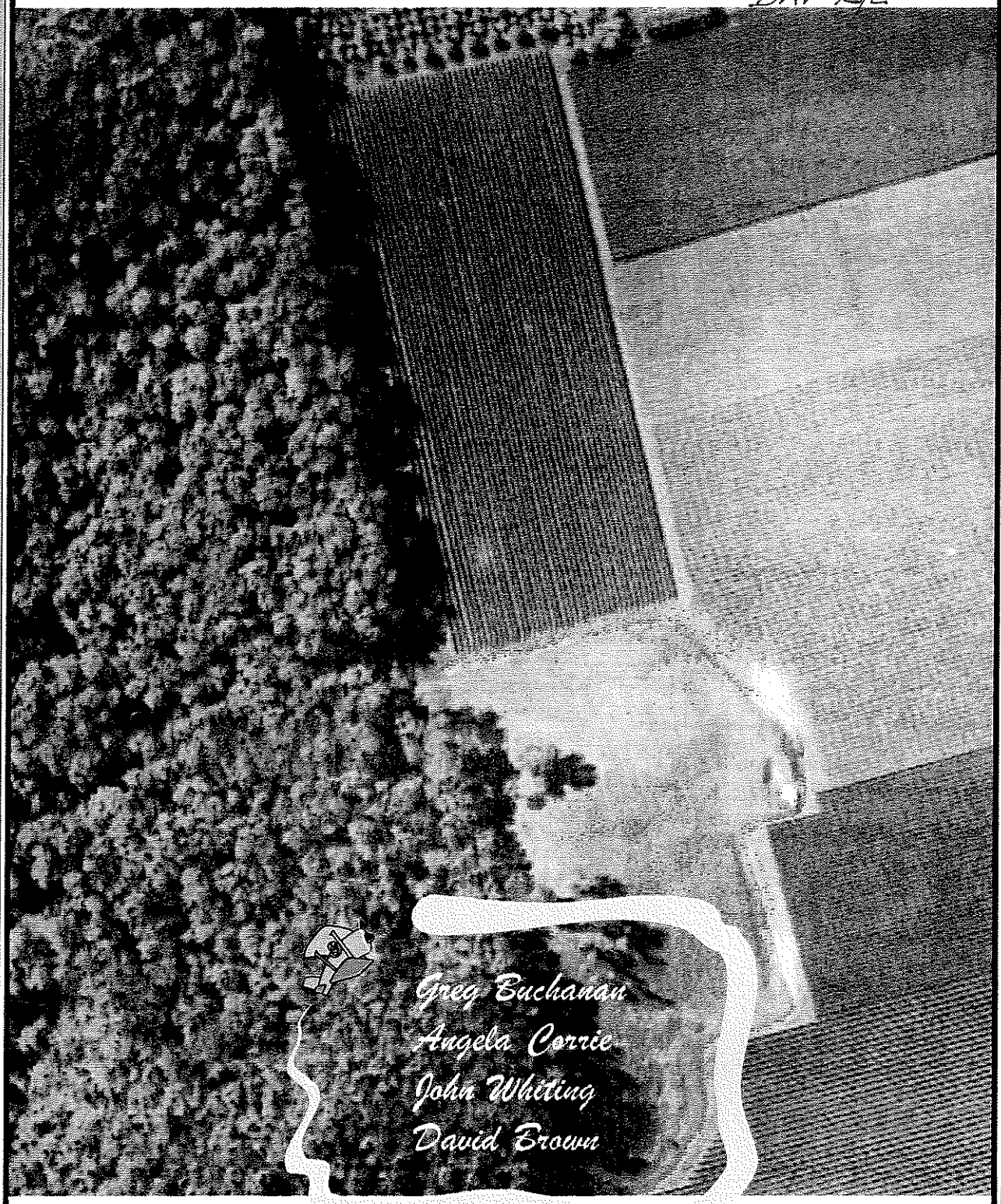
Management of grape phylloxera in Australia

DAV 93/2



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Grape and Wine Research and Development Corporation

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HORTICULTURAL RESEARCH & DEVELOPMENT CORPORATION
The Research Arm of the Australian Horticultural Industries

Phylloxera and Grape Industry Board

Management of grape phylloxera in Australia

A report on projects HRDC FR310 and GWRDC DAV 93/2

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Introduction

This is a report of phylloxera research and extension activities carried out during 1994-1996. The work was funded jointly by the Horticultural Research and Development Corporation and the Phylloxera Board of South Australia (HRDC Project FR310), the Grape and Wine Research and Development Corporation (GWRDC project 93/2), Agriculture Victoria, and New South Wales Agriculture. The objectives of the work were:

GWRDC 93/2

1. To train vineyard staff and Government officers in detection and diagnosis of phylloxera.
2. To coordinate surveys of vineyards for grape phylloxera in eastern Australia.
3. To develop collaborative research programs with European and North American scientists.
4. To develop a national policy and operational plans for future management of phylloxera.

