Natural Resources and Environment

Grape and Wine Research and Development Corporation

Phylloxera and Grape Industry Board of South Australia

Victoria The Place To Be

MANAGEMENT OF GRAPE PHYLLOXERA IN SOUTH-EAST AUSTRALIA PHASE I & PHASE II

FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **DAV 96/2 and DAV 99/2**

Principal Investigator: **Dr Kevin Powell**

Department of Natural Resources and Environment
Agriculture Victoria - Rutherglen
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AGRICULTURE VICTORIA – RUTHERGLEN
GWRDC FINAL REPORT PROJECTS DAV 96/2 & DAV99/2

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AND

MANAGEMENT OF GRAPE PHYLLOXERA IN SOUTH-EAST AUSTRALIA: PHASE II ~ THE FUTURE

A final report on projects DAV 96/2 and DAV 99/2

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EXECUTIVE SUMMARY

This report describes the research and extension activities of two GWRDC funded projects on phylloxera research and management conducted during the period 1997-2000. The combined outputs of the two projects have resulted in an increased scientific knowledge of grapevine phylloxera which has been passed on to industry and 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. This has enabled the identification of risk periods and zones within phylloxera-infested vineyards in the cool climate region of the King Valley. Phylloxera research and awareness activities have been significantly enhanced during the course of the projects through:

- A variety of extension activities including workshops, field days, seminars and industry and media articles.

- Risk assessment and disinfestation experiments providing a 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. Further development of aerial surveying techniques for early detection of phylloxera has also taken place.

- Highlighting the latest research outputs from within Australia and overseas through the organisation of the first International Symposium on Grapevine Phylloxera Management which was held in Australia allowing industry and research personnel develop networks and collaborative links.

- Development of closer international collaborative links to ensure that phylloxera research and development is of a high international standard with a global perspective and avoids the duplication of research pathways.

- The facilitation of a key workshop which formed the framework for the development of an industry driven 5-Year Plan for Phylloxera Research and Development. This will form the framework for the development of a focused, innovative phylloxera research, development and extension program in Australia for 2000-2005.

INTRODUCTION

This is a report on phylloxera research and extension activities carried out from January 1997 to June 2000. The work was funded by the Grape and Wine Research and Development Corporation (GWRDC Projects 96/2 & 99/2), the Phylloxera and Grape Industry Board of South Australia and the Department of Natural Resources and Environment (DNRE). The objectives of each project are described as follows:

Project DAV96/2 – Management of grape phylloxera in southeast Australia

Time frame: January 1997-December 1999

Project 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 by:

1. Determining the potential natural dispersal of phylloxera within and between vineyards in a cool climate (ie. King Valley). This will include studies of root and above-ground populations of phylloxera, especially in regard to the number and survival of dispersive stages during the summer and autumn.

2. Quantifying risks of transfer of phylloxera via people and machinery working within vineyards, and through harvesting operations. This will involve sampling of phylloxera from vine canopies and grape bunches, and studies of survival of phylloxera in crushed grapes and fermenting wine.

3. Facilitating and providing technical support for the implementation of agreed phylloxera management protocols, in particular the implementation of the Tri-state Phylloxera Agreement. In addition, assistance would be provided to grower groups to develop protocols appropriate to their perceived degree of risk.

4. Testing and developing methods for early detection of phylloxera in vineyards. The King Valley will be used as a testing ground for optimising detection of phylloxera with enhanced aerial photography, using information gained from ground surveys to validate the remote sensing systems. Training courses and workshops for vineyard staff will continue as the basis for improved practical knowledge of phylloxera, but will be more frequent to satisfy the demand.

5. Further develop the capacity to trace infestations, by audit trails of harvest (or other) contractors, and by use of DNA typing techniques (developed in the previous project) to provide the precise capacity to map the spread of phylloxera infestations. It will then be possible to trace infestations and thereby target the high risk methods of phylloxera transfer, and so minimise the risks of future outbreaks.

6. Helping grapegrowers to test rootstocks for phylloxera control, by providing protocols for the design and evaluation of rootstocks in their vineyards. Dependent upon the virulence of phylloxera strains, characterisation of field strains may be an essential part of this process.

7. Testing new products and approaches to control of phylloxera on ungrafted vines, so that grapegrowers are provided with sensible options for control of phylloxera. This will involve strategic testing of effects of the products in small-scale trials, rather than elaborate and expensive field trials.

Project DAV99/2: Management of grape phylloxera in south-east Australia: Phase II ~ the future

Time frame: January 2000-June 2000

Project objectives:

The overall objectives were:

8. To consolidate current industry knowledge on phylloxera management and ensure that the recommendations from recently completed research are transferred and adopted to a wider audience.

9. To identify future priorities for phylloxera management and develop a framework for Phylloxera research, development and extension in Australia for the next 5 years.

In addition further developments of Objectives 1 and 2 (DAV 96/2) to include post-harvest risk analysis and population dynamics were added to the objectives of DAV/99/2.

Project Team

The projects core activities were carried out by DNRE staff located at Agriculture Victoria - Rutherglen and additional support was provided by DNRE staff located at Tatura and Knoxfield. The project activities were carried out in close collaboration with industry bodies including the Phylloxera and Grape Industry Board of South Australia and the National Phylloxera Technical Reference Group.

Project Staff:

Agriculture Victoria - Rutherglen

Dr Kevin Powell (Project Leader post-October 1998)
Angela Corrie (Project Leader pre-October 1998)
Jo-Anne Deretic (Research Scientist post-January 2000)
Rebecca Dunstone (Research Scientist pre-January 2000)
David Brown (Research Scientist pre-April 1998)
Sarah Hetherington (Technical Officer)

Institute for Horticulture Development, Knoxfield

Richard Gardner, Plant Standards Inspector
Jane Fisher, Grapecheque Facilitator

Institute for Irrigated Sustainable Agriculture, Tatura

John Whiting, State Viticulturalist
Marcus Everett, Horticulture Industry Development Officer
Megan Hill, Grapecheque facilitator

Adherence to Quarantine Protocols:

All fieldwork was carried out under permits that specified procedures and disinfestations 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 Agriculture Victoria - Rutherglen. All field collected samples were processed in laboratory facilities at Agriculture Victoria - Rutherglen which is located within a Phylloxera Infested Zone (PIZ).

Acknowledgements

The phylloxera research and extension team would like to express their gratitude to all growers who have allowed trials to be conducted on the properties. Particular thanks is given to Dr Greg Buchanan who has provided scientific guidance and advice throughout the project period.

PHYLLOXERA POPULATION DYNAMICS AND DISPERSAL ON UNGRAFTED *VITIS VINIFERA*

Objective 1 (Projects DAV 96/2 & DAV 99/2):

Determining the potential natural dispersal of phylloxera within and between vineyards in a cool climate (ie. King Valley). This will include studies of root and above-ground populations of phylloxera, especially in regard to the number and survival of dispersive stages during the summer and autumn.

Introduction:

Two commercial vineyard sites in the cool climate region of the King Valley, north-east Victoria were selected for a 3-year detailed qualitative and quantitative studies on the population dynamics and natural dispersal rates of grapevine phylloxera in infested ungrafted *Vitis vinifera* vineyards. The seasonal population trends of phylloxera, both above-and below-ground were assessed by root surveys and a variety of trapping techniques (emergence, pitfall, trunk and aerial). Site-related factors which could influence phylloxera populations, including weather and soil conditions, were also recorded. Assessment of population and dispersal trends within infested vineyards allows the development of appropriate risk management strategies based on scientific evidence.

Methods:

Site details:

Site 1 was located 5 km east of Cheshunt, where phylloxera was first discovered in the vineyard in May 1997. Site 2 was located 5-6 km west of Whitfield and phylloxera was first recorded at the site in November 1991. Both vineyards are located in the King Valley region within a designated phylloxera quarantine zone.

Soil classification and analysis

Soil surveys were conducted at each site in 1999 and classified on the basis of soil texture and chemistry as dystrophic brown kurosols at Site 1 and mesotrophic brown chromosols at Site 2 (W. J. Slattery, Agriculture Victoria - Rutherglen, pers. comm. 1999). Soil samples (1x10 cm diameter cores) were taken at each trial site from depths of up to 1 metre in August 1999. All soils were dried immediately after field sampling in a fan-forced oven at 40°C for 24 hour, rumbled through a 2 mm sieve and stored in sealed bags at room temperature. Soil analyses were conducted at the State Chemistry Laboratories, Werribee, Victoria.

Weather data

Weather data in the form of rainfall distribution, relative humidity and max/min air temperature was recorded over 15 minute intervals for both sites throughout the trial period and was kindly supplied by Serve-Ag, Tasmania and local growers. All weather data reproduced in this report is reproduced with the permission of King Valley growers.

Experimental design

The experimental design differed for each site. Preliminary ground surveys of each site were conducted in 1997 following standard procedures as used by DNRE survey teams. Survey results formed the basis for the experimental design for each trial site.

Site 1 - Trial design

Phylloxera infested ungrafted vine study

Three adjacent rows of ungrafted *Vitis vinifera* L. cv. Sauvignon Blanc vines (designated rows 1-3) were chosen for the study block. The row spacing was 3 metres and vine spacing was 1.8 metres. A total of twelve vines were studied in the block

with 5 vines in row 1, 4 vines in row 2 and 3 vines in row 3. Every fifth vine in each row was sampled.

Site 2 - Trial design

At Site 2 three experiments were conducted in the same phylloxera infested vineyard. All three studies examined phylloxera population trends. The first study was conducted on heavily infested (based on a visual assessment of root damage and vine decline) ungrafted *Vitis vinifera*, the second on lightly infested *V. vinifera* and the third on grafted *V. vinifera*.

Heavily infested ungrafted vine study - 1997-1998 season

Eight adjacent rows of ungrafted *Vitis vinifera* L. cv. Sauvignon Blanc vines (designated rows 266-274) bordered, east and west, by blocks of *V. vinifera* grafted onto the phylloxera-resistant rootstock Schwarzmann (*V. rupestris* x *V. riparia*) were chosen for the study block. The row spacing was 1.7 metres and vine spacing was 1 metre. The block selected for study had 3 areas of unproductive vines and one relatively large area of vines with high root populations and relatively healthy vine canopy showing no visual phylloxera damage symptoms. Four alternate rows (267, 269, 271 and 273) were chosen in which to sample vines. Single sample vines were selected randomly from each of 3 panels (panels 4, 6, and 8) representing a total of 12 sample vines.

Lightly infested ungrafted vine study - 1998-2000 seasons

During the 1998-1999 season a second block was chosen for study as the infested *Vitis vinifera* L. cv. Sauvignon Blanc block was uprooted after harvest in 1998 due to severe vine decline. The second study block chosen, based on an initial root survey, was 8 adjacent rows of ungrafted *Vitis vinifera* L. cv. Chardonnay (designated rows 218-225) bordered east and west by blocks of *V. vinifera* grafted onto cv. Schwarzmann (*V. rupestris* x *V. riparia*). The row spacing was 1.7 metres and the between-vine spacing was 1 metre. The ungrafted vine block selected showed minimal above ground symptoms of phylloxera damage and roots surveys indicated a relatively low level of infestation. Four alternate rows (218, 220, 222 and 224) were chosen in which to sample vines. Single sample vines were selected from each of 3 panels (panels 4, 6, and 8) representing a total of 12 sample vines.

Grafted vine study - 1998-2000 seasons

During the 1998-1999 season a second block was chosen for study as a control block to estimate phylloxera levels and dispersal on resistant rootstocks. The study block was chosen as it was bordered to the east by an uninfested block of grafted vines and to the west by an infested block of ungrafted vines. The study block consisted of 8 adjacent rows of *Vitis vinif*era L. cv. Chardonnay grafted onto Schwarzmann (*V. rupestris* x *V. riparia*) (designated rows 226-233). The row spacing was 1.7 metres with a vine spacing of 1 metre. The block selected showed no above ground symptoms of phylloxera damage and roots surveys indicated no phylloxera presence. Four alternate rows (227, 229, 231 and 233) were chosen in which to sample vines. Single sample vines were selected from each of 3 panels (panels 4, 6, and 8) representing a total of 12 sample vines.

ESTIMATION OF PHYLLOXERA INFESTATION ON GRAPEVINE ROOTS

Method:

Root populations were monitored biweekly on vines either side of each vine used for trap sampling in order to avoid disturbing phylloxera populations. Root levels were monitored by digging and exposing vine roots (Figure 1) and using a visual damage rating scale for fibrous and storage roots separately (Table 1). The average of the two vines provided an infestation index for the sample vine. Root populations were studied on ungrafted vine blocks at both sites. The total number of vines sampled in each block was 24.

Figure 1: Visual assessment of phylloxera infestation levels on ungrafted *Vitis vinifera*

Table 1: Phylloxera infestation rating scale for *in situ* root surveys.

	SYMPTOMS	
INFESTATION RATING	**FIBROUS ROOT**	**STORAGE ROOT**
0	No nodosities, no phylloxera	No tuberosities, no phylloxera
1	Nodosities rare, isolated phylloxera	Isolated phylloxera
2	Nodosities obvious, phylloxera in groups	Phylloxera in groups
3	>20% nodosities	Reproducing colonies common
4	>50% nodosities	Phylloxera abundant

Results:

Seasonal abundance of phylloxera on grapevine roots

At both sites root infestations were characterised by single peaks of activity on both storage (main) roots and fibrous roots. Infestation levels on older storage roots were marginally higher than on young fibrous roots and the peak of activity on storage roots was later in the season than that of fibrous roots (Figure 2).

At Site 1 in the first season (1997/1998) phylloxera was detected on vine roots from early October to early May. On fibrous roots populations peaked in the spring in early January then gradually declined through February and March. On storage roots a steady increase was observed in late January peaking in the summer in late March-early April. Mean infestation levels observed on the roots were low during the first season.

At Site 2 in the 1997/1998 season phylloxera populations were detected on vine roots from late September to late May. Phylloxera populations on fibrous roots peaked in December and early January and gradually declined in late January. Populations on storage roots increased in late January and peaked in February and March. Root infestations at Site 2 were relatively high in the 1997/1998 season compared with Site 1 and the resultant vine decline in Site 2 caused the grower to remove all the infested vines at the end of the season.

Discussion:

Omer *et al.*, (1997) have shown that phylloxera population levels as determined by laboratory counts were consistently higher on tuberosities than nodosities. Phylloxera densities on tuberosities showed a single peak during the summer whilst on nodosities two yearly peaks were observed; one in the summer and one during the autumn. In our study only single peaks of activity on both nodosities and tuberosities were observed.

Fibrous roots develop twice during the season. A spring flush, which coincides with an increase in populations observed on nodosities and tuberosities arise only after an initial population on nodosities. It is important to understand the population dynamics of phylloxera existing on the roots because management practices which influence root growth, such as irrigation and fertigation, could potentially influence either the vine. Root quality and quality may also play an important role in phylloxera population levels. Other factors such as weather variables and soil characteristics will also influence root population levels. In California of soil temperatures 18°C are required for phylloxera to establish feeding sites (Turley *et al.*, 1996). The influence of weather variables is discussed later in this report.

Recommendations:

Understanding how phylloxera populations develop on roots of *Vitis vinfera* is vital to the development of phylloxera management strategies. There are many variables in the vineyard which can influence population dynamics on grapevine roots including soil variables and management practices which influence vine health. Baseline data of phylloxera development on grapevine roots under controlled environment conditions is required, where soil variables such as texture, chemical characteristics, temperature and soil moisture levels can be manipulated. Once the effect of these variables is quantified this will allow the development of improved phylloxera management practices.

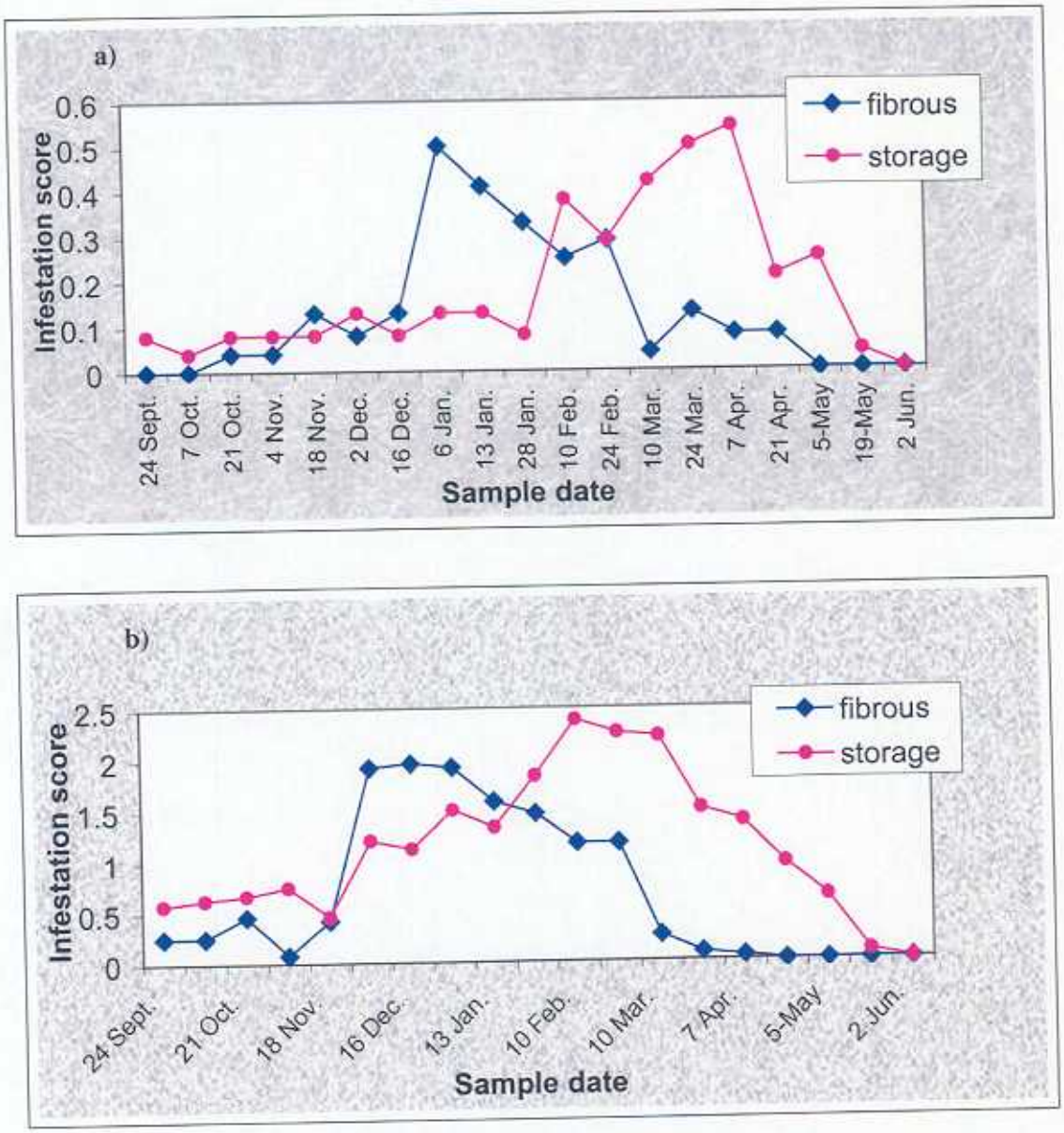

Figure 2: Seasonal variation of phylloxera infestation levels on fibrous and storage roots of *Vitis vinifera* in commercial vineyards at (a) Site 1 and (b) Site 2 in the King Valley, 1997-98.

PHYLLOXERA EMERGENCE FROM THE SOIL

Method:

The seasonal abundance of phylloxera dispersive stages from below the ground onto the soil surface was measured at both sites over three successive seasons (1997-2000) using emergence traps placed adjacent to twelve sample vines. Traps consisted of translucent plastic containers (4 litre Décor™), 22 cm diameter x 13 cm depth, open at one end and inverted onto the soil surface at a distance of 10 cm from the sample vine trunk (Figure 3). Traps were fixed flush with the soil surface using metal tent pegs. On emergence from the soil phylloxera were trapped in condensation on the container sides. At fortnightly intervals insects were removed by washing the trap with 70% ethanol and collected in plastic containers. Traps were then rinsed with tap water and replaced. Collected insects were counted using a low power binocular microscope. At Site 2 additional traps were placed at 20 cm and 40 cm from the vine trunk during the 1998-1999 and 1999-2000 seasons in order to determine the optimal trap position and quantify risk zones within a 40 cm radius from the vine trunk.

Figure 3: Insect trap positioned adjacent to infested grapevine to quantify phylloxera emergence from the soil.

Results:

Site 1 - Seasonal phylloxera emergence on ungrafted Vitis vinifera

Twelve sample vines in the infested block at Site 1 were monitored over three consecutive seasons (1997-2000). Phylloxera life stages, predominantly crawlers and to a lesser extent alates, were collected from October to May (Figure 4). Over the 3-

year trial period larger phylloxera numbers were collected from December to April but seasonal differences were observed. In the first season peak abundance of phylloxera caught in emergence traps were in January whilst during the second season peak numbers were collected in February. In the third season the greatest numbers of phylloxera were collected over a broader time period between December to April. In the third season, although more vines were infested in the study block the numbers of phylloxera emerging per vine was relatively low. The reasons for this are unclear but could be related to the overall reduction in fibrous root availability on the vines. By the end of the third season the majority of vines sampled were showing severe symptoms of vine decline and a severe lack of fibrous roots.

Site 2 - Seasonal phylloxera emergence on heavily infested ungrafted V. vinifera

Twelve sample vines in the ungrafted *Vitis vinifera* L. cv. Sauvignon Blanc infested block at Site 2 were monitored over one growing season. Phylloxera life stages, predominantly crawlers and to a lesser extent alates, were collected from November to April (Figure 5). Larger phylloxera numbers were collected from December to March and the highest population levels were observed in January coinciding with peak populations observed on vine trunks (Figure 13).

Site 2 - Seasonal phylloxera emergence on lightly infested ungrafted V. vinifera

Twelve sample vines in the *Vitis vinifera* L. cv. Chardonnay infested block at Site 2 were monitored over two consecutive seasons (1998-2000). Phylloxera life stages, predominantly crawlers and to a lesser extent alates, were collected from October to April (Figure 6). Over the 2-year trial period larger phylloxera numbers were collected from December to March but there were seasonal differences observed. In the 1998-1999 season peak abundance of phylloxera caught in emergence traps were during December to March whilst during the 1999-2000 season peak numbers were collected during January to March.

Site 2 - Phylloxera emergence risk zones on lightly infested ungrafted V. vinifera

A comparison of phylloxera numbers caught in emergence traps spaced at different distances from the vine trunk highlighted that as trapping distance from the vine increases the relative number of phylloxera trapped is reduced. In the 1998-1999 season (Figure 7) consistently higher populations were caught in traps spaced at 10 cm from the vine, compared with 20 cm and 40 cm spacings, for six consecutive months (December to May). In February there were up to ten-fold more phylloxera caught in the 10 cm traps than both the 20 cm and 40 cm traps.

In 1999-2000 season the total number of phylloxera caught in emergence traps was relatively low (Figure 8) compared to the previous season. Phylloxera numbers caught in emergence traps from over a seven-month period were again consistently higher in the traps spaced at 10 cm compared with traps spaced at 20 cm, with the exception of December when no difference was observed.

Site 2 - Seasonal phylloxera emergence on grafted Vitis vinifera

In the grafted vine block, which was established as a control site, phylloxera was unexpectedly caught in traps located on two of the twelve sample vines from December to May in the 1998/1999 season and from November to March in the 1999/2000 season. The two infested vines were located in the row nearest to the infested ungrafted block. Earlier studies (Corrie *et al.*, 1997) in the laboratory had shown that the phylloxera strain collected at Site 2 could not establish on Schwarzmann roots. Root samples of the infested vines were therefore collected in August 1999 and transported in liquid nitrogen under quarantine permit for DNA typing at the Australian Wine Research Institute, Adelaide. The samples were DNA typed using six microsatellite loci and matched the genotype Chardonnay and were therefore ungrafted vines and not grafted Schwarzmann. No phylloxera were detected in emergence traps located next to the remaining 10 grafted sample vines.

Between site emergence trends

Relative phylloxera numbers emerging at Site 1 (Figure 4) were markedly higher than those at Site 2 (Figures 5 and 6). At their peak up to 2000 phylloxera per vine were collected at Site 1 (1997/1998 season) whereas at Site 2 a maximum of 520 phylloxera per vine was recorded (1997/1998 season). The difference in emergence levels could be related to a number of site-related differences in soil type, weather, management practices and level of infestation.

Discussion:

Assessment of phylloxera emergence levels is a key factor in determining peak periods of transfer risk within infested vineyards. This study has highlighted that phylloxera emerge from the soil in the cool climate region of the King Valley from October through to May. However, higher populations can be found from December to March, which is therefore a key risk period when management practices should be modified to reduce the risk of transfer during this period. Seasonal differences in peak emergence do occur and site-related factors are likely to influence emerging population levels. In studies conducted in New Zealand and Nagambie crawler emergence was observed from November to April with peak abundance in February and March (King & Buchanan, 1986). The study has also shown that trap position is important when assessing phylloxera emergence patterns. In this particular study emergence levels were consistently higher in traps positioned 10 cm away from the vine than in traps positioned up to 40 cm away. Risk zones around infested zones can therefore be quantified using a variety of trapping protocols.

Recommendations:

Although risk periods have been documented in this study, which will assist in the development of risk management strategies for infested vineyards in the King Valley, further studies are required to determine to what extent site-related factors influence phylloxera emergence. This would involve field trials under a range of conditions over successive seasons and under controlled environment trials where emergence patterns of genetically different phylloxera strains could be quantified.

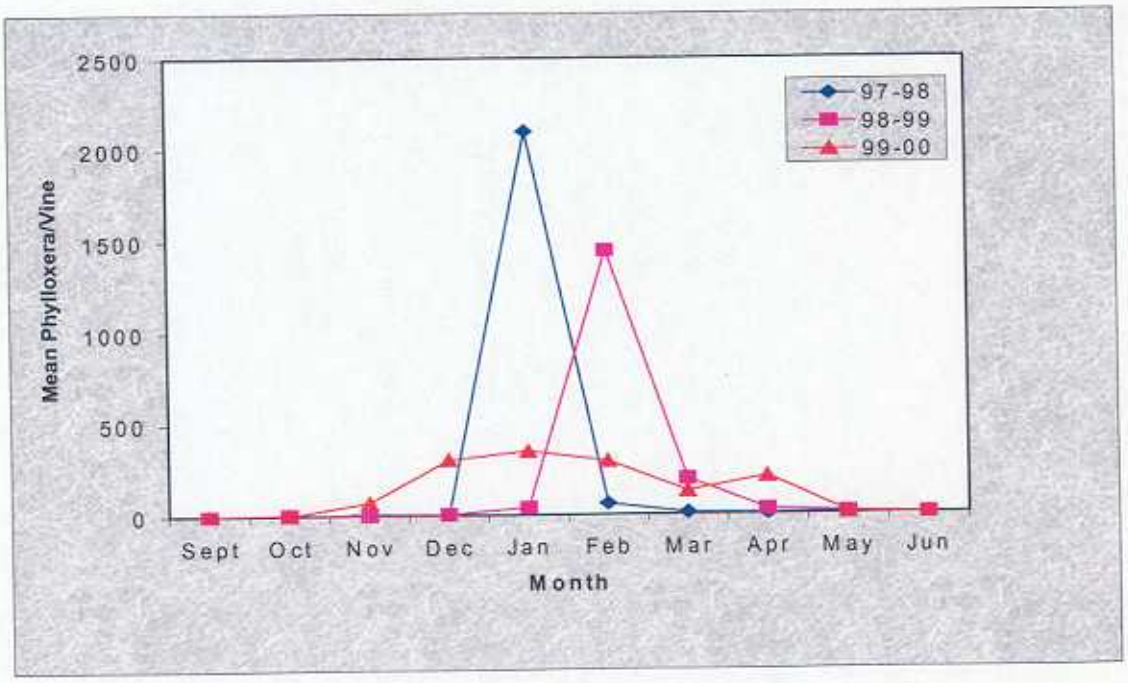

Figure 4: Phylloxera emergence over three successive seasons (Site 1: 1997- 2000)

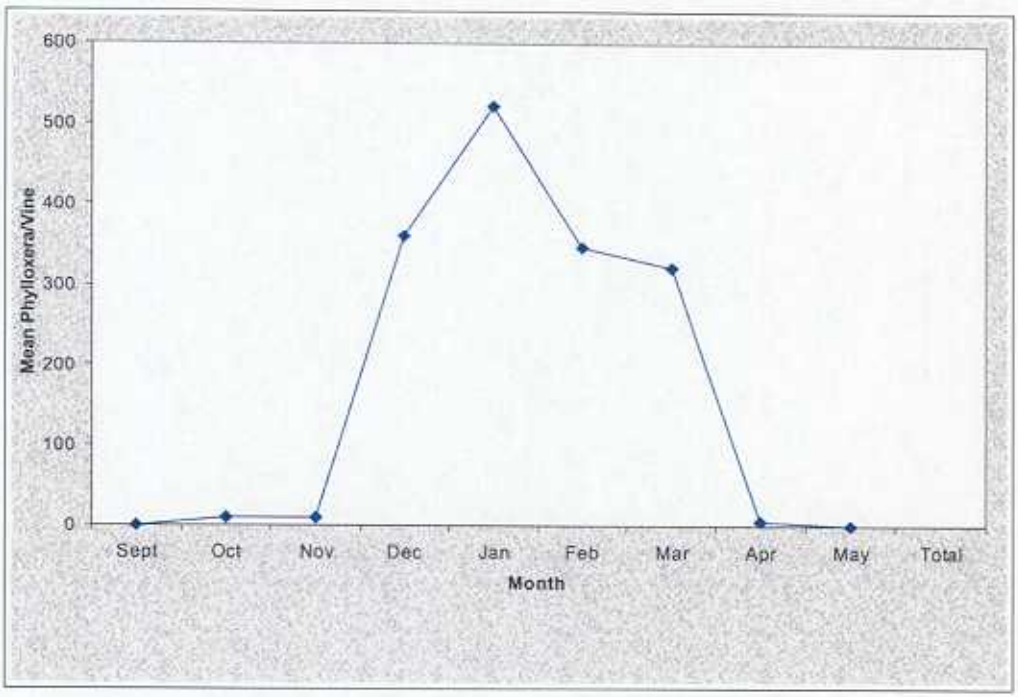

Figure 5: Phylloxera emergence on ungrafted *Vitis vinifera* (Site 2: 1997/1998)

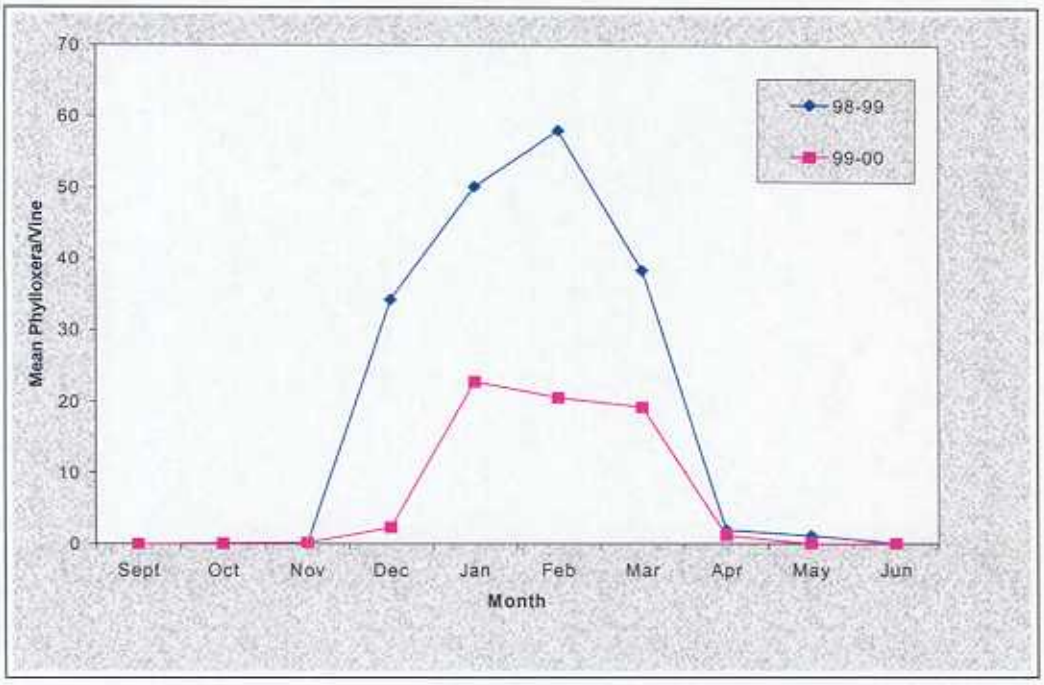

Figure 6: Phylloxera emergence on ungrafted *Vitis vinifera* over 2 successive seasons (Site 2: 1998/1999-1999/2000).

