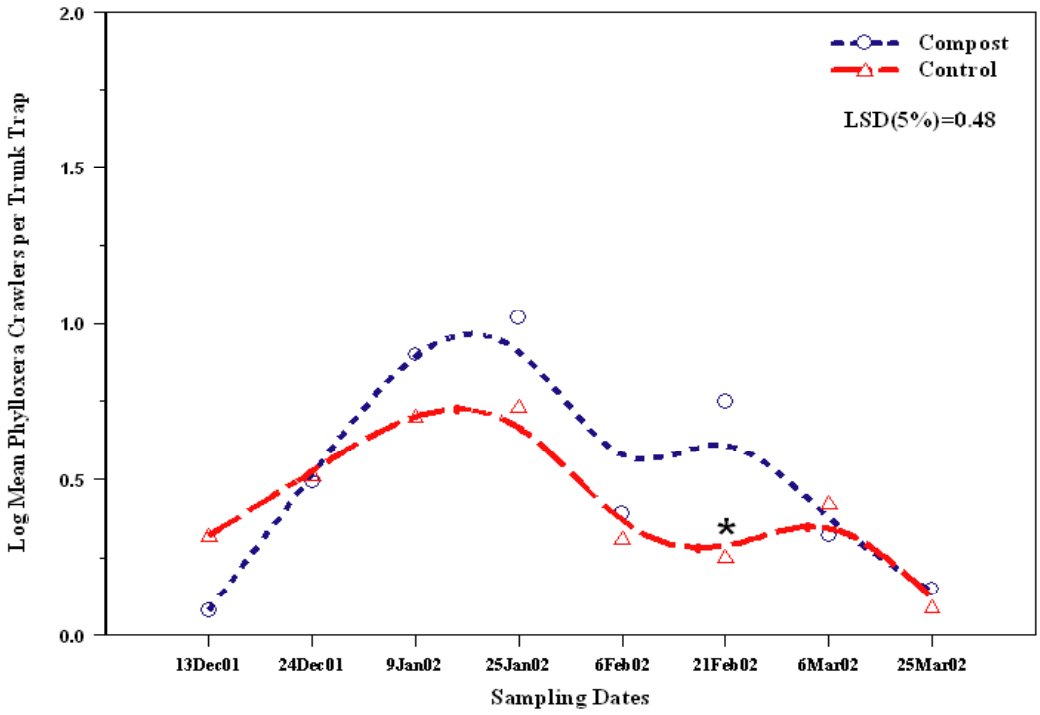
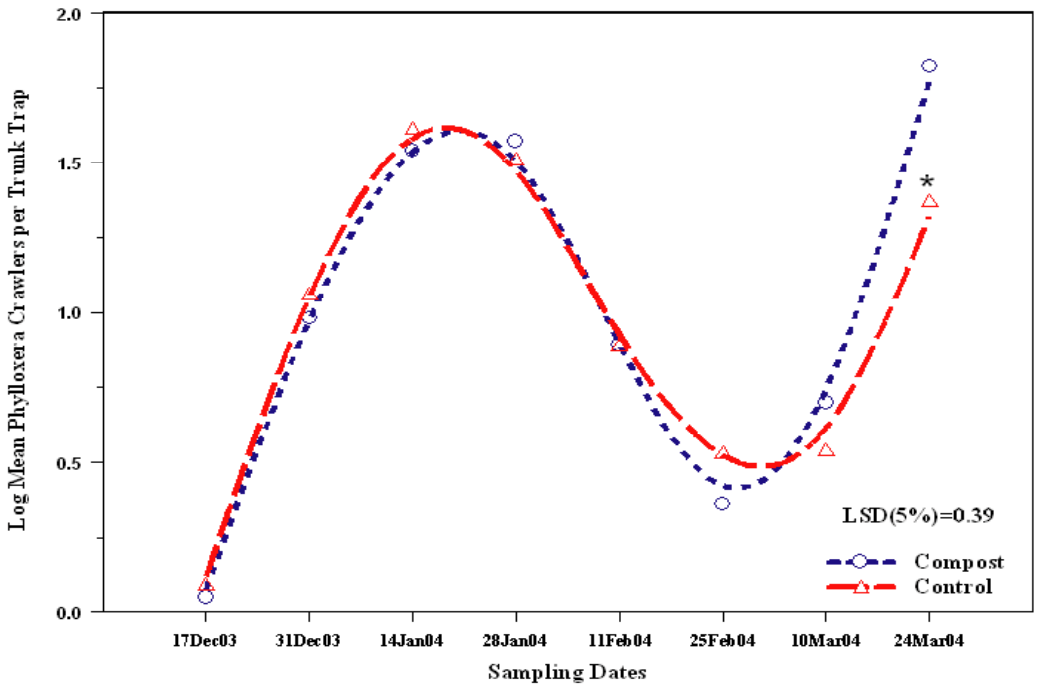


Figure 41. Seasonal first instar phylloxera abundance above-ground (log mean per emergence trap) of composted and non-composted *V. vinifera* cv. Sauvignon Blanc during (A) 2001-2002 and (B) 2002-2003 growing seasons. Asterisks denote significant differences between crawler abundance in emergence traps (*P<0.05).



A



B

Figure 42. Seasonal first instar phylloxera movement on grapevine trunks (log mean per bottom trunk trap) of composted and non-composted *V. vinifera* cv. Sauvignon Blanc during the (A) 2001-2002 and (B) 2003-2004 growing seasons. Asterisks denote significant differences between crawler abundance in emergence traps (*P<0.05).

Grapevine Performance

The data for the grape harvest in the first season of the experiment/trial could not be collected due to early harvesting by vineyard staff. Pruning weights collected in June 2001 prior to the application of treatments (in October 2001) indicated relatively vigorous vines at trial commencement. There were no significant treatment effects on pruning weights or measured grape harvest parameters (Table 28) throughout the trial periods. In Seasons 2 and 3, the mean pruning weights of the vines were approximately half that recorded the previous season, for both compost and control vines (Table 28). Whilst some significant seasonal effects between seasons were observed over the experimental block including significant differences ($P<0.001$) in mean berry weight, mean bunch weight, total bunch number, pH and ºBrix these differences are not due to treatments since the treatment x season interaction is not significant. These differences between seasons were attributed to the impact of weather variables (data not presented). The drier and warmer conditions in the second season of the trial in particular may also have reduced phylloxera populations above-ground and impacted on vine performance.

Table 28. Grape yield and quality parameters of phylloxera-infested grapevines in the presence and absence of composted green waste at the King Valley field site

Season	Treatment	Mean yield per vine (g)	Bunch number per vine	Bunch weight (g)	Mean berry weight (g)	Mean Brix (°)	Mean pH	Mean pruning weight (g)
First	Compost	-	-	-	-	-	-	965
	Control	-	-	-	-	-	-	1011
	$LSD_{0.05}$	-	-	-	-	-	-	276.1
	Significance	-	-	-	-	-	-	NS
Second	Compost	3548	18.5	185.5	1.24	25.9	3.59	522
	Control	2928	17.7	153.5	1.15	26.1	3.55	505
	$LSD_{0.05}$	879.3	5.3	563.8	0.12	0.8	0.02	180.2
	Significance	NS	NS	NS	NS	NS	NS	NS
Third	Compost	875	15.5	47.6	0.86	21.8	3.52	276
	Control	739	15.0	41.5	0.82	21.2	3.35	255
	$LSD_{0.05}$	1093.1	13.6	20.6	0.16	1.8	0.18	263.3
	Significance	NS	NS	NS	NS	NS	NS	NS

Single compost (composted green waste applied to a depth of 5cm) and control (no compost), where n=50. Least significant differences (LSD) can be used to compare significant differences between means within the column. When the difference between the means is greater than the LSD the treatments are statistically different (at the 95% confidence level).

Discussion and Recommendations

Our study used a variety of sampling techniques to assess the effect of composted green waste application on the interaction between grapevine phylloxera and its primary host plant *V. vinifera* by monitoring the population dynamics of grapevine phylloxera and assessing grapevine vigour, yield and quality characteristics.

Abundance of phylloxera life-stages, on grapevine roots, was assessed quantitatively using destructive sampling. Several authors have also shown destructive root sampling to be effective for quantifying phylloxera populations in the field (Powell et al., 2000, Porten and Huber, 2003). In our study, from root data collected early in the season (winter) prior to budburst, crawlers were the most abundant life-stage and represent a risk of transfer on root material or machinery in contact with soil around the grapevine roots. Later in the season (autumn) the risk of transfer on soil or root material was higher due to higher population numbers which included all life-stages. The application of composted green waste did not significantly affect phylloxera abundance on the roots as assessed by root samples taken in the winter and late summer. This is in contrast to Huber et al. (2003) who demonstrated that application of organic matter, in the form of spruce sawdust, reduced phylloxera root populations.

- In retrospect additional samples taken in early to mid-summer may have been more appropriate as above-ground sampling, using emergence traps, did show significant treatment effects during that period. Future studies should also include examination of the root demography as soil-dwelling insects and compost treatment may influence root longevity and the rate of root development (Hunter, 2001; Wells et al., 2002). However, excessive destructive sampling of vine roots during the growing season is likely to influence both vine health and phylloxera abundance in above-ground traps.

In California a survey, comparing conventionally managed and organically managed phylloxera-infested vineyards (Lotter et al., 1999), showed that although phylloxera population levels on grapevine roots did not differ significantly, there was significantly less related root damage, from secondary fungal pathogens, in the organically managed vineyards. In our study fungal necrosis levels on composted and uncomposted vines were not assessed. However, at the same experimental site a number of fungal pathogens, including *Fusarium oxysporum* and *Cylindrocarpon destructans* were isolated from phylloxera-infested ungrafted vines (Powell, 2003). These same fungal genera are associated with secondary root necrosis (Granett et al., 1998), changes in phylloxera development rates (Omer and Granett, 2000) and decline in vine vigour (Omer et al., 1995). Further data on the influence of fungal pathogens at this site is presented in Chapter 10.

- ❖ It is important to consider the impact on soil biota other than phylloxera when using mulch applications as this could impact on the degree of secondary root necrosis.

In our study we limited our destructive root population assessment to twice per season and obtained more frequent data using non-destructive above-ground trapping systems, such as trunk and emergence traps (Powell et al., 2003). In early to mid-summer significantly higher numbers of crawlers were collected from emergence traps located near compost-treated grapevines, in the first and second seasons. The lack of significance in the third season may be confounded by reduced irrigation inputs by the commercial grower to both treated and untreated vines, as grape yields were becoming too uneconomical to justify the associated costs. Excessive root damage may have occurred due to the high phylloxera levels, which may have also negated any treatment effects. Crawlers moving to the soil surface are more abundant on feeder roots located near the surface (Buchanan, 1990) which are likely to be influenced by irrigation levels. However, the reasons for increased phylloxera emergence in the first two seasons are unclear. This may be related to the undervine microclimate (eg soil temperature and soil moisture) and vine physiological changes associated with a mulch application. Phylloxera development is affected by vine physiology (Omer et al., 2002) and moisture and temperature changes in the environment (Granett and Timper, 1987; Turley et al., 1996). The chemical composition of the compost formulation is also likely to impact on grapevine physiological response and hence phylloxera population levels. Although green waste compost application used in this study increased the risk of phylloxera dispersal above-ground in another study using composted grape marc formulations reduced above-ground phylloxera dispersal was observed (Chapter 9).