HRDC FR310

5. To define the biotypes of phylloxera present in Australia and determine their practical significance.
6. To evaluate new rootstocks for phylloxera resistance using a rapid screening test.
7. To investigate the physiological effects of phylloxera on grapevines and the interactions with nutrition and irrigation.
8. To review the distribution of phylloxera in southeast Australia.
9. To investigate practical methods for disinfesting grapes and equipment from phylloxera.

The research objectives were focussed on the strategic issues of preventing the spread of phylloxera infestations, and on the long term viability of rootstocks recommended for its control in Australia. Both these issues required basic research on the biology of phylloxera populations. The practical aspects of the project involved applied research, extension and education on quarantine and hygiene procedures to minimise spread of the insect, and management of phylloxera infested vineyards. A phylloxera management committee including representatives from the funding organisations and State agencies guided the activities of the project.

The methods and results of the project are summarised here in the main body of the report. Details of the different experiments are contained in the appendices. Some of the information collected in surveys was confidential, and has been withheld from the report, but the conclusions obtained from the information are presented.

Methods

Project team: The project was mainly carried out by staff of Agriculture Victoria; although surveys of vineyards in New South Wales involved staff from New South Wales Agriculture. The people involved in the project were:

Sunraysia Horticultural Centre, Mildura
Dr Greg Buchanan, entomologist and project leader
Ms Miriam Pywell, technical officer
Ms Julie Hawtin, technical assistant

Institute for Irrigated Sustainable Agriculture, Tatura
Mr John Whiting, State viticulturist
Mr Damien de Castella, viticulturist
Ms Shelley Lanyon, technical officer

Institute for Integrated Agricultural Development, Rutherglen
Ms Angela Corrie, molecular biologist
Mr David Brown, entomologist
Ms Sarah Hetherington, technical assistant

Institute for Horticultural Development, Knoxfield
Mr Richard Gardner, plant standards inspector

New South Wales Agriculture
Mr Ross Turkington, viticulturist
Mr Tony Somers, viticulturist

Primary Industries, South Australia
Dr Richard Hamilton

Hygiene and quarantine protocols

Field work and vineyard surveys were carried out under permits that specified procedures and disinfestation techniques designed to prevent the spread of phylloxera by staff. Field visits were arranged so as to avoid travelling from infested to uninfested vineyards in the same day, and all equipment was cleaned and disinfested after visits to infested vineyards. Disinfestation methods were immersion in either 95% alcohol or 1% sodium hypochlorite. Phylloxera samples were stored in ethyl alcohol, or if live specimens were required they were transported from the vineyard directly to culture facilities at Rutherglen.

Sampling and culture of phylloxera populations

Sampling for phylloxera on the roots of grapevines was carried out by staff that had been trained in recognition of phylloxera, under the guidance of an experienced team leader. Typically, vine roots within 0.5 m of the trunk to a depth of 0.5m were dug with a shovel, and examined for the presence of galls on fibrous or woody roots. A positive diagnosis required sighting of at least one live phylloxera insect. Phylloxera from vineyards not previously known to be infested were collected in 70% alcohol and retained as reference specimens.

Numbers of phylloxera on roots in the various field and laboratory experiments were determined by direct counting under a binocular microscope, and where necessary categorised in the life stages of eggs, first instar (crawlers), second to fourth instar, adult, or nymphs (with wingbuds). Active stages of phylloxera were classified as live if they moved in response to prodding with a fine brush; eggs were kept to determine if they hatched. Effects of phylloxera on roots of grapevines in the field were described using a 0-4 rating system developed by Jeffrey Granett (University of California, Davis).

Phylloxera was cultured and experiments were carried out at the Institute for Integrated Agricultural Development, Rutherglen, which is within a phylloxera quarantine zone. Phylloxera

was cultured in a glasshouse on the roots and leaves of *V. vinifera*, usually Shiraz, Sultana, or Cabernet Sauvignon. The culture was maintained by transfer of phylloxera on root fragments from infested rootlings to newly propagated vines. The phylloxera culture was sourced from leaf galls on Schwarzmann rootstocks in a vineyard at Rutherglen, Victoria. This strain was later designated SRU-1. Additional strains of phylloxera were subsequently cultured separately on tissue-cultured vines within constant temperature rooms.