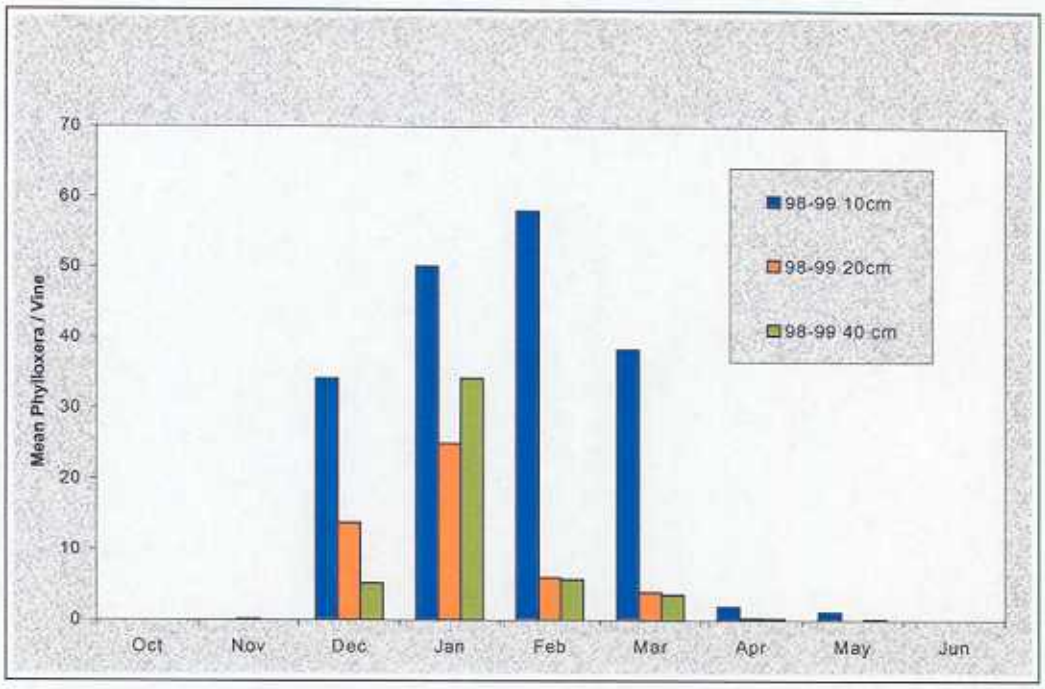

Figure 7: Comparison of emergence trap distance (Site 2: 1998-1999)

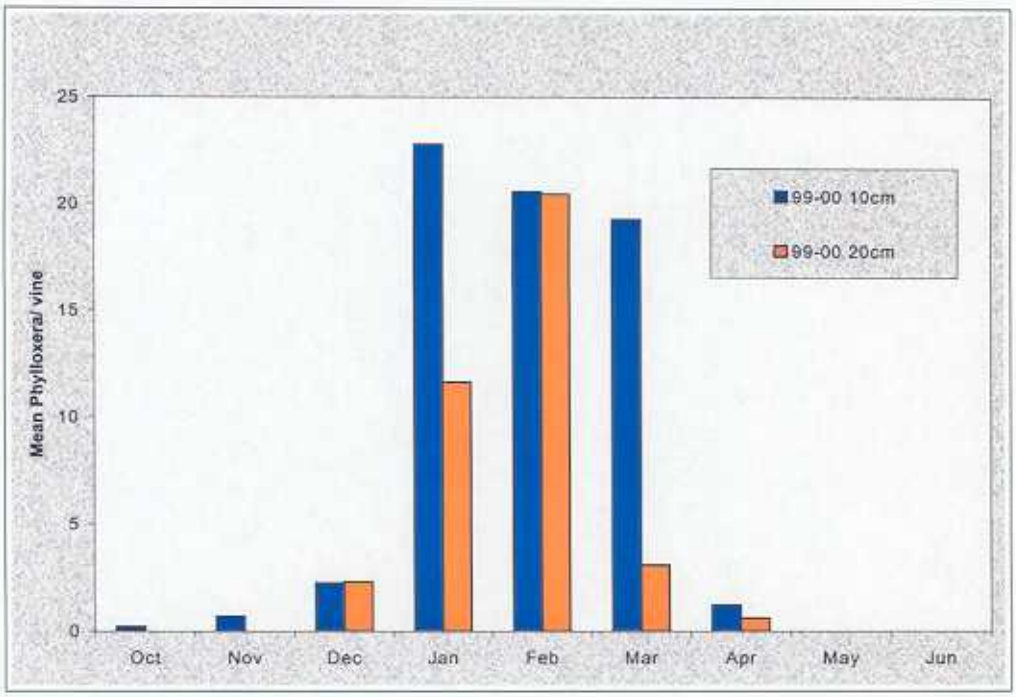

Figure 8: Comparison of emergence trap distance (Site 2: 1999-2000)

PHYLLOXERA MOVEMENT ON THE SOIL SURFACE

Method:

The seasonal abundance of first instar crawlers and alates moving across the soil surface in infested blocks of ungrafted *Vitis vinifera* was measured at both sites over two successive seasons (1998-2000) using pitfall traps (Figure 9). Single traps consisting of 250 ml plastic beakers (8 cm diameter x 9 cm depth) were placed in a hole in the ground adjacent to each of twelve sample vines. Insects were trapped in a collecting fluid of ethyl glycol and water at a 1:1 ratio. At fortnightly intervals traps were rinsed with water and collected in plastic containers. Traps were then rinsed with tap water and replaced with fresh collecting fluid. Collected insects were counted using a low power binocular microscope.

Figure 9: Pitfall trap positioned adjacent to infested grapevine to quantify phylloxera movement across the soil surface.

Results:

Site 1 - Seasonal phylloxera movement on the soil surface

Twelve sample vines in the *Vitis vinifera* L. cv. Sauvignon Blanc infested block at Site 1 were monitored over two consecutive seasons (1998/1999 and 1999/2000). Phylloxera life stages, predominantly crawlers and to a lesser extent alates, were collected from October to May (Figure 10). Over the 2-year trial period phylloxera numbers peaked from January to March but there were seasonal differences observed.

In the 1998-1999 season peak abundance of phylloxera caught in pitfall traps was January to March whilst during the 1999-2000 season peak numbers were collected in January.

Site 2 - Seasonal phylloxera movement on the soil surface

Twelve sample vines in the *Vitis vinifera* L. cv. Chardonnay infested block at Site 2 were monitored over two consecutive seasons. Phylloxera life stages, predominantly crawlers and to a lesser extent alates, were collected from November to May (Figure 11). Over the 2-year trial period larger phylloxera numbers were collected from January to March. Peak levels were recorded in January and February for the 1998/1999 and 1999/2000 seasons respectively (Figure 11).

Between site phylloxera soil movement trends

Relative phylloxera numbers caught in pitfall traps at Site 1 (Figure 10) were markedly higher than those at Site 2 (Figure 11). At both sites higher levels were recorded in the 1999/2000 season compared to the 1998/1999 season. During the 1999-2000 season at their peak up to 3,200 phylloxera per pitfall trap were collected at Site 1 whereas a maximum of 170 phylloxera per pitfall trap was recorded at Site 2. The difference in abundance levels was anticipated as infestation levels in the trial block and the number of infested vines were considerably higher at Site 1 throughout the trial period. Other site-related variables such as soil type, air temperature, rainfall and management practices may also have influenced movement across the soil surface.

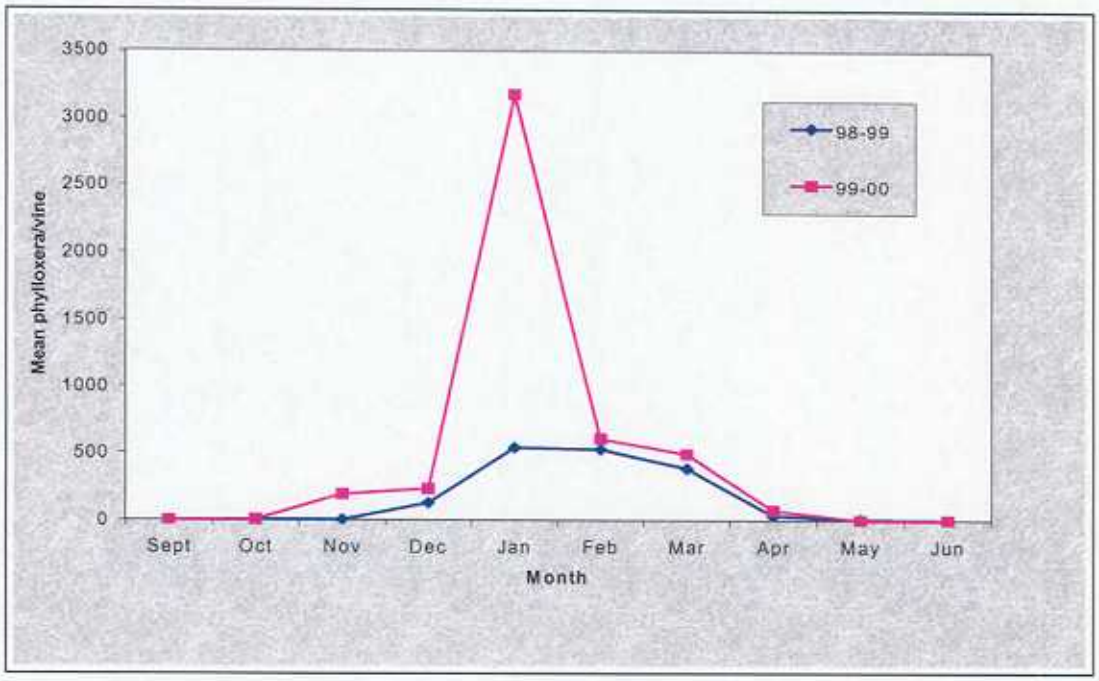

Figure 10: Estimation of phylloxera movement on soil surface, using pitfall traps, over 2 successive seasons (Site 1: 1998-2000).

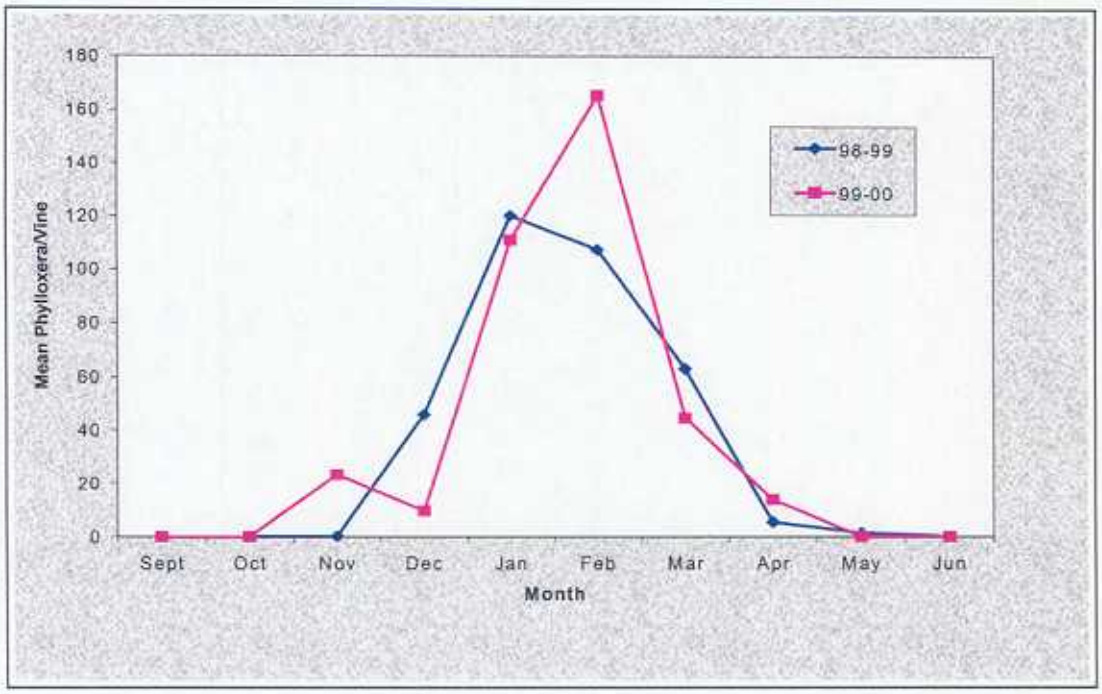

Figure 11: Estimation of phylloxera movement on soil surface, using pitfall traps, over 2 successive seasons (Site 2: 1998-2000).

Discussion:

There have been no previously published reports on the use of pitfall traps in phylloxera population studies. However, the data presented here shows that pitfall traps are useful method for population dynamics studies. Despite their relatively small size (250 ml capacity) compared to the emergence traps (4 litre capacity) higher numbers of phylloxera were collected in pitfall traps. The main reason for this is due to trap positioning. Pitfall traps are designed to collect insects moving from all directions on the soil surface and not necessarily those moving from the sample vine. Emergence traps are only designed to collect insects moving up the soil profile from underlying roots of the sample vine to the soil surface. One disadvantage in the use of pitfall traps for phylloxera sampling is they are not selective and collect a variety of soil surface vertebrates and invertebrates which then requires extensive sorting. In contrast emergence traps being inverted on the soil surface only collect arthropods that can move vertically upwards on the trap wall. Natural dispersal across the soil surface is likely to influenced by weather factors particularly temperature and the presence of natural predators.

Recommendations:

This study has highlight peak periods (December to March) of phylloxera movement across the vineyard floor, which would increase the risk of human assisted phylloxera transfer via machinery or personnel moving between the rows. There is a need to develop more extensive sampling protocols for the use of pitfall traps to estimate dispersal rates and movement directions across the vineyard floor by both natural and human-assisted dispersal.

PHYLLOXERA MOVEMENT ON THE VINE TRUNK

Method:

Phylloxera movement up and down grapevine trunks was assessed at both sites for three seasons during 1997-2000 by collecting phylloxera in sticky traps wrapped around the vine trunk of twelve sample vines. A 10 cm strip of grey duct tape was wrapped around the vine 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 could be placed the trunk traps. Trunk traps consisted of two bands of white electrical tape wrapped around the trunks of sample vines at a distance of 25 cm above the soil surface and 20 mm apart (Figure 12). Tanglefoot™ was applied evenly to the centre of the tape at a width of 1 cm using an artist paintbrush. The lower band was used to collect insects moving up the vine trunk from the soil surface whilst 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.

Figure 12: Insect trap positioned on infested grapevine to quantify phylloxera movement on vine trunk.

Results:

Site 1 - Seasonal phylloxera trunk movement on ungrafted Vitis vinifera

Twelve sample vines in the infested block at Site 1 were monitored over three successive seasons (1997/1998, 1998/1999 and 1999/2000). Phylloxera life stages, predominantly crawlers and to a lesser extent alates, were collected from October to May (Figure 13). Over the 3-year trial period larger phylloxera numbers were collected from January to March but there were seasonal differences observed. In the first season phylloxera numbers caught in trunk traps reached their peak in January whilst during the second season peak numbers were collected in February. In the third season the greatest numbers of phylloxera were collected over a broader time period between January and March.

Site 1 - Directional phylloxera trunk movement on ungrafted V. vinifera

Based on data from trunk traps strategically placed on the vine trunks it was possible to determine the relative proportions of phylloxera moving up the vine trunk (ie from the soil surface) and down the vine trunk (ie from within the canopy) (Figure 13). Over all three seasons there were more phylloxera moving up the vine trunk than down the vine trunk. In the first season (1997/1998) phylloxera movement up the vine trunk was low compared to subsequent seasons. This is probably due to the fact that over the trial block relatively few vines were heavily infested in the first season. The highest numbers of phylloxera were recorded in traps measuring upward trunk movement in the 1998/1999 season.

Site 2 - Seasonal phylloxera trunk movement on heavily infested ungrafted V. vinifera

Twelve sample vines in the ungrafted *Vitis vinifera* L. cv. Sauvignon Blanc infested block at Site 1 were monitored over one growing season. Phylloxera life stages, predominantly crawlers and to a lesser extent alates were collected from November to April (Figure 14). Larger phylloxera numbers were collected from December to March and the highest population levels were observed in January.

Site 2 - Directional phylloxera trunk movement on heavily infested ungrafted Vitis vinifera

In the 1997-98 season phylloxera movement up the vine trunk was comparatively high (Figure 14) compared to data collected in the *Vitis vinifera* L. cv. Chardonnay block (Figure 15). This was probably due to the relatively high level of infestation in the *Vitis vinifera* L. cv. Sauvignon Blanc block. Over the season phylloxera movement on the vine trunk was predominantly upwards.

Site 2 - Seasonal phylloxera trunk movement on lightly infested ungrafted Vitis vinifera

Twelve sample vines in the *Vitis vinifera* L. cv. Chardonnay infested block at Site 2 were monitored over two consecutive seasons (1998-2000). Phylloxera life stages, predominantly crawlers and to a lesser extent alates, were collected from November to April (Figure 15). Over the 2-year trial period larger phylloxera numbers were collected from early January to April but there were seasonal differences observed. In the first season peak abundance of phylloxera caught in trunk traps were during January to March whilst during the second season peak numbers were collected during February and March. In the second season greater numbers of phylloxera were collected over the whole season compared with the previous season and this coincided with wider dispersal of phylloxera within the infested block.

Site 2 - Directional phylloxera trunk movement on lightly infested ungrafted Vitis vinifera

In the first season phylloxera movement up the vine trunk was low compared to second season (Figure 15). This is probably due to the fact that over the trial block relatively few vines were infested during this season. Over both seasons there were more phylloxera moving up the vine trunk than down the vine trunk.

Site 2 - Phylloxera trunk movement on grafted V. vinifera

In the grafted vine block, which was established as a control site, phylloxera was unexpectedly caught in trunk traps located on two of the twelve sample vines from December to May in the 1998/1999 season and from November to March in the 1999/2000 season. However, roots of these two vines were later DNA-typed and found to be ungrafted *Vitis vinifera* cv. Chardonnay. On the trunks of the remaining ten grafted vines no phylloxera were recorded.

Between site trunk movement trends

Trunk data trends in terms of relative ratios moving up and down the ungrafted vine trunks were similar at both sites (Figures 13-15). However, trunk movement was consistently higher at Site 1. Phylloxera numbers trapped on the vine trunk were up to ten fold higher at Site 1 (1998-1999 season) compared to Site 2 (1998-1999 season). Similar higher population levels were observed in emergence and pitfall traps at Site 1.

Discussion:

Quantitative assessment of natural movement of phylloxera up and down the vine trunk is important in determining potential population levels in the canopy and hence determining the potential risks of transfer via human assisted dispersal such as mechanical harvesters or hand pickers that come into contact with the canopy. Trunk traps have also been used to determine the extent of downward migration of the leaf-galling form of phylloxera in Canadian vineyards (Stevenson, 1966). Our study has shown that peak population movement up and down the vine trunks is between January and April and movement of machinery or personnel in or near the canopy during this period will increase the risk of transfer. Phylloxera movement up the vine trunk was consistently higher over all seasons/sites than the movement down the vine trunk, which indicates that some factor in the canopy is affecting phylloxera survival, such as higher temperatures or natural predators. Data from aerial traps and canopy sampling provide further evidence that populations of phylloxera in the canopy are

relatively low compared with phylloxera populations moving up the vine trunk or on the soil surface.

Recommendations:

Further studies are required to determine what factors influence the upward and downward trunk movement of phylloxera and what management factors could influence this movement.

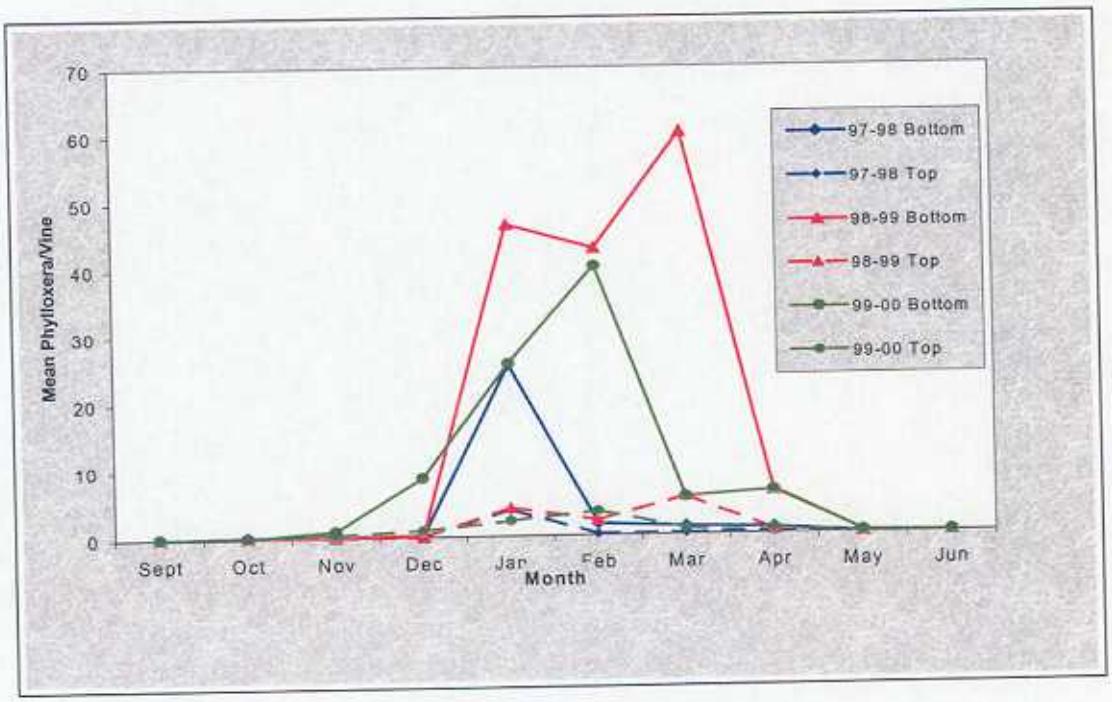

Figure 13: Phylloxera trunk movement on ungrafted *Vitis vinifera* over 3 successive seasons (Site 1: 1997-2000)

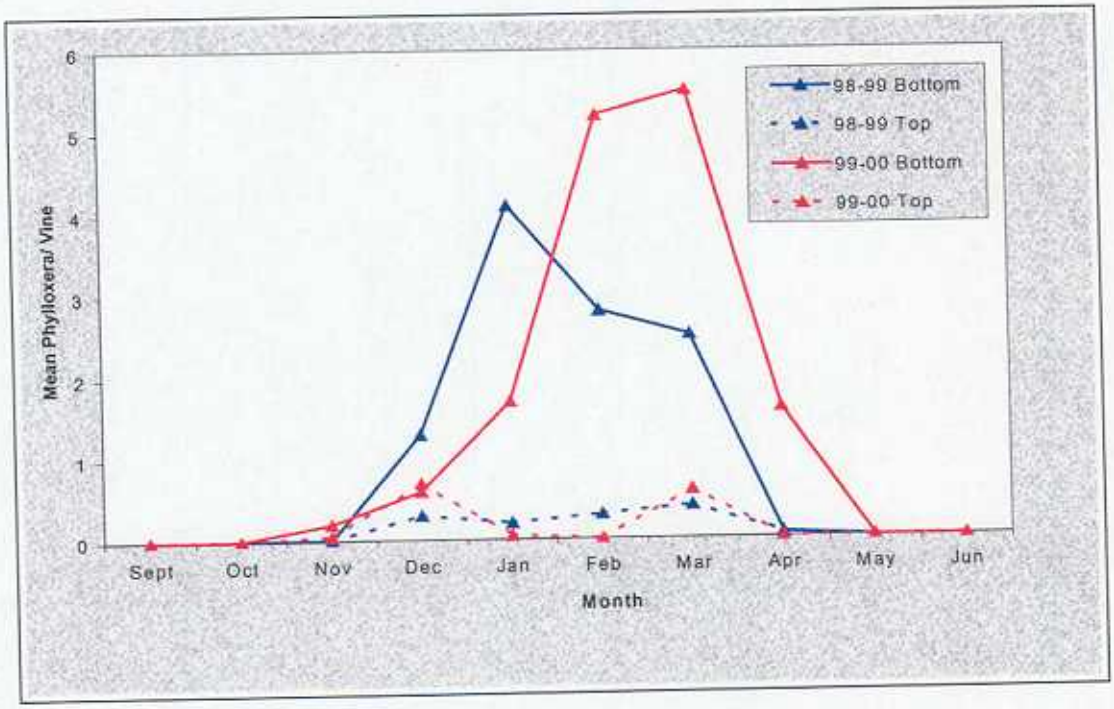

Figure 14: Phylloxera trunk movement on ungrafted *Vitis vinifera* (Site 2: 1997/1998)

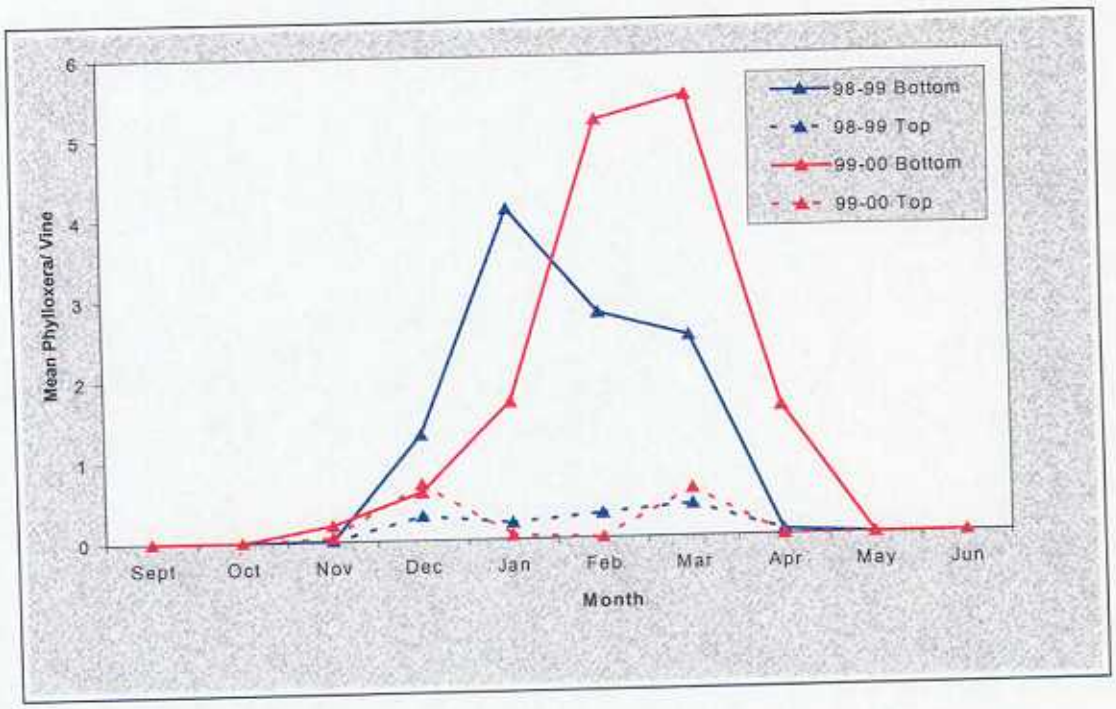

Figure 15: Phylloxera trunk movement on ungrafted *Vitis vinifera* trunks over 2 successive seasons (Site 2: 1998-2000).

AERIAL DISPERSAL OF PHYLLOXERA

Method:

Numbers of phylloxera moving in the air column were assessed at Site 2 using sticky aerial traps. Aerial traps were set-up in the first growing season (1997-98) on the borders of a heavily infested block of ungrafted vines. Following the subsequent uprooting of the infested block, the aerial traps were relocated in the second and third growing seasons on the border of a relatively lightly infested area of ungrafted vines. The trial design was also modified in the second (1998-1999) and third seasons (1999-2000).

Trial 1 - Aerial dispersal from heavily infested ungrafted Vitis vinifera

Two vine blocks positioned to the eastern and western side of a population study block were used as sampling sites to estimate aerial dispersal of phylloxera away from a heavily infested area of ungrafted *Vitis vinifera* L. cv. Sauvignon Blanc. Each aerial sampling block consisted of 14 adjacent rows of grafted vines (designated rows 247-261 and 277-291). The blocks selected were grafted phylloxera-resistant rootstock vines (*V. rupestris* x *V. riparia* cv. Schwarzmann) showing no above-or below-ground symptoms of phylloxera presence or related damage, based on pre-trial surveys. In each block three rows were selected, 7 rows apart in which to position aerial traps (eastern rows: 277, 284, and 291; western rows: 247, 254, 261). Traps were secured onto panel posts in each of 4 panels (panels 4, 6, 8 and 10) in each sample row representing a total of 12 aerial traps per sample block.

Trial 2 - Aerial dispersal from lightly infested ungrafted Vitis vinifera

In the 1998-1999 and 1999-2000 growing seasons two aerial sampling blocks either side of the population study block were designated as aerial sampling sites to estimate aerial dispersal of phylloxera away from a lightly infested area of ungrafted *Vitis vinifera* L. cv. Chardonnay. Each site consisted of 7 adjacent rows of vines (designated rows 210-216 and 235-240). The blocks selected were grafted phylloxera-resistant rootstock vines (*V. rupestris* x *V. riparia* cv. Schwarzmann) and showed no above-ground symptoms of phylloxera damage and pre-trial root surveys

indicated no phylloxera presence. Three alternate rows in each block (western block: 211, 213 and 215; eastern block: 236, 238, 240) were chosen in which to position aerial traps.

Aerial trap design and orientation

Aerial traps consisted of acetate sheets (25 cm x 25 cm) coated with Tanglefoot™ (a sticky adhesive material) attached to either side of the upper surface of an arched metal sheet painted yellow (as a visual attractant for insects). The metal arched sheet was attached to a metal stake which was secured onto trellis posts, 40 cm above the vine canopy (Figure 16), in each of 4 panels (panels 4, 6, 8 and 10) in each sample row representing a total of 12 aerial traps per sample block. In the 1997-98 traps were positioned in an east-west orientation. In subsequent seasons the traps were orientated in both east-west and north-south orientations. Sticky traps were removed at 3-4 week intervals covered with Gladwrap™ and examined for phylloxera presence using a low powered microscope.

Figure 16: Insect trap positioned above the grapevine canopy to quantify aerial dispersal of phylloxera.

Results:

Phylloxera dispersal in relation to infested block location

Over all three seasons samples were collected from sticky aerial traps located on the western and eastern sides of phylloxera-infested ungrafted *Vitis vinifera* vines. Population levels in the air column, as measured by aerial trapping, differed between seasons (Table 2 and 3) and peaked from mid-late March (Figure 17).

Although the total number of phylloxera caught per season was similar for each season (ie. within the range of 26-38) the number of phylloxera collected varied, according to trap location in relation to the infested block. In the Trial 1, which was conducted adjacent to a block of heavily infested vines, phylloxera levels in the western block were higher (18 phylloxera collected) throughout the season than the eastern block (8 phylloxera collected) (Table 2).

In Trial 2 in the 1998/1999 season phylloxera levels in the air column were evenly distributed with 21 phylloxera collected in the eastern block and 17 in the western block (Table 3). In the 1999/2000 season phylloxera levels in the air column were higher in the eastern block (18 collected) compared to the western block (11 collected).

Aerial dispersal in relation to trap orientation

Phylloxera numbers caught in aerial traps were dependent on trap orientation. In Trial 1 traps were only orientated towards the east or west and more phylloxera were trapped over the season in the east facing traps compared to west facing traps (Table 2). In Trial 2 traps were orientated in north, south, east and west directions. In the first season (Trial 2) more phylloxera were caught in the west and south facing traps than the north and west facing traps (Table 3a). In the second season (Trial 2) relatively few phylloxera were caught in the south facing traps compared to north, east or west facing traps (Table 3b).

Relative abundance of phylloxera aerial dispersive stages

Only two types of phylloxera dispersive stages, the first instar (crawler) and the winged adult (alate) were collected in aerial traps during the study period and the ratio of the two forms differed between the two seasons. In Trial 1 the ratio of alates to crawlers collected was 25:1. In Trial 2 (season 1) the ratio was more evenly distributed with 22 alates and 16 crawlers were collected; whilst in the second season there were 29 crawlers and no alates were collected. Data from emergence traps indicates that levels of alates varied between seasons at Site 2 (Figure 18) which may explain why no alates were recorded in aerial traps during the second season.

Distance of aerial dispersal spread from infested vines

If we assume that the phylloxera has spread on the wind from the infested block we can roughly estimate the minimum distance that phylloxera has been carried by the wind by measuring the distance to the nearest infested row. In the first season phylloxera were collected in traps positioned 15 rows away from the edge of the infested block. As between row width is 1.7m this roughly equates to up to 25 metres dispersal away from infested vines.

Table 2: Aerial movement of phylloxera dispersive stages in Trial 1.

Row number	East facing trap	West facing trap
Eastern Block		
247	2 alates	0
254	2 alates	1 crawler
261	3 alates	0
262-274 (infested own rooted block)	No traps	No traps
Western Block		
277	15 alates	0
284	1 alate	2 alates
291	0	0
Total	**23**	**3**

Table 3: Aerial movement of phylloxera dispersive stages in the (a) 1998-1999 and (b) 1999-2000 seasons of Trial 2.

(a)

Row number	East facing trap	West facing trap	North facing trap	South facing trap
Eastern Block				
211	0	2 alates, 1 crawler	0	3 alates, 1 crawler
213	0	1 alate, 1 crawler	1 crawler	5 alates, 1 crawler
215	1 alate	2 alates, 1 crawler	0	1 alate
217-225 (infested own rooted block)	-	-	-	-
Western Block				
236	0	0	0	1 alate
238	1 crawler	2 crawlers, 2 alates	0	1 alate, 1 crawler
240	0	2 crawlers, 1 alate	3 crawlers	2 alates, 1 crawler
Total	**2**	**14**	**4**	**21**

(b)

Row number	East facing trap	West facing trap	North facing trap	South facing trap
Eastern Block				
211	1 crawler	0	0	1
213	1 crawler	1 crawler	0	0
215	2 crawlers	1 crawlers	3 crawlers	1
218-225 (infested own rooted block)				
Western Block				
236	0	4 crawlers	3 crawlers	0
238	0	2 crawlers	2 crawlers	2 crawlers
240	3 crawlers	0	2 crawlers	0
Total	**7**	**8**	**10**	**4**

Discussion:

Aerial trapping has highlighted that two types of phylloxera life stages, alates (winged forms) and crawlers can be dispersed in the air column. Interestingly, in the first season a 25: 1 ratio of alates to crawlers was observed whilst in subsequent seasons in Trial 2 a more even ratio was observed. The reasons for this difference have yet to be determined but could be related to differences in wind direction and patterns, phylloxera infestation levels or trap layout and orientation. In Trial 1, aerial traps were established around a heavily-infested vine block, which was subsequently uprooted due to severe phylloxera damage. In conditions of overcrowding or poor nutrient availability aphids are known to produce a higher proportion of winged forms to enhance the dispersive state. Phylloxera alate populations were also estimated using emergence traps and winged populations were in higher numbers in the heavily infested block during the 1997/1998 season at Site 2, than in the lightly infested block at the same site in the 1998/1999 and 1999/2000 seasons. This higher production of alates on the heavily infested vines could have led to a higher proportion of alates being caught in the aerial traps.

The layout of the aerial sampling block and orientation of aerial traps was different in Trial 1 compared to Trial 2. In Trial 1 traps were located up to 15 rows away from the infested block whereas in Trial 2, traps were located closer to the infested block being a maximum distance of 7 rows away. Further studies would need to be carried out to determine if alates can disperse further than crawlers when wind-assisted. Trap orientation could also have influenced collection as traps were only orientated east or west in the Trial 1 as opposed to four directions (north, south, east and west) in Trial 2. Other factors such as cooler summer temperatures in the 1999/2000 season may also have influenced alate production.