- ❖ Further studies should consider how mulch application affects soil climatic conditions and consequently the degree of interaction between phylloxera and the grapevine root system.

Phylloxera crawlers have previously been found in the summer harvesting period in the canopy on foliage and harvested grapes at the experimental site (Powell et al, 2000) and on harvesting machinery (King et al., 1986) and crawlers can survive some post-harvest stages of grape processing (Deretic et al., 2003). Assessment of phylloxera crawler movement up grapevine trunks, on most sampling dates, was not significant in contrast to crawler abundance in emergence traps. The reasons for this are unclear. Emergence traps, which showed clear treatment effects on phylloxera abundance are a semi-closed trap structure, whereas trunk traps are open to the environment and microclimatic conditions and predatory invertebrates could have affected phylloxera numbers. The physical nature of the mulch could also have influenced the insects' ability to traverse the surface and restricted or slowed the insects' mobility to the trunk. Whilst no assessment of beneficials was made in this study, mulches have been shown to enhance natural predators (Mathews et al., 2004) and this could have impacted on crawler levels found on the trunks.

- Studies on the impact of natural predators for phylloxera are recommended as none have been conducted to-date in Australia and they may be able to reduce above-ground populations and hence reduce the risk of phylloxera transfer.

A change in the physical and chemical properties of the soil and the grapevine physiology could also have influenced phylloxera population levels. Compost increases moisture levels in the soil A horizon resulting in the development of feeder roots in this zone, which could support higher phylloxera population levels. Reisenzein et al. (2006) has shown that increasing humus content positively affects root nodosity formation and soil potassium to magnesium ratios can influence phylloxera abundance. In our study the potassium and magnesium levels of the compost mixture were higher than the uncomposted soil.

- No detectable grapevine response in yield or quality was observed, after application of composted green waste, and its use in phylloxera-infested vineyards at least under climatic conditions similar to the King Valley (ie cool climate) would not be considered beneficial from both a grapevine management and risk of phylloxera transfer.

This is the first study in Australia to investigate the effect of composted green waste, applied as understorey mulch to grapevines, on root dwelling (radicicole) populations of grapevine phylloxera in an ungrafted vineyard. In this study phylloxera populations as assessed by root observations did not appear to be significantly affected by management or season. However, significant differences were observed in the number of phylloxera crawlers caught above-ground highlighting that mulch application may increase the risk of phylloxera transfer.

- Further studies need to be conducted in different vineyard locations using a range of compost applications to determine whether this is a treatment specific observation. Studies should also monitor the effect that the compost application has on soil moisture and temperature and related affects on root morphology in the upper soil horizons. Buckerfield and Webster (1999) observed soil moisture increases of up to 50% under composted green waste mulch applications.

Three seasons after application composted green waste did not significantly affect yield parameters of phylloxera-infested vines. In other studies Buckerfield and Webster (1999) has shown significant increases in vigour and harvest yield parameters, from applying composted green waste to a depth of 5cm, to immature and established vines. However, their studies were not conducted in phylloxera-infested vineyards.

Whilst it is apparent that composted green waste does not appear to significantly increase phylloxera abundance on the storage roots it does increase the risk of transfer of the crawler stage during normal vineyard operations particularly during January to February.

- In a commercial vineyard which is relatively heavily infested with the G4 phylloxera genotype, under cool climate growing conditions such as the King Valley, the use of composted green waste could not at this stage be recommended.

- The increase in phylloxera levels observed in emergence traps following compost application needs to be further investigated to determine if it is due to changes in the physical (such as soil temperature or soil moisture) or chemical properties in the soil. If this is the case composted green waste could still be an option provided these other factors could be modified through a change in other management practices (eg. irrigation scheduling).

- This study was also only conducted in a site where one phylloxera genotype is known to be present. The interactions between compost application and grapevine may differ in vineyards infested with different phylloxera genotypes and this needs further investigation over a range of sites and climatic conditions.

Chapter 9. Influence of Composted Marc on Phylloxera Population Dynamics and Transfer Risk

Objective: To determine the effect of vineyard management practices on phylloxera development and risk of dispersal

Summary

A field-based study was conducted over three consecutive grapevine growing seasons to assess the effect of composted grape marc on the population dynamics and risk of dispersal of root-feeding grape phylloxera on ungrafted *Vitis vinifera* L. cv. Semillon grapevines. Grape yield and quality was also assessed to determine the effect of composted marc application on grapevine performance. Phylloxera emergence at the soil surface was significantly reduced by a single application of two composted marc formulations, combined with either pig manure or straw, at certain periods within the growing season. A third formulation consisting of pure composted marc had minimal effect on phylloxera population dynamics. The implications of these results for phylloxera management on ungrafted *V. vinifera* grapevines, and winery waste re-usage in phylloxera-infested quarantine regions is discussed.

Introduction

Recently studies have shown that phylloxera has a diversity of genetic strains which can survive on a range of 'resistant' rootstocks (Corrie et al., 2003; Viduka et al., 2003). Therefore the potential for resistance breakdown exists (Porten et al., 2000) and alternative or interim management options, including organic winery waste as composts, are being considered (Powell et al., 2006).

Grape marc consists of the residue remaining from crushing, desteming, draining and pressing stages of grapes for wine production. The residue comprises mainly grape stalks, leaves, skins and seeds. In Australia the majority of grape marc is distilled to produce alcohol but there are significant volumes of grape marc that cannot be processed for this purpose. In 2005 over 180,000 tonnes of grape marc was produced by the Australian wine grape industry (ABS, 2005). In smaller wineries marc volumes produced do not warrant collection and transportation to the distillery and in phylloxera-infested regions quarantine restrictions apply for the movement of unfermented and uncomposted marc (NVHSC, 2003) due to the risk of survival of phylloxera and transfer on grape skins and stalks (Deretic et al., 2003). There are a number of potential uses for grape marc including bedding for animals and as a component of compost. One alternative use for marc is to compost on-site in phylloxera-infested vineyards before applying as a soil mulch application to grapevines. Composting to regulatory standards (Anon, 2000) kills phylloxera due to lethal temperatures reached in the windrowing process (Bishop et al., 2002).

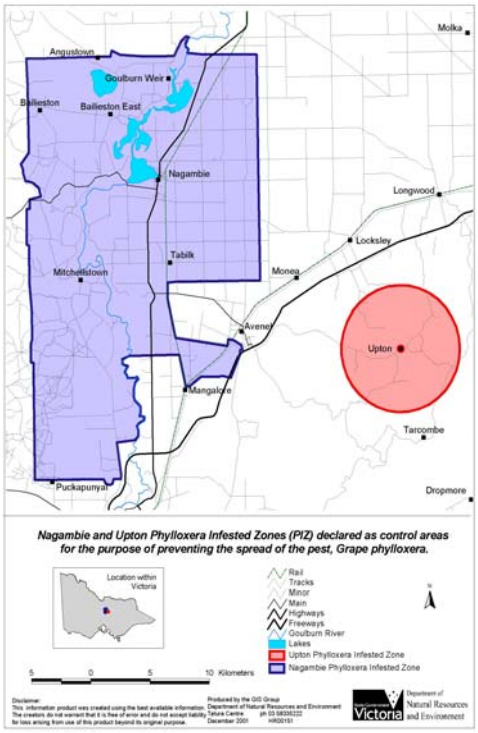


Figure 43. Nagambie and Upton Phylloxera Infested Zones

Methods

Site Details

The study site was located in a commercial vineyard, with winery on-site, 5km from Nagambie and phylloxera was first recorded at the site in 1969. The vineyard is located within the Nagambie Phylloxera Infested Zone (Fig. 43). The phylloxera genetic strain at the site was characterised as genotype G1 (Corrie et al., 2003). The experiments were conducted between October 2001 and June 2004 over three consecutive grapevine growing seasons.

A soil survey was conducted in February 2001 as described by Powell (2003). Soil in the study block was classified on the basis of soil texture and chemistry, as a dystrophic brown kurosol (Isbell, 1996). The vineyard block chosen for the study consisted of a block of twelve rows of ungrafted *V. vinifera* cv. Semillion vines. The row spacing was 3.3 metres and vine spacing was 1.8 metres.

Experimental Design

The trial was designed to examine the effect of 3 composted marc formulations on (i) phylloxera population dynamics, both above and below ground, and (ii) vine yield and vigour throughout the vine-growing season. The experiment was laid out in a randomised complete block design (RCBD) with 6 replicates (blocks). There were three compost treatments (M1, M2 and M3) and a control. Three compost formulations were used as treatments and designated as M1 (a mixture of composted marc and pig litter (3:1 ratio)), M2 (a mixture of composted marc and wood shavings

and (3:1 ratio)) and M3 (100% composted marc). All grape marc was composted on-site in windrows. In each block, there were 4 plots (one for each treatment and the control). In each plot two adjacent vines for population monitoring were selected at which a single plastic emergence trap was placed adjacent to the vine trunk to monitor phylloxera first instar. Thus 12 'monitor trap' vines were used for insect monitoring per treatment and control and 208 vines in total were used for treatment application. In each of three compost treatment plots (M1, M2 and M3), a single compost formulation was applied to a depth of 20cm at the commencement of the experiment in October 2001 just prior to budburst. No further applications were made during the course of the trial. The control plots had no compost applied to the soil surface. A chemical analysis of the compost treatments (Table 29) was conducted prior to application by the State Chemistry Laboratories (DPI-Werribee).

Phylloxera Sampling and Monitoring – Above Ground

In each plot two central grapevines were used as 'population monitor vines', to assess above-ground abundance of phylloxera life-stages. To assess phylloxera population movement above-ground a non-destructive emergence trap (Powell et al., 2000), was used for each monitor vine. Statistical analysis of emergence data covered a period of eight fortnights for each season. The seasonal abundance of phylloxera dispersive stages, (namely first instars (crawlers) and adult winged forms (alates)) emerging from below-ground onto the soil surface was measured over three successive seasons using 'emergence traps' placed adjacent to each monitor vine (as described in Chapter 4). At fortnightly intervals insects were removed by washing the trap with 70% methanol and collected in plastic containers. Traps were then rinsed with tap water and re-positioned. Collected phylloxera life-stages were identified and quantified using a low power binocular microscope. In season 2001-2002 the above-ground phylloxera counts were analysed from the 28th November 2001 to the 19th March 2002. In season 2002-2003 the phylloxera counts were analysed from the 28th November 2002 to the 26th February 2003. In season 2003-2004, phylloxera counts were analysed from the 3rd December 2003 to the 10th of March 2004.