Grapevines used in the different experiments were grown from cuttings supplied by the Victorian and Murray Valley Vine Improvement Association. The cuttings were from vines that were inspected for trueness to type and freedom from virus or phytoplasma disease symptoms. The cuttings were held in cold storage at Mildura, an area free of phylloxera, until required. Vines used in experiments were propagated at Mildura as bench grafted vines, or ungrafted rootlings, before transport to Rutherglen. Cuttings used in the glasshouse culture were rooted and grown at Rutherglen.

A technique for storing populations of phylloxera on tissue cultured grapevines was developed at Agriculture Victoria, Rutherglen. Sterile shoot cultures of the grapevine varieties Cabernet Sauvignon, Shiraz, Schwarzmann and ARG1 were established from nodal segments, and grown on solid agar-based media, or in media-coated perlite. After the roots had become established, the tissue cultured grapevines were then infested with surface sterilised eggs of phylloxera. Cultures of phylloxera from different sources could be maintained indefinitely using this technique.

DNA typing was carried out using RAPD (random amplified polymorphic DNA) analysis, which has the advantage over other DNA typing techniques that no prior knowledge of the organisms genetic makeup is required. DNA was extracted from adult females of phylloxera reared in dual tissue culture, and stored at -20°C. Amplification of phylloxera DNA sequences was performed using oligonucleotide primers, and amplification products were analysed by electrophoresis, stained with ethidium bromide, and the PCR products photographed under UV light. The bands were scored as present (1) or absent (0) and similarity coefficients were determined according to Sneath and Snokal (1973).

Results and discussion

1. Detection of phylloxera outbreaks.

1.1 Training in detection and diagnosis of phylloxera.

The project has trained vineyard staff and managers, Government inspectors, and extension officers in practical diagnosis of phylloxera through a series of intensive "hands on" workshops conducted at Rutherglen and Nagambie. Twenty to forty people attended each of the workshops, which featured microscopic examination of root and leaf galls with live phylloxera, survey techniques in phylloxera infested vineyards, examination of rootstocks for control of phylloxera, and comprehensive discussion and written information on the biology and quarantine of the insect.

The workshop participants came from every State in Australia, with both State Governments and wine companies using the workshop to train their key staff. Reactions of participants have been very positive to workshop activities, with 96% of surveyed participants rating the workshop as good or excellent. Participants valued the opportunities to view live insects in the laboratory, discussions on quarantine, and the practical experience of searching for phylloxera in vineyards.

The practical outcome of the workshops is that there are over 300 people, working in different grapegrowing regions of Australia, who are competent to diagnose grape phylloxera in vineyards and who have good understanding derived from first hand experience of the effects of phylloxera on grapevines. Some of the Government and wine company staff have subsequently surveyed vineyards in their own regions for phylloxera. The workshops will continue after this project finishes, probably on the basis of full cost recovery.

1.2 Vineyard surveys for phylloxera

Surveys of vineyards for phylloxera were carried out during November to April in each year of the project. The surveys were conducted by Government inspectors. The survey activities involve interviews with the owners or managers of the vineyard, mapping of individual vineyards and recording characteristics (variety, age, rootstocks, source of propagules, soil type) of the vines, and examination of the roots of vines according to a systematic sampling plan.

Most vineyards in and near the phylloxera infested regions of New South Wales and Victoria have been surveyed for phylloxera (Table 1). Surveys concentrated on the King valley region in Victoria due to recent discoveries of phylloxera there, but surveys were also conducted in the Yarra and Goulburn valleys, in Cambden and Cumberland near Sydney, and in northeast Victoria. The surveys have identified the infested vineyards that are potential sources of phylloxera infestation for the proliferation of new vineyards, and thus re-established the basis for quarantine of phylloxera.

Table 1. Summary of the results of vineyard surveys for phylloxera

Season	District	No. Vineyards inspected	No. Vineyards infested	Area (ha) inspected	% inspected area on rootstock	% infested area on rootstocks
1994	Ovens/ Whoroughly	16	0	129	42	-
	Heathcote/ Greytown	24	0	139	<1	-
	Central Vic	9	3	65	22	11
	Macedon	2	0	9	0	
Total		51	3	342	-	-
1995	King Valley	37	4	461.5	39	10
	Central Vic	2	0	13	62	-
Total		39	4	474.5	-	-
1996	King Valley	5	4	86.5	12	13
1997	King Valley	7	3	204.5	36	10
	Ovens/ Whoroughly	4	0	41	41	-
Total		11	3	245.5	-	-

New infestations have been found only in the King and Goulburn valleys. Part of the King Valley was first declared as a quarantine area in 1991, and the quarantine area expanded in 1995 following further discoveries of phylloxera. With the exception of the King Valley, the new infestations found during this project have been within previously existing quarantine areas. It should be noted that the systematic surveys by Government personnel were not always as efficient as surveys by vineyard managers of their own properties, but they had a synergistic effect in encouraging growers to survey and report problems in their vineyards.