This study clearly demonstrates that there is potential for phylloxera dispersive stages to spread several metres within an infested vineyard. The potential distance of aerial dispersal is likely to be influenced by the level of infestation, distance from the infested block, direction and strength of wind movement. The numbers of phylloxera caught over the length of a growing season were however relatively low in this study

compared with phylloxera numbers observed using other trapping techniques based at ground level and within the canopy. This suggests that the risk of phylloxera dispersal by aerial means is relatively low. Only one previous study on aerial dispersal of phylloxera has been conducted in Australia (Nagambie) when relatively low numbers of alates and crawlers were collected using a variety of trapping techniques (Buchanan, 1990). In that study the peak numbers were caught between late January to late March. Stevenson and Gubb (1976) also used sticky traps to estimate levels of alates in infested vineyards in Ontario and Pennsylvania and could collect alates up to 48 metres from infested vines. In their study alates were active for 2-3 months of the season and predominantly in the summer months. In our study peak abundance was between February and March and distance of dispersal was up to at least 25 metres.

Recommendations:

Continued studies are needed of aerial movement of phylloxera dispersive stages in a range of infested sites to further quantify the risks of aerial dispersal and estimate the potential distance of dispersal from infested vines.

Due to their ability to reproduce asexually on ungrafted *Vitis vinifera* phylloxera crawlers pose a greater risk than winged alates in ungrafted vineyards. However, alates could pose a potential risk in vineyards with grafted vines or in mother vine rootstock plantings. Further studies are recommended to determine what factors influence alate population levels in infested vineyards.

INFLUENCE OF SOIL AND WEATHER VARIABLES ON PHYLLOXERA POPULATION DYNAMICS AND DISPERSAL

Introduction:

Insect-life cycles can be influenced by fluctuations in temperature and humidity. Soil temperatures have been shown to influence the population dynamics of phylloxera in Californian vineyards (Omer *et al.*, 1997) and humidity influences survival and development of phylloxera (Buchanan, 1990). In conjuction with the use of trapping techniques, weather and soil data was analysed to determine if weather and soil conditions influenced phylloxera development or dispersal in ungrafted *Vitis vinifera* at the two study sites in the King Valley region.

Results:

Temperature and phylloxera emergence

Maximum and minium air temperatures were recorded for both sites for the experimental periods and mean monthly temperatures are shown in Figures 19-21. Mean temperatures throughout the growing seasons were consistently higher at Site 1 than Site 2. There were seasonal differences in temperature at each site.

At Site 1 in the 1997/1998 season the peak average temperature (25°C) was recorded in December and the following month saw a peak in phylloxera populations recorded in emergence traps (Figure 19). A similar pattern was observed in the following season (1998/1999) when the peak average temperature (25°C) was in January followed by a peak phylloxera population in February (Figure 19). In the 1999/2000 season temperatures at Site 1 were cooler throughout the summer than in previous years and reached an average maximum of only 23.4°C in February. Phylloxera populations in this season were lower than previous seasons and may have been suppressed by cooler summer temperatures.

At Site 2 in the 1997/1998 season the peak temperatures were recorded in January (mean temperature 20°C) coinciding with a peak in the number of phylloxera emerging from the soil (Figure 20). In the following season January was the warmest month, with a mean air temperature of 22°C, and peak phylloxera emergence was recorded in January and February. In the 1999/2000 season summer temperatures were cooler with a peak in February (21°C) and lower emergence levels were recorded than in the previous season.

Rainfall distribution

Rainfall distribution for both sites, for the experimental periods, are shown in Figures 22-24. Seasonal rainfall was generally higher at Site 2 than Site 1. At Site 1 in the 1997/1998 season, total rainfall from September to May was markedly lower (509 mm) compared to the 1998/9 season (777 mm) and 1999/00 season (751 mm). At Site 2 rainfall, during September to May was also lower in the 1997/1998 season (568 mm) compared to the 1998/9 season (896 mm) and 1999/2000 season (970 mm).

At both sites peak phylloxera populations emerging from the soil, moving across the soil surface or moving up the vine trunk generally coincided with months in which the lowest levels of rainfall were recorded (December to March) particularly in the 1997/1998 and 1998/1999 seasons. At Site 2 in the 1999/00 season December was a particularly wet month (187 mm) compared with the 1998/1999 season (15 mm) and the higher rainfall coincided with a lower numbers of phylloxera emerging from the soil (Figure 24).

Relative humidity

Relative humidities for the experimental periods at both sites were recorded. At both sites during the period of peak phylloxera emergence (December – March) average relative humidity was at its lowest (Figures 25-27). At Site 1 the lowest relative humidity values during this period were 50-60% during the 1997/1998 and 1998/1999 seasons but were higher (>70%) during the same period in the 1999/2000 season (Figure 25). Lower numbers of phylloxera were caught in emergence traps during the

1999-2000 season but this may be related more to the lack of fibrous roots on the infested vines than the higher relative humidity.

At Site 2 relative humidity levels were higher in the 1999-2000 season compared to the two previous seasons but the difference was not as marked as that observed on Site 1. No clear relationship between relative humidity and phylloxera population dynamics was observed at this site.

Soil characteristics

Soil texture at Site 1 varied moving down the soil profile. The A1 horizon to a depth of 0-30 cm was classed as fine sandy clay loam, the A2 horizon (30-40 cm) was classed as a sandy clay loam. The B horizon contained a higher clay content being classed as a light clay in the B1 horizon (depth 40-55 cm) and a medium clay in the B2 horizon (depth 55-100 cm).

At Site 2 the A1 horizon (depth 0-20 cm) was classed as a fine silty loam texture changing to a fine sandy clay loam in the A2 horizon (depth 20-30 cm). The B1 horizon (depth 30-70 cm) was regarded as a fine sandy clay which changed to a light clay in the B2 horizon (70-100 cm).

Soil analysis data for both sites is presented in Table 4. Carbon and nitrogen levels decreased down the soil profile at both sites with highest levels recorded at Site 2. Aluminium levels were relatively high at Site 1 corresponding with high soil acidity.

Discussion:

A variety of environmental factors including soil and weather conditions are likely to influence the population dynamics, dispersal and risk of transfer of phylloxera. In this study three above-ground weather variables (temperature, relative humidity and rainfall) were recorded to determine their influence on phylloxera. These variables are likely to influence not only phylloxera directly but also indirectly influence its relationship with the host plant *Vitis vinifera* by altering the growth and development of the vine. Buchanan (1990) has shown that temperature and relative humidity affect

phylloxera survival. Root inhabiting phylloxera cannot tolerate low (<50%rh) relative humidities and die within an hour of temperatures exceeding 40°C.

Studies conducted in Californian vineyards have shown that phylloxera populations increase as soil temperature increases during spring and early summer. Summer populations decrease as temperature exceeded 23°C and before temperatures reach below 18°C (Omer *et al.*, 1997). Temperature of >18°C is required for phylloxera to establish feeding sites (Turley *et al.*, 1996). Although soil temperatures were not recorded in our study our observations show that as mean air temperature reached >16°C phylloxera emergence levels increased markedly. Soil temperature varies according to depth and studies of soil temperature at the two sites would be recommended.

Rainfall and temperature are likely to influence the dispersal characteristics of phylloxera. Both weather factors are likely to have a direct effect on insect survival and also an indirect effect by altering the soil physical characteristics which could either impede or enhance dispersal. Dry soils are likely to crack and allow easier movement of phylloxera through the soil than wet waterlogged soils. Our observations suggest that rainfall and temperature may have some influence on phylloxera dispersal, with higher phylloxera numbers recorded emerging from the soil and moving on the soil or trunk during the drier warmer months.

Soil textures, in combination with weather variables, are likely to influence phylloxera dispersal rates. Stevenson (1963) observed that phylloxera infestations were heavier in clay and clay loam soils than in sandy loams. Both sites in our study were sandy clay loams, at least in the 'A' horizons where fibrous roots were more predominant, and a rapid spread of phylloxera was recorded over the three-year study period.

Recommendations:

This study provides preliminary evidence that weather and soil factors may influence phylloxera population dynamics and dispersal rates. However, further detailed examination of soil climatic conditions is required to determine how soil climate

influences phylloxera root dwelling populations. Further studies are also recommended to determine more clearly the relationships between weather variables and phylloxera dynamics in infested vineyards. Research activities focusing on soil and canopy climatic conditions are required so that a model for phylloxera population dynamics in the vineyard could be developed.

Table 4: Soil analysis data from population dynamics sampling sites.

Site	Horizons	Depth	Colour	Texture	Fines	pH-water	pH-$CaCl_2$	Al (mg/kg) exchange	EC (dS/m)	C (%)	N (%)	Ca (meq/100g)	Mg	Na	K	ECEC	ESP
1	A1	0-20	10YR 2/2	fine silty loam	66	5.5	4.9	130	0.06	8.2	0.43	8.1	0.79	0.07	0.22	9.2	0.8
	A2	20-30	10YR 2/2	fine sandy clay loam	74	5.6	5	88	0.05	5.8	0.3	8.1	0.99	0.05	0.18	9.4	0.5
	B1	30-70	10YR 3/2	fine sandy clay	77	5.6	5	67	0.05	4.6	0.23	7.7	1.1	0.05	0.19	9.1	0.5
	B2	70-100	7.5YR 4/4	light clay	80	5.8	5.2	10	0.05	2	0.1	7.7	1.6	0.05	0.16	9.6	0.5
2	A1	0-30	10YR 4/3	fine sandy clay loam	75	6.9	6.3	0	0.08	2.3	0.15	8.9	1	0.05	0.43	10	0.5
	A2	30-40	10YR 4/2	sandy clay loam	74	5.4	4.6	110	0.05	3.4	0.2	4.3	0.69	0.05	0.28	5.4	0.9
	B1	40-55	10YR 4/3	light clay	77	4.9	4.2	270	0.05	1.2	0.09	0.99	0.32	0.05	0.18	1.6	3.1
	B2, 1	55-65	10YR 4/6	medium clay	83	4.8	4	330	0.05	0.5	0.07	1.1	0.61	0.05	0.2	2	2.5
	B2, 2	65-90	10YR 5/6	medium clay	82	4.7	4	320	0.05	0.3	0.06	0.61	0.67	0.05	0.17	1.5	3.3
	B2, 3	90-100	10YR 5/5	medium clay	82	4.7	4	330	0.05	0.3	0.06	0.22	0.63	0.05	0.18	1.1	4.5

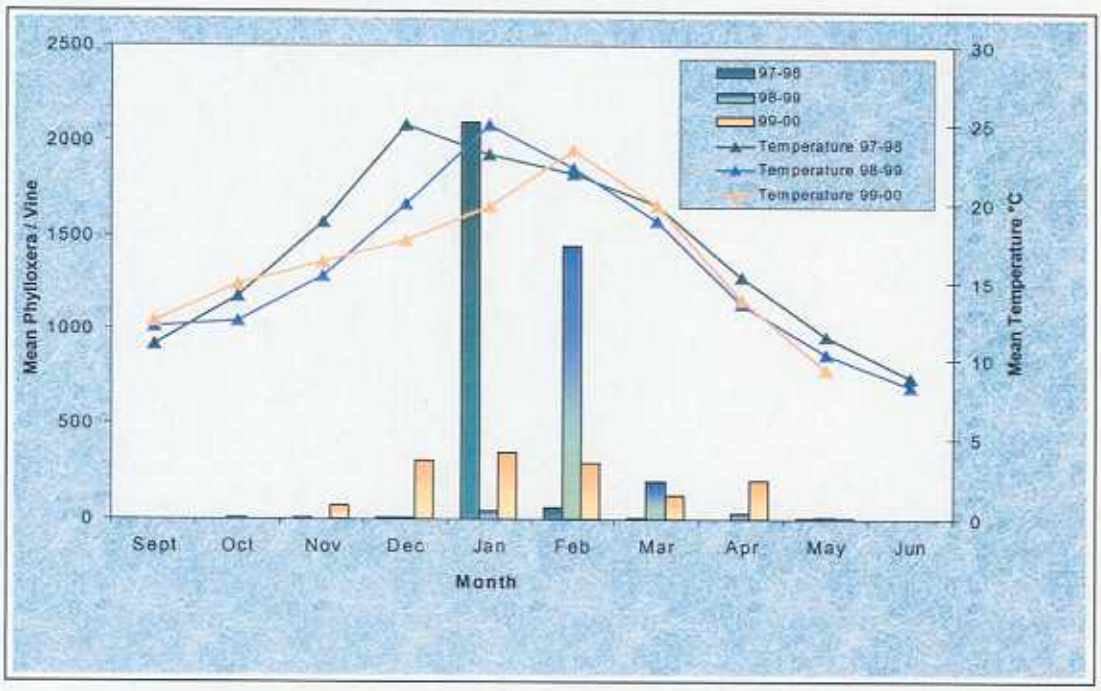

Figure 19: Influence of temperature on phylloxera emergence over 3 successive seasons (Site 1: 1997-2000)

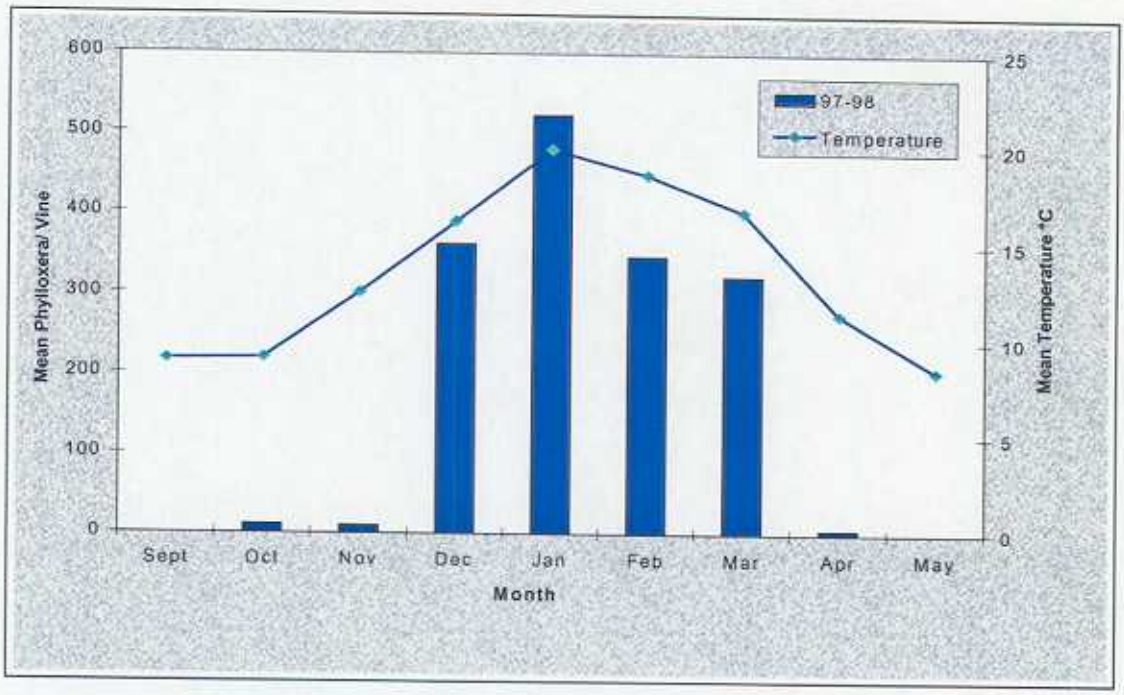

Figure 20: Influence of temperature on phylloxera emergence (Site 2: 1997-1998)

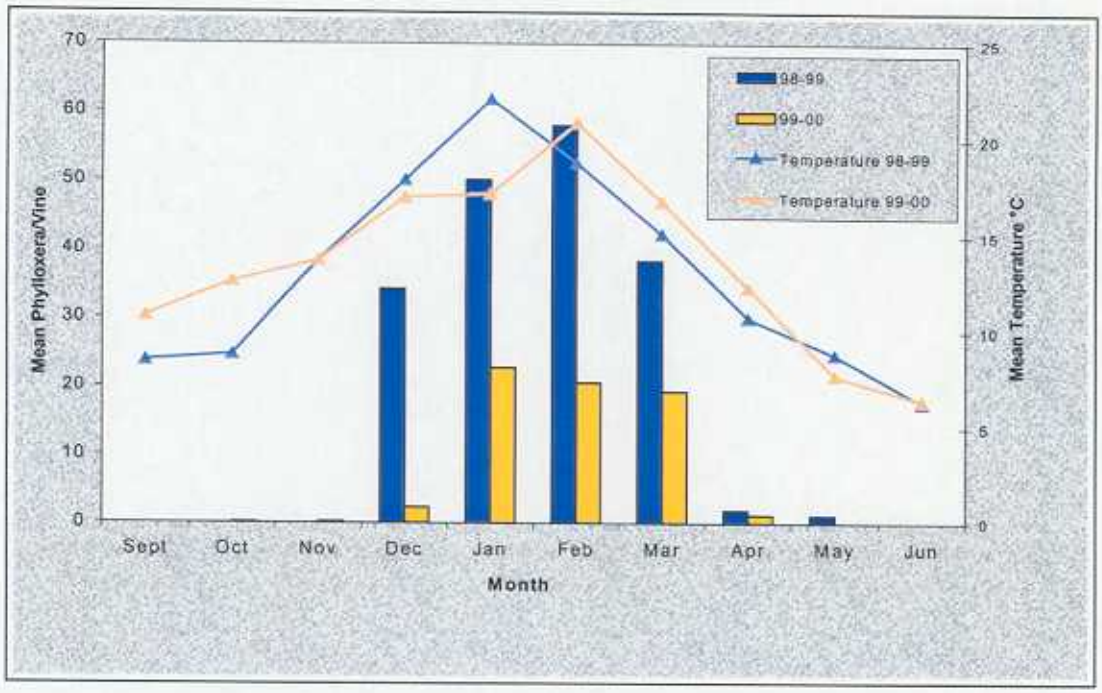

Figure 21: Influence of temperature on phylloxera emergence over 2 successive seasons (Site 2: 1998-2000).

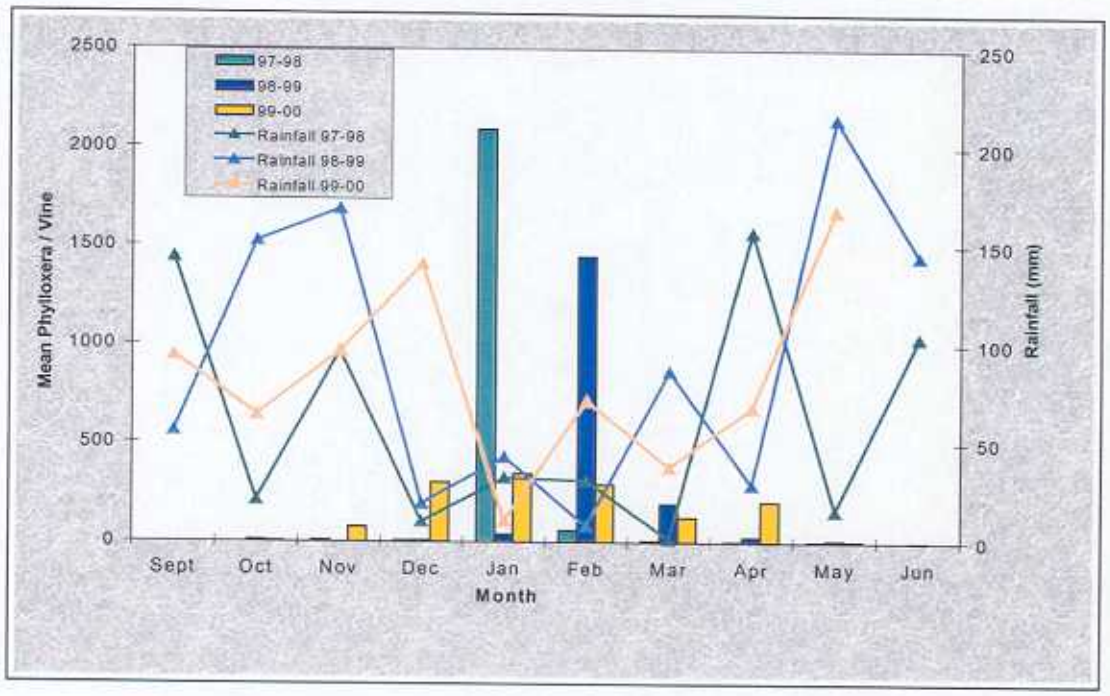

Figure 22: Influence of rainfall on phylloxera emergence over 3 successive seasons (Site 1: 1997-2000)

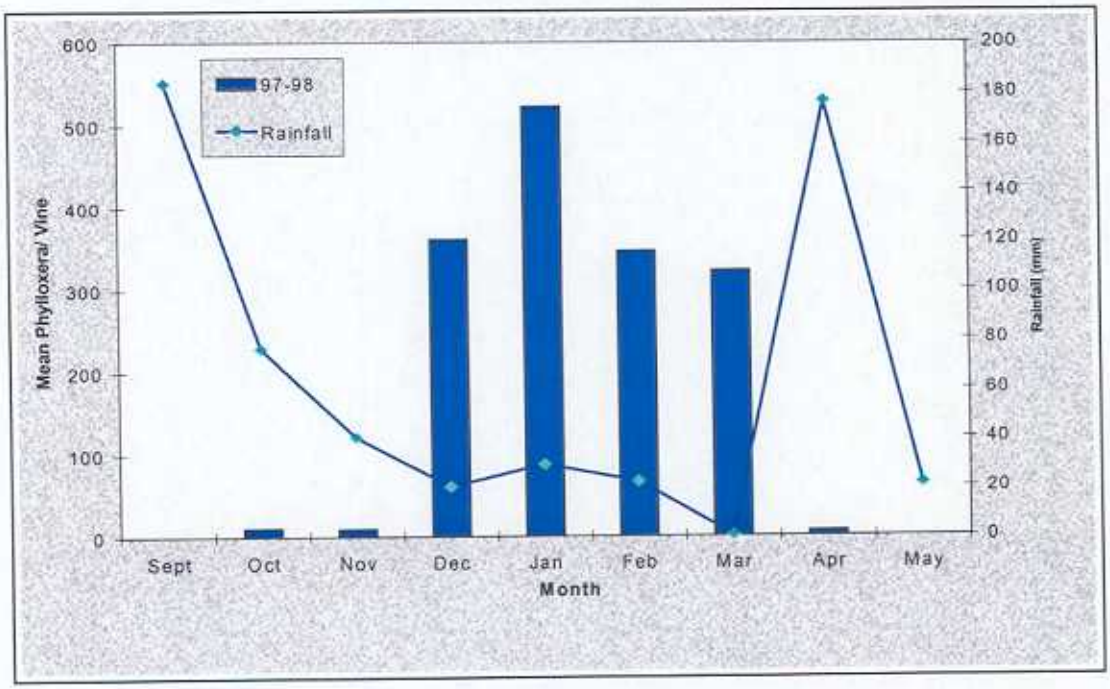

Figure 23: Influence of rainfall on phylloxera emergence (Site 2: 1997-1998)

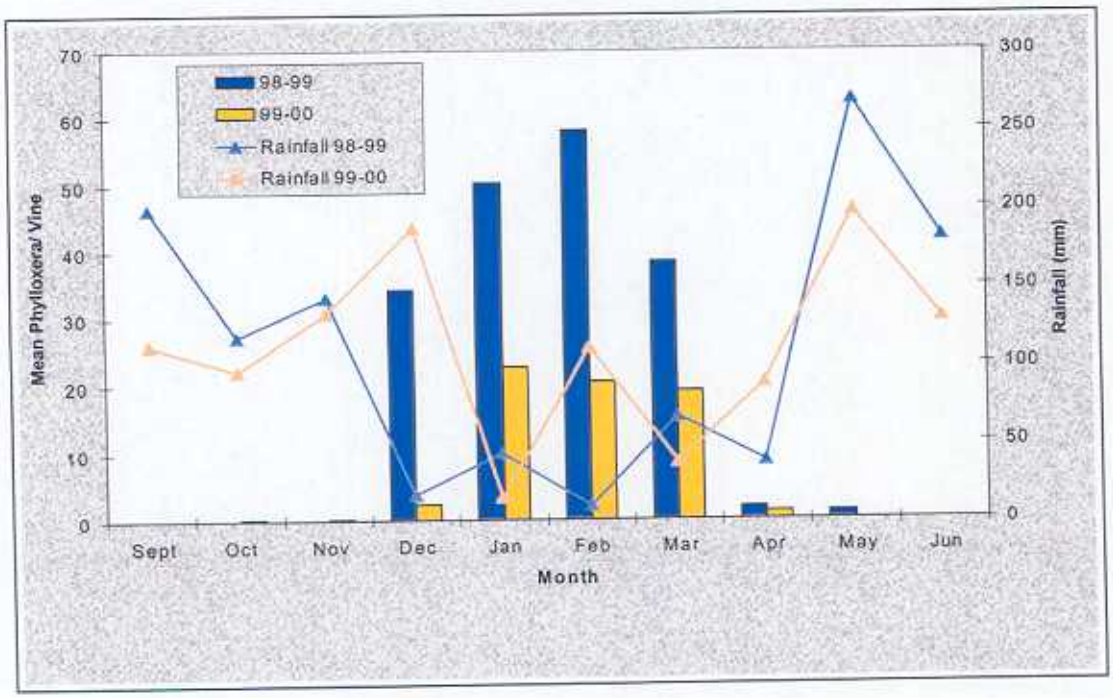

Figure 24: Influence of rainfall on phylloxera emergence over 2 successive seasons (Site 2: 1998-2000).

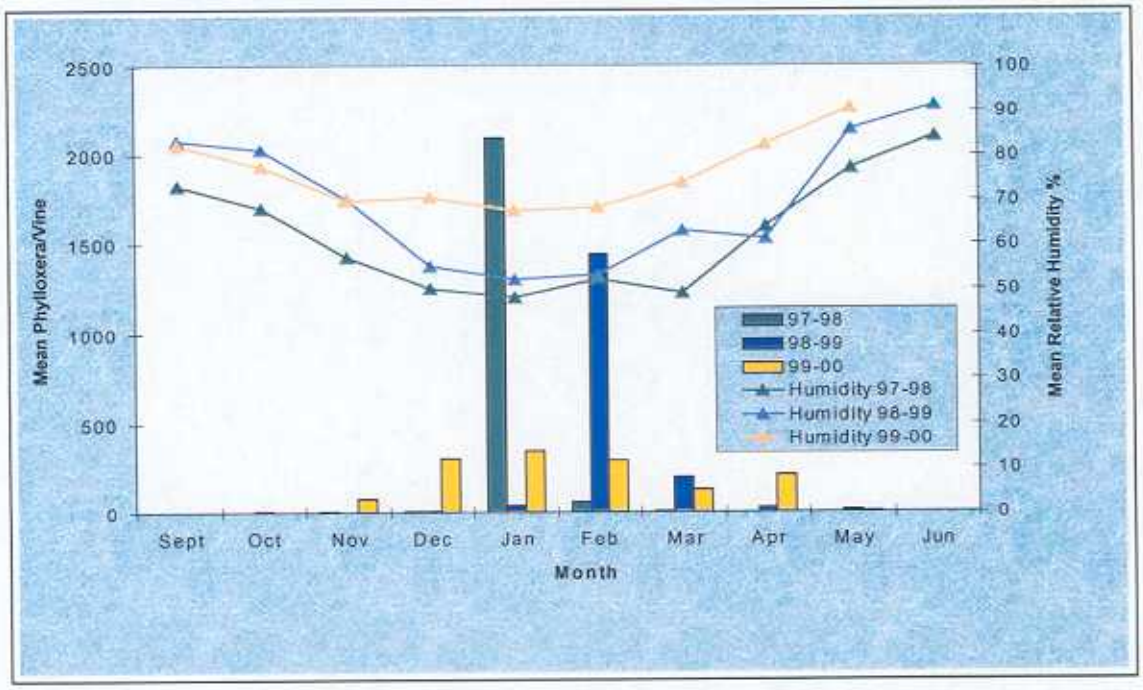

Figure 25: Influence of relative humidity on phylloxera emergence over 3 successive seasons (Site 1: 1997-2000).

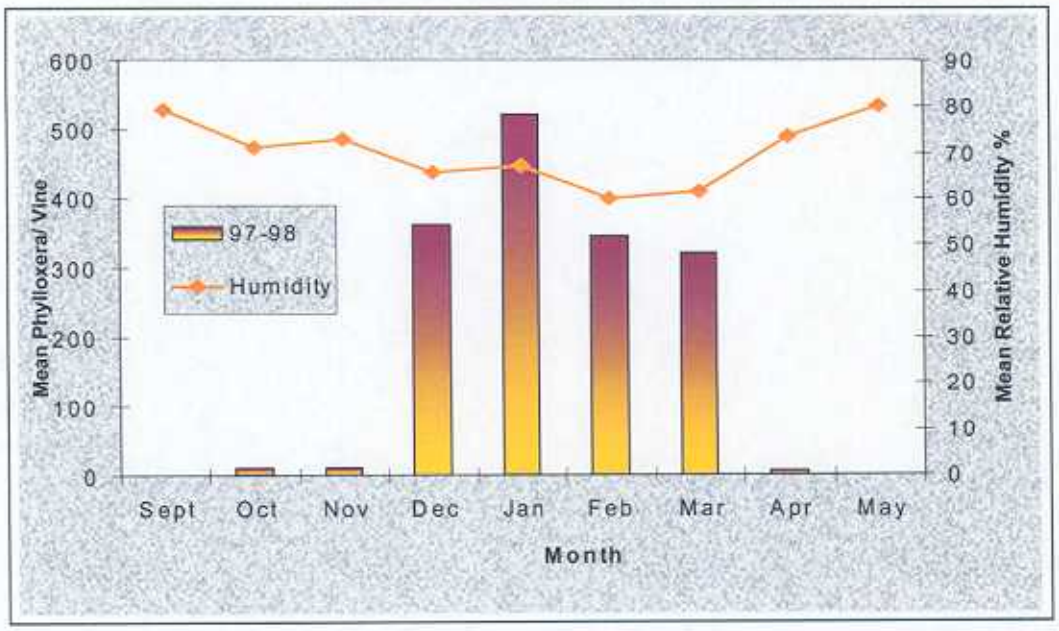

Figure 26: Influence of relative humidity on phylloxera emergence (Site 2: 1997-1998)

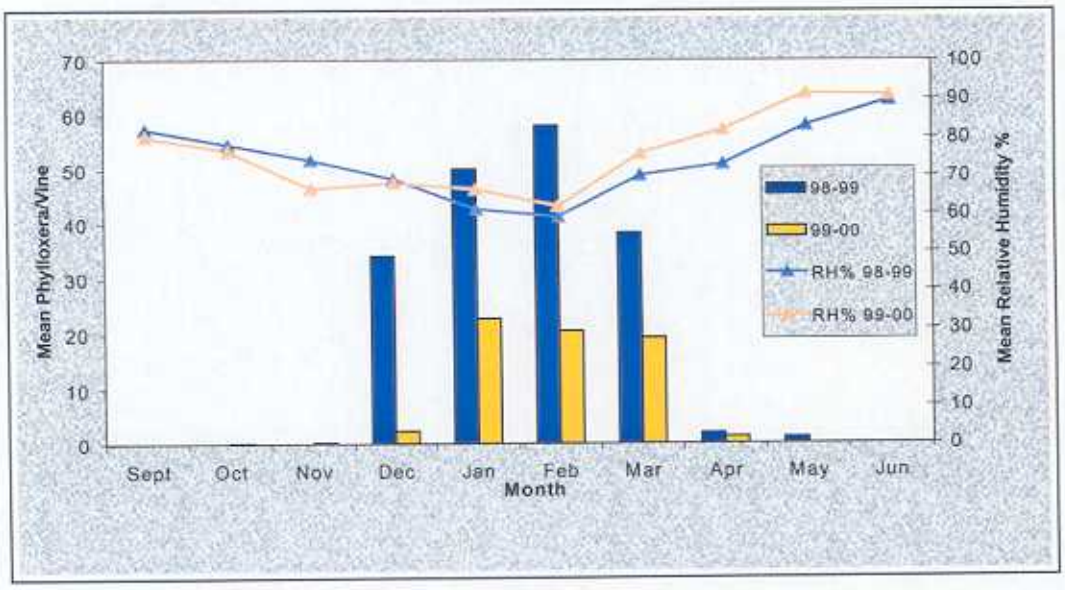

Figure 27: Influence of relative humidity on phylloxera emergence over 2 successive seasons (Site 2: 1998-2000).

RATE OF PHYLLOXERA SPREAD IN UNGRAFTED VINEYARDS

Introduction:

It is important to assess the general rate of phylloxera spread within an infested vineyard as this information will influence the management decision in determining the timeframe for implementing a rootstock replanting scheme or interim management strategies. The rate of phylloxera spread within an infested vineyard is a multi-mechanistic process depending on a number of factors including the initial degree of infestation, the method of dispersal (ie natural or human-assisted), site-related factors including soil characteristics, below- and above-ground weather variables and management practices within the vineyard (eg. vine spacing, inter-row management, vine management etc).

Method:

The overall rate of phylloxera spread and the degree of infestation was determined at Sites 1 and 2 over three and two years respectively. Monitoring of population dynamics using the techniques as described earlier enabled the number of infested vines and the degree of infestation to be quantified. Initially visual root assessments were used to determine if vines were infested. Quantitative assessment of the degree of infestation was determined by monitoring the emergence of phylloxera from infested vines using a rating scale based on numbers trapped (Table 5).

Table 5: Quantitative assessment scale to determine the degree of phylloxera infestation.

Infestation scale	Phylloxera number per vine per season
None	0
Low	1-10
Medium	11-100
High	>100

Results:

There were differences between the rate of phylloxera dispersal at the two vineyards studied (Table 6). However, direct comparisons of the spread rate at each site cannot be made due to differences in both vineyard management, in particular vine spacing, and edaphic and climatic conditions.

At Site 1, nine of the sample vines were found to be infested with phylloxera in the first year of study (1997/1998), however only two of the sample vines were moderately to heavily infested. In the second year of study the number of infested vines had increased to 11, and 5 of those infested vines were showing heavy to medium infestation levels. In the third year every vine sampled was infested and 9 of the sample vines showed medium to heavy infestation levels.