Table 29. Chemical analysis of composted marc treatments conducted in October 2001

Treatment *Analysis*	M1	M2	M3
pH (water)	7.6	7.7	8.1
pH ($CaCl_2$)	7.2	7.4	7.8
Salt EC (DS/m)	1.73	3.81	2.81
Dry matter (%)	54	55	65
Organic carbon (%)	10.4	12.6	9.4
C:N ratio	8:1	9:1	8:1
Nitrogen (%)	1.36	1.39	1.2
Phosphorus (%)	0.20	0.35	0.21
Potassium (%)	1.88	2.34	2.16
Sulphur (%)	0.15	0.22	0.13
Calcium (%)	0.64	0.73	0.36
Magnesium (%)	0.17	0.25	0.15
Copper (mg/kg)	71.7	118.0	54.3
Zinc (mg/kg)	43.6	73.0	35.6
Manganese (mg/kg)	106.2	149.9	143.0
Iron (mg/kg)	3139	4607	4326

Where M1= composted marc/wood shavings (3:1), M2= composted marc/pig litter (3:1), M3=100% composted marc.

Phylloxera Sampling and Monitoring – Below Ground

To assess population numbers below-ground a destructive root sampling technique was used twice per growing season in spring and summer. Root populations of grapevine phylloxera were monitored at six-monthly intervals, over three successive seasons, in October and February-March. Root samples (2-8g dry weight) were taken from a single vine adjacent to each monitor trap vine. The roots were transported under quarantine conditions to a quarantine laboratory, where they were washed and examined, under a dissecting microscope, to record the number of each phylloxera life-stage. The roots were weighed to calculate wet weight, then oven-dried at 50°C for 48 hours and reweighed to determine dry weight.

Grapevine Performance

The grapevine response to treatment application and phylloxera presence was assessed twice per season once as a winter pruning and again during the summer at harvest. Vines were pruned by hand (Tassie and Freeman, 1992), in June (winter) of three consecutive seasons (2001-2003), for each treatment and control vine. The prunings were weighed on a portable field balance. Grape yield and quality assessments were taken at harvest in March (autumn) for all treatment and control

vines (n=12). Total grape bunches were counted per vine and individual bunches weighed in the field using a portable balance. Random samples of 50 berries were collected from each vine and weighed to determine mean berry weight. The berry samples were then transferred in a plastic clip seal bag to a quarantine laboratory where they were manually crushed and the juice tested for sugar level (ºBrix) by a hand-held Atago™ refractometer and for pH by electrode.

Statistical Analyses

All plot-level data were analysed using Genstat 8.1 (Payne et al., 2006). Data that were collected as a one-off event (harvest data and root data) were analysed using standard ANOVA whereas data that were collected over time (emergence traps) were analysed using repeated measures ANOVA. Data on insect abundances from emergence trapping and root data were $\log_{10}$ transformed prior to analysis in order to satisfy the assumptions underlying the ANOVA procedure. Fisher's unprotected least significant differences (LSD at 5%) were used to calculate significant differences between treatments. Post-ANOVA residuals-based diagnostic plots in GenStat showed that, for each characteristic, the data reasonably met the ANOVA assumptions.

Results

Phylloxera Sampling and Monitoring - Below Ground

In winter of each season, relatively low numbers of phylloxera life-stages were recorded on grapevine roots (Table 30). Crawlers and eggs represented the predominant life-cycle stage in winter. Intermediate instars and winged adults were observed, but no winged adults. Due to low numbers of some life-stages statistical analysis was conducted for crawlers, eggs and pooled data (total) or all life-stages. There was no significant difference in crawler, egg or total life-stage abundance on the roots of compost treated and control vines in the winter sampling in Season 2001-2002. Later in the season (summer) phylloxera life-stages were more abundant on the roots and eggs and crawlers were the most abundant life-stages. There were relatively few intermediate instars and wingless adults, but more than in the winter samples. This difference in crawler, egg or total life-stage abundance was not significant. In this first season there was no significant difference between treatments and no significant difference between time of collection (ie winter or summer).

Overall in season 2002-2003 again there was no significant difference in the number of phylloxera between treatments. There was a significant ($p<0.05$) time interaction with summer collected root samples having significantly more phylloxera present on roots than winter collected samples. There was also a significant ($p<0.05$) treatment by time interaction for eggs, crawlers and total phylloxera. The control vines had higher phylloxera numbers in winter than summer compared with all three treatments. Vines treated with M1, M2 or M3 had higher phylloxera numbers in summer than winter (ie the opposite to the untreated control vines. In the 2003-2004 season there

were no significant differences between treatments and no significant treatment by time interaction. There was a significant ($p<0.05$) difference between winter and summer with higher phylloxera numbers in the summer sampling.

Table 30. Seasonal phylloxera life-stage abundance on grapevine roots (g/dry weight root), over three seasons, with and without composted grape marc (M1, M2 and M3) applications

a) Summary of results for Season 2001-02

Treatment	**Eggs**	**Crawlers**	**Total**
Control	0.10	0.61	0.65
M1	0.20	0.64	0.71
M2	0.12	0.43	0.51
M3	0.16	0.53	0.60
Time			
T1	0.13	0.47	0.53
T2	0.16	0.63	0.70
Treatment x Time			
Control X T1	0.09	0.66	0.67
Control X T2	0.12	0.55	0.64
M1 x T1	0.24	0.61	0.72
M1 x T2	0.16	0.67	0.71
M2 x T1	0.11	0.29	0.34
M2 x T2	0.14	0.57	0.67
M3 x T1	0.10	0.33	0.40
M3 x T2	0.23	0.73	0.80
[1]LSD (Treatment)	0.14	0.23	0.26
[1]LSD (Time)	0.12	0.20	0.22
[1]LSD (Treatment x Time)[a]	0.22	0.36	0.39
[1]LSD (Treatment x Time)[b]	0.25	0.41	0.43

1 = LSD at 5%, a = at different level of treatment, b = at the same level of treatment, where T1 =winter and T2 = summer

b) Summary of results for Season 2002-03

Treatment	Eggs	Crawlers	Total
Control	0.21	0.21	0.39
M1	0.28	0.32	0.58
M2	0.24	0.24	0.47
M3	0.17	0.16	0.33
Time			
T1	0.13	0.18	0.29
T2	0.32	0.29	0.59
Treatment x Time			
Control x T1	0.23	0.26	0.42
Control x T2	0.19	0.15	0.36
M1 x T1	0.08	0.21	0.28
M1 x T2	0.48	0.43	0.88
M2 x T1	0.13	0.18	0.31
M2 x T2	0.34	0.30	0.63
M3 x T1	0.08	0.06	0.16
M3 x T2	0.27	0.27	0.51
[1]LSD (Treatment)	0.23	0.24	0.35
[1]LSD (Time)	0.12	0.10	0.16
[1]LSD (Treatment x Time)[a]	0.28	0.28	0.40
[1]LSD (Treatment x Time)[b]	0.24	0.21	0.32

c) Summary of results for Season 2003-04

Treatment	Eggs	Crawlers	Total
Control	0.02	0.18	0.26
M1	0.04	0.10	0.18
M2	0.00	0.12	0.30
M3	0.00	0.03	0.19
Time			
T1	0.00	0.05	0.09
T2	0.03	0.17	0.38
Treatment x Time			
Control x T1	0.00	0.10	0.12
Control x T2	0.05	0.25	0.41
M1 x T1	0.01	0.08	0.10
M1 x T2	0.08	0.12	0.25
M2 x T1	0.00	0.00	0.00
M2 x T2	0.00	0.24	0.60
M3 x T1	0.00	0.02	0.12
M3 x T2	0.00	0.05	0.26
[1]LSD (Treatment)	0.07	0.16	0.27
[1]LSD (Time)	0.05	0.10	0.15
[1]LSD (Treatment x Time)[a]	0.09	0.21	0.34
[1]LSD (Treatment x Time)[b]	0.09	0.19	0.31

1 = LSD at 5%, a = at different level of treatment, b = at the same level of treatment, where T1 =winter and T2 = summer

Phylloxera Sampling and Monitoring – Above Ground

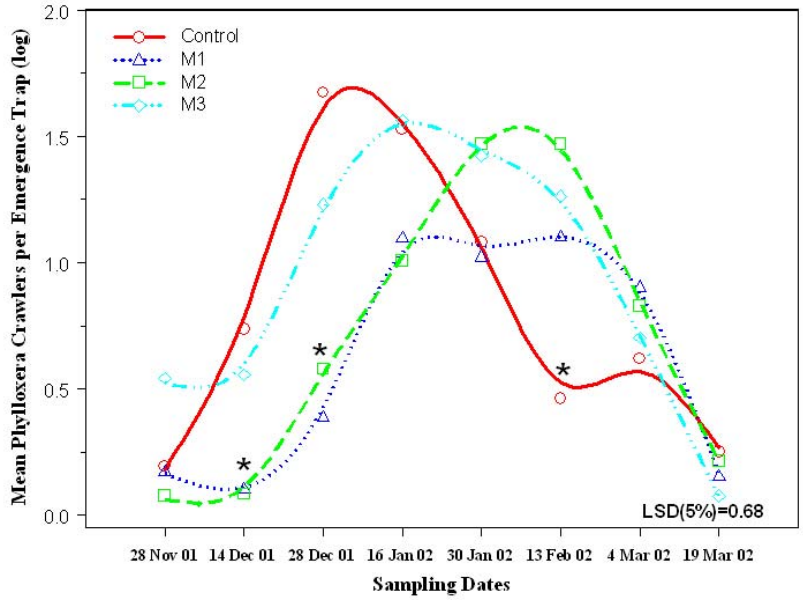
Phylloxera first instars were monitored using emergence traps from late November to early March for all experimental seasons (Fig. 44). Data were analysed from 8 consecutive fortnightly samples to avoid large numbers of zero counts in both the control and compost in other periods. In the first season (2001-2002) peak crawler abundances differed between treatments with the control vines having an earlier peak than all other treatments (Fig. 44a). Over the whole sampling period there was no significant difference between the $\log_{10}$ number of crawlers in the compost treated vines (M1, M2 and M3) and the control. There were significant differences in the log number of crawlers between counting periods ($P<0.001$). On two consecutive December sampling dates the M1 and M2 treatments showed significantly lower emergence levels than both the non-compost and the M3 treatment (Fig. 44a). On one sampling date, later in the season (13^{th} February), the control had significantly lower levels than all three compost treatments ($P<0.05$). Overall there was a significant interaction between treatment and time ($P<0.05$). There was a significant interaction effect between treatment and time ($P<0.01$), that is, the trend in the number of log crawlers in each treatment appeared to be significantly different over time. The control and M3 appeared to have a similar trend. M1 and M2 also appeared to have similar trends but different from those of the control and M3.