1.3 Aerial photography for detection of infestations

A series of colour and infrared aerial photographs have been taken to evaluate their potential for early detection of phylloxera. The infrared photographs were more useful than colour photographs, particularly when taken with the sun at an oblique angle. In 1997 infrared photographs were taken at two heights. Best results were obtained at 3000m (10,000ft) at a cost of $114/vineyard; at 6000m (20,000ft) it cost $42/vineyard but resolution was not satisfactory.

The photographs allowed weak vines or groups of vines to be identified and assisted in planning of further survey activities, but ground examination of vines was necessary to establish the cause(s) of weak growth. One of the intrinsic problems was that aerial photography needed to be done in mid-March when stressed vines showed typical oil-spot symptoms of phylloxera infestations, leaving little time in that season for followup ground surveys.

Sequential photographs of particular vineyards have provided useful information on the pattern and rate of spread of phylloxera symptoms. Digitised infrared photographs of infested vineyards have been recorded on a compact disc, as a reference for future survey activities. However, it was concluded that aerial photographs were not a good basis for an early warning system without further development.

1.4 Database of infested vineyards

Detailed information on the surveys have been stored on an a database, held by Agriculture Victoria. Information related to individual vineyards is confidential, but regional summaries can be provided to stakeholders.

Records of vineyard characteristics and interactions with other vineyards helped to trace potential pathways of phylloxera infestations. Circumstantial evidence suggests that movement of people and machinery may be important in spreading infestations to new vineyards. Windblown dispersal of phylloxera crawlers appears responsible for spread within vineyards and is possibly another avenue of infestation to adjacent vineyards.

2. Preventing spread of grape phylloxera

2.1 Quarantine zones

The main result of phylloxera surveys carried in this project has been to redefine the King valley quarantine area, which occurred in 1995. No other changes to boundaries of quarantine areas have been necessary as a result of new phylloxera infestations.

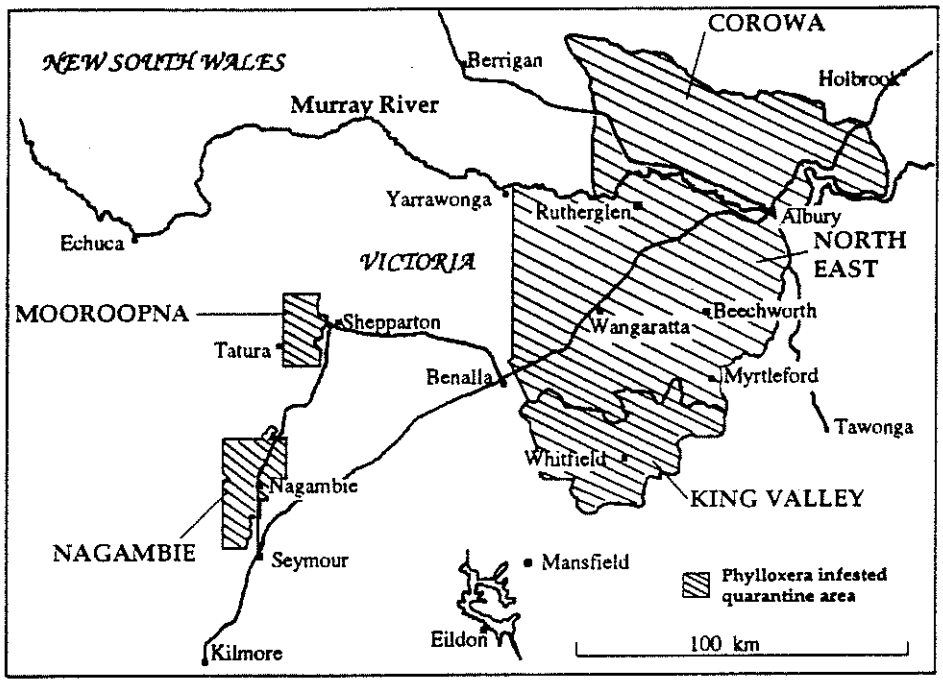


Figure 1. Map of phylloxera quarantine zones in northeast Victoria and southeast New South Wales.

2.2 Disinfestation techniques

The survival of phylloxera insects (eggs, crawlers, or active stages) was determined for several commonly used disinfestation practices, and in grape juice or must, in a series of 8 experiments. In each experiment, phylloxera insects were confined to a vial with fine stainless steel mesh on both ends, and immersed in the test liquid for a specified period.