At Site 2 in the first year of study the level of infestation was low with only 3 sample vines infested but after the second year this had increased three-fold. The number of medium to heavily infested vines remained stable over both years but the number of lightly infested vines had increased from zero in the first year to six in the second year.

Table 6: Levels of phylloxera infestation in ungrafted *V.vinifera* vineyards in the King Valley during consecutive sampling seasons (1997-2000).

	% sample vines infested (n=12)		% sample vines with medium -high infestation	
Season	Site 1	Site 2	Site 1	Site 2
1997/8	75	NR*	17	NR*
1998/9	92	25	42	25
1999/00	100	75	75	25

**NR= not recorded*

Discussion:

Phylloxera movement and the degree of infestation across the infested blocks was high at both sites. Although site-related factors particularly soil characteristics and weather affected the population dynamics, conditions at both sites were clearly

conducive to the rapid natural spread of the insect over a 2-3 year period. Even where the phylloxera infestation was relatively low (Site 2; 25% infested vines) the number of infested vines in the study block increased by 50% over one growing season. A further year of population monitoring will be carried out at Site 2 to obtain three years of dispersal data. In King and Buchanan's studies (1986) the rate of spread on phylloxera was assessed over a 3-4 year study period in New Zealand and Australian (Nagambie) vineyards and the spread changed from initial low levels of infestation to the majority of vines being infested by the end of the study period.

A range of natural and human assisted dispersal has undoubtedly occurred at both sites monitored in our study. Natural dispersal of phylloxera crawlers across the soil surface and in the vine canopy was recorded at both sites yet this may not account for the relatively rapid spread within each infested block. Davidson and Nougaret (1921) suggested crawlers only move 1-2 vines away from the infested vines in one year. Aerial dispersal of phylloxera crawlers could have played a role in the rapid spread as crawlers were recorded, at Site 2, in aerial traps up to 25 metres from infested vines. The likelihood of human-assisted dispersal via machinery and on infested personnel should also be considered.

Recommendations:

Further quantitative data on phylloxera populations *in situ* in a range of sites is required to understand rates of spread and damage in the field and identify limitations to the development and spread of populations. This data will assist in the development of appropriate management strategies for infested vineyards.

Summary:

This study has quantified phylloxera population dynamics within the season, between seasons, within different stratigraphic zones both below and above-ground. This has identified peak population periods of phylloxera dispersive stages to be identified and key risk zones which can be utilised in the development of risk management strategies. It has also examined population dynamics at sites with different edaphic and climatic conditions and tentatively identified site-related factors which influence not only population dynamics but also dispersal rates of grapevine phylloxera.

There are clearly quarantine risks associated with the peaks of phylloxera population growth. In this study dispersive stages (namely crawlers and alates) were quantified emerging from the soil, moving across the soil surface and in the canopy. At the levels found during this study phylloxera, transfer via human assisted vectors is likely via machinery and personnel's footwear and clothing, especially at times of increased vineyard traffic such as at harvest .

However, from canopy and aerial population studies conducted over a diverse range of trial sites over two growing seasons the population levels in the canopy appeared to be relatively low. The reasons for this are yet to be elucidated but could be due to microclimate factors within the canopy such as high temperatures and low humidity and the level and diversity of natural predators which may be restricting populations.

ASSESSING THE RISK OF PHYLLOXERA TRANSFER ON THE CANOPY OF UNGRAFTED *VITIS VINIFERA*

Objective 2 (DAV 96/2) & (DAV 99/2):

Quantifying risks of transfer of phylloxera via people and machinery working within vineyards, and through harvesting operations. This will involve sampling of phylloxera from vine canopies and grape bunches, and studies of survival of phylloxera in crushed grapes and fermenting wine.

Introduction:

Quantifying canopy populations of phylloxera

To assess the transfer risk of grapevine phylloxera from the vine canopy by machinery or personnel working, particularly near vintage, a three-year study was conducted. The study's aim was to develop the most appropriate techniques to determine phylloxera levels within specific areas of the canopy. In the first year (1997) a pilot study was conducted at four infested vineyards in order to develop appropriate sampling protocols. Detailed quantitative assessment of phylloxera populations in the vine canopy were then conducted over two successive seasons (1998/1999 and 1999/2000) at a number of sites in the King Valley and Rutherglen in north-east Victoria. Phylloxera levels were assessed on bunches, shoots, stems, leaves and whole canopies of ungrafted *Vitis vinifera* varieties between February and April. Determination of phylloxera levels in the vine canopy was later used in the development of protocols for post-harvest risk assessment experiments.

Methods:

Site selection

Sites selected for study in the 1998/1999 and 1999/2000 seasons were in four commercial vineyards in north-east Victoria and included a range of phylloxera

infestation levels (based on visual inspection of roots), soil types and ungrafted *Vitis vinifera* varieties (Table 7).

Table 7: Sampling sites used to estimate pre-harvest phylloxera population levels in the vine canopy.

Site	*Infestation level*	*Date vineyard infestation first detected*	*Variety*	*Location*	*Sample dates*
1	High	May 1997	Sauvignon blanc	Cheshunt, King Valley	23/3/99 3/3/99 8/3/2000
2	Low-Medium	November 1991	Chardonnay	Whitfield, King Valley	22/2/99 25/3/99 8/3/2000 22/3/2000
3	High	February 1995	Shiraz	Cheshunt, King Valley	6/4/99
4	Low	Early 1900's	Cabernet sauvignon	Rutherglen	1/3/99 31/3/99

Soil classification and analysis

Soil samples (1 x 10 cm diameter cores) were taken at each trial site from depths of up to 1 metre in August 1999. All soils were dried immediately after field sampling in a fan-forced oven at 40°C for 24 hours, rumbled through a 2 mm sieve and stored in sealed bags at room temperature. Soil analyses were conducted at the State Chemistry Laboratories, Werribee, Victoria.

Trial layout

Four adjacent rows of ungrafted *Vitis vinifera* were sampled at each trial site (Table 7). Phylloxera populations were monitored within the canopy early in the morning to ensure that high temperatures did not influence population levels. A standard set of samples were taken at each site, including destructive and non-destructive sampling.

Destructive sampling techniques

1. Leaf populations

Five leaves were removed from forty vines (n=200) in the sampling area (2 vines x 5 panels x 4 adjacent infested rows). Leaves were primarily removed from the centre of the canopy. However, at some sites, depending on the canopy management strategy employed, strategic sampling was conducted at three heights within the canopy (ie: low, medium and high, n=600). Leaves were carefully cut using secateurs to avoid dislodging insects and placed in a sealed plastic bag.

2. Grape bunch populations

One grape bunch was removed from each of twenty vines in the sampling area (1 vine x 5 panels x 4 adjacent infested rows). Bunches were carefully cut using secateurs to avoid dislodging insects and placed in a sealed plastic bag.

3. Vine shoot populations

Using hand-held secateurs, 2 vine shoots were removed from single vines (2 vines x 5 panels x 4 adjacent infested rows) and placed into sealed plastic bags.

Non-destructive sampling

1. Whole vine canopy populations

Using a battery operated D-Vac™ Knapsack Insect Sampling Device whole canopy samples were analysed non-destructively. The whole canopy of 20 vine panels was sampled using the suction apparatus (5 panels x 4 rows) (Figure 28). Insects were preserved in 70% ethanol in a screw-top plastic container.

Figure 28: Non-destructive sampling of whole canopy phylloxera populations using a D-Vac™ suction device.

2. *Vine stem populations*

Two vine stems from each sampled vine (2 vines x 5 panels x 4 adjacent infested rows) were vigorously tapped twice with the back of a pair of hand-secateurs to remove any insects which were collected on a white plastic beating tray held below the stem. The tray was rinsed after each sampling with 70% ethanol and the insects were preserved in a stoppered plastic container

3. *Leaf populations*

Using a modified Black and Decker™ Dustbuster whole leaf samples were analysed non-destructively. A total of ten leaves per vine (1 vine x 5 panels x 4 rows) were sampled by placing the whole attached leaf into the suction end of the device (Figure 29). Insects were preserved in 70% ethanol in a screw-top plastic container.

Figure 29: Non-destructive sampling of phylloxera from single leaves using a modified Dustbuster suction sampler.

Post-sampling assessment of canopy populations

All samples were transported to the laboratory at Agriculture Victoria - Rutherglen in sealed containers under permit. On arrival at the laboratory, within 24 hours all plant samples were weighed to give a fresh weight. All samples were gently rinsed three times in a mixture of 1% Teepol™ sieved through a 60 µm brass sieve and examined microscopically for the presence of phylloxera life stages. After rinsing leaf and stem area was quantified using a LiCor ™ Electronic Planimeter. Leaf, shoot and stem dry weights were also recorded after 24 hours at 45°C. All ethanol-preserved samples were examined microscopically for the presence of phylloxera life stages.

Results:

Soil classification and analysis

Soil classification for Sites 1 and 2 are described in the Population Dynamics section of this report. The soil type at Site 3 was classed as a dystrophic brown chromosol. At Site 4 the soil type was a subnatric brown sodosol (W. J. Slattery Agriculture Victoria-Rutherglen 1999, pers.comm). Sodosols show a clear textural B horizon in which a major part of the upper 0.2m of the B2 horizon is sodic and not strongly acidic. In subnatric soils a major part of the upper 0.2m of the B2 horizon has an exchangeable sodium percentage (ESP) of between 6 and <15. Soil analysis data highlight differences in soil texture and chemical characteristics at each site (Table 8).

exchangeable sodium percentage (ESP) of between 6 and <15. Soil analysis data highlight differences in soil texture and chemical characteristics at each site (Table 8).

Estimation of canopy populations

Over two sampling seasons the number of phylloxera life stages recorded was low for all sites. On four out of the ten sampling dates phylloxera could not be found in the samples collected (Table 9) indicating that date of sampling is an important consideration when estimating population levels. Destructive sampling techniques were the most successful method for collecting phylloxera from the canopy and showed that phylloxera can be found on grape bunches, vine leaves and shoots during February to March. Only two forms of phylloxera dispersive stages, crawlers (first nymphal stage) and alates (adult winged form), were recorded in the canopy and 80% of the total number collected were crawlers.

Table 8: Soil analysis data from four canopy sampling sites.

Site	Horizons	Depth	Colour	Texture	Fines	pH-water	pH-$CaCl_2$	Al (mg/kg) exchange	EC (dS/m)	C (%)	N (%)	Ca (meq/100g)	Mg	Na	K	ECEC	ESP
1	A1	0-20	10YR 2/2	fine silty loam	66	5.5	4.9	130	0.06	8.2	0.43	8.1	0.79	0.07	0.22	9.2	0.8
	A2	20-30	10YR 2/2	fine sandy clay loam	74	5.6	5	88	0.05	5.8	0.3	8.1	0.99	0.05	0.18	9.4	0.5
	B1	30-70	10YR 3/2	fine sandy clay	77	5.6	5	67	0.05	4.6	0.23	7.7	1.1	0.05	0.19	9.1	0.5
	B2	70-100	7.5YR 4/4	light clay	80	5.8	5.2	10	0.05	2	0.1	7.7	1.6	0.05	0.16	9.6	0.5
2	A1	0-30	10YR 4/3	fine sandy clay loam	75	6.9	6.3	0	0.08	2.3	0.15	8.9	1	0.05	0.43	10	0.5
	A2	30-40	10YR 4/2	sandy clay loam	74	5.4	4.6	110	0.05	3.4	0.2	4.3	0.69	0.05	0.28	5.4	0.9
	B1	40-55	10YR 4/3	light clay	77	4.9	4.2	270	0.05	1.2	0.09	0.99	0.32	0.05	0.18	1.6	3.1
	B2, 1	55-65	10YR 4/6	medium clay	83	4.8	4	330	0.05	0.5	0.07	1.1	0.61	0.05	0.2	2	2.5
	B2, 2	65-90	10YR 5/6	medium clay	82	4.7	4	320	0.05	0.3	0.06	0.61	0.67	0.05	0.17	1.5	3.3
	B2, 3	90-100	10YR 5/5	medium clay	82	4.7	4	330	0.05	0.3	0.06	0.22	0.63	0.05	0.18	1.1	4.5
3	A1	0-13	5YR 3/4	fine sandy clay	44	6.3	5.6	<10	0.05	2.5	0.14	5.9	1.1	0.05	0.61	7.7	0.6
	B2	13-100	7.5YR 3/6	fine sandy light clay	63	5.1	4.5	<10	0.05	0.4	0.05	3.5	0.99	0.05	0.57	5.2	1
4	A1	0-12	10YR 4/4	fine sandy clay loam	43	5.8	5.2	10	0.06	0.82	0.08	4	0.46	<0.05	0.65	5.2	1
	B1	12-40	7.5YR 4/6	sandy light clay	50	5.9	5.4	<10	0.05	0.35	<0.05	4.3	0.75	<0.05	0.54	5.7	1
	B1,1	40-80	10YR 4/6	sandy clay	48	6.2	5.7	<10	<0.05	0.2	<0.05	3.6	1.4	0.06	0.32	5.4	2
	B1, 2	80>	10YR 4/6	heavy clay	68	7	6.1	na	0.1	0.22	<0.05	3.9	7.5	1.2	0.53	13	10

Table 9: Relative distribution of phylloxera life stages sampled in the canopy of ungrafted *Vitis vinifera* at four infested vineyards in NE Victoria.

		Sample type/size					
Site	*Sample date*	*Bunches (n=20)*	*Leaves (n=200-600)*	*Shoots (n=80)*	*Beat (n=80)*	*Dustbuster (n=200)*	*D-Vac (n=20)*
1	23/2/99	0	1crawler, 1 alate	1 crawler	1 crawler	1 crawler	0
1	3/3/99	0	0	2 crawlers	0	0	0
1	8/3/00	1 crawler	2 alates	0	1 crawler	0	0
2	22/2/99	3 crawlers	1 crawler	0	0	0	0
2	25/3/99	0	0	0	0	0	0
2	8/3/00	3 crawlers	0	0	1 crawler	0	0
2	22/3/00	0	0	0	0	0	0
3	7/4/99	0	0	0	0	0	0
4	1/3/99	0	0	0	0	0	0
4	31/3/99	0	0	1 alate	0	0	0
TOTAL		**7**	**5**	**4**	**3**	**1**	**0**

Discussion:

This study clearly highlights that during peak periods of activity in the vineyard in the summer months phylloxera dispersive stages can be found on the vine canopy and pose a risk of transfer on viticulture machinery (such as machine harvesters), personnel (particularly grape pickers) and harvested grape material. The presence of phylloxera on the vine canopy will also enhance the likelihood of aerial dispersal of the insect. The results from the study allowed an estimation of phylloxera numbers in the canopy, which were used in the development of risk assessments for post-harvest processing of grape products.

Whilst phylloxera presence has been recorded in this study in low numbers on the canopy bunches, leaves and shoots of grapevines contained relatively high numbers of phylloxera that were observed either emerging on to the soil surface or moving across the soil surface. This data highlights the potential risk of phylloxera transfer

on machinery, clothing and footwear. There is only one report in the literature of quantifying phylloxera presence on grape harvesters where a single phylloxera crawler was recovered from a grape (King & Buchanan, 1986).

Recommendations:

In the King Valley it is most likely that in some cases phylloxera has been inadvertently transferred from infested to uninfested vineyards on machinery or clothing and footwear of personnel. Further studies using modified sampling methods to quantify phylloxera survival on machinery, clothing and footwear would enable a more thorough risk assessment to be conducted.

PEST RISK ANALYSIS - FROM GRAPE TO WINE

Objective 2 (Project DAV 96/ & DAV 99/2):

Quantifying risks of transfer of phylloxera via people and machinery working within vineyards, and through harvesting operations. This will involve sampling of phylloxera from vine canopies and grape bunches, and studies of survival of phylloxera in crushed grapes and fermenting wine.

Introduction:

Phylloxera population dynamics and dispersal studies, conducted as a core component of projects DAV 96/2 and DAV 99/2, have highlighted that phylloxera life stages are found not only below-ground, on the vine roots and soil, but also above-ground on the vine canopy. The presence of phylloxera crawlers and alates on grape bunches and foliage have highlighted the need for detailed experimental studies to quantify the risks of phylloxera transfer on post-harvest grape materials as they pass through each phase of wine-grape processing. Quarantine restrictions currently exist for the movement of grape products from phylloxera-infested vineyards and are designed to minimise the risk of movement of the insect outside existing quarantine zones. Buchanan *et al* (1996) conducted preliminary studies on the survival of phylloxera life stages in grape juice and must. Their observations showed that active stages and eggs of phylloxera can survive in unfermented must and grape juice for at least 48 hours. However, prolonged immersion in grape juice or must does reduce phylloxera survival levels.

Further investigations were conducted in order to assess the risk of phylloxera survival through a number of post-harvest processing stages including:

1. transportation of grapes to the processing centre
2. must and unclarified juice
3. grape crushing and destemming
4. grape pressing
5. juice filtering

A pilot laboratory-based study, consisting of five experiments, was conducted during Vintage 2000. The experiments were designed to simulate winery processing conditions in the laboratory and optimise experimental procedures, so that the resultant data could be used to form the basis of a comprehensive pest risk assessment package for the industry. This risk assessment process could be used to refine the existing National Phylloxera Management Protocols where necessary.

Experiment 1 - Survival of phylloxera during post-harvest transport

Introduction:

Phylloxera crawlers have been observed in low levels on grape bunches and foliage prior to harvesting and are therefore likely to be transported on grape bunches after harvest. The temperature during transport and transport duration are highly variable depending on variety and distance from the vineyard to the processing centre. This experiment examined phylloxera survival over a temperature range of 15-25°C and duration of 2-4 hours.

Methods:

Experiment 1a - Quantifying the risk of phylloxera survival, over a 2-hour period, following transport to the winery in grape bins

Phylloxera crawlers were collected from a VWL phylloxera population (see GWRDC 93/2) reared on excised root pieces in a controlled environment room (25°C±2°C). Twenty crawlers were placed on 1 kg of machine-harvested red grapes. Phylloxera infested grapes were placed in 4-litre lidded stainless steel bins and incubated for two hours at 15, 20 and 25°C in a cooled illuminated incubator. After 2 hours grapes were washed three times in detergent/water, to remove phylloxera, then rinsed in tap water and sieved through a 65 µm sieve three times. Sieved samples were examined using a low-power light microscope and surviving phylloxera crawlers were counted. Each treatment was replicated four times.

Experiment 1b - Quantifying the risk of phylloxera survival, over a 4-hour period, following transport to the winery in grape bins

The same procedure was conducted as in Experiment 1a but incubation time was increased to 4 hours duration.

Results:

Experiment 1a - Post harvest survival over a 2-hour period

The results show that up to 70% of phylloxera crawlers survived 2 hours incubation (Table 10a). Incubation temperature over the range tested, 15-25°C, had no significant effect on survival ($P>0.05$).

Experiment 1b - Post harvest survival over a 4-hour period

The results show that up to 50% phylloxera crawler survived 4 hours incubation. Incubation temperature over the range tested, 15-25°C, had no significant effect on survival ($P>0.05$).

Table 10: Phylloxera crawler survival on harvested grapes over (a) a 2-hour period and (b) a 4-hour period at a range of incubation temperatures.

(a)

Survival (%) at 15°C	Survival (%) at 20°C	Survival (%) at 25°C
64.25±11.24a	69.75±10.30a	68.00±5.46a

(b)

Survival (%) at 15°C	Survival (%) at 20°C	Survival (%) at 25°C
45.00±8.17a	50.00±10.00a	42.5±14.51a

Discussion:

The data presented indicates that phylloxera crawler survival rates on harvested grapes, during transport to the winery for processing, are likely to be relatively high in grape bins over a 2 to 4 hour period within a transport temperature range of 15-25°C. Whilst the results highlight the fact that duration of transport in grape bins may have an effect on phylloxera survival, temperatures within the range 15-25°C have no significant effect on survival over a 2-4 hour period.

Experiment 2 - Survival of phylloxera during the crushing and destemming process

Introduction:

If phylloxera crawlers can survive during transportation in grape bins to the processing winery, the next stage of processing for hand-harvested grapes is the destemming procedure. This is a mechanical process where grapes are tipped into a destemmer/crusher and grapes are removed from the stems and partially crushed grapes are separated from the stems. Levels of phylloxera on grape bunches at harvest will vary depending on the level of infestation in the vineyard, temperature at time of harvest etc. This experiment quantified phylloxera survival during this process

hand operated crusher/destemmer (Figure 30) and was conducted at two rates of phylloxera infestation.

Figure 30: Mechanical crusher/destemmer used to assess phylloxera survival

Experiment 2a - Survival of phylloxera through a crusher/destemmer on 1 kg grape samples

Method:

Phylloxera crawlers were collected from population reared on excised root pieces in controlled environment room (25°C). Twenty-five crawlers were placed on 1 kg grape bunches and infested bunches were placed through a manually operated Enolitalia crusher destemmer. Processed grapes and stem were collected separately in plastic containers, washed in a detergent mixture and passed through a 65µm sieve three times. Sieved samples were examined using a low power light microscope and surviving phylloxera crawlers were counted. Two controls were used to determine the percentage recovery pre-treatment by placing 25 crawlers on destemmed grapes and grape stems. Each treatment and control was replicated five times.

Experiment 2b - Phylloxera survival through a crusher/destemmer on 2 kg grape samples

Method:

Phylloxera crawlers were collected from population reared on excised root pieces in controlled environment room (25°C±2°C). Ten crawlers were placed on 2 kg grape bunches. Phylloxera-infested grape bunches were placed through a manually

operated Enolitalia™ crusher/destemmer. Processed grapes and stem were collected separately in plastic containers, washed in a detergent mixture and passed through a 65µm sieve three times. Sieved samples were examined using a low power light microscope and surviving phylloxera crawlers were counted. Two controls were used to determine the percentage recovery pre-treatment by placing 10 phylloxera crawlers on destemmed grapes and grape stems. Each treatment and control was replicated five times.

Results and discussion:

Experiment 2a - Phylloxera survival post crusher/destemmer (1 kg grape samples)

The results show that 22% of the phylloxera crawler population survived following the standard crushing destemming process (Table 11). In the treated samples survival on the destemmed grapes was three-fold higher than on stems. This highlights the risk of phylloxera survival on marc and unfermented must. The suitability of this method for assessing the risk of crawler survival through the grape pressing process was demonstrated by the relatively high crawler retrieval levels on control samples; with 58% and 68% on destemmed grape and stem samples respectively which were not passed through the crusher/destemmer.

Experiment 2b - Phylloxera survival post crusher/destemmer (2 kg grape samples)

The results show that 38% of the phylloxera crawlers survived following the standard crushing destemming process (Table 11). In the treated samples survival on the destemmed grapes was sixteen-fold higher than on stems. This again highlights the risk of phylloxera survival on marc and unfermented must. The suitability of this method for assessing the risk of crawler survival through the grape pressing process was demonstrated by the relatively high crawler retrieval levels in controls with 76% and 36% recovered from untreated destemmed grape and stem samples respectively.

Table 11: Phylloxera crawler survival on (a) 1 kg samples and (b) 2 kg samples of hand harvested grapes passed through a hand-operated mechanical crusher/destemmer.

(a)

Survival (%) on untreated destemmed grapes	Survival (%) on post-treatment destemmed grapes	Survival (%) on untreated grape stems	Survival (%) on post-treatment grape stems
57.60±5.74	16.80±5.85	68.0±10.35	5.6±3.71

(where sample size =25 crawlers/kg)

(b)

Survival (%) on untreated destemmed grapes	Survival (%) on post-treatment destemmed grapes	Survival (%) on untreated grape stems	Survival (%) on post-treatment grape stems
76.00±9.27	32.00±3.74	36.00±7.48	2.00±2.00

(where sample size =5 crawlers/kg)

Experiment 3 - Phylloxera crawler survival following mechanical pressing of destemmed grapes

Introduction:

Following destemming, either mechanically in the vineyard or at the winery, grapes are crushed mechanically to separate grape material from the unclarified juice. As phylloxera can survive the destemming process, it is important to determine survival rates post-destemming through the pressing process.

Method:

Phylloxera crawlers were collected from population reared on excised root pieces in controlled environment room (25°C±2°C). Twenty-five crawlers were placed on 1 kg machine harvested destemmed whole red grapes. Phylloxera infested grapes were left

to stand at room temperature for one hour and pressed using a stainless steel mini basket press (Figure 31). Processed grapes and grape juice were collected separately in plastic containers, washed in a detergent mixture and passed through a 65µm sieve three times. Sieved samples were examined using a low power light microscope and surviving phylloxera crawlers were counted. Two controls were used to determine the percentage recovery pre-treatment by placing 25 phylloxera crawlers on destemmed grapes and in pressed juice. Each treatment and control was replicated five times.

Figure 31: Basket press used to assess phylloxera survival on pressed grapes.

Results and discussion:

The results show that up to 82% of the phylloxera crawlers survived the standard crushing process (Table 12). In the treated samples crawler survival in the pressed juice was seven-fold higher than in the pressed grape sample indicating that in the separation process significantly more phylloxera ($P<0.05$) is collected in the juice than in the crushed grape pressings. This highlights the risk of phylloxera survival on marc and unfiltered juice. The suitability of this method, for assessing the risk of crawler survival through the grape pressing process, was demonstrated by the relatively high crawler retrieval levels in the pre-treatment controls with 58% and 73% recovered, from crushed grape and juice samples respectively.

Table 12: Phylloxera crawler survival in pressed grapes and grape juice.

Survival (%) on untreated crushed grapes	Survival (%) on post-treatment crushed grapes	Survival (%) in untreated grape juice	Survival (%) in post-treatment grape juice
58.4±9.6a	10.4±3.7b	72.8±10.5a	72.0±6.1a

Experiment 4 - Phylloxera crawler survival following prolonged immersion in unclarified grape juice and fermenting grape must

Experiment 4a - Estimation of phylloxera survival over a 24-hour period

Method:

Phylloxera crawlers were collected from a population reared on excised root pieces in controlled environment room (25°C±2°C). Twenty crawlers were placed in either a 2 litre sample of unclarified Semillon grape juice or fermenting red must. Treatments were placed in 4 litre lidded stainless steel bins. Two controls of 20 crawlers on either moistened filter paper in a standard sized petri-dish or in 2 litres of tap water were used. All treatments and controls were incubated for 24 and 48 hours at 25°C in a cooled illuminated incubator. After incubation treatment samples were washed 3 times in detergent/water, to remove phylloxera, then rinsed in tap water and sieved through a 65µm sieve three times. Sieved samples and controls were examined using a low power light microscope and surviving phylloxera crawlers were counted. Each treatment was replicated five times.

Experiment 4b - Estimation of phylloxera survival over a 48-hour period

Method:

The same experimental procedure was followed as in Experiment 4a but the incubation period was increased to 48 hours.

Results and discussion:

Experiment 4a - Survival over a 24 hour period

The retrieval rates from the samples was very high being 95% from juice and 74% from fermenting must respectively indicating that the methodology was suitable (Table 13). The results show that 100% mortality was observed after 24 hours in both unclarified juice and fermenting must samples. The relatively low rates of phylloxera survival in the control and the observation that some phylloxera appeared to survive in the juice sample led to modified version of this experiment.

Experiment 4b - Survival over a 48 hour period

The retrieval rates from the samples were significantly higher from the water control with 96% retrieval compared to 82% from the unclarified juice treatment. From unclarified juice one hundred percent mortality was observed from the retrieved crawler sample compared to zero mortality on the controls. Unclarified juice over a 48-hour period clearly has a significant effect on phylloxera mortality.

Table 13: Phylloxera crawler survival in (a) unclarified juice and fermenting must over a 24 hour period and in (b) unclarified juice over a 48 hour period.

(a)

% crawlers retrieved from petri-dish (24 hrs/25°C)	% crawlers retrieved from unclarified juice (24 hrs/25°C)	% crawlers retrieved from fermenting must (24 hrs/25°C)	% survival in control (24 hrs/ 25°C)	% survival in fermenting must (24 hrs/25°C)	% survival in unclarified juice (24 hrs/25°C)
70±3.16	95.00±2.74	74.00±7.14	21.4±3.6	0	0

(b)

% crawlers retrieved from water (48 hrs/25°C)	% crawlers retrieved from unclarified juice (48 hrs/25° C)	% survival in water (48 hrs/25°C)	% survival in unclarified juice (48 hrs/25°C)
96±2.92a	82.00±4.06b	100	0

Experiment 5 - Quantifying the risk of phylloxera survival in unclarified grape juice post-filtering

Method:

Phylloxera crawlers were collected from a population reared on excised root pieces in controlled environment room (25°C±2°C). Ten crawlers were placed in a 2 litre sample of unclarified white grape juice. Treatments were filtered using an Enolmaster 3 head filler and cartridge filter (0.25 micron) connected to an Enolmatic vacuum filler (Figure 32). The control consisted of ten crawlers in unclarified white grape juice which was not filtered. Post treatment all samples were sieved through 65µm sieve and surviving phylloxera crawlers examined using a low power light microscope. Each treatment was replicated five times.

Figure 32: Filter apparatus used to assess phylloxera survival post-filtering.

Results and discussion:

The retrieval rates from control samples was 90% and all of the retrieved phylloxera survived 1 hour immersion in grape juice (Table 14). No crawlers were recovered from the 0.25 micron pore filtered sample. This study has shown that no phylloxera crawlers can survival the juice filtering process.

Table 14: Phylloxera survival rates following filtering.

% crawlers retrieved from unfiltered juice (control)	% crawlers retrieved from filtered juice (0.25μ filter)
90.00±6.32a	0b

Summary:

This study was conducted in order to develop laboratory protocols to assess phylloxera survival through various stages of wine grape processing. Preliminary results have shown that a variety of processes can be simulated under laboratory conditions and early indications are that phylloxera can survive some mechanical processing procedures including crushing, destemming and pressing. Further studies are required in order to develop a rigourous quantitative risk assessment for post-harvest survival of phylloxera through each stage of wine grape processing.

PHYLLOXERA DISINFESTATION PROTOCOLS

Objective 3 (DAV 96/2):

Facilitating and providing technical support for the implementation of agreed phylloxera management protocols, in particular the implementation of the Tri-state Phylloxera Agreement. In addition, assistance would be provided to grower groups to develop protocols appropriate to their perceived degree of risk.

Introduction:

Effect of sodium hypochlorite on phylloxera crawler survival

Phylloxera, particularly the first instar (crawler) can reach high levels in and on the soil surface of infested vineyards and can therefore be spread by machinery or footwear which may carry phylloxera infested soil particles. Sodium hypochlorite is currently used as a disinfestation treatment to prevent the spread of phylloxera on footwear (Dunstone *et al.*, 1998). Sodium hypochlorite was tested under laboratory conditions to determine the optimal concentration and duration required to cause 100% mortality of first instar (crawlers) phylloxera.

In 1991, phylloxera was identified in a vineyard in the King Valley region of Victoria and as a result, in 1995, the King Valley Grower Association initiated and published the first draft document of *Phylloxera Quarantine Protocols*. The document provided employees in the grape growing industries protocols to minimise the risk of phylloxera spread to uninfested vineyards and regions of Australia. More recently National Phylloxera Management Protocols (2000) have been developed in a coordinated effort by the National Vine Health Steering Committee. One of the national management protocols recommends the use of household bleach, with an active ingredient of sodium hypochlorite (NaOCl), as a disinfestation treatment for footwear to reduce the risk of phylloxera spread from vineyard to vineyard. The

recommendation specifies a concentration of 1% active chlorine of the disinfestation solution and immersion for 30 seconds.

Method:

The phylloxera population used for this experiment was collected from a vineyard in Nagambie, Victoria. Phylloxera were maintained under controlled environment conditions (25°C±2°C) on excised roots (adapted from Granett *et al.*, 1987). Phylloxera eggs were collected using a small artist's brush and placed on moist filter paper in a sealed petri dish until use. Ninety eggs were counted and transferred into a 30ml plastic vial that had been sealed, top and bottom, with wire mesh discs (100µm aperture). The vial was placed into a humidifier (95% relative humidity) for four days to allow eggs to hatch.

Insects were treated by immersing vials in three sodium hypochlorite solutions of the following concentrations; 0.5%; 1% and 2% (v/v). Each vial was immersed for duration of 30, 60, 300 or 600 seconds. After treatment, the crawlers and eggs were collected onto moist filter paper on petri dishes. The recovery and survival rates of crawlers were determined immediately after treatment. Crawlers were classified as living or dead under a dissecting microscope and were scored as alive if movement was apparent or could be elicited by gentle prodding with a paintbrush. Eggs were maintained on moist filter paper in sealed petri dishes for 10 days then examined for percentage hatch.

Immersion of phylloxera in distilled water for 300 and 600 seconds was used as a control in addition to phylloxera survival without any immersion treatment. The trial included a total of 15 treatments each replicated 3 times. Significant differences in the treatments were determined using analysis of variance with Genstat™ software (Rothamsted Experimental Station, 1998).

Results:

All sodium hypochlorite treatments had a significant effect ($p<0.001$) on the survival of first instar phylloxera when compared with both water immersed and no immersion controls. Total mortality of first instars was obtained in 2% sodium hypochlorite (Figure 33). Treatments with 2% sodium hypochlorite were significantly different from the 0.5% NaOCl with 30 and 600 second immersion times. There was no significant difference between all other sodium hypochlorite treatment combinations.

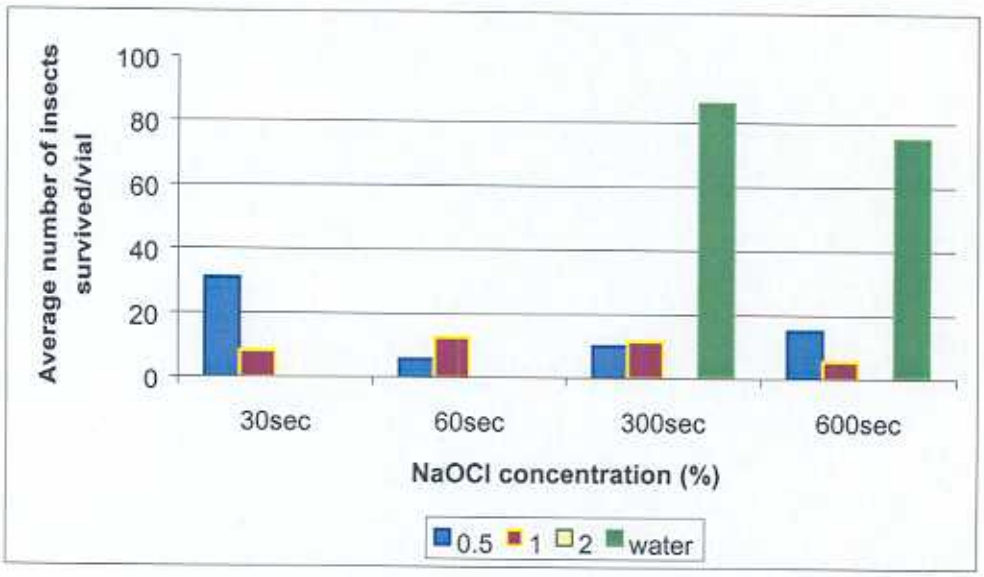

Figure 33: Phylloxera crawler survival when treated with a range of sodium hypochlorite concentrations over time.