In the second season abundance of phylloxera crawlers (Fig. 44b) was lower than the first season (Fig. 44a). Over the entire sampling period there was no significant difference between the log number of crawlers in the compost treated vines (M1, M2 and M3) and the control. However, there were significant differences in the log number of crawlers between counting periods ($P<0.001$) with higher numbers recorded generally during the summer period (January-February). With the exception of the control, which showed an additional emergence peak in spring (November-December), this was the peak period for all compost treatments (M1, M2 and M3). On three sampling dates in significantly lower phylloxera abundance was recorded from each compost treatment (M1, M2 and M3) compared to the control ($P<0.05$). Later in the season on one sampling date the M3 treatment vines showed significantly higher numbers than either the M1 or M2 treatment but not the control. Overall there was a significant interaction between treatment and time ($P<0.01$).

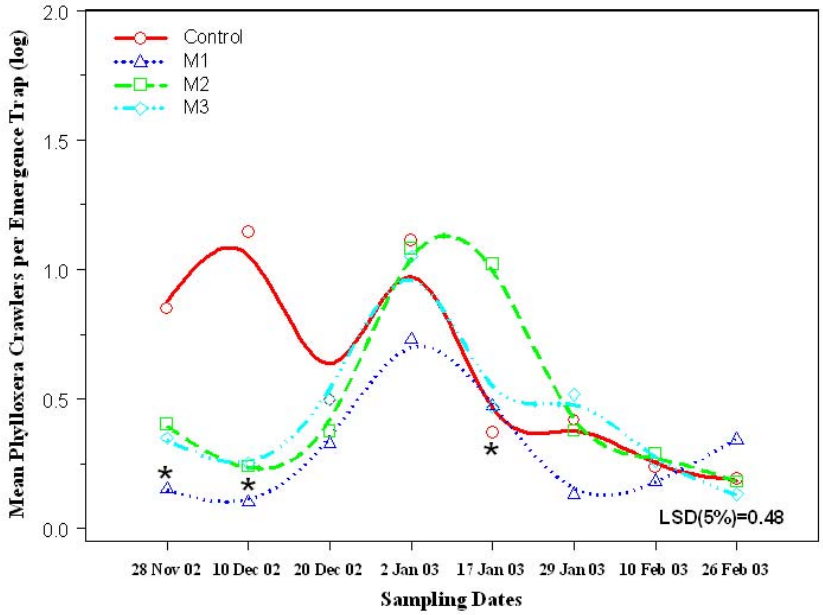
In the third season after treatment application crawler abundance was (Fig. 44c) was higher than the previous season (Fig. 44b). Over the entire sampling period, in the third season, significant differences were observed between the log number of crawlers in the compost treatments (M1 and M2) and the control and M3 treatment ($P<0.01$). There were also significant differences in the log number of crawlers between the counting periods ($P<0.001$). The highest numbers of crawlers caught in the emergence traps was during the summer months (January-February). On three consecutive sampling dates significantly lower crawler numbers were recorded ($p<0.05$) from the M1 and M2 treated vines than both the M3 and the no-compost treatments. Overall there was no significant interaction between treatment and time ($P<0.05$).

At the commencement of the counting period, phylloxera emergence levels were significantly lower than previous seasons this may be due to drier weather conditions in the previous season (data not presented) impacting on phylloxera development.

(A)



(B)



(C)

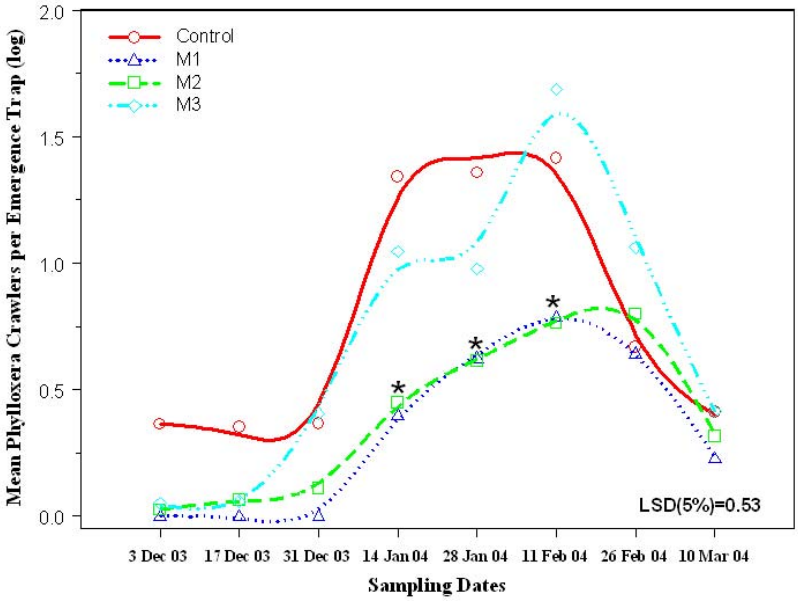


Figure 44. Seasonal first instar phylloxera abundance above-ground (log mean per emergence trap) of composted, M1, M2 and M3 and non-composted *V. vinifera* cv. Semillon during (A) 2001-2002, (B) 2002-2003 and (C) 2003 and 2004 growing seasons. Asterisks denote significant differences between crawler abundance in emergence traps (*P<0.05).

Grapevine Performance

Unfortunately pruning data could not be collected in June 2003 due to earlier than anticipated pruning by vineyard staff.

There were no significant treatment effects on pruning weights or measured grape harvest parameters throughout the trial periods. Some significant seasonal effects were observed over the experimental block including reduced pruning weights (P<0.001), and significantly lower grape yields and Brix and higher pH in season 2 (Table 31). These were attributed to the impact of weather variables (data not presented).

Table 31. Harvest data from a phylloxera-infested rootstock trial at Nagambie trial site. Summary results from ANOVA.

(a) Summary for season 2001-02

	Control	M1	M2	M3	LSD (5%)	Significance
Mean berry weight (g)	1.41	1.45	1.56	1.47	0.46	NS
Mean bunch weight (g)	95.8	102.4	108.6	99.4	39.0	NS
Total bunch number	32.8	34.1	36.2	36.1	14.6	NS
pH	2.78	2.79	2.80	2.73	0.13	NS
ºBrix	24.21	23.83	24.25	23.54	1.66	NS

(b) Summary for season 2002-03

	Control	M1	M2	M3	LSD (5%)	Significance
Mean berry weight (g)	1.00	1.27	1.38	0.93	0.47	NS
Mean bunch weight (g)	35.4	43.6	64.4	37.7	35.5	NS
Total bunch number	27.8	34.0	53.8	26.8	26.4	NS
pH	3.56	3.64	3.66	3.65	0.12	NS
ºBrix	21.50	23.58	22.46	23.00	2.92	NS

(c) Summary for season 2003-04

	Control	M1	M2	M3	LSD (5%)	Significance
Mean berry weight (g)	1.31	1.39	1.49	1.39	0.35	NS
Mean bunch weight (g)	64.2	74.4	70.6	56.2	29.1	NS
Total bunch number	44.1	58.0	58.2	62.1	47.8	NS
pH	3.16	3.54	3.57	3.60	0.45	NS
ºBrix	22.33	21.96	22.00	22.04	3.50	NS

(d) Summary for all season (overall)

	Season 2001-02	Season 2002-03	Season 2003-04	LSD (5%)	Significance
Mean berry weight (g)	1.47	1.14	1.40	0.12	*
Mean bunch weight (g)	101.5	45.3	66.4	9.3	*
Total bunch number	34.8	35.6	55.6	10.4	*
pH	2.77	3.63	3.47	0.14	*
ºBrix	23.96	22.64	22.08	1.14	*

Where M1= Pig litter + composted marc (3:1); M2= wood shavings + composted marc (3:1) M3=pure composted marc and control =no compost. Least significant differences (LSD) can be used to compare significant differences between means within the column. When the difference between the means is greater than the LSD the treatments are statistically different (at the 95% confidence level), n=12 per treatment and control, NS = not significant.

Conclusion

This study demonstrated some composted marc formulations applied as mulch can be a beneficial management option in phylloxera-infested vineyards at certain times within the growing season. Active phylloxera life-stages stages (ie crawlers) emerging from the soil, were predominantly reduced in summer by two compost formulations, M1 (pig litter + composted marc (3:1)) and M2 (wood shavings + composted marc (3:1)). More variable results were observed with treatment M3 (pure composted marc). However, this was dependent on duration after treatment application. In the four-month sampling period after the initial application there were no significant differences in phylloxera emergence between treatments and control. Therefore there was no increased risk of phylloxera spread during this period. This supports the root population data, where no significant effects were observed six months after treatment application.

However, significant treatment effects were observed, during the summer of each season, with M1 and M2 compost treated vines having generally lower crawler levels emerging. This highlights the risks of phylloxera dispersal are lowered when these composted marc formulations are applied. This is an important consideration when assessing the risk of transfer of phylloxera on machinery and personnel during periods of routine vineyard operations which are at their peak in the summer months.

This suggests the risks of phylloxera dispersal are lower when some composted marc formulations are applied. Interestingly however, the peaks of phylloxera abundance are affected by compost application and season. This is an important consideration when assessing the risk of transfer of phylloxera on machinery and personnel during periods of routine vineyard operations. This observation emphasises the need for long-term monitoring of compost applications. None of the composted marc treatments significantly affected harvest yield, vigour and quality and therefore at least at the rates applied had no beneficial effects on vine performance.

Recommendations

- Based on only three years trial data at only one field site only tentative conclusions can be drawn at this stage but nevertheless the findings that compost application has the potential to reduce the risk of spread from infested vineyards warrants further investigation and may provide an alternative use for winery waste.

In relation to the use of composted marc in vineyards the results from this trial provide preliminary evidence that phylloxera population dynamics is significantly reduced by composted marc treatments.

- Further trials should be conducted under controlled conditions to investigate the mechanism of action of the compost treatments on phylloxera development and dispersal characteristics within the vineyard.
- Root damage in phylloxera-infested vineyards is reported to be associated with secondary fungal invasion of feeding sites resulting in necrosis. Further studies could investigate the influence of compost treatments on associated fungal necrosis.
- Further chemical analysis of composted marc would be advisable to determine if any chemical constituents in the marc have pesticidal properties.
- Before recommendations on the potential use of marc formulations can be made further investigations need to be carried out to determine the long-term effects of composted marc applications on other vineyard management parameters such as soil condition, soil nutrients, moisture retention, weed control, erosion control, grape yield and quality, and vine vigour.

Chapter 10. Fungal Pathogens Potentially Associated With Phylloxera Damage

Objective: To determine the tritrophic interactions between phylloxera, grapevine root and fungal pathogens

Introduction

Research in California has highlighted that wounds in grapevine roots, caused by phylloxera feeding, may be associated with secondary infection by a range of fungal pathogens (Granett et al, 1998). Omer et al. (1995) associated the rate of vine decline in phylloxera-infested vineyards as being due to the secondary invasion of roots by fungal pathogens. Granett et al. (1998) highlighted *Fusarium roseum, F. oxysporum* and *Phythium ultimum* as fungal pathogens of *Vitis vinifera* roots in Californian vineyards. Studies have also shown that there is variability in the pathogenicity of *F. oxysporum* to different American rootstocks with the more phylloxera-susceptible rootstocks showing more signs of fungal root damage (Omer et al., 1999). A preliminary survey was conducted by Powell (2003) to identify potential fungal pathogens associated with root damage in phylloxera-infested vineyards. This was the first study of its kind in Australia. It highlighted the presence of *Phaeoacremonium*, *Fusarium oxysporum* and *Cylindrocarpon destructans* which are regarded as pathogenic and have caused vine decline and root rot in Californian vineyards (Gubler et al., 2006).