The results showed that most of the disinfestation techniques were sound, ie:

- Hot water (52 ^{0}C for 5 minutes) killed all eggs and active stages of phylloxera.
- A 1% solution of sodium hypochlorite killed active stages of phylloxera within 1 minute, but had no negative effects on eggs.
- Most of the active stages of phylloxera were killed by immersion in 70% ethyl alcohol, although a small proportion survived. Eggs immersed for short periods (5 and 1 minute, respectively) survived 70% alcohol.
- Phylloxera adults, crawlers, and eggs did not survive freezing at -16^{0}C for 24 hours, or liquid nitrogen (-196^{0}C) for 5 minutes.

In contrast, the various stages of phylloxera were tolerant of submersion in water, detergent and metabisulphite solutions. Survival was significantly reduced in grape juice or must, in comparison to controls (air) or immersion in water at room temperature. It was shown that:

- Active stages of phylloxera tolerated submersion in water for up to 48 hours in one experiment. Eggs were even more tolerant than active stages, with a high proportion of eggs surviving immersion for 48 hours.
- The addition of detergent or potassium metabisulphite had no discernible effect on survival of phylloxera in aqueous solutions.
- Immersion in grape juice reduced survival of both active stages and eggs of phylloxera. However, in one experiment 3.5% of phylloxera crawlers and 19% of eggs survived immersion for 48 hours.
- Crawlers of phylloxera showed a maximum 1.6% survival in grape must after 24 hours immersion, and 4.9% of eggs were alive after 48 hours immersion.

These data have been used by individuals and companies to develop protocols for disinfestation of equipment used in vineyards, and to determine the potential risks in transfer of grape juice or must for winemaking purposes. A systematic approach of Pest Risk Assessment is being used to develop policies for Governments and industry bodies, that will subsequently translate into quarantine legislation and industry recommendations.

2.3 Tristate quarantine protocols

One of the key objectives of the GWRDC project was to develop a national policy and operational plans for future management of phylloxera. This involved assessment of risks of phylloxera transfer, and development of quarantine protocols and action plans to minimise the risks. A key issue was that the phylloxera quarantines would be accepted and supported by the viticultural industries, rather than be a Government responsibility.

A phylloxera "Code of Practice" was prepared by Agriculture Victoria and the Victorian Wine Industry Association, and printed in March 1995. The document explained the legislation and procedures involved in phylloxera quarantine, particularly for Victoria and New South Wales, and provided practical advice on methods of disinfesting vine material or viticultural equipment. The Code of Practice was subsequently used by major wine companies and grapegrowers as the basis for their operating procedures. Consideration of the Code of Practice by the "Interstate Plant Health Regulation Working Group", and of the differences between States in phylloxera quarantine, resulted in the formation of a "Tristate Working Group" to harmonise phylloxera regulations across Australia. The initial Tristate working Group comprised Greg Buchanan and John Whiting (Agriculture Victoria), Richard Hamilton and David Cartwright (Primary Industries SA), and Ross Turkington (NSW Agriculture). Ross Turkington retired in January 1996, and was replaced by Colin Bower.

A discussion paper, "Coordinating quarantine strategies for phylloxera" was prepared and circulated to state and national viticultural associations in October 1995. The document was presented and discussed in several industry forums, including a tristate workshop at Mildura in October 1995. Responses were received from a wide range of industry and Government people (Appendix 5). Many of the recommendations in the paper were agreed, but contentious issues such as distances of quarantine boundaries from infested vineyards, and movement of processed grapes, were not resolved.

A second draft of the discussion paper was circulated in January 1996, which again aroused intense interest from industry. This interest centred around the issue of movement of grape must, particularly from northeast Victoria into the Murrumbidgee Irrigation Area. The Victorian Wine Industry Association sponsored an industry forum in February 1996 to discuss the issues raised in the paper, and a national meeting was held in August 1996. After further modification, the paper was used as an interim working document for 1997 (Appendix 5).

There have already been several outcomes from the activities of the Tristate Working Group, some of them unexpected. Industry awareness of phylloxera has never been greater, because of the realisation that phylloxera quarantine protocols have a fundamental effect on the commercial operations of the wine industry. Grapegrower associations and wine companies have adopted part or all of the phylloxera protocols, and included them in their own standard operating procedures. New investors in vineyards and wineries are now accounting for phylloxera quarantines in their development plans. The technical basis of phylloxera quarantine obviously requires further investigation, and is being addressed in a new GWRDC funded project which commenced in 1997.