There was no significant difference between the three controls; water immersion for 300 and 600 seconds and no immersion treatment.

Discussion:

Sodium hypochlorite, at the three concentrations used, demonstrated the effectiveness of the chemical as a phylloxera disinfestation treatment for footwear in the viticultural industry. To obtain 100% mortality of first instar phylloxera, the data presented indicates a minimum of 2% sodium hypochlorite solution is required for duration of 30 seconds immersion.

The results reported here show that whilst the current recommendation of 1% active chlorine would significantly reduce phylloxera survival if used as a disinfestation solution, it would not be as effective as the 2% sodium hypochlorite solution which achieves 100% mortality.

When using sodium hypochlorite as a chemical for disinfesting footwear in the vineyard, occupational heath and safety precautions should be considered.

Recommendations:

Based on this study, the use of 2% sodium hypochlorite (2% active chlorine) solution as a footwear disinfestation treatment would be recommended to provide 100% kill of first instar phylloxera. Current recommendations of 1% active chlorine disinfestation solution reduce first instar phylloxera survival significantly. Sodium hypochlorite has been used successfully for surface sterilisation of phylloxera eggs (Askani and Beiderbeck, 1991; Grzegorczyk and Walker, 1997). Phylloxera eggs are susceptible to treatment with hypochlorite with reduced hatching recorded due to increased concentration of hypochlorite and treatment duration (Grzegorczyk and Walker, 1997). Data has not yet been published regarding the effect of sodium hypochlorite on active stages of phylloxera. We report here the results of treatment of first instar phylloxera with sodium hypochlorite. This work provides scientific basis for phylloxera management protocols.

EARLY DETECTION OF GRAPEVINE PHYLLOXERA

Objective 4 (DAV 96/2):

Testing and developing methods for early detection of phylloxera in vineyards. The King Valley will be used as a testing ground for optimising detection of phylloxera with enhanced aerial photography, using information gained from ground surveys to validate the remote sensing systems. Training courses and workshops for vineyard staff will continue as the basis for improved practical knowledge of phylloxera, but will be more frequent to satisfy the demand.

Introduction:

Since the outbreak of phylloxera in the King Valley there has been increasing concern about further spread of the insect. In Victoria vineyards are not congregated in discrete areas, but are widely scattered across various regions. This makes the task of surveying vineyards difficult. Some vineyards cover relatively large areas of 40 hectares or more and are time-consuming to survey thoroughly using ground-based techniques. In addition the rate of spread of phylloxera within vineyards has primarily been monitored in the Nagambie area and rates of spread under other climatic situations need to be determined. The use of aerial photography is one way of tracking spread and was evaluated during the course of the project.

Phylloxera survey information compiled by survey teams remains as hard copy documents in central file systems. With existing computer programs much of the information can be condensed and provided on CD format. These files would contain survey results over a number of years and aerial photographs can be scanned in to provide a record that is relatively complete for each vineyard.

Infra-red aerial photography techniques had been developed during a previous GWRDC project (DAV 93/2). The aim of the DAV 96/2 project was to extend the photography to other areas at risk and to revisit areas photographed earlier. Several vineyards were selected for sequential photography to ascertain rates of spread particularly under different climatic conditions and of growers using different strategies for replanting their vineyards.

Methods:

Evaluation of infra-red photography and remote sensing

The aerial photography was undertaken by the South Australian Department of Environment, Heritage and Aboriginal Affairs – Resource Information Group. Copies of the photographs were passed on to the Plant Standards group (Knoxfield) to assist them with their ground surveys. Aerial infra-red photographs have been taken annually during the course of the project. Photographs were taken in late March-early April at a time when vines were likely to be at full canopy and stressed through crop load. Photographs were taken at about 3,000 m which gave roughly a 1:10,000 scale. The optimum timing was mid-morning or afternoon. Photographs were examined with a hand lens for weak patches of vines and these are identified for further field surveying.

A number of weak patches of vines were selected on the photograph where field surveys had determined whether the weak areas were caused by phylloxera or other soil related problems. In order to explore the remote sensing capabilities of the infra-red photographs, digitised versions of the aerial photographs were prepared. The spectral signatures of each of these weak spots were then determined from the digitised photographs. Three digital data sets were used, corresponding to the red, green and near infra-red spectral bands. The data sets were processed using ERDAS IMAGINE software provided by the Geographic Information System group at the Department of Natural Resources and Environment (DNRE) Institute at Tatura.

Database of infested/non-infested vineyards

The results of the field surveying conducted by the Plant Standards group in DNRE was developed into a database format. As each vineyard is surveyed, data was entered onto a record sheet. The survey information was recorded on a standardised survey form and a copy provided to ISIA, Tatura for entry of data onto a Microsoft Access database. Any hand written maps and the aerial photographs were also scanned and linked to the database. As the database was updated it was also copied to CD. The

information on the database and various reports can then be printed off. In recent years DNRE have commenced scanning aerial photographs and linking them to survey records. The database was updated on an annual basis after the seasonal surveys were completed.

Results:

Evaluation of infra-red photography and remote sensing

With appropriate timing, orientation and resolution, weak areas in vineyards could be detected, although the cause of the poor vine growth could not be ascertained from the photograph (see GWRDC project DAV93/2 final report). Weak areas of the vineyard surveyed were able to be identified by image classification (Figure 34) but it was not possible to distinguish between weak areas caused by phylloxera and other weak areas caused by other soil issues, eg. shallow top soil, waterlogged soil. This apparent lack of distinction may be influenced by other factors such as the quality of the photographs or corruption of the spectral bands during digitising. The average annual costs of the aerial surveys was $11, 200 at a cost of approximately $40 per hectare. A total of 133 vineyards were surveyed using aerial photography from 1998-2000.

Figure 34: Aerial photograph showing weak spots in phylloxera infested vineyard.

Database of infested/non-infested vineyards

The database contains information on surveys collected during the project period. During the 1997/1998 season a total of 24 vineyards (total area 273 hectares) were surveyed of which 5 were infested. In the following season 6 vineyards (total area 16 hectares) were surveyed, none of which were found to be infested. An increased number of vineyards were surveyed during the 1999/2000 season including a number of vineyards in the Upton region following the discovery of an infestation in April 2000. A total of 65 vineyards covering an area of around 450 hectares were surveyed in the 1999/2000 season.

The database contains the following fields of data information:

- Property details - name, location, manager
- Owner details - name, contact details, easting and northing of vineyard, other vineyards owned
- Irrigation type - drip, overhead, other
- Source of planting material - name of nursery, variety, year obtained
- Nursery details - name, contact details, location
- Where fruit marketed - name, contact details, location
- Harvest method - mechanical/hand
- Contractor details - what work done, who carried out the work, contact details
- Inspection of vineyard - date, inspector, remarks, sketch, aerial photo
- Sampling of vineyard - variety, area, soil type, result, number of vines checked
- ArcView - image of region showing infested and uninfested vineyards

Discussion:

In summary the aerial photography was useful for visual interpretation of weak areas within vineyards which could then be targeted during field survey work. The remote sensing studies did not clearly define weak areas due to phylloxera and the extra expense of digitising and identifying spectral band signature sets could not be justified. The study was limited to the three spectral bands used for infra-red

photography. Other spectral bands could be explored that may identify signature sets for phylloxera infested vines.

Aerial photographs have assisted ground survey teams in targeting vineyards to detect and contain phylloxera. Using aerial photography to determine the rates of spread and patterns of spread within vineyards will help growers determine management strategies to minimise the economic impact of a phylloxera infestation. By collating the information onto a central database, it is then available for others to access and use.

PHYLLOXERA EDUCATION AND AWARENESS ACTIVITIES

Objective 4 (Project DAV 96/2):

Testing and developing methods for early detection of phylloxera in vineyards. The King Valley will be used as a testing ground for optimising detection of phylloxera with enhanced aerial photography, using information gained from ground surveys to validate the remote sensing systems. Training courses and workshops for vineyard staff will continue as the basis for improved practical knowledge of phylloxera, but will be more frequent to satisfy the demand.

Objective 8 (Project DAV 99/2):

To consolidate current industry knowledge on phylloxera management and ensure that the recommendations from recently completed research are transferred and adopted to a wider audience

Introduction

Throughout the project period, 1997-2000, phylloxera extension encompassed a range of activities. Annual phylloxera workshops, field days, seminars, industry and media articles and the development of a comprehensive set of printed and audio visual material all helped highlight phylloxera awareness and latest research outputs. Conference presentations and the facilitation of the First International Symposium on Grapevine Phylloxera Management increased industry awareness of phylloxera research highlights both nationally and internationally. The effectiveness of phylloxera extension activities was assessed at a workshop level, through workshop evaluation surveys, and state-wide, through a telephone survey of Victorian grapegrowers.

Annual phylloxera identification and management workshops

Introduction:

Annual Phylloxera Identification and Management Workshops aim 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 about the risk periods and methods to reduce the risk of phylloxera movement, how the insect is spread, and how to detect early phylloxera infestations.

Methods:

The workshop format has predominantly followed a one–day format although two-day bus tours have also been organised in conjunction with the Phylloxera and Grape Industry Board of South Australia. Workshops are conducted within phylloxera-infested zones and have been held in Nagambie and Rutherglen in Victoria and Penrith in NSW. Phylloxera workshops are held for all industry personnel. The workshops are publicised annually through industry publications, local and interstate newspapers, mail-outs to regional organisations, and through the Department of Natural Resources and Environment Grapecheque groups. The program is revised each year to include new information as it emerges from phylloxera research activities.

The workshops 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 infestations, as well as the galls produced as a result of phylloxera feeding
- **Microscopic examination of phylloxera**
- **Population dynamics and distribution of phylloxera** - on the vine and potential methods of spread
- **Quarantine and disinfestation protocols** - to restrict phylloxera spread

- **Outbreak hypotheticals** - phylloxera outbreak scenarios to stimulate people to think about what actions are needed and the consequences for a viticultural business
- **Discussion** - with local growers and viticulturalists on the management of vineyards infested with phylloxera
- **Inspection of rootstock trials**

Each workshop participant was also provided with a Phylloxera Identification and Management Workshop Booklet which provided further details on a range of phylloxera-related topics. The Workshop Booklet has been updated annually and a table of contents is provided in Appendix 1 as an example. Workshop evaluation surveys were also introduced in 1997 to assist with the ongoing improvement of the workshop program. A standard evaluation form is shown in Appendix 2.

Results:

Workshop delivery

The first phylloxera workshop, organised by Agriculture Victoria - Rutherglen, was held in 1994 and workshops have continued on an annual basis since then. The number of workshops and participants has increased over time. Due to popular demand, the number of workshops held in 1999 increased to six and consequently the number of participants almost doubled that of the previous year (Table 15). The workshops have attracted a wide range of participants from different viticultural regions of Australia. In total, almost 500 people have attended the 15 workshops held between 1997-2000.

Table 15: Phylloxera workshops delivered by Agriculture Victoria - Rutherglen.

Year	Number of workshops held	Location of workshops	Number of participants	% evaluation survey responses
1994	1	Rutherglen	30	-
1995	1	Rutherglen	40	-
1996	1	Rutherglen	35	-
1997	1	Rutherglen	80	63
1998	2	Nagambie	80	75
1999	6	Penrith, Nagambie, Rutherglen,	150	80
2000	6	Nagambie, Rutherglen	104	100

Workshop evaluation surveys

The aim of the anonymous workshop evaluation surveys was to gather information about occupational status of participants, most interesting sessions, suggestions for future workshops, research and educational issues that the industry should be addressing and an overall rating for the workshop attended. The survey information has been used to improve the workshop program, and to assist in the development of future research and educational programs. Local growers who have spoken at the workshops are an important component of the workshop program and are always well received by participants. They provide insights into the personal experiences of growers dealing with phylloxera in different regions. These sessions also help to dispel the overwhelming fear and panic sometimes associated with phylloxera which can be counter productive when attempting to implement workable quarantine protocols.

Over the last three years, there has been a change in the occupations of workshop participants. In 1997, most workshop participants were grapegrowers (Table 16). Since then, a large proportion of participants have included company-employed vineyard workers and managers. Miscellaneous occupations (Table 16) included employees of Government departments (quarantine and regulatory officers, extension

viticulturalists), private industry (waste managers, real estate valuers and cellar door staff) and viticultural students.

Table 16: Occupation (% of total) of phylloxera workshop participants from 1997 to 2000.

Occupation	1997	1998	1999	2000
Grapegrower	78	27	34	19
Vineyard worker	6	14	17	15
Vineyard manager	0	20	18	1
Consultant	0	6	8	7
Contractor	0	0	1	9
Nursery staff	0	2	3	0
Winemaker	0	4	2	0
Miscellaneous	16	27	17	43

Recommendations:

The need for continuation of phylloxera workshops on annual basis has been highlighted, not only by an increase in the number of annual workshops and participants since 1994, but also by the increased demand for ad-hoc phylloxera seminars, field day presentations and information sessions run through the Grapecheque program. Phylloxera workshops, unlike other forms of educational awareness programs, also 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 also highlighted that a more diverse audience could be attracted to the workshops in the future. Although there were some contractors, winemakers and nursery owners who attended phylloxera workshops in the last three years, the numbers have not been substantial. In future, a more concerted effort will be made to attract more people from these areas of the viticultural industry to the workshops.

Field days and seminar activities

A wide range of formal seminars and field day displays relating to the phylloxera research and education program have been conducted on a national basis during 1997-2000. Several hundred people attended these statewide activities which are highlighted in Appendix 3. These included a poster display at the Margaret River Field Day in Western Australia (1999) and an oral presentation at Vititech (1999). Research seminars were presented at DNRE institutes (Rutherglen, Mildura and Knoxfield), to grower groups (Perricoota, Geelong, King Valley, Colbinabbin and Central Victoria High Country), at educational institutes (Dookie Agricultural College, Wangarratta TAFE, University of Adelaide), to wine companies (Southcorp, Orlando Wyndham) and to industry bodies (National Phylloxera Technical Reference Group, Adelaide Regional Phylloxera Board Committee). In July 1998 phylloxera research highlights were presented as part of two one-day seminars in Wangaratta and Mitchelton as part of a workshop on "Vineyard Planning, Establishment and Management".

Conference presentations and information distribution

Notable extension activities included the presentation of two research posters at the 10th Australian Wine Industry Technical Conference, Sydney, in August 1998. A poster was also presented at the Fifth International Symposium on Cool Climate Viticulture and Oenology and the First International Symposium on Grapevine Management, Melbourne in January 2000. The poster presentations were:

- "Seasonal variation in phylloxera (*Daktulosphaira vitifoliae*) population behaviour and its impact on national quarantine"
- "Phylloxera biotypes in Australia"
- "Use of sodium hypochlorite as a disinfestation treatment for phylloxera"

Three hundred copies of the Proceedings of the International Symposium on Grapevine Phylloxera Management, containing 12 refereed papers were printed and a paper entitled " Population dynamics of phylloxera in Australian vineyards and

implications for management" was presented at the Symposium in Melbourne in January 2000 (Appendix 4).

In addition, 7,000 phylloxera brochures " A guide to the identification, prevention and control of grape phylloxera" and 1,000 phylloxera research brochures "Taking Aim at Phylloxera" were reprinted for distribution through mail-outs and workshops during 1999-2000 (Appendix 5). Two information brochures on future research directions, entitled "Alternative Management of Phylloxera" and "Rootstock Control of Phylloxera" and were also printed and distributed in collaboration with the Phylloxera and Grape Industry Board (Appendix 6).

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 – 1999-2000) and as a component of the SBS documentary entitled "Wine Lovers Guide to Australia" which was broadcast nationally in 1999.

Phylloxera annual general meeting and quarterly newsletter

Phylloxera research and extension activities are highlighted in Phylloxera Annual General Meetings (held since 1997) where extension officers and researchers from a number of research and extension programmes meet to discuss and present their annual progress reports. Meetings have been held since 1997 in Rutherglen, Knoxfield, Mildura and Adelaide. The objective of the meetings is to keep staff informed of each groups latest research and extension activities. A less formal method of communication between phylloxera research groups and extension officers has been through the production of a Quarterly Phylloxera Newsletter which commenced in September 1997 as an outcome of the first AGM.

Educational modules

An Educational Package aimed at a target audience of both growers and viticulture students composed of a video, an interactive CD-ROM and a set of Practical Guidelines for Phylloxera Management commenced in 1999. The package covers a broad range of phylloxera education and management aspects and is currently being developed in close collaboration with industry partners. The video is composed of three-10 minute sections focusing on phylloxera history, life-cycle and management and includes segments on latest research outputs, phylloxera management protocols and revised quarantine zones. The video is in its final editing stage and should be available for distribution by December 2000. The CD-ROM and Practical Guideline Advisory Leaflets are in the developmental stage and are expected to be completed by December 2000. Delays in the development of the educational package arose primarily due to increased extension activities arising from the International Phylloxera Symposium and the Upton phylloxera outbreak and the addition of a series of Pest Risk Analysis experiments to the project outputs of DAV 99/2.

Phylloxera awareness - evaluation survey

An evaluation of phylloxera extension activities was conducted in 2000, in Victoria, with the development of a combined telephone survey in a collaborative venture between phylloxera and Grapecheque teams. The overall survey evaluated the level of awareness of both phylloxera and Grapecheque amongst growers.

Method:

Development of the survey commenced in September 1998, when a workshop was held at DNRE Tatura involving phylloxera research and Grapecheque teams, and statisticians from the Australian Bureau of Statistics (ABS). The survey questions are shown in Appendix 7. The survey was conducted in January 2000 and 384 growers from six regions of Victoria (Central, Great Western, Murray Valley, North East, South Coast and South East) participated.

The sample number was derived from a sample size formula for simple proportions in large populations (ABS, 1998):

$$n \geq \frac{1 - p}{p[RSE\,(p)]^2}$$

Where n = sample size, p = sample proportion, RSE = required relative standard error of the sample proportion. Data analysis was completed in June 2000.

Results:

Three hundred and thirty-two growers participated in the survey representing an 85% response rate. Every respondent was aware of phylloxera and 55% had attended a phylloxera workshop. Of those who had had attended a phylloxera workshop 65% had attended workshops in the last 5 years.

Phylloxera quarantine awareness and practices were evaluated in the survey. Over 70% of vineyards reported that they used some precautionary quarantine measures in their vineyards such as the use of footbaths, signs, clean vine material, monitoring traffic in vineyards and non-sharing of machinery. Thirty-eight percent of respondents had erected quarantine signs in their vineyards supplied either by grower associations (47%) or DNRE (44%). The use of quarantine protocols varied from region to region with 90% growers in the Central Victoria using protocols compared to 40% in the Murray Valley region.

To target a wider audience with phylloxera awareness and research articles, growers were asked which industry publications they read regularly. The Australian Grapegrower and Winemaker was the most popular industry journal with 88% readership. Other widely read industry journals included Victorian Viticulture News (67%), Australian Viticulture (64%), South Australian Grapegrowers (26%) and GWRDC Highlights (22%). Other sources of information included other grapegrowers (91%) and the internet (30%). A complete report on the survey data is being prepared in collaboration with Grapecheque for publication in a wine industry journal.

DNA BIOTYPING OF PHYLLOXERA

Objective 5 (Project DAV96/2):

Further develop the capacity to trace infestations, by audit trails of harvest (or other) contractors, and by use of DNA typing techniques (developed in HRDC project FR310) to provide the precise capacity to map the spread of phylloxera infestations. It will then by possible to trace infestations and thereby target the high risk methods of phylloxera transfer, and so minimise the risks of further outbreaks.

Objective:

To determine the strain of phylloxera present in Victorian vineyards from infestations detected between 1996 and 1998 by ground survey teams.

Methods:

A total of eight new phylloxera infested vineyards were detected in north-east Victoria during the growing seasons of 1996/97 and 1997/1998 by ground survey teams (*Objective 4; DAV 96/2).* Phylloxera from each infested site were collected by researchers from Agriculture Victoria - Rutherglen, to be cultured and maintained in the dual tissue culture system as described in Corrie *et al* (1997). Phylloxera populations were maintained on the vine species they were originally collected from in the vineyard. The collected populations screened in this experiment can be seen in Table 17. All populations were sourced from *Vitis vinifera* cultivars excepting SRU-1, which was sourced from infested Schwarzmann vines (leaf galls).

Table 17: Sources of populations of phylloxera.

Population	Location
SRU-1	Rutherglen
VNA-2	Nagambie
VWF-1	Whitfield
VWF-5	Whitfield$^{\Psi}$
VWF-6	Whitfield$^{\Psi}$
VWF-7	Cheshunt$^{\Psi}$
VWF-8	Cheshunt$^{\Psi}$
VWF-9	Whitfield$^{\Psi}$
VWF-10	Whitfield$^{\Psi}$
VWF-11	Cheshunt$^{\Psi}$
VMY	Myrrhee$^{\Psi}$

$^{\Psi}$Denotes population identified between 1996 and 1998

DNA was extracted from adult females from the above populations using the grinding methods of Lin and Walker (1996). DNA was stored in TE buffer (10 mM Tris-HCl, pH 7.5, and 0.1 mM EDTA) frozen at -20°C.

Random Amplified Polymorphic DNA (RAPD) analysis was performed to amplify phylloxera DNA sequences using 10-mer oligonucleotide primers OPC2, OPC19, OPA3 and OPA4 (Operon Technologies, Alameda, CA, USA) as described by Williams *et al.* (1990). The primers used included those already identified by previous experiments to amplify polymorphism's in phylloxera populations (Corrie *et al.*, 1997).

Each reaction contained 3.0 mM $MgCl_2$, 500 mM KCl, 100 mM Tris-HCl pH 8.3, 0.01% (m/v) gelatine, 0.2 mM each of dATP, dGTP, dCTP and dTTP (Pharmacia), 16.5 ng primer, 0.625 units Amplitaq DNA polymerase (Perkin Elmer, USA), and approximately 30 ng of template DNA.

The polymerase chain reaction (PCR) was performed in a Perkin Elmer 480 DNA thermal cycler with the following amplification conditions: 4min at 92°C, 35 cycles of 1min at 92°C, 1min at 35°C, 30secs at 45°C, and 2min at 72°C, followed by a final extension cycle of 3min at 72°C. Amplification products were electrophoretically analysed in 1.8% (m/v) agarose gels in TAE buffer (40 mM Tris-acetate and 2 mM

Na_2EDTA). Gels were then stained with ethidium bromide and PCR products were photographed under UV light.

Replicated bands were scored as present (=1) or absent (=0) and similarity coefficients were determined according to Sneath and Sokal (1973), for the eleven populations analysed.

Results:

A total of 31 bands were scored, 21 of which were polymorphic for one or more populations. The RAPD band patterns for the phylloxera populations detected between 1996 and 1998 were identical to the Whitfield population identified in previous studies. The RAPD band patterns of SRU-1, VNA-2 and VWF populations were descriptive of three different strains as supported by similarity coefficients (Table 18).

Table 18: Similarity coefficients for the eleven populations of phylloxera, calculated using the method outlined by Sneath and Sokal (1973).

Phylloxera population	VWF-1	VNA-2	SRU-1
SRU-1	0.52	0.48	1.00
VNA-2	0.69	1.00	-
VWF-1	1.00	-	-
VWF-5	1.00	-	-
VWF-6	1.00	-	-
VWF-7	1.00	-	-
VWF-8	1.00	-	-
VWF-9	1.00	-	-
VWF-10	1.00	-	-
VWF-11	1.00	-	-
VMY	1.00	-	-

Figures 35 and 36 show different sized DNA fragments obtained with the RAPD technique for the eleven populations (with OPC2 and OPA3 respectively).

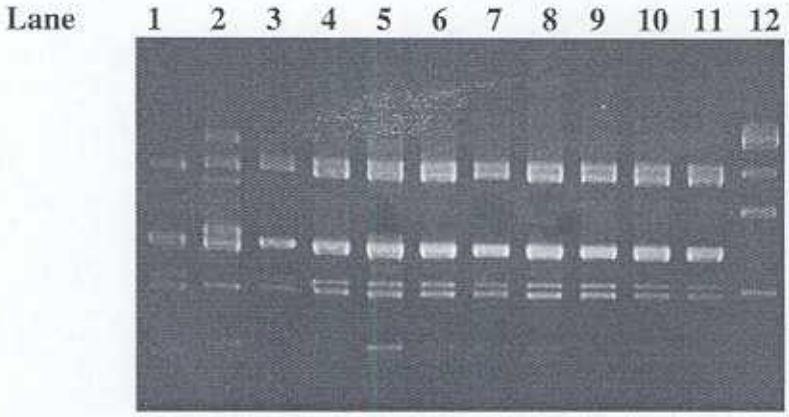

Figure 35: RAPD-PCR amplified phylloxera DNA using primer OPC2. Lane identification: 1 = SRU-1, 2 = VNA-2, 3 = VWF-1, 4 = VWF-5, 5 = VWF-6, 6 = VWF-7, 7 = VWF-8, 8 = VWF-9, 9 = VWF-10, 10 = VWF-11, 11 = VMY, 12 = 1kB ladder (molecular weight marker, Gibco-BRL), bp = base pairs.

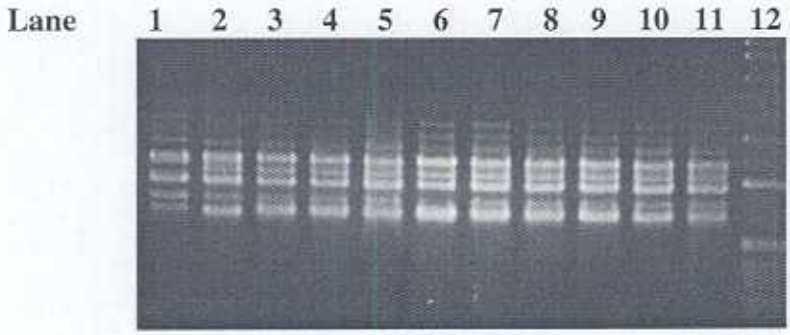

Figure 36: RAPD-PCR amplified phylloxera DNA using primer OPA3. Lane identification: 1 = SRU-1, 2 = VNA-2, 3 = VWF-1, 4 = VWF-5, 5 = VWF-6, 6 = VWF-7, 7 = VWF-8, 8 = VWF-9, 9 = VWF-10, 10 = VWF-11, 11 = VMY, 12 = 1kB ladder (molecular weight marker, Gibco-BRL), bp = base pairs.

Discussion:

The objective of this experiment was to identify different strains of grape phylloxera. RAPD analysis can detect the presence or absence of loci in a genome, but not the multiple alleles at each locus. When studying population genetics, the RAPD technique is suitable for the analysis of genomes of uniform species, with predominantly asexual reproduction such as phylloxera.

Similarity coefficients for SRU-1, VNA-2 and VWF-1 were concurrent with those obtained in previous studies (Corrie *et al* 1997). The similarity coefficients obtained

for the phylloxera populations detected between 1996 and 1998 indicate the populations isolated are the same strain as already identified in the King Valley region (VWF-1). This similarity is supported by the physical location of the newly infested vineyards within the current Phylloxera Infested Zone that encompasses the King Valley region.

The experiment reported here achieved the objective to determine the strain of phylloxera from vineyards identified with phylloxera infestations between 1996 and 1998. The results indicate the phylloxera strain collected from newly infested vineyards is the same as that found commonly found in the King Valley suggesting that the source of infestation is from within the region. The implications of this research are that we now have the ability to track the progress of new infestations and having a tool to determine whether phylloxera is staying within the PIZ boundaries.

SCREENING ROOTSTOCKS FOR PHYLLOXERA RESISTANCE

Objective 6 (Project DAV 96/2):

Helping grapegrowers to test rootstocks for phylloxera control, by providing protocols for the design and evaluation of rootstocks in their vineyards. Dependent upon the virulence of phylloxera strains, characterisation of field strains may be an essential part of this process.

Introduction:

Phylloxera has been present in Australia for over 100 years. In that time, the use of rootstocks with resistance to phylloxera has increased. Most rootstocks used today have been screened for phylloxera resistance overseas, with different genetic strains of phylloxera to those observed in Australia. New rootstocks being developed for use need to be screened against populations found in Australia which have been shown to behave differently in their survival and development on different vine species. Access to phylloxera strains, the facilities and expertise for rootstock screening and the location of the Agriculture Victoria - Rutherglen in the centre of a Phylloxera Infested Zone (PIZ), provide the opportunity for rootstock screening to be conducted under controlled environment conditions. Before rootstocks can be screened for field resistance to phylloxera they need to be evaluated using different genetic strains of phylloxera in the laboratory. In collaboration with CSIRO Merbein, the phylloxera team at Agriculture Victoria - Rutherglen have screened new rootstock hybrids for phylloxera resistance using the excised root bioassay system.

Methods:

Main roots of rootstock hybrids were collected from the field. Bioassays were performed on excised root pieces based on the method of Granett *et al.* (1987). Initially 8 rootstocks were screened (Experiment 1) and a further 5 rootstocks were screened at a later date (Experiment 2).

The roots, 3-5 mm in diameter, were cut into 6 cm long sections. They were washed with sterile distilled water and dipped into a 300 mg/l chlorothalonil solution

(Bravo™). The proximal end of the blotted dry sections were wrapped with cotton wool (moistened with sterile water) and placed into petri-dishes lined with filter paper. Three, and two, root sections were placed into each petri-dish in Experiments 1 and 2, respectively. The petri-dishes were aerated via a 1 cm diameter hole in each lid, with the hole covered with 60 µm cloth (Sigma), to prevent the build up of high humidities and the development of fungal and bacterial organisms. The petri-dishes were stored in plastic containers at 23°C± 1°C.

Ten, two-to-five day old eggs from a dual tissue culture population of phylloxera were placed on each root piece. In Experiment 1, there were 7 replicates of each vine type, where a replicate consisted of a petri-dish containing three excised roots (total of 30 eggs per replicate). In Experiment 2, there were 10 replicates of each vine type, each consisting of two excised roots (20 eggs per replicate). The phylloxera strain VWL-1was used in both bioassays (Corrie *et al.*, 1997).

The controls consisted of the vine type Shiraz (*V. vinifera*), which is susceptible to phylloxera (demonstrates vine decline), and the resistant rootstock Schwarzmann (*V. rupestris* x *V.riparia*). Schwarzmann has been used extensively in previous bioassays undertaken at Agriculture Victoria, Rutherglen. The phylloxera strain VWL-1 does not survive or establish on Schwarzmann. (Corrie *et al.*, unpublished data).

Twenty days after the phylloxera eggs had been introduced to the root pieces, the rate of phylloxera development was assessed, and eggs removed. This was not done in Experiment 2. After another 10 days, the numbers of surviving phylloxera were recorded, noting their stages of development and their positions on the root pieces. The survival data was analysed by analysis of variance using Genstat ™ statistical software.

Results:

The phylloxera survival data show that two of the hybrid rootstocks (MS 10-03, MS 25-16) were very similar to the susceptible Shiraz (*V. vinifera*) vine type, while six other hybrid rootstocks were similar to the resistant Schwarzmann rootstocks (Tables

19 and 20). Phylloxera survival on the remaining five hybrid rootstocks were intermediate to those of Shiraz and Schwarzmann.

Table 19: Average number of phylloxera survivors (n = 30) for vine types in Experiment 1.

Vine type	No. of phylloxera survivors	No of phylloxera survivors established on main roots
Shiraz (*V.vinifera*)	20.71	20.14
Schwarzmann	0.00	0.00
MG 61-04	3.71	3.71
MS 10-03	19.86	19.57
MG 54-89	3.29	2.86
MI 09-05	0.86	0.86
MS 10-79	15.00	11.29
MG 56-80	2.43	2.14
MS 25-16	22.71	22.00
MG 60-30	7.29	2.00
$LSD_{0.001}$	5.69	5.50

Table 20: Average number of phylloxera survivors (n = 20) for vine types in Experiment 2.

Vine type	No. of phylloxera survivors	No of phylloxera survivors established on main roots
Shiraz (*V. vinifera*)	14.9	14.3
Schwarzmann	0.0	0.0
MS 37-2 (2)	13.8	10.6
MS 37-12 (6)	13.1	8.6
MS 37-33 (4)	10.4	5.7
MS 37-59 (7)	13.2	4.3
MV 46-21 (5)	0.0	0.0
$LSD_{0.001}$	2.44	2.88

Discussion:

Boubals (1966) noted that the presence of phylloxera galls (tuberosities) on main roots was the most important indicator of phylloxera damage. This corresponds with field observations that phylloxera populations, established on the fibrous roots of Schwarzmann rootstock, have caused fleshy galls (nodosities), even though no vine decline has been observed on this rootstock.

The rootstocks MS 10-03 and MS 25-16 would not be recommended for use as phylloxera-resistant rootstocks as phylloxera survival in Experiment 1 was not significantly different ($P<0.001$) from the susceptible variety, Shiraz.

Both MS 10-79 (Experiment 1) and MS 37-33 (Experiment 2) would also not be recommended. While significantly fewer phylloxera survived on these two rootstocks than on Shiraz, phylloxera were however able to establish on the majority on their main roots, which is associated with vine decline.

MG 60-30 (Table 19) was significantly different to both the susceptible and resistant vine varieties. In Experiment 2, MS 37-2, MS 37-12 and MS 37-59 were not significantly different to Shiraz in total phylloxera survival but all three differed from both Shiraz and Schwarzmann in numbers of phylloxera that established on main roots (Table 20). As some phylloxera established on the fibrous or callus material of these rootstocks, further testing is recommended with alternate phylloxera strains to determine an overall phylloxera resistance rating. Pot trials are also recommended to determine the effects of phylloxera infestation on the yields of these rootstocks.