In April 2004 two field surveys and preliminary laboratory experiments were conducted, in collaboration with Visiting Professor Jeffery Granett (UC Davis, Department of Entomology, California) and Dr Jacky Edwards (DPI-Knoxfield) to examine the relationships between phylloxera genotype, fungal pathogens, root necrosis and vine vigour.

Method

Two vineyard sites of ungrafted *V. vinifera* cv. Cabernet Sauvignon affected by phylloxera were chosen. One site was located within the King Valley Phylloxera Infested Zone (PIZ) and another located within the Upton PIZ. At each site single phylloxera genotypes, G1 (Upton site) and G4 (King Valley site), had previously been characterised. Two trials (one for each genetic strain) were set up to examine the relationship between plant vigour and phylloxera as well as phylloxera abundance, degree of root necrosis and associated fungal pathogens. This can be considered as a two-phase experiment in which the first phase is the field trial and the second phase the laboratory trial.

Phase 1 - Field Study

At each site, 3 adjacent phylloxera-infested vine rows (blocks) were selected as trial plots. A visual vigour assessment was conducted by a single observer and three sample vines were selected from each of three vigour levels (low, medium and high; Fig. 45) within each row (Fig. 46). At each sample vine, lignified root samples were excised and transported under quarantine to the DPI-Rutherglen laboratory for analysis (Fig. 47).

In the laboratory the phylloxera insects were removed by root washing and each life-stage counted using a microscope. Roots were dissected with a sterile razor into equal sized sections (3-8 mm diameter), assessed for the degree of necrosis and categorised visually as 0%, 10, 30, 70, and 100% necrotic. Then necrotic root sections were surface-sterilized for 2 minutes in 1% sodium hypochlorite, then rinsed for 2 minutes in Ultrapure water and plated onto agar and incubated 22.5 degrees for 2 days to isolate fungal pathogens. The isolated fungi were identified to genus level, and those from genera known to contain pathogenic species were used in a subsequent fungal inoculation root bioassay (Fig. 48).

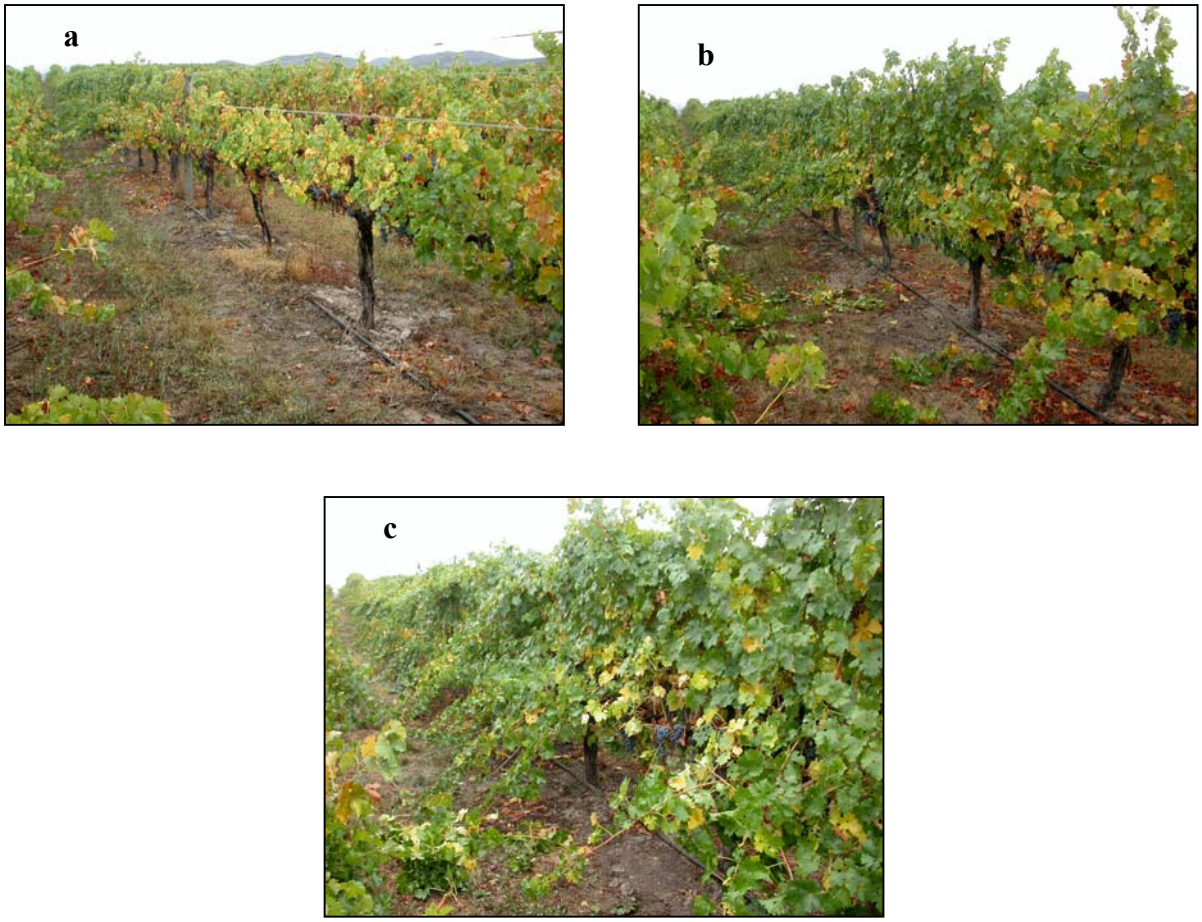


Figure 45. Visual plant vigour assessment showing (a) low (b) medium and (c) high vigour in phylloxera-infested grapevines at the King Valley field site.

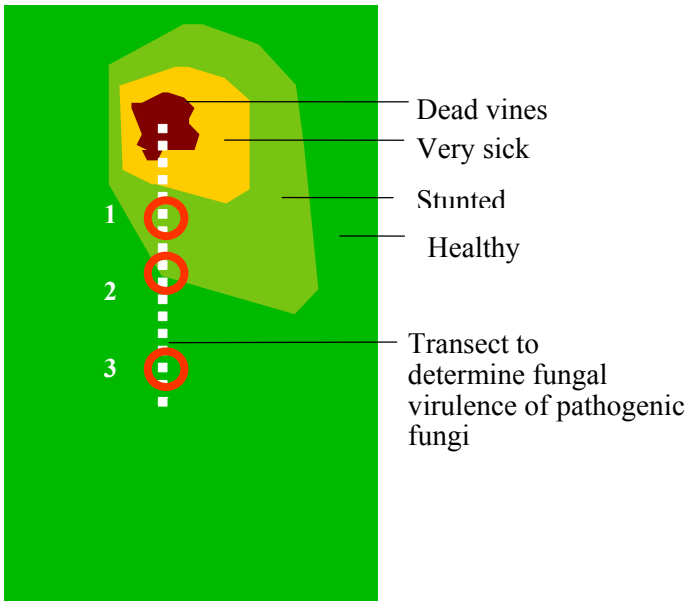


Figure 46. Diagrammatic representation of the transect used to sample roots from each row of phylloxera-infested grapevines, exhibiting low vigour (ie. very sick), medium vigour (ie visibly stunted) and high vigour (healthy) vigour. 1 = stunted or low vigour; 2 = medium vigour; 3 = healthy or high vigour.



Figure 47. Removing infested root material from phylloxera-infested grapevines for insect, root and fungal analysis.

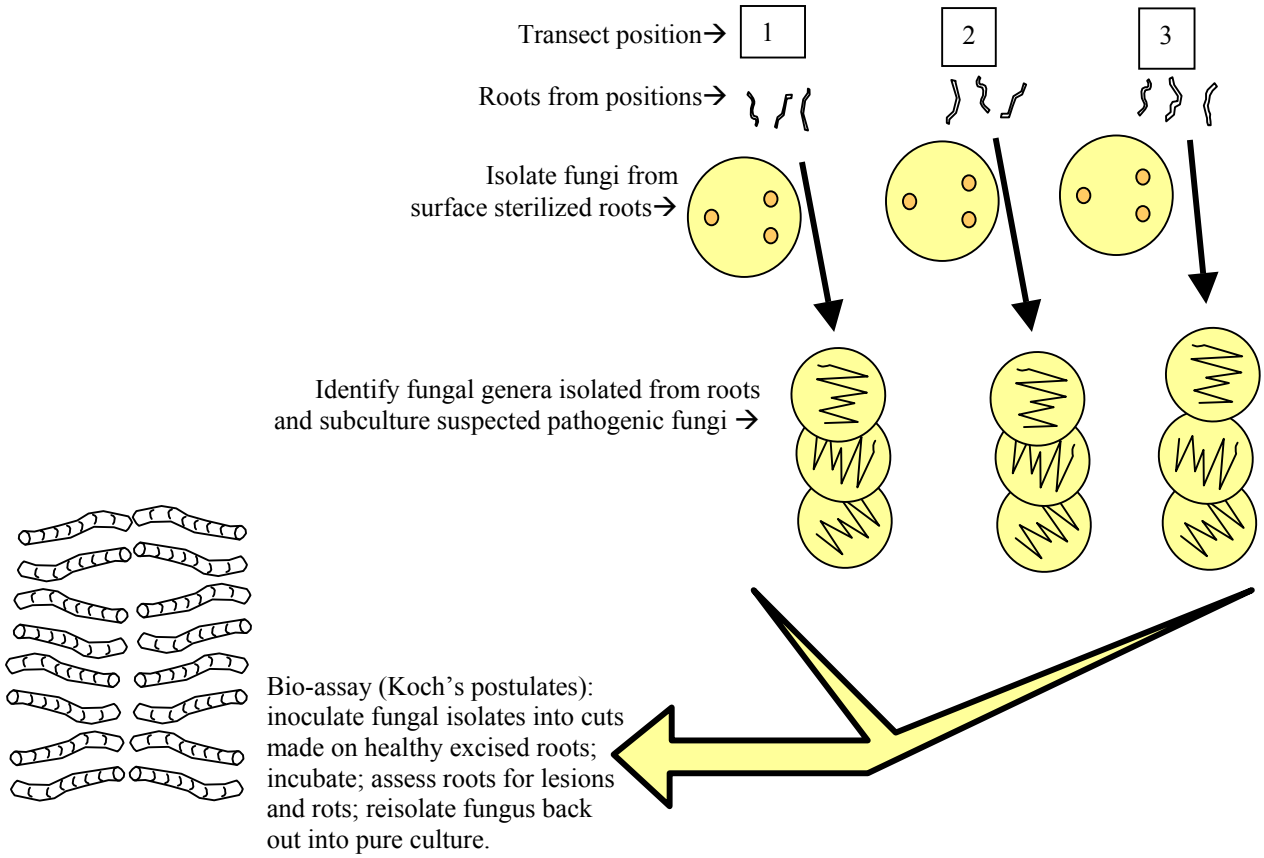


Figure 48. Schematic diagram of root bioassay to determine whether pathogenic fungi are associated with the root damage found on phylloxera-infested grapevines.