Further actions required to implement the recommendations of the tristate paper include a formal "pest risk analysis" that applies risk analysis principles to the outstanding contentious issues in phylloxera quarantine, the formation of national and State industry groups with responsibilities for collecting funds and implementation of quarantine and control strategies, and the development of State legislation that empowers and supports the industry bodies. An interim "National Phylloxera Council" has been formed to progress the legislative and operational basis of the tristate protocols.

2.4 Public awareness and education

Public awareness of phylloxera and its impact has been facilitated by direct dissemination of information to the scientific and grapegrowing industry, and by providing the technical information and tools for public awareness and education activities undertaken by other groups or organisations. Staff from the program have presented information and results to scientific and educational forums including the Cooperative Research Centre for Viticulture, University of Adelaide and Latrobe University seminar series, and adult education classes (CAE).

A major presentation of phylloxera research was organised by program staff during September 1996, in conjunction with the Rutherglen Wine Show Society. Over seventy people attended from four Australian States. The theme "A century of phylloxera: Management for the future" focused on the history of phylloxera in Australia, and current approaches to its management. Four speakers presented topics on phylloxera including an international speaker, Dr Helen Fisher (Ontario, Canada), who spoke of the problems (or lack of problems) involved with producing wine in phylloxera's native habitat. Earlier in 1996 another overseas visitor, Dr Jeffrey Granett (University of California, Davis), also presented research results to industry in Wangaratta (Vic.) and Adelaide (SA), whilst undertaking collaborative field work in Australia with staff from this project.

An active program of extension activities directed at grower associations and groups has been conducted in all States, usually in collaboration with local staff. In addition, the interest in the Tristate protocols has generated a further demand which has been met by news and magazine articles, seminar presentations to growers, radio interviews, TV interviews and distribution of phylloxera colour brochures. The colour brochure (Appendix 9) has been the most useful information source. It was updated and reprinted during 1996, and over 6000 have since been

distributed to grower associations and Government agencies in all States. Phylloxera stickers, produced by the Phylloxera and Grape Industry Board of South Australia, have been widely distributed and are a common sight on farm vehicles throughout Victoria and the rest of Australia.

The most important aspect of communication has been the flow on effect of providing information to other organisations. The successful IPM Research to Practice education module now includes a comprehensive section on phylloxera based upon our slides and notes. The Phylloxera and Grape Industry Board of SA use project material for their publicity, and University of Adelaide, Melbourne University, and Charles Sturt University all use phylloxera material in their undergraduate courses. It is noteworthy that videos, produced from the previous HRDC project, continue to be widely used, including for ABC open learning and various TAFE courses. The Victorian Wine Industry Association and NSW Wine Industry Association have also conducted extensive phylloxera awareness campaigns in their respective states. Newsletters of these associations and commercial industry magazines regularly feature articles based on the phylloxera project and its activities. Service providers to the grape industries are also becoming aware of their need to establish and observe quarantine protocols (Appendix 9).

3. Control of phylloxera on ungrafted vines.

3.1 Management techniques to reduce effects of phylloxera on grapevines

An experiment with container grown vines was conducted in an attempt to determine the interaction of nutrition and irrigation with the effects of phylloxera. This experiment aimed to determine the relative effects of additional irrigation and fertilisers on the growth of vines grafted to resistant (Kober 5BB), tolerant (ARG1), or susceptible (*V.vinifera*) rootstocks, either infested or not infested by phylloxera. The rationale for the experiment was that water stressed grapevines show above ground symptoms of phylloxera damage earlier and to a greater extent than well watered grapevines, suggesting that frequent, light watering may reduce the effects of phylloxera. Similarly, additional fertiliser is often applied to phylloxera affected vines to stimulate canopy growth.

Unfortunately, the level of infestation by phylloxera in this experiment was low, with only Cabernet Sauvignon vines infested. Consequently, any interactions between phylloxera and water or irrigation could not be determined, even on the Cabernet Sauvignon vines. It is possible that phylloxera infestations had been assessed too late in the season to obtain meaningful estimates of populations and root damage, as there were several Cabernet Sauvignon vines with dead galls and decayed roots. There were significant effects of rootstock, and phylloxera infestation, on root weight, eg., phylloxera infested Cabernet Sauvignon vines had a mean root mass of 8.91 gm compared to uninfested vines with 12.54 gm. However, in the absence of clear relations between phylloxera numbers and root damage, the data must be treated with caution.

The application of fertilisers had highly significant effects ($p<0.001$) on shoot weight, with no interactions with other factors. Fertilised vines had shoots of 25.93 gm dry weight compared to unfertilised vines with 4.55 gm. The vines with extra water had increased root weights ($p=0.05$), but the irrigation treatment did not significantly affect shoot weight in this experiment.