Phylloxera survival on rootstocks MG 61-04, MG 54-89, MG 56-80 and MI 09-05 (Experiment 1) were not significantly different from that on Schwarzmann. They do, however, exhibit varying levels of resistance. Due to some phylloxera survival on these rootstocks in Experiment 1, further evaluation is recommended, using alternate strains of phylloxera and pot trials, to determine the impact of phylloxera on the yield of each vine variety. Phylloxera did not establish on MV 46-21 from Experiment 2. Further study of the survival of different phylloxera strains on MV 46-21 is recommended to ensure that its resistance is not specific to the Whitlands strain of phylloxera.

Recommendations:

Rapid screening of hybrid rootstocks for phylloxera resistance was performed on a total of 13 rootstocks, which were screened during May to July 1998, in two separate experiments. The phylloxera resistance of the rootstocks MG 54-89, MG 56-80, MG 61-04, MI 09-05 and MV 46-21 were not significantly different to that of

Schwarzmann. MG 60-30 showed some resistance to phylloxera with phylloxera establishing on the fibrous roots. It should be noted that phylloxera establishing on fibrous roots has not yet been directly linked with vine decline.

Further testing is required as this screening was conducted using only one of the phylloxera strains found in Australia. This population, sourced from a vineyard in the King Valley, has survived very well on the susceptible *Vitis vinifera* varieties, whilst none have survived on the Schwarzmann rootstock. Other phylloxera strains are known to behave differently on different vine varieties. It is recommended that rootstocks are screened against other phylloxera strains to ensure that any resistance is not strain specific, before a phylloxera resistance rating can be determined. Future evaluation should also include pot trials to determine effects of the different phylloxera genetic strains on the crop yields of tested rootstocks.

ALTERNATIVE MANAGEMENT OF PHYLLOXERA

Objective 7 (Project DAV 96/2):

Testing new products and approaches to control of phylloxera on ungrafted vines, so that grapegrowers are provided with sensible options for control of phylloxera. This will involve strategic testing of effects of the products in small-scale trials, rather than elaborate and expensive field trials.

Introduction:

The International Symposium on Grapevine Phylloxera Management in January 2000 highlighted that whilst the use of resistant rootstocks is the main form of phylloxera control other approaches to management are being examined. These include the use of chemical insecticides and fumigants, nutrient and irrigation management, organic vineyard management and an integrated phylloxera management approach. Phylloxera outbreaks and infestations in Victoria have highlighted the industries vulnerability and the need for vigilance, awareness and on-going research into early detection and phylloxera management. The use of grafted grapevines and phylloxera-resistant rootstocks is currently the only effective method known to control phylloxera. Over 85% of Australian vineyards are planted to ungrafted *Vitis vinifera* which is susceptible to phylloxera. Due to the cost of rootstock material, shortage of supply and the extra cultural management required for grafted vines, the proportion of total vineyard area in Australia planted as own-rooted vines is unlikely to decrease appreciably over the next 20 years.

In order to minimise the effects of phylloxera management strategies must be applied as soon as an infestation is detected. To date this has consisted solely of replanting with resistant rootstocks. A potential alternative approach could be the use of either chemical insecticides or biofumigants, enabling continued production at economically sustainable levels without the need or delaying the need for replanting. Chemical control methods have been trialed in Australia, Europe and the USA, and although they appear to reduce phylloxera populations none have prevented grapevine decline due to the damage caused by the insect. One of the main restrictions to the

development of these new methods has been a lack of knowledge of the insect-plant interaction, and subsequent inability to target control methods for maximum effect.

Methods:

The development of alternative approaches to phylloxera management is a relatively complex issue. It requires an extensive knowledge of insect-host plant interactions, phylloxera population dynamics in order to optimise the efficacy of an alternative management approach and studies on the mode of action of the management technique. In 1998-1999 background data was gathered on existing and novel treatments which could be trialed against phylloxera. There then followed negotiations with two agrochemical companies to identify and obtain a group of systemic insecticides which could be trialed to determine their efficacy against phylloxera. Based on unpublished data provided by the companies involved modified protocols for detailed screening of two insecticides were developed.

Rather than conducting small-scale trials with limited resources a full proposal was submitted to the CRCV for a PhD studentship entitled *"Early detection and alternative management of phylloxera on ungrafted vines* ". This proposal was subsequently funded and a PhD student based at Agriculture Victoria - Rutherglen commenced her research studies in January 2000 in collaboration with the University of Adelaide. A major focus of the alternative management approach to phylloxera management will involve:

- Determining the key period in the growing season when the phylloxera population is most susceptible to control agents by identifying critical population growth phases and their relationship with the grapevine growth phases.

- Assessing the feasibility of alternative/novel methods of control, which may include the use of systemic chemical insecticides and biofumigants, utilising a targeted approach based on the results of insect population studies.

Discussion:

The CRCV-funded project on early detection and alternative management of phylloxera in ungrafted vines will be completed in June 2003 and outputs from the project will be made available to industry during the course of the project.

FUTURE PHYLLOXERA RESEARCH ACTIVITIES

Objective 9 (DAV 99/2):

To identify future priorities for phylloxera management and develop a framework for Phylloxera research, development and extension in Australia for the next 5 years

Introduction:

The Australian viticulture industries main approach to phylloxera management relies on a combination of rootstock and quarantine management. This combined approach is somewhat unique, compared to other grape-growing countries because phylloxera is restricted to clearly defined quarantine zones. Yet over 85% of vines are still planted on ungrafted *Vitis vinifera* and remain susceptible to the insect and recent outbreaks have highlighted the industries vulnerability. There are relatively few research groups worldwide focussing on phylloxera management and each has its own approach to solve the problem. In order to focus future phylloxera research activities in Australia a 3-phase approach was implemented. Firstly to organise a workshop bringing together industry personnel and researchers to outline past and current phylloxera research activities and set priorities for future research. The second phase was to increase the industries awareness of current international phylloxera research activities. This was achieved through the organisation of the first International Symposium on Grapevine Phylloxera Management. The final phase, which drew on the outcomes of the workshop and symposium, was to develop and prioritise future phylloxera management in Australia through the development of a 5-Year Phylloxera Research and Development Plan.

Research and development planning workshop

A two-day research and development workshop was held in Rutherglen and Albury in October 1999. The workshop was attended by over forty industry personnel and researchers. Researchers provided summaries of phylloxera research findings which was then followed by a process to set priorities for future research, development and

extension for phylloxera management. This process, combined with the outputs of the International Symposium on Grapevine Phylloxera Management (January 2000) established the framework for the development of a 5–Year Research and Development Plan for Phylloxera Management.

International symposium on grapevine phylloxera management

The First International Symposium on Grapevine Phylloxera Management was organised and convened in Melbourne, January 2000. The one-day symposium was attended by over 80 national and international industry and research personnel. A post-symposium tour to the King Valley region and Agriculture Victoria - Rutherglen was organised to give presenters the opportunity to meet with growers from a phylloxera-infested region and to allow overseas researchers to discuss future collaborative links with Australian researchers.

Contributions from international phylloxera research groups in Australia, USA, Germany, Russia, Canada and South Africa were presented at the Symposium (Appendix 4). Travel sponsorship for four international presenters was provided by GWRDC (Project DAV 99/4) and the Phylloxera and Grape Industry Board of South Australia. Three papers were presented by Australian researchers including one paper arising from research conducted as a component of Projects DAV 96/2 and DAV 99/2 (see Appendix 8).

The symposium proceedings, comprising 12 papers, was edited and published in January 2000 and three hundred copies were printed for distribution. Key research topics covered in the symposium included phylloxera population dynamics and dispersal, rootstock management, integrated pest management, phylloxera awareness, alternative management, resistance and susceptibility mechanisms (Appendix 4). The symposium was highly successful commanding media coverage on national radio and in industry journals and local press (Appendix 3). A series of technical articles arising from the Symposium has also been produced in the *National Grapegrowers* and the *Australian Grapegrower and Winemaker*.

Feedback from both national and international symposium presenters was highly complimentary:

"I have just finished reading the Symposium Proceedings. Congratulations for a job well done and one that has been sorely needed for some time.

Your paper was very clear and it was fun to think of all those yellow bugs drowning in ethyl glycol, getting stuck to glue, or being dust-busted back to the lab!"

Lucie Morton, Canada

"Allow me to congratulate you successful completion of Symposium. I am very much obliged to you for the copy of the Proceedings for the International Phylloxera Symposium, for printing of our theses."

Sabina Agapova,
Novocherkassk,
Russia

"Just received a notice about availability of the proceedings on your recent symposium on management of grape phylloxera. I wish to obtain a copy for our reference library.
Grape phylloxera has been present in some vineyards throughout the Okanagan Valley for several years but has had minimal detectable impact on production. However that can change as growers in California found out."

Hugh Philip
Extension Entomologist
British Columbia Ministry of Agriculture & Food
Canada

"I'd love to have a copy of the International Phylloxera Symposium Proceedings."

Kevin Chambers
Oregon Vineyard Supply Co.
USA

As a result of this first symposium a second is to be held in August 27-28th 2001 in Geisenheim, Germany initiated by researchers who presented in Melbourne.

Five year research, development and extension plan

Following the Planning Workshop and Symposium the 5-Year Phylloxera Research and Development Plan was developed by a key group of phylloxera researchers. The plan took eight months to develop and went through a rigorous process of re-editing with substantial input from researchers and key industry personnel. It was presented to an industry body the National Phylloxera Technical Reference Group for endorsement in June 2000. Key activity areas and outcomes of the plan were prioritised by the reference group and the plan was finalised in August 2000. Further details of the plan are provided in Appendix 9.

The key objectives of the plan are:

- the early detection and management of phylloxera outbreaks;
- to reduce the potential for spread of phylloxera;
- to ensure the long-term viability of rootstocks.

Discussion:

The overall process of developing a framework for future phylloxera research and development has provided a unique opportunity to bring industry and researchers, both nationally and internationally, together with one primary focus: the future management of phylloxera. It became evident at the International Symposium on Grapevine Phylloxera Management that Australian researchers are at the forefront of certain aspects of phylloxera research. This research emphasises the development of risk management strategies based on a scientific knowledge of molecular approaches to phylloxera management, understanding the phylloxera genetic variability and quantifying phylloxera population dynamics and dispersal. The symposium highlighted alternative approaches to phylloxera management and stimulated new ideas and directions for research activities. It also provided the opportunity for growers and scientist to network both nationally and internationally and had a major impact on increasing phylloxera awareness worldwide as well as in Australia.

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APPENDIX 1:

Phylloxera Workshop Booklet (Front Cover/Table of Contents)

PHYLLOXERA WORKSHOP 2000

Phylloxera and Grape Industry Board of South Australia

Grape and Wine Research and Development Corporation

Natural Resources and Environment

AGRICULTURE • RESOURCES • CONSERVATION • LAND MANAGEMENT

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APPENDIX 2: Phylloxera Workshop Evaluation Form

2000 PHYLLOXERA WORKSHOP: SURVEY

The Department of Natural Resources and Environment are keen to offer programs which meet your needs. By completing the following survey you will help to achieve this. *(please tick appropriate box)*

1. Have you attended a phylloxera workshop in the last 3 years?

☐ Yes ' ☐ No

2. How did you first find out about this workshop?

☐ Australian Grapegrower and Winemaker ☐ Word of mouth
☐ Local organisation ☐ DNRE staff
Newspaper (please specify) .....
Other (please specify).....

3. I am

☐ Self employed ☐ Company employed
☐ Government employed (please specify) .....

4. My role is best described as a

☐ Grape grower ☐ Potential investor
☐ Vineyard worker ☐ Nurseryman
☐ Vineyard manager ☐ Student
☐ Vineyard consultant ☐ Winemaker
☐ Contractor/supplier to the grapegrowing industry (please specify).....
☐ Other (please specify).....

5. Before attending this workshop, my knowledge of the following topics was

	none	basics only	average	advanced
Phylloxera biology	☐	☐	☐	☐
Vineyard survey techniques	☐	☐	☐	☐
Quarantine procedures	☐	☐	☐	☐
Phylloxera management	☐	☐	☐	☐
Current research on phylloxera	☐	☐	☐	☐

6. After attending this workshop, my knowledge of the following topics was

	none	basics only	average	advanced
Phylloxera biology	☐	☐	☐	☐
Survey techniques	☐	☐	☐	☐
Quarantine procedures	☐	☐	☐	☐
Phylloxera management	☐	☐	☐	☐
Current research on phylloxera	☐	☐	☐	☐

7. Tick the 3 sessions most valuable to you today

- ☐ Introduction/biology of phylloxera
- ☐ Vineyard survey techniques
- ☐ Rootstock trial
- ☐ Phylloxera "hypothetical"
- ☐ Quarantine procedures
- ☐ Lunch
- ☐ Other.....

8. Have you suggestions for things we could do to better/include in the workshop?

.....
.....
.....
.....
.....

9. Have you suggestions for issues that you would like to be addressed in future phylloxera research and education programs?

.....
.....
.....
.....
.....

10. Is there anything you will do differently as a result of this field day?

☐ YES ☐ NO

If yes what will you do differently?

.....
.....
.....
.....

11. What is your overall rating for the day?

☐ Poor ☐ Average ☐ Good ☐ Excellent

Please give completed evaluation form to a presenter.

Thankyou for your participation!

APPENDIX 3: Phylloxera extension activities and technical reports

Referred Journal Articles:

Title:	DNA typing of populations of phylloxera (*Daktulosphaira vitifoliae* (Fitch)) from Australian vineyards
Authors:	A.M. Corrie, G.A. Buchanan and R. van Heeswijck
Journal	Australian Journal of Grape and Wine Research 3(2):50-57

Industry Journal Articles:

Title:	Phylloxera: What does it mean for Sunraysia winegrape growers?
Authors	Yasmin Wilson, Winegrape Industry Development Officer, SHC Mildura
Journal:	Murray Valley Wine Grape Grower Vol 3 (4), September 1997

Title:	Phylloxera: where are the risks?
Author:	G.A. Buchanan
Journal:	The Australian Grapegrower and Winemaker No. 411 March 1998 pg. 28-29

Title:	Early Detection of Grape phylloxera
Author:	Megan Hill, Angela Corrie, John Whiting DNRE Tatura and AV-Rutherglen
Journal:	The Australian Grapegrower and Winemaker No. 412 April 1998

Title:	Excellence Award for phylloxera researcher
Author:	Joanne Bates, IHD Knoxfield
Journal:	The Australian Grapegrower and Winemaker No. 409 January 1998 pg 10

Title:	Preventing spread of phylloxera: providing the scientific basis for quarantine protocols
Author:	Angela Corrie AV-Rutherglen
Journal:	Victorian Viticultural News Spring 1997

Title:	Interstate research staff discuss progress on phylloxera
Author:	David Braybrook IHD Knoxfield
Journal:	Victorian Viticultural News Spring 1997

Title:	Viticultural researcher takes out award
Author:	Joanne Bates, IHD Knoxfield
Journal:	Victorian Viticultural News Spring 1997/98

Title:	Spread vines, not phylloxera
Author:	David Braybrook, IHD Knoxfield
Journal:	Victorian Viticultural News Spring 1997

Title:	Phylloxera and its transmission- some facts and fiction
Author:	Megan Hill, Angela Corrie, John Whiting, illustrations by Peter Cole
Journal:	Victorian Viticultural News Autumn 1998

Title:	Fighting phylloxera
Author:	Joanne Bates, IHD Knoxfield
Journal:	Shepparton News Vol 121 No. 39 pg 18

Title:	War of the Vineyard
Author:	Viv Burnett AV-Rutherglen
Journal:	Shepparton News Monday April 28 1997

Title:	Phylloxera and its transmission - facts and fiction
Author:	Megan Hill, Angela Corrie and John Whiting
Journal:	The Victorian Viticulture News, autumn 1998

Title:	The early detection of phylloxera
Author:	Megan Hill, Angela Corrie and John Whiting
Journal:	The Australian Grapegrower and Winemaker, April 1998

Title:	Know thine enemy- the 1999 phylloxera workshops.
Author:	Rebecca Dunstone, Kevin Powell and Megan Hill
Journal:	The Australian Grapegrower and Winemaker March 1999

Title:	Preventing the spread of phylloxera
Author:	Rebecca Dunstone, Angela Corrie, and Kevin Powell
Journal:	The Australian Grapegrower and Winemaker, December 1998

Seminars:

Title/topic:	Phylloxera: Biology, research and quarantine
Presenter:	Angela Corrie, John Whiting, David Brown
Location:	Yarra Glen Hall 30th October 1997
Audience:	Yarra Valley Wine grower Association, Vineyard Winery owners, contractors.

Title/topic:	Phylloxera Biology and management
Presenter:	Angela Corrie
Location:	AV- Rutherglen 28th October 1997
Audience:	25 Attendants. Viticultural students (2nd year) from Dookie Agricultural College

Title/topic:	Phylloxera: Biology, research and quarantine
Presenter:	Angela Corrie, David Brown
Location:	AV-Rutherglen, 11th July 1997
Audience:	20 growers from Tumbarumba and Tooma NSW (Southcoup growers)

Title/topic	Phylloxera: Research Highlights
Presenter:	Angela Corrie, David Brown, John Whiting, Robyn van Heeswijck
Location:	SHC Mildura

Audience:	50 attendaes including researchers, industry research collaborators and growers from Sunraysia region
Title/topic	Phylloxera: research highlights
Presenter:	Angela Corrie, David Brown, John Whiting, Greg Buchanan and Malcolm Campbell
Location:	Wangaratta TAFE College 17th September 1997
Audience:	70 growers from North East Victoria
Title/topic:	Phylloxera: Biology and management: component of Viticultural Course, The Centre Wangaratta
Presenter:	Angela Corrie
Location:	DNRE Ovens Research Station
Audience:	20 growers/students from North East Victoria
Title/topic:	Phylloxera: Biology and management: Component of viticultural Course the Centre Wangaratta
Presenter:	David Brown
Location:	Whitfield Victoria
Audience:	20 growers/students from North East Victoria
Title/topic:	Phylloxera: Biology and management
Presenter:	Jane Fisher, DeAnne Glen
Location:	Geelong 4th March 1998
Audience:	15 Growers from Geelong region
Title/topic	Phylloxera: Biology and management
Presenter:	Angela Corrie, Rebecca Dunstone
Location:	Yarra Valley Grapegrowers Expo. 16th May 1998
Title/topic:	Phylloxera biotypes and Rootstocks
Presenter:	Angela Corrie
Location:	Hunter Valley Vineyard Annual Seminar 27th May 1998
Title/topic:	Phylloxera awareness
Presenter:	David Brown, Rebecca Dunstone
Location:	Avenel, Mansfield Centre 21st March 1998
Audience:	30 potential vineyard investors/managers

Conference Posters/presentations:

Title:	Management of grape phylloxera in Australia
Authors:	David Brown and Angela Corrie
Conference:	The 28th Annual General Meeting and Scientific Conference of the Australian Entomological Society University of Melbourne 28th September- 3rd 1997. Looking ahead
Title:	Variability of phylloxera in Australian vineyards
Authors:	Angela Corrie, Greg Buchanan and Robyn van Heeswijck
Conference:	South Australia Viticultural Technical Conference September 1997

Title: Grapevine rootstock performance in some regions of Victoria.
Authors: Whiting, J. and D de Castella
Conference: Poster in Proceedings of the Tenth Australian Wine Industry Technical Conference. Eds R. J Blair , A N Sas, P F Hayes and P B Høj. Sydney, Australia, 2-5 August 1998. Australian Wine Industry Technical Conference Inc., Adelaide. p 266-7.

Title: Seasonal variation in phylloxera population behaviour and its
Authors: David Brown, Rebecca Dunstone and Angela Corrie
Conference: Tenth Australian Wine Industry Technical Conference, 3rd August 1998

Authors: Whiting, J., R Gardner, A Corrie and G Buchanan
Title: Monitoring phylloxera with aerial photography.
Conference: Poster in Proceedings of the Tenth Australian Wine Industry Technical Conference. Eds R. J Blair , A N Sas, P F Hayes and P B Høj. Sydney, Australia, 2-5 August 1998. Australian Wine Industry Technical Conference Inc., Adelaide. p 289.

Authors: Whiting, J.
Title: Monitoring phylloxera with aerial photography.
Conference: Breakout session at the Tenth Australian Wine Industry Technical Conference. Sydney, Australia, 2-5 August 1998.

Authors: Whiting, J. Rootstocks in southern Victoria.
Title: Workshop on Rootstocks for Cool Climates.
Conference: At Fifth International Symposium on Cool Climate Viticulture and Oenology. Melbourne, Australia. 16-20 January 2000.

Authors: Whiting, J. and M Everett.
Conference: Grapevine Improvement Workshops- Rootstocks Binder. Four workshops in June 1999.

Authors: Powell, K.S. and J. Whiting (Eds)
Title: Proceedings of the International Symposium on Grapevine phylloxera Management, January 21st 2000, Melbourne, Australia.

Authors: Powell, K.S., D. Brown, R. Dunstone, S.C. Hetherington, & A. Corrie.
Title: Population dynamics of phylloxera in Australian vineyards and implications for management.
Conference: *In:* Proceedings of the International Symposium on Grapevine Phylloxera Management, January 21st 2000, Melbourne, Australia.

Media articles:

Title:	Phylloxera Fight
Journal:	National Grapegrowers
Date:	August 2000
Title:	Phylloxera management under the microscope
Journal:	North East and Goulburn Murray Farmer
Date:	May 2000
Title:	Phylloxera Symposium
Journal:	National Grapegrowers
Date:	March 2000
Title:	Phylloxera talks for Melbourne
Journal:	The Weekly Times
Date:	January 2000
Title:	Phylloxera Symposium
Published	National Grapegrowers
Date:	March 2000
Title:	UK entomologist takes on Vic project to study phylloxera's life cycle
Journal:	National Grapegrowers
Date:	March 2000
Title:	Interaction with roots
Published	National Grapegrowers
Date:	March 2000
Title:	Australian Research
Journal:	National Grapegrowers
Date:	March 2000
Title:	New Scientist joins fight
Journal:	National Grapegrowers
Date:	March 2000
Title:	Phylloxera symposium highlights need for more research into vine-pest interactions
Journal:	The Australian Grapegrower and Winemaker
Date:	February 2000
Title:	Vine killer targeted
Journal:	The Land
Date:	February 2000
Title:	Aventis Crop Science represented at phylloxera workshop
Journal:	Barossa Valley Local paper
Date:	June 2000

Title:	Grapevine pest research should look at understanding vine-pest interaction
Journal:	North East Goulburn Murray Farmer
Date:	February 2000
Title:	Grape pest push- research to focus on phylloxera biology
Journal:	The Chronicle, Wangaratta
Date:	January 2000
Title:	War of the vineyard
Journal:	Country News
Date:	April 1997
Title:	Fighting Phylloxera
Journal:	Shepparton News
Date:	February 1998
Title:	Fighting Phylloxera
Journal:	Country News
Date:	February 1998
Title:	Wine growers warned of Pest
Journal:	Yarra Ranges Post
Date:	December 1997

APPENDIX 4: International Symposium on Grapevine Phylloxera Management (Presentation List)

Proceedings of the
International Symposium on
Grapevine Phylloxera Management

Melbourne, Victoria, Australia

January 21st, 2000

Edited by K.S. Powell and J. Whiting

SESSION 1: PHYLLOXERA RESEARCH IN AUSTRALIA

1. Population dynamics of phylloxera in Australian vineyards and implications for management

Kevin Powell, David Brown, Rebecca Dunstone, Sarah Hetherington and Angela Corrie

DNRE, Agriculture Victoria, Rutherglen, Victoria, Australia

2. Analysis of the interaction of phylloxera with susceptible and resistant grapevines using *in vitro* bioassays, microscopy and molecular biology

Kellow, A.V., Sedgley, M., McDonald, G and van Heeswijck, R.

Department of Horticulture, Viticulture and Oenology, Waite Campus, University of Adelaide, PMB 1 Glen Osmond; Cooperative Research Centre for Viticulture, Glen Osmond and Agriculture Victoria, Rutherglen, Australia.

3. Keeping Phylloxera at Bay: enhancing protective behaviours and industry practices in a phylloxera free region

R. Reynolds

Flinders University of South Australia

SESSION 2: PHYLLOXERA RESEARCH IN THE USA

4. Status and progression of infestations and management of the grape phylloxera in the Pacific Northwest, USA.

Fisher JR and Hellman E.

USDA,ARS,HCRL, Corvallis, Oregon,USA & NWREC, Oregon State University, Aurora, Oregon, USA

5. Interactions between grape phylloxera and fungal infections of grape roots

A. Omer

Entomology Department, University of California, Davis

6. Progress toward phylloxera IPM

J. Granett

Entomology Department, University of California, Davis

SESSION 3: PHYLLOXERA RESEARCH IN EUROPE

7. Grape cultivar and phylloxera isolate as two factors of vine susceptibility in Hungary

Kocsis, L. Horvath, L.; Kozma, P. jr. and Pinter, Cs.

University of Agricultural Sciences, Georgikon Faculty of Agronomy, Dept. of Horticulture, Dept. of Entomology and Viticultural and Enological Research Station, Eger, Hungary

8. Influence of N-fertilization on the development of Phylloxera root damage in laboratory and field trials

Kopf, A & K.J Shirra

Staatliche Lehr - und Forschungsanstalt für Landwirtschaft, Weinbau und Gartenbau („Neustadt Research Center"), Neustadt/Weinstraße, Germany.

9. Current Problems With Phylloxera On Grafted Vines In Germany And Ways To Fight Them

M. Porten, J. Schmid, Ernst H. Rühl.

Institute for Viticulture and Grapevine Breeding, Geisenheim Research Centre, Eibinger Weg 1, 65366 Geisenheim - Germany

SESSION 4: PHYLLOXERA RESEARCH IN SOUTH AFRICA

10. A Review Of Phylloxera Research In South Africa

C.A. De Klerk

ARC-Fruit, Vine and Wine Research Institute, P/Bag X5026, 7600 Stellenbosch, South Africa

REVIEW PAPERS*

11. Phylloxera Problem On The Don

Agapova S.I and Kostrikin I.A.

All-Russia Research Institute for Viticulture and Winemaking, Baklanovsky av., 166., Rostov region, Novocherkassk, Russia

12. The grape phylloxera, *Daktulosphaira vitifoliae*, in Ontario

A.B. Stevenson

Agriculture and Agrifood Canada, Vineland Station, Ontario - (retired) 26 McKenzie St., St. Catharines, Ontario, Canada, L2M 2N1, asteven1@becon.org

** PAPERS NOT PRESENTED*

Appendix 5: Taking Aim at Phylloxera (research Leaflet)

TAKING AIM AT PHYLLOXERA

PHYLLOXERA RESEARCH ACTIVITIES AT AGRICULTURE VICTORIA - RUTHERGLEN

TEAM

- Kevin Powell
- Jo Deretic
- Sarah Hetherington

OBJECTIVES

- To make a significant contribution to the growth and long term viability of the Australian viticultural industry by minimising the potential impact of grape phylloxera.
- To provide a means for the sustained control of phylloxera through durable resistant rootstocks.
- To minimise the spread of phylloxera between vineyards and districts through understanding modes of dispersal of the pest.

RESEARCH FOCUS

The focus of the team is to provide a scientific basis for quarantine protocols, and to develop early detection procedures and tactical management options. The research aims to ensure that grapevine resistance remains a durable means of phylloxera control through understanding the molecular basis of grapevine resistance and immunity, and the potential of the insect to overcome resistance mechanisms within rootstocks.

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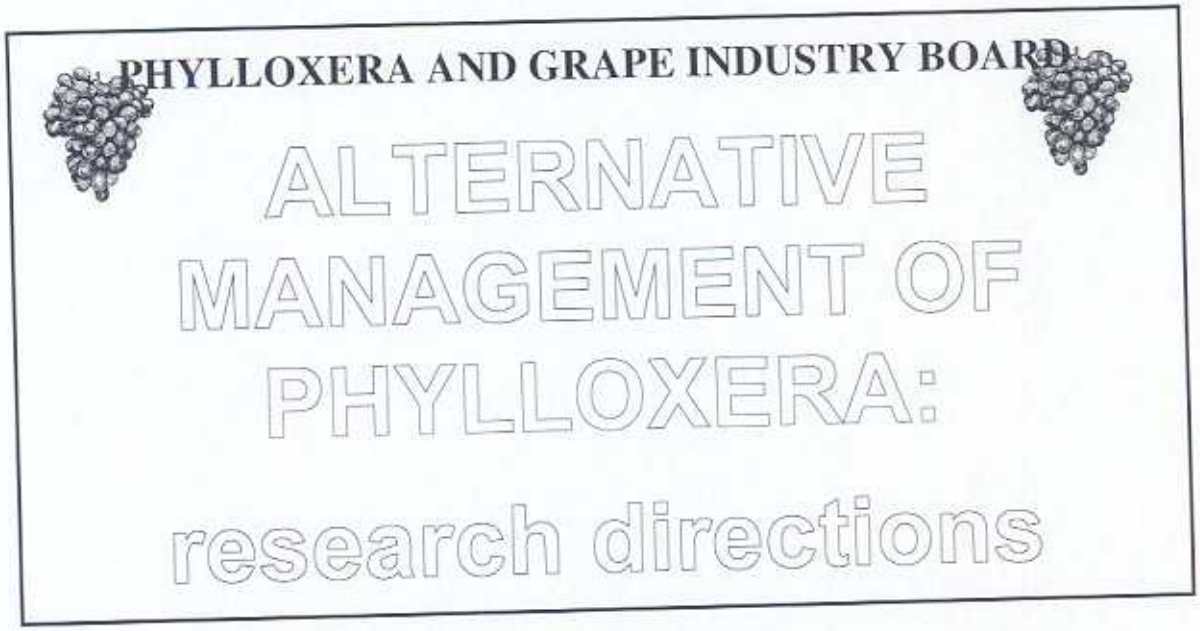
Appendix 6: Alternative Management Leaflets



Angela Corrie, the project Leader of Agriculture Victoria Rutherglen's Phylloxera Research Program, conducted a study tour of Western Europe, Eastern Europe and the USA during 1998. The main aim of her tour was to review the research that had been conducted in these countries into phylloxera control. This fact sheet summarises her key findings in the area of alternative management for phylloxera and will be of particular interest to the Australian grape industry. A complementary fact sheet covers recent research directions in rootstock control of phylloxera.

Alternate methods of phylloxera management

The success of rootstocks in preventing damage by phylloxera has resulted in limited research in the past into other possible control methods. However, there has been some recent research into alternative management options, which could be very important for Australia given that it has large areas of ungrafted vines protected from phylloxera by quarantine measures only.

The alternative measures include chemical, biological and cultural methods.

Chemical control

Chemical control of phylloxera has been unsatisfactory in the past, primarily because of the poor penetration of the chemical into the soil.

Carbofuran is one of the few chemicals that have substantial impact on phylloxera. It is being used near Salinas in California to prevent phylloxera damage on both ungrafted and ARG#1 rootstock. Two applications are commonly used, one in early spring coinciding with the initial flush of root growth, and the other post harvest.

There are currently field trials under way at the University of California, Davis examining a new chemical for the control of phylloxera. The chemical's name and active ingredient cannot be reported

due to confidentiality agreement restrictions. Preliminary results however indicate that the chemical is having a large impact on phylloxera. Whether the chemicals will actually slow vine decline has not yet been determined, and their effect on other soil invertebrates and soil microbial populations is unknown.

With increasing public scrutiny on the use of agricultural chemicals, any future developments will need to rely on a detailed knowledge of phylloxera biology and the phylloxera-plant interaction. This will ensure the chemicals can be targeted to maximise control of the pest, while minimising chemical costs and use.

Biological control

There appears to be very little detailed research on potential control agents for phylloxera. The only known laboratory trial on biological agents currently is with nematodes at Cornell University (with limited success).

Cultural management

Several research projects are currently being conducted world wide focusing on reducing phylloxera damage in infested vineyards by manipulating the soil environment.

Although most of these projects are still not conclusive and need further clarification some interesting preliminary findings have been made.

Research with potted grapevines at the University of California, Davis, suggested that secondary fungal infections of phylloxera infested vine roots accelerated vine decline. The increased level of vine damage is thought to be due to insects causing wounds, providing non- pathogenic fungi and other microbes an entry point in the plant and reducing growth. It is believed that the impact of secondary infections becomes intensified as phylloxera-stressed vines need more water, which in turn favours the development of these pathogens.

These preliminary results have triggered a series of large-scale field trials to determine whether differences in phylloxera populations, or phylloxera-related damage were based on manageable soil characteristics. These types of studies are logistically difficult and to date have not obtained statistically significant results.

PHYLLOXERA AND GRAPE INDUSTRY BOARD

ROOTSTOCK CONTROL OF PHYLLOXERA: research directions

Angela Corrie, the project Leader of Agriculture Victoria Rutherglen's Phylloxera Research Program, conducted a study tour of Western Europe, Eastern Europe and the USA during 1998. The main aim of her tour was to review the research that had been conducted in these countries into phylloxera control. This fact sheet summarises her key findings in the area of rootstock control for phylloxera and will be of particular interest to the Australian grape industry. A complementary fact sheet covers recent research directions in alternative management of phylloxera.

Rootstocks for control of phylloxera

Breeding programs seeking high phylloxera resistance are concen-trating on the use of members of the *Vitis* species other than *V. vinifera*. *Vitis cinerea* and *Vitis rotundifolia* are recognised for their high resistance /immunity to phylloxera and other soil borne pests including *X. index*. Unfortunately these *Vitis* species are also well known for their poor propagation (poor rooting, callus formation and cane yield), lime intolerance and grafting incompatibility.

Breeders throughout Europe have constructed numerous combinations of *Vitis* hybrids including some *V. vinifera* because it improves grafting compatibility of the rootstock with the *V. vinifera* scion, and rootstocks with *V. vinifera* in their parentage are easier to propagate. However, they have concluded that rootstocks with any *V. vinifera* in their parentage should not be used in phylloxera infested soils due to the risk that their resistance to phylloxera will fail (as occurred with AXR (*V. vinifera X V. Rupestris*) in California in the early 1990s.

The majority of rootstocks available today exhibit tolerance rather than resistance. This is a major concern as tolerant rootstocks allow phylloxera to infect the fibrous root system. Although such plants are largely asymptomatic, the ongoing presence of insects provides a source of phylloxera that can spread and infest new vineyards. Additionally such infestations provide the opportunity for more virulent strains to develop.