Phase 2 - Laboratory Inoculation Study

The chosen isolates, from the field study, were inoculated into clean 3-year old *V. vinifera* cv. Cabernet Sauvignon 4-8 cm long and 3-7 mm diameter root pieces, which had been surface sterilised with 1% sodium hypochlorite and maintained in moist incubation chambers for 6 weeks. The roots were then assessed for necrosis, and then plated onto agar to confirm that the fungus was still present. The reisolated fungi were then identified to species level and data analysed to determine the level of necrosis associated with individual fungal species.

Results

Plant Vigour and Phylloxera Genotype

From the field study all phylloxera life-stages were observed on the roots sampled, with the exception of alates, at both sites. Of the two genetic strains G4 was more abundant than G1 (Table 32).

At the Upton site (G1 present) the highest number of phylloxera observed were on roots of medium vigour vines where all life-stages including apterous adults where observed and first instar (crawlers) were the predominant life-stage. On the high vigour vines again crawlers were the predominant life-stage but no adult phylloxera were recorded. By far the lowest numbers of insects were recorded on the low vigour vines.

At the King valley site (G4 present) again the highest total number of phylloxera observed were on roots of medium vigour vines where all life-stages where observed and crawlers were the predominant life-stage (Table 32). On both the low and high vigour vines crawlers were the predominant life-stage. The lowest numbers of insects were recorded on the low vigour vines.

Table 32. Mean number of each phylloxera life-stage per gram of root mass (excluding alate adults) by plant vigour of genotypes G1 and G4

Vigour	***Low***		***Medium***		***High***	
Phylloxera life-stage	**G1**	**G4**	**G1**	**G4**	**G1**	**G4**
Eggs	0.12	0.77	0.13	0.56	0.02	0.46
1st instars	0	7.69	1.05	22.65	0.64	9.34
2nd-4th instars	0	0.99	0.21	2.31	0.06	0.71
Apterous adults	0	0.04	0.05	0.21	0	0.01
Total (all life-stages)	0.12	9.50	1.45	25.72	0.71	10.51

Table 33 shows that despite the general trends observed there was no statistically significant difference between insect population (eggs, 1st instars, 2nd – 4th instars, apterous adults and total) in terms of plant vigour for both G1 and G4 strains. However, apterous adults (G4 strain) showed significance difference between plant vigour (Medium<Low<High (0.2067, 0.0437, 0.0045).

Table 33. Non-parametric analyses for differences between plant vigour (high, medium, low vigour) for the two phylloxera genotypes, G1 and G4

Genotype	**G1**		**G4**	
Phylloxera life-stage	**P-value**	**Test statistic**	**P-value**	**Test statistic**
Eggs	0.387	1.896	0.172	3.52
1st instars	0.161	3.654	0.411	1.78
2nd-4th instars	0.156	3.721	0.203	3.18
Apterous adults	0.125	4.154	0.041	6.40
Total (all life-stages)	0.236	2.890	0.357	2.06

H_o = all 3 treatments are identically distributed. H_a = all 3 treatments are not identically distributed.

After dissection the level of root necrosis was monitored by visual assessment of cut root sections. For the G1-infested roots the highest level of root necrosis was observed on the low vigour vines

which also recorded the lowest numbers of phylloxera per gram of root (Table 34). On the G4 infested vines the highest degree of root necrosis was observed on the high vigour vines.

Table 34. Mean insect, necrosis, pathogens, non-pathogens and total fungal genera by plant vigour for genotypes G1 and G4

Vigour	***Low***		***Medium***		***High***	
Genotype	**G1**	**G4**	**G1**	**G4**	**G1**	**G4**
Insect (per gram)	0.12	9.50	1.45	25.72	0.71	10.51
Necrosis	19.44	6.44	11.44	7.44	12.89	9.44

Pathogens and Genotype

The initial observation from the field assessment of necrosis suggested that we found more necrosis on G1-infested vines than G4-infested vines. This leads us to expect that there would be more fungi (pathogens) on G1 vines than G4 in the re-isolation experiment. When the pathogens were identified from each site at the Upton (G1-infested) site was *Cylindrocarpon destructans* and at the King Valley site the predominant pathogen was *Fusarium oxysporum* (Table 35). However, there was no statistically significant relationship between phylloxera genotype and either central necrosis or circumference necrosis (Tables 5and 6).

Table 35. Relative number of pathogenic isolates from each of two phylloxera-infested field sites Upton (G1) and King Valley (G4)

***Pathogen*/Genotype**	**G1**	**G4**
Cylindrocarpon destructans	88	0
Fusarium oxysporum	45	141
Macrophomina phaseolus	22	33
Epicoccum purpurascens	4	0
Clonostachys rosea	0	16
Mucor sp.	22	0

Statistically the degree of root circumference necrosis was shown to be closely associated with fungal pathogen and vine vigour (Table 36). There was a significant difference between the pathogens in terms of the level of circumference necrosis ($P<0.001$). With significantly more fungal necrosis observed in the presence of *Fusarium oxysporum*. There was also a significant difference between plant vigour in terms of the level of circumference necrosis ($P<0.001$) with more necrosis in low vigour vines.

Table 36. Wald tests for fixed effects (circumference necrosis)

	Df	Wald statistic	P value
Genotype	1	2.70	0.100
Pathogens	5	36.74	<0.001
Vigour	2	14.30	<0.001

Statistically the degree of central root necrosis was shown to be closely associated with fungal pathogen and vine vigour (Table 37). There was a significant difference between the pathogens in terms of the level of central necrosis (P<0.001), with significantly more fungal necrosis observed in the presence of *F. oxysporum*. There was also a significant difference between plant vigour in terms of the level of circumference necrosis (P<0.021) with more central necrosis in high vigour vines.

Table 37. Wald tests for fixed effects (central necrosis)

	Df	Wald statistic	P value
Genotype	1	0.02	0.884
Pathogens	5	27.64	<0.001
Vigour	2	7.70	0.021

Results Summary

Initial Field Isolation Experiment

1. There was a trend in the number of insects and plant vigour, that is, medium vigour plants had the highest population of phylloxera. This is consistent for both G1 and G4 strains.
2. There is no obvious relationship between insects and pathogens (consistent for both strains). However, *Fusarium oxysporum* is consistently present at high levels.
3. There is a relationship between pathogens and necrosis for G4 but no relationship for G1. However, it is interesting to note also that there was more necrosis and pathogens on G1.
4. There appears to be no relationship between number of insects present and level of necrosis for both G1 and G4 strains. However, in low vigour vines, where root necrosis was high, the root quality may have deteriorated to such an extent that phylloxera may have moved elsewhere in search of a more suitable feeding site.
5. It appears that low vigour plants also had highest level of necrosis (certainly significant for G1). This was not evident with G4 infested vines, since there were many G4-infested vines that had no necrosis.

Re-Isolation Laboratory Experiment

1. There were more fungi isolated from G1-infested vine roots than G4 infested vines. G1 had *Cylindrocarpon destructans*, *Epicoccum purpurascens*, *Fusarium oxysporum*, *Macrophomina phaseolus*, *Mucor sp.* Isolated, whereas G4 had *Clonostachys rosea*, *F. oxysporum*, and *M. phaseolus* re-isolated.
2. In terms of the circumference necrosis, the order of pathogenic virility were as follows: (HIGH necrosis) *Fusarium oxysporum* < *Mucor sp.*< *Macrophomina phaseolus* < *Clonostachys rosea* < *Cylindrocarpon destructans* < *Epicoccum purpurascens* (LOW necrosis).
3. In terms of the central necrosis, the order of pathogenic virility were as follows: (HIGH necrosis) *Fusarium oxysporum* < *Clonostachys rosea* < *Macrophomina phaseolus* < *Mucor sp.*< *Cylindrocarpon destructans* < *Epicoccum purpurascens* (LOW necrosis).

Conclusion

Several pathogenic fungi were isolated from the two infested vineyards *Fusarium oxysporum* was isolated from both infested vineyard sites, within the lignified roots, suggesting that it contribute to root necrosis. *Fusarium oxysporum* is not generally regarded as a significant pathogen of vines, although it has been associated with some damage in roots already damaged by phylloxera (Granett *et al*., 1998, Omer *et al*., 1999). *Cylindrocarpan destructans* which was isolated form one of the sites is a common invader of roots, with relatively little impact, but with sufficient inoculum or sufficient stress on the vine, it invades the stem and causes rapid death of the vine. It also causes black foot disease and is a more aggressive pathogen of vines than *F. oxysporum*. In New Zealand black foot is a major problem where new vines have been interplanted into phylloxera-infested vineyards (Ian Pascoe, DPI-Knoxfield, pers.comm).

A variety of fungal strains were observed in this study between sites and site-related variables such as soil type, soil moisture and temperature and vine variety may influence their ability to be either pathogenic or beneficial. Further studies are required in order to test the hypothesis that soil-borne pathogens may be associated with vine decline in phylloxera-infested vineyards.

In the future if we are able to show a clear association between the level of phylloxera infestation and secondary root damage induced by secondary fungal invasion this could have important implications for alternative management of phylloxera. Lotter *et al* (1999) have shown that in surveys of conventionally managed vineyards in California levels of *Cylindrocarpon* and *Fusarium* are higher than in organically-managed vineyards and damage to vines is lower in the organically managed vineyards. Compost application has been shown to induce protection against *Fusarium oxysporum* in other crops (Reuveni et al., 2002). It has also been shown to either increase (Chapter 8) or decrease phylloxera abundance (Chapter 9) depending on the formulation. Omer et al., (1995) has demonstrated that application of conventional fungicide applications may reduce root damage in phylloxera-infested vines by reducing populations of secondary fungal pathogens.

Recommendations

Whilst this study shows that some fungal genera such as *Fusarium* and *Cylindrocarpon* can be found in phylloxera-infested vineyards it does not demonstrate conclusively that these fungi cause significant secondary root damage.

- A larger survey sample coupled with controlled-environment studies are required to fully elucidate between the interactions that may exist between fungi, phylloxera and grapevines. Potentially this may lead to alternative management strategies for grapevine phylloxera.

Chapter 11. Phylloxera Education and Awareness Activities

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

Summary

A key component of any research project is to ensure that research outputs are provided to the stakeholders in a timely, accessible and coordinated manner. Throughout the project period, July 2003-June 2006, phylloxera extension encompassed a range of activities. Previous phylloxera extension activities conducted by DPI-Rutherglen included the production and release of a Phylloxera Awareness Video (Powell, 2001), the facilitation of the First International Grapevine Phylloxera Management Symposium (Powell and Whiting 2000) and organisation of the first phylloxera workshop, held in 1994. Subsequently phylloxera workshops have continued on an annual basis and the number of workshops held and participant levels have increased over time (Dunstone et al., 1999; Powell, 2000).