3.2 Effectiveness of Cinnacure™ in control of phylloxera on Pinot Noir grapevines

A new systemic chemical, Cinnacure™ (active ingredient denoted as CNMA), was tested in Victoria during November 1995 to March 1996, as part of collaborative research with Dr. Jeffrey Granett of University of California, Davis. This product had been tested on container grown vines in California with promising results. Subsequently, field trials were established in Australia and California. The experiment was conducted in a phylloxera infested Pinot Noir vineyard in the King Valley of Victoria.

Treatments consisted of single or multiple applications of the chemical at various growth stages (pre bloom, bloom, and veraison). The effects of the treatments were assessed on the basis of phylloxera populations on the roots, bioassays of phylloxera on root pieces from different plots, rating of shoot vigour, and yield measurements. None of the treatments resulted in a substantial decrease in ratings of phylloxera populations, with the majority of replicates increasing their population rating from the pre treatment assessment. The population ratings averaged 2.31±0.89 pre treatment and 3.40±0.54 pre verasion, with no significant differences due to the Cinnacure treatment. Similarly, bioassays showed no consistent differences between the treated or untreated vines. The ratings of shoot growth demonstrated the majority of the vines were poor to very poor in vigour (rating 1-2), emphasising the severity of the phylloxera infestation in this vineyard. It was concluded that the Cinnacure treatments did not control the phylloxera populations, prevent root damage, or have beneficial effects on vine health or yields.

4. Control of phylloxera with resistant rootstocks

4.1 Screening of rootstocks for phylloxera resistance

The resistance of rootstocks to phylloxera can be defined as the relative growth of the rootstock (or grafted grapevine) infested by phylloxera compared to uninfested vines. Testing procedures that are in accord with the definition have the advantage that they measure resistance, without confounding factors such as adaptation to soils, water, or inherent vigour of the vines. An experiment was conducted to measure phylloxera resistance of several rootstocks by comparing the growth of a range of infested and uninfested grafted vines grown in pots (Appendix 6).

The growth of the vines and levels of phylloxera infestation was assessed in March 1997, and again in May 1997. On each occasion, one vine from each plot was destructively sampled to measure mass of shoots and leaves, root mass, phylloxera populations, and root galls. Higher numbers of phylloxera and galls were found in either or both assessment times on Cabernet Sauvignon, and the rootstocks J17-48, J17-69, K51-32, K51-40 and SO4 than on ARG1, or Ramsey. Over all rootstocks tested, the infested vines had significantly lower root weights and shoot weights than uninfested vines.

The relatively high populations of phylloxera on the J and K series of rootstocks indicate that these rootstocks are not sufficiently resistant to phylloxera. The performance of SO4 rootstock is also of concern, since this rootstock showed both high populations of phylloxera and significantly reduced root and shoot growth of infested vines relative to uninfested vines. Conversely, the relatively good performance of ARG1 is not a cause for optimism, given the susceptibility of this rootstock to phylloxera in both California and Australia.

This experiment, infested with VRU-1 strain of phylloxera, had phylloxera infestations lower than desirable for a severe test of the rootstocks. The levels of phylloxera on the control (Cabernet Sauvignon) vines suggest that a more virulent strain of phylloxera (eg. VNA-1) may have given different results, but again indicate that some strains of phylloxera can survive and develop on several different rootstocks to a similar extent as on *V.vinifera*.

5. Characterisation of phylloxera strains

5.1 DNA typing of phylloxera populations in Australia

The identification of so-called biotype B phylloxera by Granett et al. (1985) provided the first clear evidence that the increased virulence and/or aggressiveness of phylloxera strains could lead to the breakdown of resistance of a rootstock, in this case ARGI (a *V. vinifera x V. rupestris* hybrid). However, Californian workers have so far been unable to define genetically different strains of phylloxera associated with the biotypes.

Inconsistencies have been noted in the behaviour of phylloxera between trials at Nagambie and Rutherglen, but in the absence of other information they were attributed to environmental effects (Whiting et al. 1987, Buchanan 1990). The current project was the first serious attempt to investigate the status of phylloxera biotypes or strains in Australia, and their practical significance.

A dual tissue culture technique was developed to allow the simultaneous culture of different phylloxera populations confined to tissue cultured vines grown on agar or agar-perlite based substrates. This technique was developed independently but in parallel with similar techniques in California. The dual culture technique has proved suitable for maintaining phylloxera populations on both *V.vinifera* and various rootstocks including ARG1 and Schwarzmann. Phylloxera populations were collected from the roots of grapevines from several vineyards representing the major phylloxerated districts of Australia.