There is currently a great deal of interest in rootstocks with *V. cinerea* in their parentage as this vine type expresses "true" resistance to phylloxera in that no phylloxera develops to reach adulthood.

Boerner, a hybrid of *V. cinerea* var. Arnold*V. riparia* is the only commercial rootstock available with very high resistance to phylloxera.

Boerner performs particularly well under dry conditions and appears to express chlorosis under very wet soil conditions. As Boerner is becoming more widely planted and exposed to different environmental conditions and possibly different strains of phylloxera there have been some reports of

phylloxera root and leaf galls. This galling however seems to be very uncommon and strongly linked to locations where there is a lot of cloud cover, and restricted hours of sunlight.

At present Boerner is not commercially available in Australia. Negotiations between the Australian Vine Improvement Association (AVIA) and the Geisenheim Research Station, Germany, are currently underway for its use by the Australian viticulture industry

Preliminary research undertaken in Australia indicates that the phylloxera biotype tested cannot establish on the roots of Boerner. Some cultures of different phylloxera types from Australia have been left in Germany so that Giesenheim can assist in screening potential new hybrids for use in Australia using tissue culture techniques.

Screening for rootstock resistance

The various screening methods used for assessing rootstock resistance to phylloxera include overall vine health, root evaluation, leaf evaluation and root health.

However, there are inconsistencies between the screening methods used in different countries. This highlights the danger of relying solely on overseas screening results for selecting rootstocks for use in Australia and identifies the need to screen all new rootstocks used under local conditions, particularly against strains of phylloxera identified in Australia.

Strains of grape phylloxera

To ensure that rootstocks are a long term option it is important to establish if the genetic variation observed in commercial vineyards is stable or changing. Understanding the potential of phylloxera populations to evolve is also critical to the successful development and implementation of alternative management options for phylloxera on ungrafted vines.

Several research projects have been established to look specifically at the mechanisms by which genetic variation occurs and at what stages of the insect's lifecycle it occurs in commercial vineyards. Most findings to date have been inconclusive although there are indications that there are different strains and biotypes of phylloxera.

Samples from the *vitis* hybrid Schwarzmann in France indicate a very similar DNA pattern to phylloxera sourced from Schwarzmann in Rutherglen, Australia. Although the study is limited and requires further research it does indicate that France was the most likely source of infestation in Rutherglen.

Copies of Angela Corrie's Overseas travel report titled "Grape Phylloxera, Overseas Travel Report June 26-October 15, 1998" and the complementary fact sheet on alternative management of phylloxera are available on request from the Phylloxera and Grape Industry Board of SA. Telephone: 8226 0430

APPENDIX 7: Phylloxera & Grapecheque Telephone Survey Form

Interview Number ☐

GRAPECHEQUE/ PHYLLOXERA TEAM EVALUATION SURVEY.

Printed name, address and phone number goes here. ←

Good evening, my name is ..... from the Department of Natural Resources and Environment (Department of Agriculture). Could I speak with the person who manages the vineyard please?

Male

Female

If a new person comes to the phone, repeat the introduction.

We recently sent you a letter informing you about a phone survey we are conducting to determine if our viticultural programs are meeting your needs.

To help us plan our future work we would appreciate it if you could spend about 10 minutes answering some questions. Any information you give us will be kept confidential.

Are you available to do that now?

Yes ☐ Go to Q1.

No or hesitate **Would you prefer us to call at another time?**

Yes. When?.....

No. *Finish.* **Thank you for your time.**

Phylloxera

First I would like to ask you some questions about grapevine phylloxera. Are you familiar with this pest? *If the answer is yes, go to question 1, if respondent doesn't know what phylloxera is, and asks for further information, read the following;*
Phylloxera is an aphid-like insect, which originated from North America. Phylloxera lives on the roots and sometimes the leaves of grapevines, causing the formation of galls. Phylloxera is a devastating insect to own-rooted grapevines, causing vine decline and eventually death. Go to Q 7.

1. Has anyone from your vineyard have attended a phylloxera field workshop or seminar?
   - ❑ Yes **Go to Q2**
   - ❑ No **Go to Q5**
   - ❑ Don't Know **Go to Q5**

   Detail _____

2. How many people have attended a workshop?
   Number _____ **Go to Q3**
   Don't know _____ **Go to Q3**

3. What year was the workshop held?
   - ❑ Year_____ **Go to Q4**
   - ❑ Years ago ____ **Go to Q4**
   - ❑ Don't know **Go to Q4**

4. What benefit, if any, did you get from attending the phylloxera workshop?

   _____
   _____
   _____
   _____

   Go to Q7

5. Is there any reason that you haven't attended a workshop? (*Tick more than one answer if applicable*)
   - ❑ Too expensive **Go to Q7**
   - ❑ Workshops held too far away **Go to Q6**
   - ❑ Timing **Go to Q6**
   - ❑ Not needed **Go to Q7**
   - ❑ Didn't know about them **Go to Q6**
   - ❑ Other (please specify) **Go to Q6**
   - ❑ No **Go to Q6**

6. Would you attend a workshop held in your region?
   - ❑ Yes
   - ❑ No
   - ❑ Maybe **Go to Q7**

7. Do you have quarantine signs erected at your vineyard?
   - ❑ Yes **Go to Q8**
   - ❑ No **Go to Q11**
   - ❑ Don't know **Go to Q11**

8. Where did you get your quarantine sign?
   - ❑ DNRE **Go to Q9**
   - ❑ PGIBSA **Go to Q12**
   - ❑ Home made/local association **Go to Q12**
   - ❑ Other (please specify) **Go to Q12**

9. Are you satisfied with the DNRE sign?
   - ❑ Yes **Go to Q10**
   - ❑ No **Go to Q10**

10. Do you have any suggestions to improve the signs? _____
_____
_____
_____
_____

Go to Q12

11. Is there a reason you don't have any quarantine signs? (*tick more than one answer if applicable*)
   - ❑ Too expensive
   - ❑ Didn't know they existed
   - ❑ Didn't like the designs available
   - ❑ Lack of availability
   - ❑ Other (please specify) _____
   - ❑ Don't need them **Go to Q12**

12. What precautions do you take to prevent the introduction of phylloxera into your vineyard? (*prompt, tick more than one answer if applicable*)
   - ❑ Monitor traffic onto and within the vineyard **Go to Q13**
   - ❑ Footbath **Go to Q13**
   - ❑ Other (please specify) _____
_____
_____ **Go to Q13**
   - ❑ None **Go to Q15**

(If they mention any precautions, slip it into the space in the question below)

13. You mentioned _____, when did you implement this practice at the vineyard?
   - ❑ Year _____
   - ❑ No. of years ago _____
   - ❑ Don't know

14. What events prompted you to implement this practice? _____

_____

_____

_____

_____

15. What area of vines do you currently have planted? _____Ha/Ac
(circle which)

16. When were the first vines planted? _____Years ago/Date
(circle which)

17. How much of your vineyard is planted with phylloxera-resistant rootstock?
_____ Ha/Ac *(circle which)* or _____%
Go to question 18 if greater than 0, question 19 if =0

18. When were rootstocks first planted in the vineyard? _____ Year/Date
Don't know

19. Are you planning to expand the vineyard in the next 2 years?
- ❑ Yes **Go to Q20**
- ❑ No **Go to Q21**
- ❑ Maybe **Go to Q20**

20. Will further plantings be on phylloxera tolerant rootstock? (*prompt*)
- ❑ All
- ❑ None
- ❑ Some
- ❑ Don't know

21. Do you want more information on phylloxera?
- ❑ Yes *if yes, read below before going onto grapecheque*
- ❑ No *Go to next section*

I'll send you out an information package on phylloxera.

Grapecheque information.

The next group of questions I would like to ask you are about an extension program called grapecheque.

22. Are you aware of the DNRE grapecheque viticulture extension program?
- ❑ Yes **Go to Q23.**
- ❑ No **Go to Q30**

If the respondent asks for more information about grapecheque- read out the following:

Grapecheque is a state funded viticultural extension program. Groups of growers from different regions get together and address current production issues. The growers identify specific areas of grape production they would like more information on, such as crop forecasting and irrigation. Experts and guest speakers are brought to the group to present information.

23. Do you participate in Grapecheque activities?
    - ☐ Yes **Go to Q24**
    - ☐ No **Go to Q33**

24. How many grapecheque meetings have you attended in the last 12 months?
    - ☐ None **Go to Q33**
    - ☐ 1-2 **Go to Q25**
    - ☐ 3-4 **Go to Q26**
    - ☐ 5 or more **Go to Q26**

25. What benefit, if any, did you get from attending grapecheque meetings?

    _____
    _____
    _____
    _____
    _____

26. What part of Grapecheque meetings do you prefer? *(prompt)*
    - ☐ Vineyard walk
    - ☐ The guest speaker
    - ☐ Meeting and chatting with the other growers
    - ☐ Other (please specify)

27. If Grapecheque became a commercial service how much would you be prepared to pay to attend Grapecheque meetings?
    - ☐ $_____
    - ☐ $0

28. Is there anything that could be improved with the Grapecheque meetings?

    _____
    _____
    _____
    _____
    _____
    _____

More general background information

Before we finish, I'd like to ask you some general questions about your vineyard.

29. How many years of experience do you have at growing vines?.....years

30. Do you read industry publications?

- ☐ Yes **Go to Q35**
- ☐ No **Go to Q37**
- ☐ Sometimes **Go to Q35**

31. I'm going to list some viticultural publications, could you check off which ones you read please?

- ☐ Australian Grape Grower and Winemaker
- ☐ South Australian Grapegrowers
- ☐ Australian Viticulture
- ☐ Australian and New Zealand Wine Industry Journal
- ☐ Victorian Viticulture News
- ☐ Grape and Wine R & D Corporation Highlights
- ☐ Others *(please specify)* _____

32. Do you use a professional adviser or consultant?

- ☐ Yes **Go to Q37**
- ☐ No **Go to Q38**

33. Which advisers do you use?

- ☐ Consultants
- ☐ Government staff
- ☐ Other *(please specify)* _____

34. I'm going to list some other sources of information, could you check off those you use?

- ☐ Local association
- ☐ Fellow growers
- ☐ Industry reps
- ☐ University/ TAFE
- ☐ Company viticulturalist
- ☐ Internet
- ☐ Other (*please specify*) _____

A summary of results of this survey will be compiled and published in the Australian Grapegrower and Winemaker in the near future. Thankyou very much for your time and assistance.

Finish.

Appendix 8: Phylloxera paper presented at the International Symposium on Grapevine Phylloxera Management

Population dynamics of phylloxera in Australian vineyards and implications for management

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Abstract

Field monitoring was conducted in commercial vineyards over successive seasons to determine the population dynamics and potential for spread of grapevine phylloxera *Daktulosphaira vitifoliae* (Fitch) in the King Valley region of NE Victoria. Populations were relatively low in the spring (September-November), reached a peak during early summer (January-February) and declined from mid to late summer (April-May). Populations were monitored both below and above ground using a variety of techniques. To quantify below ground populations, root surveys were conducted based on a visual estimate of phylloxera infestation levels. Phylloxera dispersive stages, in the form of crawlers and alates, were monitored using emergence, pitfall, trunk and aerial traps to quantify above ground levels throughout the season. This enabled the identification of critical periods when management practices could be modified in order to prevent man-assisted dispersal within and between vineyards. Canopy populations were also monitored extensively during February to March (veraison) using both destructive and non-destructive techniques. Canopy levels were relatively low in comparison with phylloxera levels recorded emerging from the soil and moving up the vine trunk. The significance of this study in relation to phylloxera management is discussed.

Introduction

Grapevine phylloxera (*Daktulosphaira vitifoliae* Fitch) has been present in Australia since 1877 (Buchanan, 1987) and has successfully been contained in discrete regions of the country. Phylloxera is currently only found within Phylloxera Infested Zones (PIZs) in the SE states of Victoria and New South Wales. Up until very recently phylloxera was only found in quarantine areas termed phylloxera infested zones or vine disease districts. Since the establishment of quarantine boundaries they have been redefined based on ground surveys (Buchanan, 1987) and the discovery of new infestations. In 1991 in a vineyard in the King Valley region of NE Victoria

phylloxera appeared outside the existing vine disease district resulting in an extension of the quarantine boundaries.

In Australia the asexual radicicolae or root dwelling stage of the phylloxera life cycle is the predominant form and although the sexual leaf galling or gallicolae stage has been observed in discrete regions, its occurrence is relatively rare. The main dispersive stages of phylloxera are the first instar (crawler) and alates. The spread of phylloxera within and between vineyards is associated with the movement of these dispersive stages either naturally or through man-assisted movement, for example on vine planting material, equipment or personnel.

Australia is one of the few wine producing countries which remains predominantly (around 85%) on ungrafted vineyards making them potentially a target for spread of phylloxera should existing quarantine protocols breakdown. Understanding the population dynamics and rate of spread of phylloxera infestations is essential for the effective development of quarantine protocols and management strategies. This paper forms part of a three-year study and preliminary results are presented here on the population dynamics of phylloxera over two successive seasons. The study was conducted within two infested ungrafted vineyards in the King Valley region of Australia. This research is a sub-component of a larger research project focussing on the management of phylloxera in SE Australia.

Field based research on phylloxera population dynamics in Australian vineyards has been scarce with studies confined to areas in the Nagambie and Milawa regions of Victoria (King and Buchanan, 1986; Helm, 1983; 1991) and the Sydney region of NSW (Helm, 1983; 1991). This is the first reported study describing the seasonal abundance of phylloxera over consecutive seasons on commercial vineyards in the King Valley region.

Materials and Methods

1. Site selection

Studies on seasonal population dynamics were conducted at two established commercial vineyards in Cheshunt and Whitlands in the King Valley region of Victoria. Studies commenced in September 1997. The vineyard at Cheshunt was planted with ungrafted *Vitis vinifera* and the infestation was estimated to have been around two years old. The Whitlands vineyard was planted in blocks of ungrafted *Vitis vinifera* and *V.vinifera* grafted onto phylloxera-resistant rootstocks and phylloxera was present in the vineyard since the late 1980s. Phylloxera populations at the two sites were studied for two successive seasons during 1997-1999. Studies on canopy population levels prior to harvesting were also conducted at both field sites during the second season. Relative canopy levels were determined by two days sampling at each site using destructive and non-destructive techniques. Soil cores were taken at both trial sites and characterised as a mesotrophic brown chromosol at the Whitlands site, and as a dystrophic brown chromosol at the Cheshunt site (Slattery, pers. comm.). Metrological data was collected over the trial periods at both sites.

2. *Experimental design*

All trials were conducted in study blocks of own rooted (ungrafted) *Vitis vinifera* vines. At the Cheshunt site three adjacent rows of ungrafted *Vitis vinifera* L. cv. Sauvignon Blanc were selected. Inter-row spacing was 3m and inter-vine spacing 1.8m. Every fifth vine in each row was selected for sampling and a total of five vines in row 1, four in row 2 and three in row 3 were sampled.

At the Whitlands site during the first season a study block of eight rows of ungrafted *Vitis vinifera* cv. Sauvignon Blanc, bordered east and west by blocks of *V.vinifera* grafted onto the phylloxera-resistant rootstock Schwarzmann (*V. rupestris* x *V. riparia*), was selected. Inter-row spacing was 1.7m and inter-vine spacing 1.0m. Four alternate rows were selected and a single vine from panels 4, 6 and 8 in each row was sampled. Each panel contained 7 vines. At the end of the first season at the Whitlands site the block was up-rooted and a block of ungrafted Chardonnay (*Vitis vinifera*), bordered east and west by blocks of *V.vinifera* grafted onto Schwarzman, was selected for the second seasons study.

Sampling techniques

1. *Estimation of root infestation levels*

Root populations were monitored biweekly on single vines either side of each sampled vine in order to avoid disturbing phylloxera populations. Root levels were monitored by digging and exposing vine roots and using a visual damage rating scale for fibrous and storage roots separately (Table 1). The average of the two vines provided an infestation index for the sample vine.

Table 1: Phylloxera infestation rating scale for *in situ* root surveys.

Infestation rating	Fibrous root symptoms	Storage root symptoms
0	No nodosities, no phylloxera	No tuberosities, no phylloxera
1	Nodosities rare, isolated phylloxera	Isolated phylloxera
2	Nodosities obvious, phylloxera in groups	Phylloxera in groups
3	>20% nodosities	Reproducing colonies common
4	>50% nodosities	Phylloxera abundant

2. *Emergence sampling*

The seasonal abundance of first instars (crawlers) and alates emerging from below ground onto the soil surface was measured at both study sites. Twelve emergence traps were placed adjacent to the trunk of each sample vine. Traps consisted of translucent plastic containers, 22cm diameter and 13cm deep, open at one end, which was inverted onto the soil surface. Emerging phylloxera were trapped in condensation on the container sides. At fortnightly intervals insects were removed by washing with 70% ethanol and collected in plastic containers. Traps were then rinsed with tap water and replaced. Collected insects were counted using a low power binocular microscope.

3. *Soil surface sampling*

Throughout the second season of the study, at both sites, phylloxera dispersal over the soil surface was quantified with the use of pitfall traps. Single traps, consisting of 250ml plastic beakers (8cm diameter and 9cm deep) were placed in a hole in the ground adjacent to each of the twelve sample vines. Phylloxera were trapped in a 1:1 collecting fluid solution of ethyl glycol and water. At biweekly intervals traps were removed, rinsed with 70% ethanol and fresh collecting fluid was added.

4. *Vine trunk sampling*

A study of phylloxera movement up and down vine trunks was carried out at both sites over two seasons. Trunk traps consisted of two bands of white electrical insulation tape, 1.5cm wide and 17cm long, coated with Tanglefoot™ wrapped around each of the twelve vine trunks. The bands were placed on the trunks 25cm from the vine base to collect phylloxera moving up the vine trunk and 35cm from the vine base to collect phylloxera moving down the vine trunk off the canopy. At biweekly intervals trunk traps were removed and covered with clear plastic wrap (Gladwrap™) to prevent contamination and facilitate handling and storage. Collected trunk bands were examined using a low power binocular microscope.

5. *Aerial sampling*

Aerial traps were used at the Whitlands site, over two successive seasons, to quantify the risk of wind dispersal of phylloxera life stages. Twelve traps were arranged in grafted vine blocks on either side of the own rooted sample block to determine dispersal away from the infested sample area. Double traps were set up in four alternate rows located at panels 6, 8 and 10. Each trap consisted of an arch-shaped metal sheet, painted yellow and covered on each surface with an acetate sheet (21cm x 29cm) coated with Tanglefoot™. Traps were mounted 40cm above the vine canopy on a metal stake fixed onto a trellis post. Traps were orientated alternately within each row in either an EW or NS direction. Acetate sheets were removed at three weekly intervals, placed on grid-lined paper, covered with Gladwrap™ and examined microscopically.

6. Destructive canopy sampling

Foliar and bunch samples were collected randomly from four infested vine rows at each site. A total of 200 grape bunches, 600 leaves and 100 shoots were collected in clip seal plastic bags. Samples were collected early in the morning and transported in sealed containers to the laboratory for same-day analysis. Samples were washed in four washes of water (+1% Teepol) and washings were sieved and examined microscopically for the presence of phylloxera. The surface area and fresh/dry weight of sampled leaves and shoots was calculated using a Paton electronic planimeter and by oven drying samples for 24 hours at 50°C. Fresh weight of grape bunches was also recorded.

7. Non-destructive canopy sampling

Three methods of non-destructive sampling were carried out using a D-Vac Knapsack suction sampler; a modified hand-held Black and Decker Dustbuster suction sampler and canopy beating. Using the hand-held device a total of 200 leaves were sampled randomly from within 5 infested panels of four vine rows. Using the D-Vac sampler 5 panels of vine canopy in each of four infested rows were sampled. The canopy beating method involved gently tapping 2 vine branches in each of 20 infested panels and collecting dislodged insects on a white plastic beating tray. All canopy samples were collected into 70% ethanol and examined using a low powered binocular microscope.

Results

1. Root infestation levels

At both sites root infestations were characterised by single peaks of activity on both storage (main) roots and fibrous roots (Figure 1). Infestation levels on storage roots were marginally higher than on fibrous roots and the peak of activity on storage roots was later in the season than for that of fibrous roots. On fibrous roots the peak infestation level was in the spring (December -January) whereas on the storage roots it occurred in the summer months (February-April). Infestation levels were higher at the Whitlands site during the 1997-98 season with all sample vines suffering phylloxera damage. The study block was so badly infested that it was removed by vineyard management. In the first season at the Cheshunt site only 42 percent of the sample vines showed visible signs of infestation but during the second season phylloxera had spread to all sample vines and root damage was extensive (data not presented).

2. *Emergence of phylloxera from the soil*

At both sites dispersive stages, predominantly crawlers and to a lesser extent alates, were trapped between September-June. The duration of phylloxera emergence was longer at the Whitlands site than the Cheshunt site. At the Whitlands site during both seasons phylloxera emergence from the soil commenced in early to mid December (Figure 2a) corresponding with an increase in root infestation levels (Figure 1a). By mid-March levels had declined dramatically, corresponding with a decline in infestation levels on fibrous roots. In the second season phylloxera levels caught in emergence traps were relatively low because the Chardonnay block was not as badly infested as the Sauvignon block with only 69% sample vines infested compared with 100% in the Sauvignon block the previous season.

In the first season at the Cheshunt site phylloxera emergence from the soil commenced in late December (Figure 2b), corresponding with an increase in fibrous root infestation (Figure 1b). By mid-February emergence levels had declined dramatically, corresponding with the decline in infestation levels on fibrous roots. In the following season emergence levels increased later in the season (early January) followed by a subsequent decline by mid-March (Figure 2b).

3. *Movement of phylloxera over the soil surface*

During the second season, at both sites, phylloxera dispersal (predominantly in the form of crawlers) over the soil surface was quantified with the use of pitfall traps. Movement over the soil surface at both sites occurred from early January to early April (Figure 3). At the Cheshunt site, where the block was more heavily infested (all sample vines infested), the population of trapped phylloxera at its peak was up to six-fold higher than at the lesser-infested (69% sample vines infested) Whitlands site.

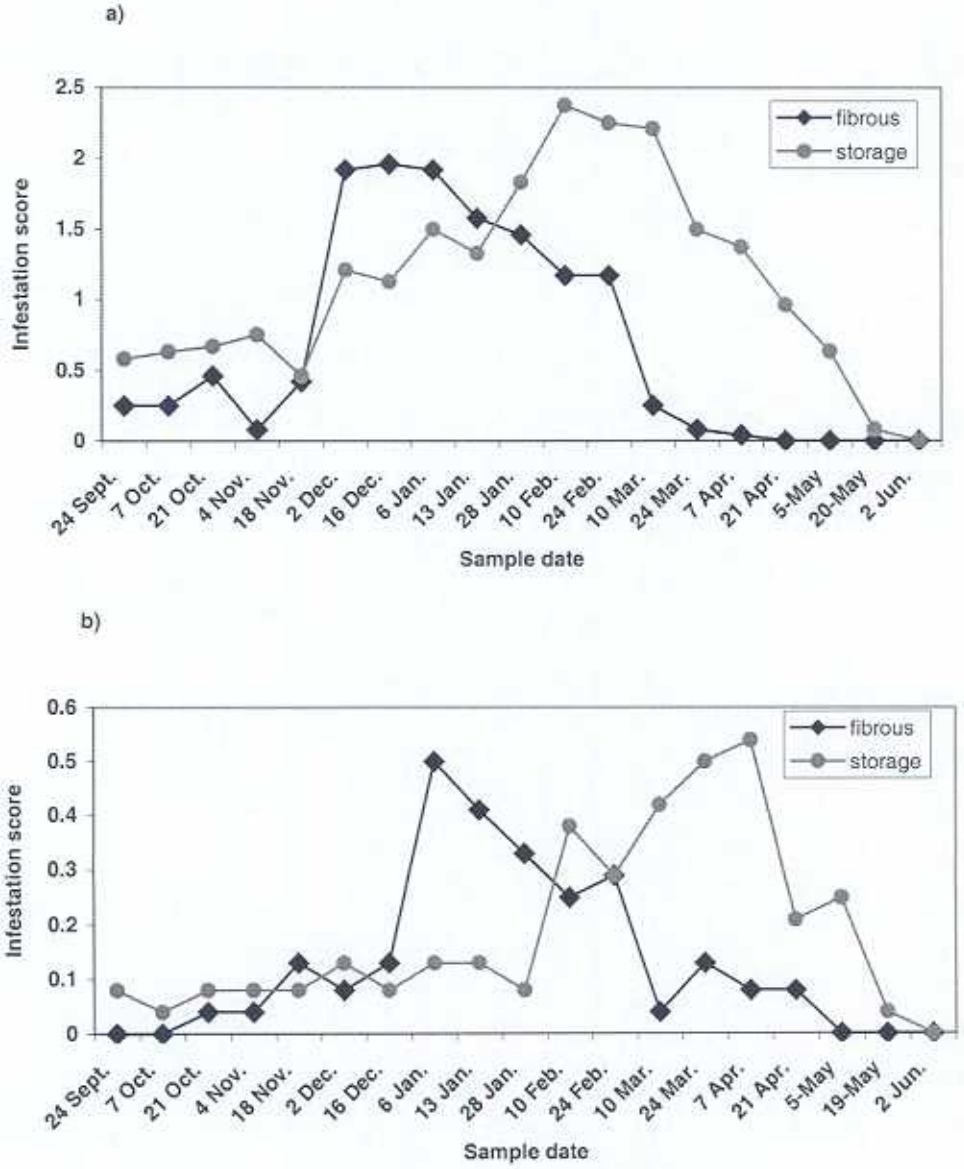

Figure 1: Seasonal variation of phylloxera infestation levels on fibrous and storage roots of *Vitis vinifera* in commercial vineyards at (a) Whitlands and (b) Cheshunt in the King Valley, 1997-98.

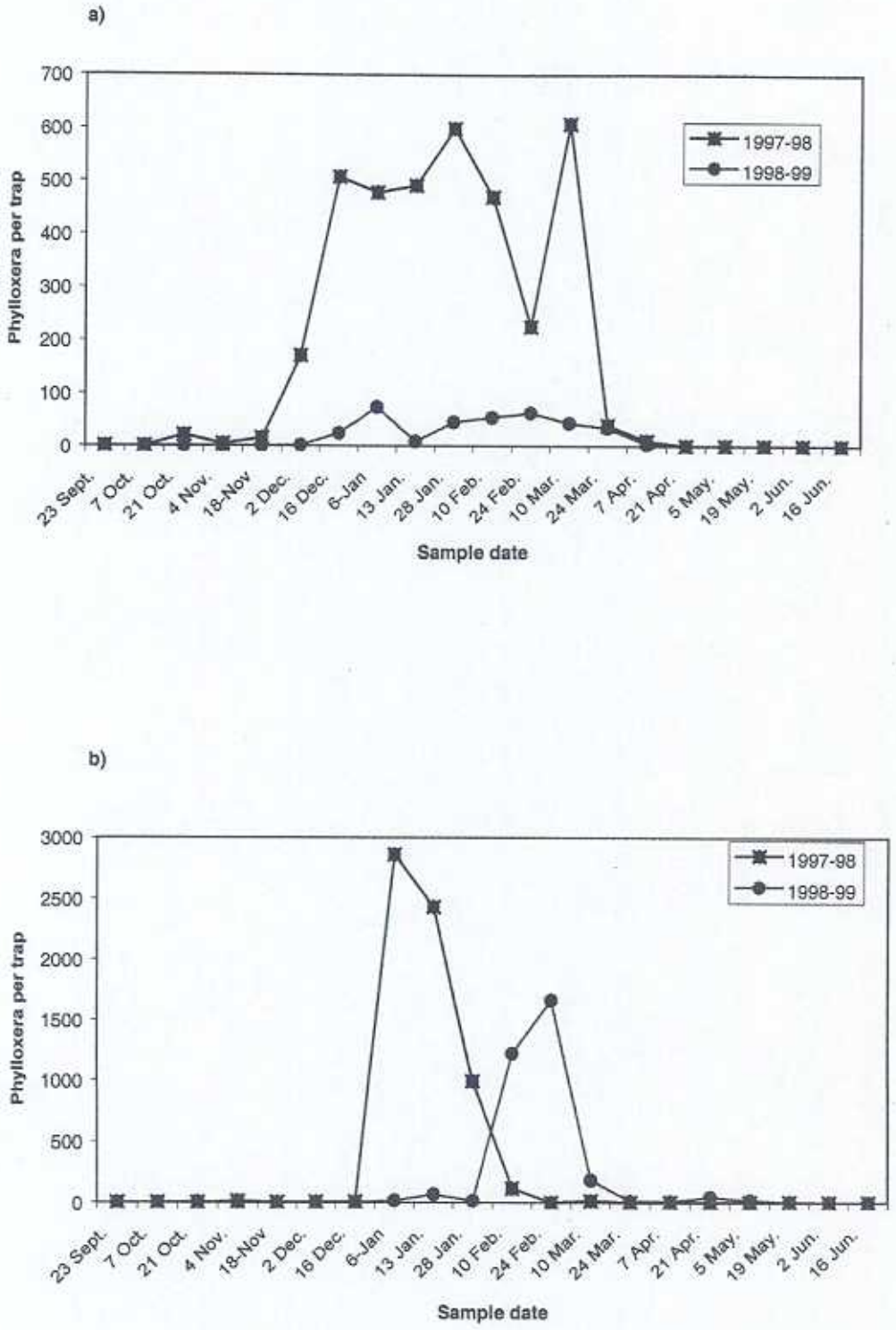

Figure 2: Seasonal variation of phylloxera dispersive stages emerging from the soil in phylloxera infested commercial vineyards at (a) Whitlands and (b) Cheshunt in the King Valley over two consecutive seasons 1997-99.

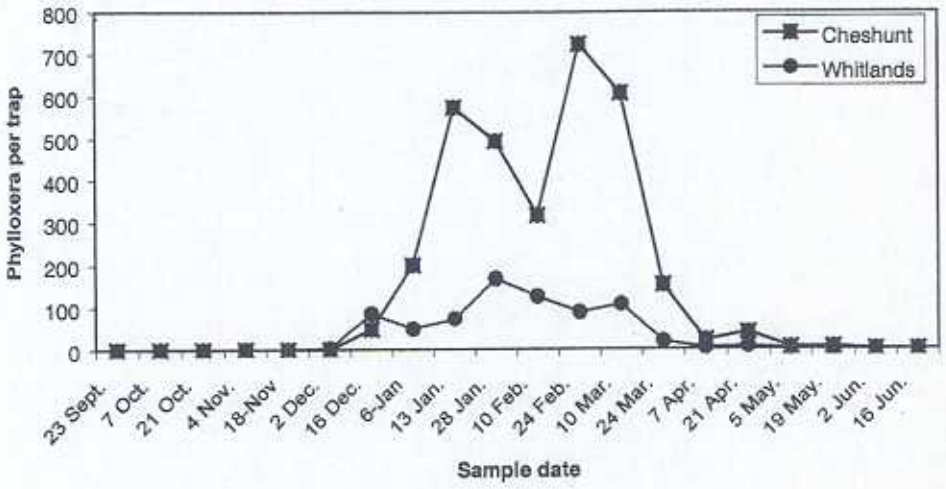

Figure 3: Seasonal variation of phylloxera dispersive stages moving across the vineyard soil surface in phylloxera infested commercial vineyards at Whitlands and Cheshunt in the King Valley during the 1998-99 season.

4. Movement of phylloxera on vine trunks

Vine movement from the soil surface up the vine trunk was markedly higher than movement down the vine trunk at both sites over both seasons (data not presented). At the Whitlands site, over both seasons, movement up the vine trunk, towards the canopy, commenced in early-December (Figure 4a) and steadily declined by early April. This correlates with the emergence of phylloxera from the soil (Figure 2a). Phylloxera movement on vine trunks at the Cheshunt site in the first season was later (Figure 4b) commencing in late December/early January and declining by early April. Occurrence of phylloxera on the trunk correlated well with emergence, pitfall and root infestation data (Figures 1, 2 and 3). Phylloxera levels recorded on vine trunks at the Cheshunt site were higher than those recorded at the Whitlands site due to higher levels of infestation at this site.

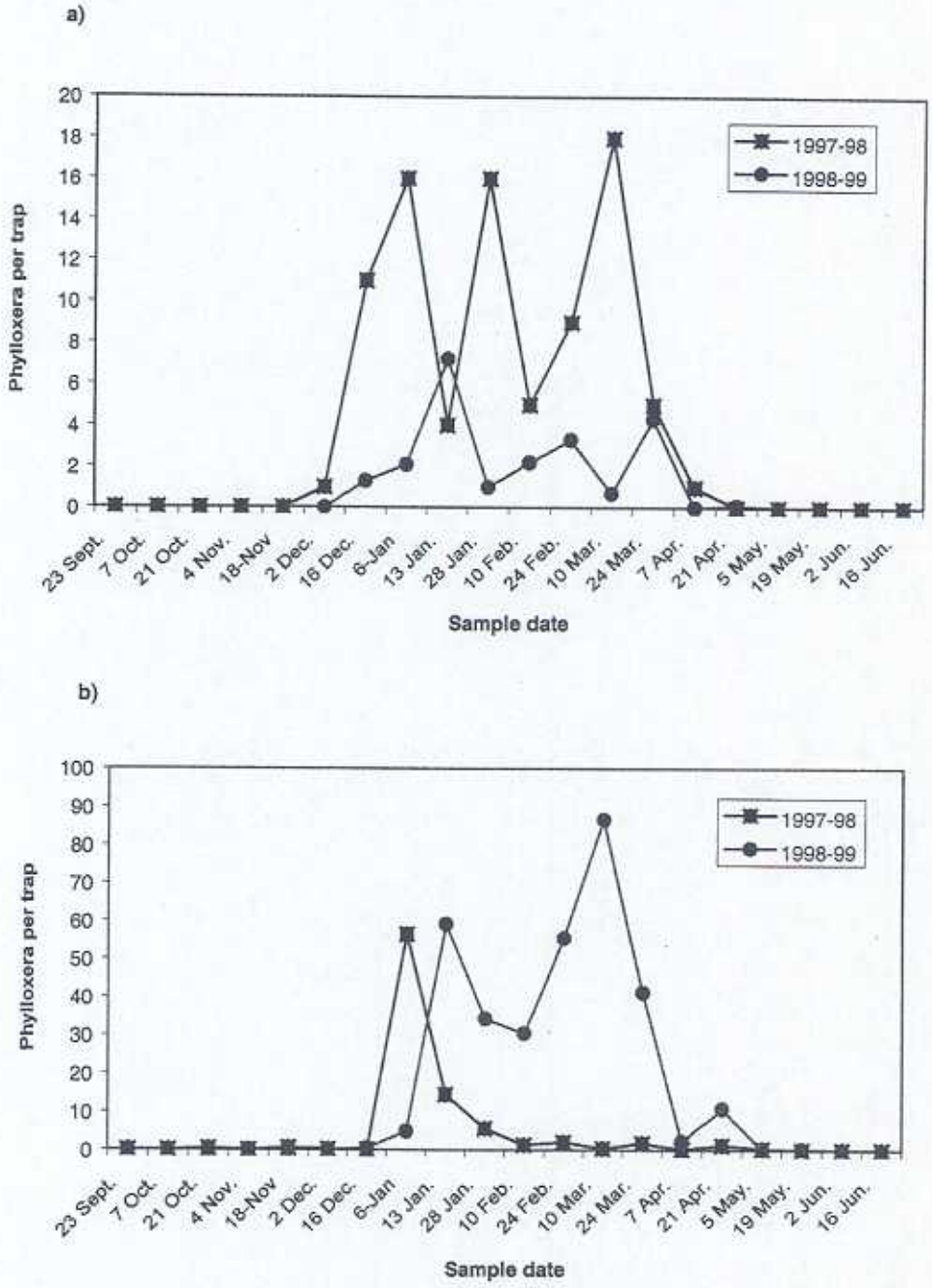

Figure 4: Seasonal variation of phylloxera dispersive stages moving up the vine trunk in phylloxera infested commercial vineyards at (a) Whitlands and (b) Cheshunt in the King Valley over consecutive seasons, 1997-99.