Introduction

Annual Phylloxera Identification and Management Workshops aim to inform viticulture industry personnel of ways to manage phylloxera and highlight protocols to reduce the risk of phylloxera movement to uninfested vineyards and regions. At the workshops industry personnel are provided with up-to-date practical information and advice about phylloxera risk management, how the insect is spread, and trained in detection techniques and disinfestation and quarantine protocols. The participants are also provided with research updates from a range of related research project activities.

Method

Workshop Facilitation and Format

Annually the workshop format predominantly followed a one–day format, with the addition of a two-day bus tour format for South Australian participants which was organised in conjunction with the Phylloxera and Grape Industry Board of South Australia. Workshops were conducted within Phylloxera Infested Zones and were held in Milawa, Nagambie, Glenrowan, Porepunkah, Bright, Buckland Valley, King Valley and Rutherglen in Victoria, with the cooperation of local growers within the Phylloxera Infested Zones. Phylloxera workshops were targeted for all viticulture-related industry personnel. The workshops were publicised annually through industry publications, local and interstate newspapers, mail-outs to regional organisations, and through the Department of

Primary Industries-Victoria facilitated Grapecheque groups. The program was revised annually to include new information as it emerged from national and international phylloxera research activities and based on feedback provided in workshop surveys.

The comprehensive workshop format covered the following topics:

- **Introduction** - the history of phylloxera outbreaks, insect biology and geographical distribution across Australia and the world.
- **Field examination of phylloxera** - field survey techniques for identifying phylloxera infestation symptoms above and below ground in commercial vineyards were demonstrated.
- **Microscopic examination of phylloxera -** familiarising the participants with all phylloxera life-stages and associated root gall morphology and damage.
- **Population dynamics and distribution of phylloxera** - on the vine and potential methods of spread.
- **Quarantine and disinfestation protocols** – a comprehensive overview of the National Phylloxera Management Protocols and methods to restrict phylloxera spread.
- **Genetic diversity and life cycle -** familiarising the participants with phylloxera life-cycle variations and significance of genetic strains and their virulence on ungrafted and grafted grapevines under Australian conditions.
- **Interim management –** covering the limitations of cultural approaches to manage phylloxera in infested vineyards.
- **Early detection –** highlighting the traditional ground and aerial surveying techniques and novel approaches including research being conducted at DPI-Rutherglen to investigate potential for phylloxera-specific DNA probes for detection in soil, chemical and pigment fingerprinting and the use of hyperspectral imagery techniques**.**
- **Outbreak hypotheticals** - phylloxera outbreak scenarios to stimulate people to think about what actions are needed and the consequences for a viticultural business in the event of an outbreak.
- **Discussion** - with local growers and viticulturists with first-hand experience in managing phylloxera-infested vineyards.
- **Rootstock management** – providing up-to-date information on rootstock recommendations.

Workshop Reference Material

Each workshop participant was provided with a Phylloxera Identification and Management Workshop Booklet, which gave further details on a range of phylloxera-related topics including additional reference material. The Workshop Booklet was updated annually. Workshop evaluation surveys, which were originally introduced in 1997 as a component of project DAV 96/2 (Powell,

2000), were continued and provided useful feedback to assist with the ongoing improvement of the workshop program.

Results

Workshop Delivery

In 2003-2004, the number of workshops conducted was three in the following 2 years requests for study tours to the Research Centre for updates on phylloxera research activities were incorporated into the extension component of the project (Table 38). The requests for study tours came from local grower groups, Swinburne University, DPI-Mildura and VAMVIA, DPI Cadet program, Victorian Wine Industry and the Alpine Valley Growers group (following the Buckland valley phylloxera outbreak). The workshops and study tours attracted a wide range of participants from different viticultural regions of Australia. In total, over 240 people have attended the 9 workshops and 4 study tours held between 2003-2006, compared to around 200 participants attending 13 workshops over the preceding 3-year period (Powell, 2003).

Table 38. Phylloxera workshops and participation levels over the three-year project period (July 2003-June 2006)

Year	2004	2005	2006
Number of workshops held	3	3 + 1 study tour	3 + 3 study tours
Location of workshops	Nagambie, Rutherglen, Glenrowan, Milawa Bright, Porepunkah	Nagambie, Rutherglen, Milawa	Rutherglen, King Valley, Milawa
Number of participants	51	112	80

Workshop Evaluation Surveys

At the conclusion of each one-day or two-day workshop (but not study tour as the format of each differed depending on the target audience) the majority of participants completed evaluation forms. The aims of the anonymous workshop evaluation surveys were to (i) gather information about the participants demographics (ii) evaluate the effectiveness of individual workshop components (iii) obtain feedback on potential workshop improvements and (iv) obtain an overall rating for the workshop attended. The survey information was used to improve the workshop program, and to assist in the development of future research and educational programs. Local growers who spoke at the workshops were an important component of the workshop program and were well received by participants. They provide insights into the personal experiences of growers dealing with phylloxera in different regions. These sessions also helped to dispel some misconceptions and reduce the often heightened anxiety relating to phylloxera management, which can be counter-productive when attempting to implement workable quarantine and management protocols.

Staff directly involved in daily vineyard operations (ie grapegrowers, vineyard workers and managers) represented, over the 3-year period in which workshops were conducted, made-up the majority (over 50%) of workshop participants (Table 39). Those involved in seasonal or transient visits to the vineyard represented the remainder and included quarantine and regulatory officers, phylloxera survey team members, extension viticulturalists, lecturers, researchers, cellar door staff, winemakers and contractors. There was a gradual decline in the number of day-to-day vineyard staff, consultants and students attending the 1-2 day workshops during the three-year period. In 2005 there was a slight decrease in the number of grapegrowers, winemakers and contractors attending but their percentage jumped to even higher by 2006. However, several growers did attend the half-day study tours which provided a modified workshop program. The number of transient staff attending the workshops did increase in the second and third years of the project as this coincided with an upsurge in phylloxera survey activity particularly in New South Wales and South Australia. Thirty-four personnel from New South Wales and Queensland attended workshops specifically for training in survey techniques.

Not surprisingly company-sponsored participants from the majority (65%) of the workshop audience, with the remaining being equally split between self-employed and government-employed participants (Table 40). However, the number of company-sponsored participants gradually decreased over the three-year period. There was less then 10% government employed participants in 2004, while in 2005 self-employed participants dropped below 10%. By 2006 the number of participants from both of these occupational groups increased to almost half of all participants. This evenly balanced ratio of participants from different backgrounds enhances the diversity of the workshop groups and improves interaction particularly in the hypothetical discussion sessions.

Workshop participants were asked to nominate the three most valuable sessions during the workshop (Table 41). The most valuable sessions to growers were phylloxera biology, survey techniques and quarantine procedures. There was a steady increase in interest for the rootstock management session, which almost doubled in the three years. In rating phylloxera biology in the top three sessions, workshop participants clearly realised the importance of understanding the pest's biology and behaviour. It was pleasing to note that the interest shown by growers to emerging research remained stable though out the 3-year period. Workshops are a direct outlet that researchers have to industry and it is very important to engage industry representatives at this level. Phylloxera outbreak scenarios did not rate very high among the participants, which is of concern as this area of the workshop is very important. Overall we can conclude that participants enjoyed the balance of presentations and hands-on experience of the workshops.

Table 39. Occupation (% of total) of phylloxera workshop participants over the three-year project period (July 2003-June 2006)

Occupation	2004	2005	2006	Mean
Grapegrower	18.6	4.9	22.2	15.2
Viticulturist	0	2.4	0	0.8
Vineyard worker	30.3	14.6	11.1	18.7
Vineyard manager	14.0	31.7	11.1	18.9
Day-to-day vineyard operations (Total)	**62.9**	**53.6**	**44.4**	**53.6**
Consultant	11.6	12.2	3.7	9.2
Contractor	7.0	0	7.4	4.8
Nursery staff	0	0	3.7	1.2
Winemaker	4.7	2.4	3.7	3.6
Student	2.3	0	0	0.8
Other*	11.6	31.7	37.0	26.8
Transient vineyard involvement (Total)	**37.2**	**46.3**	**55.5**	**46.4**

*predominantly survey team members – 40 phylloxera survey team personnel trained

Table 40. Occupational status of workshop participants (% of total) over the three-year project period (July 2003-June 2006)

Occupation	2004	2005	2006	Mean
Company employed	73.0	68.3	54.3	65.1
Self-employed	18.9	7.3	25.0	17.1
Government employed	8.1	24.4	20.8	17.8

Table 41. Most valuable sessions of the phylloxera workshops voted by the participants over the three-year project period (July 2003-June 2006)

Session	2004	2005	2006	Mean
Introduction and phylloxera biology	18.9	20.2	16.7	18.6
Vineyard survey techniques	22.5	21.8	28.3	24.2
Quarantine procedures	22.5	21.0	23.3	22.3
Rootstock management	8.1	12.9	15.0	12.0
Information on current research	11.7	12.1	13.3	12.4
Other (talk by vineyard manager, laboratory time)	13.5	5.7	1.7	6.9
Phylloxera hypotheticals	2.7	6.5	1.7	3.6

In addition workshop participants were asked to rate their knowledge on the individual topics presented before and after the workshops (Figs 49 and 50). Prior to attending the workshops the participants had limited knowledge on current phylloxera research, vineyard survey techniques and the biology of phylloxera. Few participants rated their knowledge on quarantine procedures average to advanced before the workshops and over 60% of participants rated their knowledge as advanced after the workshops. This topic appeared the most applied and relevant to the participants. Post-workshop over 40% of participants achieved advanced knowledge on phylloxera management, new phylloxera research and vineyard survey technology. Slightly less then 40% of the participants rated their knowledge on phylloxera biology as advanced after the workshops. Overall 90% of the participants improved their knowledge in all topic areas.

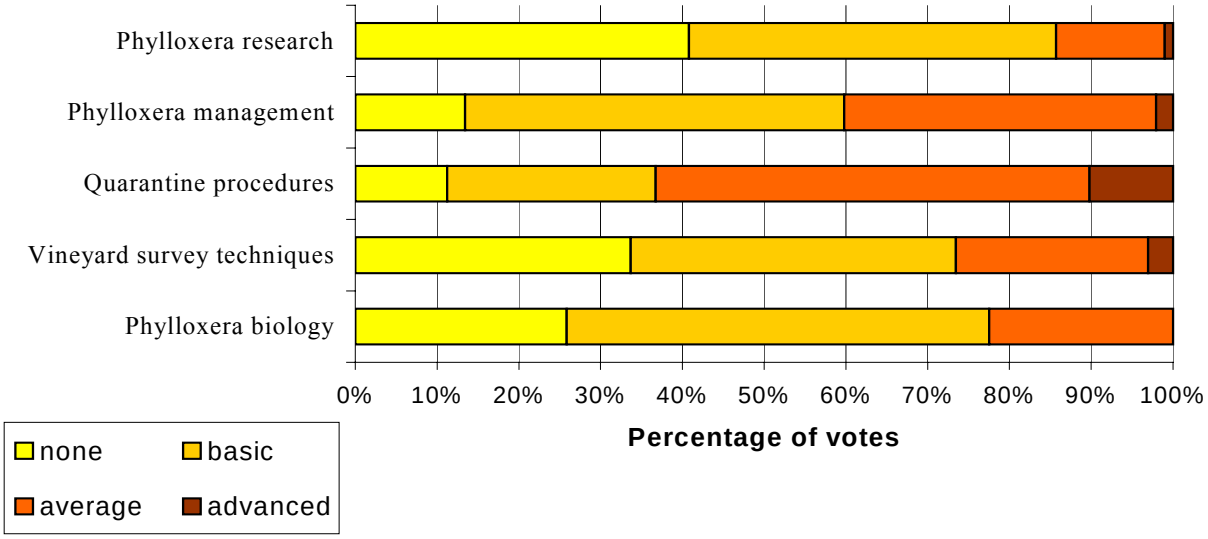


Figure 49. Workshop participants' knowledge before the workshops on the topics presented; voted by the participants over the three-year project period (July 2003-June 2006).

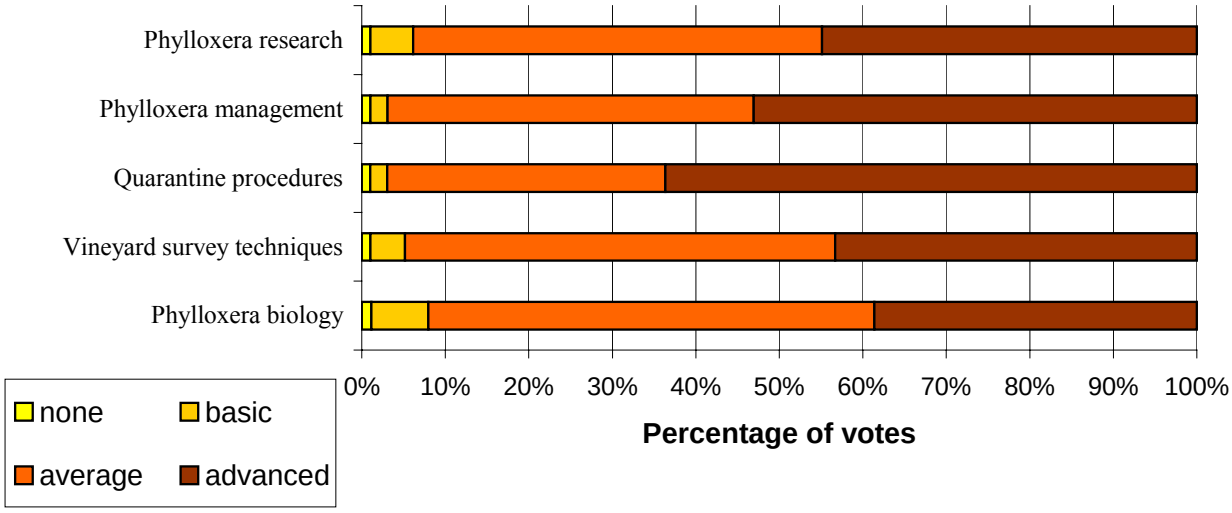


Figure 50. Workshop participants' knowledge after the workshops on the topics presented; voted by the participants over the three-year project period (July 2003-June 2006).

Workshop participants were also asked their opinions on the quality of the workshops. Overall from the survey responses received the majority of participants rated the workshops as good (32%) to excellent (68%) with the responses improving from good to excellent with each year (Table 42).

Table 42. Overall rating of workshop by participants (% of total) over the three-year project period (July 2003-June 2006).

Occupation	2004	2005	2006	Mean
Poor	0	0	0	0
Average	0	0	0	0
Good	47.4	34.2	15.8	32.4
Excellent	52.6	65.9	84.2	67.6

Quotes

A selection of comments from workshop participants is provided to highlight the effectiveness of the workshops and to show what participants will do differently after the workshops.

Workshop participants 2004:

"Will be more strict with persons and equipment moving between vineyards."

"Ensure new employees to the company are well informed of the implications and quarantine requirements for phylloxera."

"Having had limited knowledge on phylloxera I am now fully aware of all consequences."

Workshop participants 2005:

"Implement proper protocols instead of 'she'll be right'."

"Thought the field exercise and actually finding the phylloxera was most beneficial."

"Update handout information to contractors and also have training session with staff and contractors."

"Spend 30 seconds in the footbath, ensure it is changed regularly and the concentration of the active ingredient is correct."

Workshop participants 2006:

"I will be more careful with visiting personnel to ensure no introduction of the pest to my vineyard."

"Better clean machinery."

"I will be more aware/vigilant of quarantine protocols."

Recommendations

The need for continuation of phylloxera workshops on annual basis has been highlighted, with the maintenance of the number of annual workshops and participants since 1997, but also by the increased demand for ad-hoc and target audience specific workshops. Phylloxera workshops,

unlike many other forms of educational awareness programs, give the growers direct access to the researchers conducting the work and provide a unique opportunity for participants to identify the insect and observe damage symptoms in the field situation.

The evaluation exercise of the workshop program has highlighted that a more diverse audience could be attracted to the workshops in the future. A more concerted effort will be made to attract more people from these areas of the viticultural industry, less well represented to the workshops. Although there has been a small decline in attendance of some target occupations (eg; contractors, consultants and nursery owners), who attended phylloxera workshops in the last three years, those involved in daily vineyard operations, have remained at a consistently high level. Over 240 industry participants have been given new knowledge in the field of phylloxera management during the project period.

National and International Conference Presentations and Information Distribution

Notable extension activities included oral and poster presentations (Appendix 1) at a number of national and international conferences to both industry and scientific target audiences.

Presentations were made at the following conferences:

2003

International Plant Protection Conference, Beijing, China, July 2003 - two oral presentations.

2004

12th Australian Wine Industry Technical Conference, Melbourne, Australia, July 2004 - four poster presentations.

22nd International Congress of Entomology in Brisbane, Australia, August, 2004 - two oral presentations

2005

3rd International Phylloxera Symposium in Fremantle, Australia in October 2005. Two oral presentations.

7th International Aphid Symposium, Fremantle, Australia in October 2005. Three oral presentations and six poster presentations.

15th Biennial Australasian Plant Pathology Society Conference, Geelong, Australia, September 26 - 30, 2005. Three poster presentations.

2006

6th International Cool Climate Symposium on Viticulture and Oenology. Three poster presentations.

Media Activities

Phylloxera awareness and research activities have also been highlighted with members of the research team being interviewed for local television and radio channels (WIN News, ABC radio – 2003-2006).

Educational Material Distribution

Five hundred copies of the video Taking Aim at Phylloxera and 500 copies of the Educational CD-ROM Managing Grapevine Phylloxera were prepared for distribution through Winetitles, specialist multimedia publishers.

In addition, 200 phylloxera brochures "A guide to the identification, prevention and control of grape phylloxera" for distribution through mail-outs and workshops during 2003-2006.

Awards and recognition

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

2005 - Project Leader presented with International Society of Horticultural Sciences medal.
2006 - Team Finalists in Daniel MacAlpine Outstanding Achievement Award-DPI Science Awards.

Conclusion

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 and organisation of the 3rd International Phylloxera Symposium in Fremantle, Australia 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 surveys and demonstrated an improved awareness of phylloxera management issues.

Recommendations

- The need for continuation of phylloxera workshops has been highlighted by (i) a steady increase in the number of participants in the annual workshops and (ii) specific requests

coming from industry groups such as educational institutes for tailor-made workshops/study tours.

- Phylloxera is an important biosecurity issue, to the whole Australian Viticulture industry, that the target audience needs to be broadened to include more contractors, winemakers and nursery owners from a variety of viticulture regions and states. This may require a change in timing, advertising procedure or location for the workshops.

Appendix 1. Communications

Oral presentations conducted during the course of the project included:

Burns, A.E., Powell, K.S. (2004) A novel approach for rapid screening of grapevine rootstocks for resistance to Grape Phylloxera *Daktulospharia vitifoliae* Fitch (Hemiptera: Phylloxeraidae). Brisbane.

Macklin, H., Herbert, K., Corrie, A., Powell, K.S., Hoffmann, A.A. (2005) The use of DNA markers for pest management: The interaction between phylloxera clonal lineages and vine types in Australia. Fremantle.

Powell, K.S., Burns, A., Deretic, J. (2003) Field-based risk analysis and management of grapevine phylloxera *Daktulosphaira vitifoliae* (Fitch) in Australia vineyards. IPPC, Bejing, China.

Powell, K.S. and J. Deretic (2003) Post-harvest risk analysis and quarantine management of grapevine phylloxera *Daktulosphaira vitifoliae* (Fitch) in Australia. IPPC, Bejing, China.

Poster presentations conducted during the course of the project included:

Burns, A.E., Powell, K.S. (2004) A new method for rapid screening of grapevine rootstocks for resistance to grape phylloxera. AWITC, Melbourne.

Korosi, G.A., Trethowan, C.J., Powell, K.S. (2005) Improved screening of grapevine rootstock resistance to phylloxera genotypes under controlled conditions. Third Phylloxera Symposium, Fremantle.

Macklin, H., Herbert, K., Corrie, A., Powell, K.S., Hoffmann, A.A. (2005) The use of microsatellite markers for phylloxera management. Third Phylloxera Symposium, Fremantle.

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Appendix 2. Intellectual Property

The Corporation and the Research Organisations agree that the Corporation's share of title to all intellectual property and project income will be allocated on a percentage basis according to the proportion of funding for the project provided by the Corporation in relation to the funding provided by the Research Organisation.

Appendix 3. References

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Appendix 4. Staff

Project Activities

The projects core activities were carried out by DPI-Rutherglen staff under the supervision of the project Leader Dr Kevin Powell. 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), Anna Burns, Ginger Korosi, Annette Blanchfield and Carolyn Trethowan. In addition soil characterisation was conducted by Dr Mark Imhof (DPI-Werribee), new rootstock trials were established by John Whiting (DPI-Tatura).

CESAR staff involved in genotyping work included Kathy Viduka, Vanessa White, Paul Umina and Heidi Macklin.

Adherence to Quarantine Protocols

All fieldwork was carried out under quarantine 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 the DPI-Rutherglen Centre, which is located within the North-East Victoria Phylloxera Infested Zone (PIZ). All field-collected samples were processed in laboratory facilities at the DPI-Rutherglen Centre. Insect and plant materials, which required genetic characterisation, were transferred to the CESAR laboratory complex under quarantine regulations under permit issued by Plant Standards-Victoria.

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