5. *Wind assisted phylloxera dispersal*

Phylloxera stages collected in aerial traps were relatively low over both seasons with a total of 24 insects identified during the first season and 39 during the second season. Sixty percent of dispersive stages collected were alates, the remainder being crawlers. The peak occurrence was between January and March.

6. *Canopy population levels*

A range of sampling techniques were used to estimate canopy population levels including destructive and non-destructive techniques. At both sites canopies were sampled in late February and early-mid March. Data from the first sample date are presented (Table 2). Although phylloxera numbers recovered were very low the destructive canopy sampling techniques proved the most successful (Table 2a), with crawlers being collected from bunches, leaves and shoots and alates recovered from leaves. The use of non-destructive sampling techniques enabled the recovery of crawlers from only one of the infested sites and no alates were recovered from either site (Table 2b).

Table 2: Selected canopy population data from two phylloxera infested vineyards.

(a) Destructive sampling

Site	Sample	Crawlers	Alates	Total
Cheshunt	*Shoot*	1	0	1
	Leaf	1	1	2
	Bunch	0	0	0
Whitlands	*Shoot*	0	0	0
	Leaf	0	1	1
	Bunch	3	0	3

(b) Non-destructive sampling

Site	Sample	Crawlers	Alates	Total
Cheshunt	***Dustbuster***	1	0	1
	D-Vac	0	0	0
	Beat	1	0	1
Whitlands	***Dustbuster***	0	0	0
	D-Vac	0	0	0
	Beat	0	0	0

Discussion

Seasonal population dynamics

Phylloxera population dynamics as observed in the King Valley follows similar seasonal trends to other phylloxera studies in the USA and Australia. Omer *et al.* (1997) showed in field studies in commercial Californian vineyards that very low phylloxera populations occurred in early spring rising exponentially to peak during mid-summer and declining to low levels in mid-late summer. The emergence of phylloxera through soil cracks onto the soil surface is of prime concern when managing infested vineyards as this increases the potential for dispersal both naturally and artificially via machinery and personnel. When comparing the King Valley emergence data over the two seasons there is clearly 'between season' and 'between site' variation in both peak phylloxera emergence and duration of phylloxera emergence. Our preliminary findings have identified that late-December to mid-March are periods when above ground phylloxera activity is at its peak and management during this period should take this into account. Studies have shown that peak phylloxera emergence was observed from mid-January to mid-March in Nagambie, Australia (King and Buchanan, 1986).

Omer *et al.* (1997) suggest that soil temperature and root quality and abundance are factors which may affect phylloxera population dynamics. Although soil temperatures were not recorded in this study, temperature thresholds do influence the development of phylloxera. When temperatures exceed 18°C in the laboratory phylloxera establish feeding sites (Turley *et al.*, 1996). This would coincide with the increase in phylloxera levels observed in spring at the Cheshunt site in the King valley in the 1997-98 season where temperatures exceeded 18°C (data not presented). In the following season mean monthly air temperatures during November and December were 5-7°C lower than the previous season which could have influenced the delay in phylloxera emergence from the soil. The decline in King Valley phylloxera populations in the late summer is unlikely to be due to a temperature effect, as temperatures rarely fell below 18°C and further studies are needed to determine whether soil borne, climatic or host plant factors influence this decline. Earlier studies and anecdotal evidence suggest that soil characteristics play a role in phylloxera population dynamics and dispersal (Buchanan, 1990). Preliminary soil analyses have been carried out at both sites and will be used in further studies to determine which soil characteristics may be influencing phylloxera population dynamics in the King Valley region.

The potential for spread of phylloxera on vineyard machinery is clear. This study has highlighted that phylloxera is found in the soil for most of the growing season and above ground either on the soil surface or in the canopy from November through to May. Transfer onto machinery is therefore likely and in an earlier study a single crawler has been identified on harvesting machinery (King and Buchanan, 1987). The risk of transporting phylloxera on spraying, pruning and cultivation equipment although not yet quantified is evident. In infested vineyards precautions should be taken to ensure that infested areas are treated separately to uninfested areas to reduce the potential for spread by mechanical means and ensure machinery is disinfested according to state or national quarantine protocols.

The potential for aerial dispersal of phylloxera was observed in this study with both alate and crawler stages being dispersed several metres from the infested vine blocks. Aerial movement of phylloxera up to 20 metres away from infested vines has been shown in other studies in both Australian and New Zealand vineyards (King and Buchanan, 1986). Alates were the predominant form of dispersive stage collected in this study in aerial traps. However, in Australia alates are not considered a significant agent of phylloxera spread due to their limited capacity to produce progeny, which need then to develop leaf galls on American rootstocks. Leaf galling has been observed infrequently in New South Wales (Helm, 1983) and the Rutherglen and Glenrowan areas of Victoria (Buchanan, 1990) but not yet recorded in the King Valley. However, the occurrence of crawlers in aerial traps in this and other studies is a cause for concern as these can increase the natural spread of phylloxera within infested vineyards.

Canopy population dynamics

Determining the canopy population of phylloxera is important as this can potentially act as a source for contamination of grapes or must and for further dispersal when dislodged from the canopy by natural (eg: wind) or man assisted (eg: machinery) movement. In this study, relatively low phylloxera population levels were found in the canopy in February, despite high numbers caught in emergence and pitfall traps. Canopy sampling studies carried out in New Zealand and Nagambie, Australia (King and Buchanan, 1986) have recovered similar low numbers of crawlers and alates on leaves, canes and shoots. This indicates that canopy-related factors influence crawler and alate survival. Two factors which could influence canopy levels are temperature and predation. High temperatures in the canopy during the summer months in the King Valley may have influenced canopy population levels in this study, with air temperatures reaching a maximum of 34°C in January and 30°C in February 1999 at both trial sites. Granett and Timper (1987) showed that at temperatures above 28°C nymph survival rapidly declines and at 36°C the development of phylloxera to adulthood is prevented. There are no published studies on the potential influence on the effect of natural predators in the canopy and this requires further investigation.

As phylloxera is found above ground prior to harvest it is important to ensure that vineyard personnel, in particular hand-harvesters, follow recommended disinfestation procedures (Dunstone *et al.*, 1998) to ensure that phylloxera is not inadvertently transferred to other vineyards on footwear or clothing. It is important to quantify the relative numbers of phylloxera present in the canopy at or near to harvest, to address industry concerns over the transport of potentially contaminated grapes, juice and must from phylloxera infested regions into non-infested wineries. The movement of wine grapes in Australia from a phylloxerated region to a non-phylloxerated region is currently prohibited, whilst the movement of must and juice is only allowed with a permit under protocol (National Phylloxera Management Protocols, 1999). Quantification of canopy levels of phylloxera, particularly on grape bunches, is the first stage in the development of a series of trials to develop a pest risk analysis protocol, from grape to wine. These protocols are currently being developed by DNRE, Agriculture Victoria - Rutherglen in collaboration with the Australian wine industry.

Phylloxera biotypes

This study concentrates on phylloxera populations located in the King Valley region of Victoria. In Australia at least three distinct strains of phylloxera have been identified from six different geographical locations (Corrie *et al.*, 1997). Population studies have now been carried out to varying degrees at five of the six locations. The strain identified in the Rutherglen region is genetically distinct from the other two strains and in excised root bioassays appears to differ in its ability to survive on both *Vitis vinifera* and certain rootstocks (Corrie *et al.*, 1998). Detailed population dynamics studies on the Rutherglen strain have not been carried out and would certainly add to our knowledge of biotype behaviour *in situ* and improve management practices in this region. In conclusion, this study has identified periods of above and below ground phylloxera activity in King Valley vineyards and will assist in the further development of management strategies and quarantine protocols for growers in the region.

Acknowledgments

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Appendix 9: 5 year plan for phylloxera research and development

DRAFT ONLY

FIVE YEAR RESEARCH AND DEVELOPMENT PLAN

2000-2005

MANAGEMENT OF GRAPEVINE PHYLLOXERA

Introduction

The Australian Viticulture Industry recognises that grape phylloxera is the major insect pest threat to sustainable long-term production. Over 85% of Australian vineyards are on ungrafted susceptible *Vitis vinifera* roots. Phylloxera outbreaks and infestations in recent years, such as those in the King Valley and Upton, highlight the industries vulnerability and the potential economic impact of the insect pest in susceptible vineyards. Phylloxera outbreaks reduce marketability, investor confidence and cause severe financial and emotional stress for growers whose livelihood depends on grape growing.

This document has been developed as a component of the Grape and Wine Research and Development Corporation project DAV 99/2 – "Management of Grapevine Phylloxera in South East Australia - Phase II - The Future". It prioritises phylloxera-related research and development directions, on an industry needs basis, for the next 5 years. The plan was developed with industry and phylloxera researchers (Appendix 1) following discussion at two key phylloxera research planning meetings; the Phylloxera Research and Development Planning Workshop (Rutherglen/Albury, October 1999) and First International Symposium on Grapevine Phylloxera Management (Melbourne, January 2000). This consultative process set phylloxera research and development priorities for the next five years by highlighting of key issues, opportunities and constraints to these activities. Industry and Government stakeholders will be asked to support, implement and develop elements of the plan together with organisations with phylloxera research and extension expertise (Appendix 2).

The overall aim of the five-year plan is to maintain a sustainable viable viticulture industry through the development of strategies that reduce the risk of phylloxera spread in Australia and minimise the impact of phylloxera in infested vineyards. The plan has three main objectives:

1. **EARLY DETECTION AND MANAGEMENT OF PHYLLOXERA OUTBREAKS**

2. **REDUCING THE POTENTIAL FOR SPREAD OF PHYLLOXERA**

3. **ENSURING LONG-TERM VIABILITY OF ROOTSTOCKS**

OUTCOME 1

EARLY DETECTION AND MANAGEMENT OF NEW OUTBREAKS OF PHYLLOXERA

Background:

Aerial photography has been used in California to monitor the impact of phylloxera on vineyards. In Australia this method has been extended to detecting vines with reduced growth and possibly early stages of a phylloxera infestation. By using infra-red photography at particular times of the day weaker growth areas can be detected. These areas can then be ground surveyed for phylloxera. Differences between the ratios of the intensity of the wavelengths may also be used to highlight weaker vines through image enhancement. To-date satellite imagery has not had the degree of resolution required to detect small areas of weak vines and hence has the potential to miss phylloxera infested vines. Other detection methods may have application, eg. video imagery. Recognition of changes to the composition of the leaf canopy arising from feeding by phylloxera offer further research opportunities. Early detection of phylloxera is vital as it allows quarantine protocols and management practices to be implemented rapidly to ensure that the risk of phylloxera spreading to uninfested vineyards is reduced.

If quarantine should fail and a vineyard becomes infested with phylloxera, the only way that viticulture can continue in the long-term is if vines are replanted to phylloxera-resistant rootstocks. In the event of a large-scale phylloxera outbreak, or an outbreak in a large but previously infested area, the industry will require interim management strategies in order to maintain market stability. Financial constraints or shortages of rootstock material will mean that a transition period from managing phylloxera-affected vines to replanting on phylloxera- resistant rootstocks may be unavoidable. The effectiveness of cultural or chemical management techniques as an interim option to keep vines productive has received little attention in Australia but has been considered in Europe and the USA (Fisher and Hellman, 2000; Schmid and Ruhl, 2000).

Objectives:

1. To improve phylloxera detection (methods), ensure the effective containment of new outbreaks of phylloxera and the early implementation of interim phylloxera management strategies

2. To develop interim management strategies for phylloxera-infested ungrafted vineyards to maintain vine vigour and prolong productive life of vines whilst gathering resources to implement replanting on grafted vines

Industry Outcomes:

1. Verification of phylloxera free and phylloxera infested zones
2. New improved early detection techniques for widespread industry use
3. Increased industry awareness of and use of early detection techniques resulting in a reduction in the cost of phylloxera to the industry
4. Improved management practices to contain the spread and reduce the impact of phylloxera
5. Increased industry awareness and adoption of appropriate strategies to manage infested ungrafted vines
6. Cost-benefit analysis of extended/prolonged production from infested vineyard compared to a rootstock replanted vineyard in different vine-growing regions
7. Reduced impact and cost of phylloxera outbreaks

Activity areas:

A. Development of early detection technologies

- ➢ Development of simple practical and appropriate early detection methods for phylloxera on both grafted and ungrafted vines
- ➢ Awareness and use of early detection and outbreak protocols at the grower and national level
- ➢ Development of an early detection training module for adult education and tertiary teaching institutions
- ➢ Development of phylloxera information packages for a range of industry sectors, eg. contractors, tourists, nurseries, supermarkets, etc.
- ➢ Development and delivery of effective national training of growers and the wider industry on recognition of phylloxera infestation symptoms within the vineyard and the use of early detection techniques for phylloxera

B. Surveillance and surveillance management

- ➢ Maintenance and regular updating of a database on phylloxera surveys and infested vineyards at a national level
- ➢ Implementation of a surveillance and monitoring program based on risk assessment

- In the event of an outbreak provision of technical support for appropriate fruit marketing arrangements and conducting tracebacks

- Development and delivery of focussed national training of growers on management and quarantine protocols to minimise the risk of phylloxera spread

C. Developing and managing phylloxera protocols

- Development of vineyard and district phylloxera survey protocols

- Regular review and update (with appropriate industry consultation and agreement) of action and contingency plans for phylloxera outbreaks

- Registration of growers and geo-coordinates of vineyards in each region for purposes of distributing information about phylloxera

- Community awareness of phylloxera boundaries through signposting and general awareness of quarantine boundaries and protocols and assisting with vineyard signage distribution and design

- Assisting regional groups with the development of phylloxera codes of practice

Development of interim management strategies that enable vineyard viability during the transition from ungrafted to grafted vines

- Determination of the effects of foliar and soil-applied fertiliser (particularly nitrogen and potassium) and irrigation strategies on phylloxera damage and vine vigour

- Screening of novel and conventional chemical control methods including systemic acquired resistance, biofumigants, novel and conventional chemical insecticides to reduce phylloxera populations in infested vineyards

Performance Indicators:

- Number, age and area of infested vineyards discovered within and outside quarantine zones

- Percentage of vineyards monitored using early detection technologies

- Percentage of phylloxera affected vineyards implementing strategies that maintain grape production and extended productive life of infested vineyards

- The extent to which interim management strategies are used in infested vineyards

- A decrease in the number of new phylloxera outbreaks

OUTCOME 2

REDUCING THE POTENTIAL FOR SPREAD OF PHYLLOXERA

Background:

Understanding the population dynamics and rate of spread of phylloxera infestations, and factors that influence these two processes, is essential for the effective development of quarantine protocols and management strategies. Field-based research on phylloxera population dynamics in Australian vineyards has been limited. To date two major studies in Victoria limited to areas in Nagambie region (Buchanan, 1990) and a recently completed 3-year study in the King Valley (Powell *et al.*, 2000). These initial studies have highlighted risk periods and risk zones within infested vineyards. The spread of phylloxera within and between vineyards is associated with the movement of phylloxera life stages either naturally or through assisted movement for example on vine planting material, harvested grapes, equipment or personnel, or birds and animals. A recent pilot study conducted by Powell *et al.*, (in prep) has attempted to quantify the risks of phylloxera transfer from harvesting through to the unfermented product. Quantifying the risks and economic costs associated with human-assisted phylloxera transfer is vital to the industry. Australia is one of the few wine grape-producing countries which remains predominantly (around 85%) on ungrafted vineyards making it highly vulnerable should existing phylloxera quarantine protocols breakdown.

Objectives:

1. To understand the fundamental processes of phylloxera population biology and dispersal under a range of environmental and management conditions

2. To incorporate the enhanced understanding of phylloxera population studies into risk assessments and quarantine protocols

3. To determine the economic impact of a grapevine phylloxera infestation and to analyse the risks associated with phylloxera

Industry Importance:

1. Improved management of phylloxera through a greater understanding of the biology and behaviour of phylloxera in both grafted and ungrafted vineyards

2. Improved industry knowledge of the risk of phylloxera transfer

3. Understanding the economic impact of phylloxera in terms of lost production and replanting or costs associated with the use of preventative methods in different regions

Projects and Activities:

A. Applied research on phylloxera population biology and dispersal

- Quantification of phylloxera dispersal rates and monitoring of population dynamics under a range of field conditions in geographically different phylloxera infested zones
- Identification of natural factors which influence phylloxera dispersal including the role and suitability of natural control predators and pathogens
- Identification of site-related factors which influence phylloxera dispersal
- Determination of the effect of root zone and canopy zone management practices on phylloxera population dynamics and dispersal traits
- Assessment of predominant reproductive mode and life cycle of phylloxera

B. Risk Analysis – dispersal and grape processing

- Quantification of phylloxera survival and the risk of transfer through human-assisted vectors (including footwear/clothing, machinery, vine material) and non-human assisted (eg. wind, water, birds, animals) vector routes during the grapevine growing season
- Quantification of phylloxera survival through various stages of grape processing from harvesting to post fermentation
- Development of a scientifically based pest risk analysis model

C. Economic model of phylloxera risks

- Development of a desktop economic model on phylloxera risks and losses associated with phylloxera and map different risk zones based on historical records of phylloxera outbreaks

Performance Indicators:

- The number of infested sites examined and risk factors examined over successive seasons
- Pest Risk Analysis document quantifying the risk of transfer through grape processing
- Pest Risk Analysis document quantifying the risk of transfer by various vectors
- Recommendations incorporated into National Phylloxera Management Protocols

OUTCOME 3

ENSURING LONG-TERM VIABILITY OF ROOTSTOCKS

Background:

Assessment of phylloxera resistance of rootstocks has been carried out since the late 1800's using a variety of methods that are relatively laborious, yet which remain essentially unchanged today. In general, they have involved inoculating potted or field-grown vines with phylloxera and observing the number of root galls formed after one or more seasons growth. Some of the common rootstocks used in Australia have been assessed using this procedure in one or more sites and the results generally match with those published from overseas trials. For others, only overseas information is available. The recent identification of different phylloxera 'biotypes' in Australia may require a re-examination of rootstock x biotype interactions, given that some 'resistant' rootstocks appear to support significant populations of some 'biotypes' of phylloxera. Optimisation of *in vitro* co-cultivation methods and characterisation of the mechanisms of rootstock resistance are required for development of more facile and rapid tests for rootstock resistance.

Few studies worldwide have examined the fundamental host plant-pest interaction between the grapevine and phylloxera and the biotic and abiotic factors which may influence this interaction. A recent study by Kellow *et al.* (2000) highlighted the physiological differences apparent at the molecular and cellular level between susceptible and resistant grapevine roots subjected to phylloxera attack. Within the vineyard, phylloxera population size and dispersal rates are likely to be significantly affected by not only by the soil environment but also by the grapevine physiology, particularly root growth and composition. In California, recent studies suggest that interactions between phylloxera and soil-borne fungal pathogens result in increased root damage with the extent of damage influenced by both the vine parentage and vineyard management practices (Omer, 2000). Development of sustainable phylloxera management practices requires a better understanding of the factors which influence the level of association between phylloxera and the grapevine in infested vineyards. Further, adequate information on suitability of rootstocks *per se* to various site aspects (climate, soil, etc.) is important for their successful adoption. Information on rootstock performance has been gathered from field trials in sites of major phylloxera presence but is otherwise incomplete in other regions of Australia.

Phylloxera population genetics studies help to determine their potential to spread to uninfested regions and also their ability to evolve and adapt to control strategies. Initial research utilised the Random Amplified Polymorphic DNA typing technique (RAPDs) to demonstrate the presence of more than one genetic type of phylloxera in Australia vineyards. Three different genetic strains of phylloxera were identified from four geographic regions (Corrie *et al.*, 1997). More recently a far more informative DNA marker system for population studies has been developed. These DNA markers, termed microsatellites, have greatly enhanced our knowledge of the number and composition of different genetic strains in the majority of Australian infested vineyards. This information is being used to determine the mode of reproduction and the spread of the insect by examining the type and frequency of different strains.

Objectives:

1. To determine the extent of genetic variation within Australian phylloxera populations, understand the fundamental processes influencing genetic variation and highlight the effect that the variation may have on phylloxera management practices in grafted and ungrafted vineyards

2. To understand the fundamental host plant characteristics that determine resistance and susceptibility of *Vitis* species to grapevine phylloxera and make appropriate rootstock recommendations based on this knowledge together with information on agronomic performance.

3. To understand which factors influence phylloxera-vine interactions and develop phylloxera management strategies based on this knowledge

Industry Outcomes:

1. Improved management through an understanding of phylloxera genetics

2. Ensure long-term viability of phylloxera-resistant rootstocks

3. Clear recommendations on rootstock choice in Australia

4. Wider improved rootstock selection by industry

5. Vineyard management strategies available to improve vine health and reduce the impact and cost of phylloxera infestations

Activities:

A. ***Rootstock development and evaluation***

➢ Development and evaluation of phylloxera-resistant vines/rootstocks suited to Australian conditions, in a range of laboratory and field trials

➢ Evaluation of new and existing rootstocks in phylloxera-infested and phylloxera-risk areas

➢ Assistance with the supply of rootstocks through the Australian Vine Improvement Association and its state affiliates

➢ Promotion of the use of grafted vines to industry

B. Phylloxera population genetics

- Assessment of the level of genetic variation within and between phylloxera populations, based on microsatellite markers and mitochondrial DNA, and mechanisms by which this variation is generated
- Determination of regional biotype variation and linkages
- Determination of phylloxera biotype-rootstock interactions under controlled environment conditions and different soil types
- Development and completion of a comprehensive database on genetic variation using multiple polymorphic markers and cluster analysis to ascertain linkages
- Development of a comprehensive genetic data base for mapping new infections, assessing rates of phylloxera movement, and linking with overseas phylloxera research and linked to surveillance database

C. Resistance and susceptibility mechanisms

- Develop an understanding of the mechanisms of susceptibility and resistance of grafted and ungrafted vines, and the interactions with different biotypes of phylloxera
- Determine modes of inheritance of susceptibility and resistance and develop molecular markers
- Develop novel genes for tolerance and/or resistance and understand inheritance and resistance mechanisms
- Quantify the effect of vineyard management practices including fertilisers, irrigation and organic mulches on vine health and susceptibility to phylloxera damage
- Quantify the significance of fungal interactions on secondary root rot damage of phylloxera infested vines and determine management strategies which may influence levels and diversity of soil microflora.

Performance Indicators:

- The level of genetic variability of phylloxera quantified from all infected vineyards, using at least eight polymorphic markers, and interpreted in terms of population processes
- A genetic database that will enable new outbreaks to be linked to existing infestations
- Characterisation of mechanisms of resistance and susceptibility to phylloxera of *Vitis* species

- Proportion of vineyards planted on resistant rootstocks
- Yield and quality of vines grafted to rootstocks compared with own-rooted vines in phylloxera infested vineyards
- New genetically improved vines/rootstocks for production of phylloxera resistant planting material
- Characterisation of factors and implementation of management practices which reduce or disrupt the interactions between phylloxera and grapevines

APPENDIX ONE

This plan has been facilitated by Agriculture Victoria–Rutherglen in consultation with the following industry and research personnel

Name	*Location*
Avery, Angela	DNRE, Rutherglen Research Institute, Vic
Buchanan, Greg (Dr)	DNRE: Sunraysia Horticulture Centre, Mildura, Vic
Clingeleffer, Peter (Dr)	CSIRO, Merbein, Vic
Cornish, John	Primary Industries, SA
Corrie, Angela	La Trobe University, Bundoora, Vic
Darling, Guy	Darling Estates & King Valley Grape Growers Association, Vic
Dunstone, Rebecca	DNRE, Rutherglen Research Institute, Vic.
Englefield, Brian	Victorian & Murray Valley Grape Growers Council; Australian Vine Improvement Association; Robinvale & District Winegrape growers Association
Everitt, Marcus	DNRE, Institute for Sustainable Irrigated Agriculture, Tatura
Fischer, Jane	DNRE, Knoxfield, Vic
Franks, Tricia (Dr)	University of Adelaide, SA
Gardner, Richard	DNRE, Knoxfield, Vic
Hamilton, Richard	Southcorp Wines;
Hardie, Jim (Dr)	CRC for Viticulture, Adelaide, SA
Heeswijck, Robyn van (Dr)	University of Adelaide, SA
Heinze, Ross	Phylloxera and Grape Industry Board; Southcorp Wines, SA
Hilder, Richard	*Richard Hilder, Rosemount Estate, Vic*
Hill, Megan	DNRE, Tatura,Vic
Hoffman, Professor Ary	La Trobe University, Bundoora, Vic
Leamon, Keith	DNRE, Mildura, Vic
Lester, Don (Dr)	Phylloxera and Grape Industry Board; Orlando Wyndham Wines, SA
Murtagh, Michael	Rutherglen Vineyard Services, Rutherglen, Vic
Nettlebeck, Robyn	S. Smith and Sons, SA
O'Conner, Jan	O'Conner Harvesting, Robinvale, Vic.
Park, Rod	Park Wines, Vic.
Paton, Bob	NSW Agriculture, Orange, NSW
Planck, James Dr	DPI, Queensland
Pech, Leo	South Australian Farmers Federation (Grapes Section), SA
Powell, Kevin (Dr)	DNRE, Rutherglen Research Institute, Vic
Read, Peter	King Valley, Vic.
Smith, Mark (Dr)	Southcorp Wines, SA
Strachan, Stephen	Winemakers Federation of Australia, SA
Turkington, Ross	Miranda Wines, Griffith, NSW
Whiting, John	DNRE, Tatura, Vic.
Walpole, Mark	Brown Brothers, Milawa, Vic.

APPENDIX TWO

Organisations with phylloxera research and extension expertise

1. **Department of Natural Resources and Environment:**

- Rutherglen Research Institute, Rutherglen, Victoria
- Sunraysia Horticulture Centre, Mildura, Victoria
- Tatura, Victoria
- Institute for Horticulture Development, Knoxfield, Victoria

➢ DNRE has a broad range of skills including National Phylloxera Workshop and International Symposium organisation, rootstock screening, phylloxera population dynamics and dispersal, insecticide screening, early detection and surveying, DNA biotyping and a broad network of extension teams.

2. **La Trobe University, Department of Biochemistry and Genetics Bundoora, Victoria**

➢ Research expertise in the use of molecular markers to understand genetic variation and life cycle of phylloxera.

3. **University of Adelaide, Department of Horticulture and Oenology, Adelaide, South Australia**

➢ Research expertise in pest-host plant interactions, vine physiology, regulation of nitrogen metabolism in grapevines & characterisation of grape berry proteins.

4. **Phylloxera and Grape Industry Board, Adelaide, South Australia**

➢ Phylloxera awareness and education programs.

Industry priority rating of project activities – August 2000

Each project has been rated out of 5 for relevance, competence and value. The total rating is the sum of the three component ratings. The ratings represent the average of those given by individual raters.

PROJECT ACTIVITY	Rele-vance	Compet-ence	Value	Total
OUTCOME 1: EARLY DETECTION AND MANAGEMENT OF NEW OUTBREAKS				
A. Development of early detection technologies				
Development of simple practical and appropriate early detection methods for phylloxera on both grafted and ungrafted vines	4.25	3.33	3.50	11.08
B. Awareness and use of early detection and outbreak protocols				
Development of an early detection training module for adult education and tertiary teaching institutions	3.75	3.00	3.50	10.25
Development of phylloxera information packages for a range of industry sectors, eg. contractors, tourists, nurseries, supermarkets	4.00	2.33	2.50	8.83
Development and delivery of effective national training of growers and industry on recognition of phylloxera infestation symptoms within the vineyard and the use of early detection techniques for phylloxera	5.00	4.33	4.75	14.08
C. Surveillance and surveillance management				
Maintenance and regular updating of a database on phylloxera surveys and infested vineyards at a national level	4.25	4.00	3.50	11.75
Implementation of a surveillance and monitoring program based on risk assessment	5.00	4.67	4.50	14.17
In the event of an outbreak provision of technical support for appropriate fruit marketing arrangements and conducting tracebacks	4.75	4.00	4.50	13.25
Development and delivery of focussed national training of growers on management and quarantine protocols to minimise the risk of spread	4.75	4.33	4.25	13.33
D. Developing and managing phylloxera protocols				
Development of vineyard and district phylloxera survey protocols	5.00	4.00	4.25	13.25
Regular review and update (with industry consultation and agreement) of action and contingency plans for phylloxera outbreaks	3.75	3.33	2.75	9.83
Registration of growers and geo-coordinates of vineyards in each region for purposes of distributing information about phylloxera	4.50	3.67	4.00	12.17
Raise awareness of phylloxera boundaries through signposting and education and assisting with vineyard signage distribution and design	3.25	3.33	3.25	9.83
Assisting regional groups with development of codes of practice	3.33	3.50	3.33	10.17
E. Development of interim management strategies for infested ungrafted vines				
Determination of the effects of foliar and soil-applied fertiliser (particularly nitrogen and potassium) and irrigation strategies on phylloxera damage and vine vigour	3.00	3.33	3.00	9.33
Screening of novel and conventional chemical control methods including systemic acquired resistance, biofumigants, novel and conventional chemical insecticides to reduce phylloxera populations	2.00	2.33	2.00	6.33

OUTCOME 2: REDUCING THE POTENTIAL FOR SPREAD OF PHYLLOXERA				
A. Applied research on phylloxera population biology and dispersal				
Quantification of phylloxera dispersal rates and monitoring of population dynamics under a range of field conditions in geographically different phylloxera infested zones	3.75	3.67	3.50	10.92
Identification of natural factors which influence phylloxera dispersal including the role and suitability of natural control predators and pathogens	4.50	3.67	3.75	11.92
Identification of site-related factors which influence phylloxera dispersal	3.50	3.33	3.00	9.83
Determination of the effect of root zone and canopy zone management practices on phylloxera population dynamics and dispersal traits	2.75	3.00	2.75	8.50
Assessment of predominant reproductive mode and life cycle of phylloxera	4.00	3.67	3.75	11.42
B. Risk analysis – dispersal and grape processing				
Quantification of phylloxera survival and the risk of transfer through human-assisted vectors and non-human assisted vector routes during the grapevine growing season	5.00	4.67	4.25	13.92
Quantification of phylloxera survival through various stages of grape processing from harvesting to post fermentation	4.75	4.33	4.25	13.33
Development of a scientifically based pest risk analysis model	5.00	5.00	4.75	14.75
C. Economic model of phylloxera risks				
Development of a desktop economic model on phylloxera risks and losses associated with phylloxera and map different risk zones based on historical records of phylloxera outbreaks	3.75	3.00	3.75	10.50
OUTCOME 3: ENSURING LONG-TERM VIABILITY OF ROOTSTOCKS				
A. Rootstock development and evaluation				
Development and evaluation of phylloxera-resistant vines/rootstocks suited to Australian conditions, in a range of laboratory and field trials	4.50	4.33	4.00	12.83
Evaluation of new and existing rootstocks in phylloxera-infested and phylloxera-risk areas	4.50	4.33	3.75	12.58
Assistance with the supply of rootstocks through the Australian Vine Improvement Association and its state affiliates	3.50	4.00	3.50	11.00
Promotion of the use of grafted vines to industry	4.75	5.00	4.75	14.50
B. Phylloxera population genetics				
Assessment of the level of genetic variation within and between phylloxera populations, based on microsatellite markers and mito-chondrial DNA, and mechanisms by which this variation is generated	3.50	4.00	3.50	11.00
Determination of regional biotype variation and linkages	3.50	3.67	3.75	10.92
Determination of phylloxera biotype-rootstock interactions under controlled environment conditions and different soil types	4.00	4.00	4.00	12.00
Development of a comprehensive database on genetic variation using multiple polymorphic markers and cluster analysis to ascertain linkages	2.75	2.67	3.00	8.42
Development of a comprehensive genetic data base for mapping new infections, assessing rates of phylloxera movement, and linking with overseas phylloxera research and linked to surveillance database	3.75	3.33	3.75	10.83

C. Resistance and susceptibility mechanisms				
Develop an understanding of the mechanisms of susceptibility and resistance of grafted and ungrafted vines, and the interactions with different biotypes of phylloxera	4.50	4.33	4.50	13.33
Determine modes of inheritance of susceptibility and resistance and develop molecular markers	4.25	4.00	4.00	12.25
Develop novel genes for tolerance and/or resistance and understand inheritance and resistance mechanisms	4.00	3.67	3.50	11.17
Quantify the effect of vineyard management practices including fertilisers, irrigation and organic mulches on vine health and susceptibility to phylloxera damage	3.00	3.00	3.00	9.00
Quantify the significance of fungal interactions on secondary root rot damage of phylloxera infested vines and determine management strategies which may influence levels and diversity of soil microflora	3.75	3.67	3.50	10.92