DNA typing was carried out using RAPD (random amplified polymorphic DNA) analysis. Genetic differences between phylloxera populations were readily apparent from the different sizes of DNA fragments obtained with the RAPD technique. The phylloxera populations analysed fell into three distinct groups based on their RAPD band patterns. Two of these groups were similar, the third was quite distinct. The populations from Nagambie (VNA-1) and Orchard Hills (VOH-1) demonstrated no differences. Likewise the populations collected from Milawa (VMW-1), Whitfield (VWF-1) and Whitlands (VWL-1) demonstrated 100% similarity over the range of primers used. The population collected from Rutherglen demonstrated only 48% and 51% similarity with the other two groups. The three population groups are designated VRU-1 (collected at Rutherglen), VMW-1 (Milawa) and VNA-1 (Nagambie), based on the type populations collected in these three locations.

The origin of the three distinct population groupings of phylloxera identified in this study is unclear. They may have originated through separate introductions into Australia of infested vine material, occurring on several occasions. Further work is necessary to determine if other strains of phylloxera exist in Australia, and to relate the Australian strains to overseas populations. Further knowledge of Australian strains could prove valuable in determining factors involved in transfer or dispersal of phylloxera to uninfested vineyards.

No significant differences were found between RAPD band-patterns obtained for the phylloxera populations described above and those obtained for single adult insects obtained from these same populations. Comparisons of the results of RAPD analyses of an additional population from *V. vinifera* with that of the original population at Rutherglen (VRU-2 *versus* VRU-1) and at Nagambie (VNA-2 *versus* VNA-1) failed to show any differences between the two in each case. A comparison of a phylloxera population derived from leaf galls of Schwarzmann in Rutherglen (SRU-1) showed minor genome differences to VRU-1, sourced from the adjacent vineyard. This indicated that there is conservation of genetic types within geographic regions. If the different phylloxera strains demonstrate differences in their behaviour, especially virulence to rootstocks or ungrafted vines, there may be cause to review approaches to quarantine between infested regions.

5.2 Survival of phylloxera biotypes on rootstocks and *V.vinifera*

The demonstration that different genetic strains of phylloxera are present in Australia (Corrie et al. 1997), together with the Californian experience of biotypes that are virulent against ARG1 rootstock, provides reasons to investigate the biological attributes of phylloxera in Australia. Survival of phylloxera has been found previously to be the most important parameter used to differentiate phylloxera biotypes (Granett et al.1985, Song and Granett 1990), and can be used as an indicator of the level of plant resistance. Consequently, a bioassay was conducted to determine the survival of three different biotypes of phylloxera on susceptible vines (*V. vinifera*), a "tolerant" rootstock (ARG1), and a "resistant" rootstock (Schwarzmann).

The bioassay showed significant differences in the survival of the phylloxera strains on the different rootstocks. The main difference was that phylloxera strain SRU-1 survived and developed on Schwarzmann, whereas the other phylloxera strains did not. In addition, strain VNA-1 survived significantly better on *V. vinifera* than strain SRU-1. The populations VNA-1 and VWF-1 behaved similarly in respect to survival on *V. vinifera* and ARG1, the majority of the phylloxera settling on tuberosities on the main root. In contrast, most of the SRU-1 individuals were feeding on the callus tissue or fibrous roots of both ARG1 and Schwarzmann. Results from DNA typing of the phylloxera strains are consistent with the biological relationship, in that VWF-1 and VNA-1 are closely related genetically, but SRU-1 is distinct (Corrie et al. 1997).

The differences observed in the bioassays are consistent with field observations in the regions from which the phylloxera was sourced. Strain VNA-1 was sourced from a vineyard at Nagambie where phylloxera devastated ungrafted vines. Sampling of a rootstock trial in the same vineyard showed high populations of phylloxera on ungrafted vines and ARG1, but no phylloxera were recovered from rootstocks including Schwarzmann (Buchanan 1990). Strain VWF-1 is genetically identical to phylloxera at Milawa, Victoria, where sampling of rootstocks showed high populations on ungrafted *V.vinifera* and significantly lower numbers on the roots of Schwarzmann (ARG1 was not included in this trial). A rootstock trial at Rutherglen, where strain SRU-1 was present, had similar numbers of phylloxera on the roots of ungrafted vines, Schwarzmann, and ARG1.

The biological differences between phylloxera strains are likely to be reflected in the effects of phylloxera on the vigour and yield of ungrafted *V.vinifera* vines. Boubals (1966) noted that the presence of phylloxera galls (tuberosities) on secondary roots was the most important indicator of phylloxera damage. Most individuals of phylloxera strains VNA-1 and VWF-1 were feeding on tuberosities on *V.vinifera* and ARG1, whereas SRU-1 individuals were relatively less abundant and were on both nodosities and tuberosities of *V vinifera* (Figure 2).

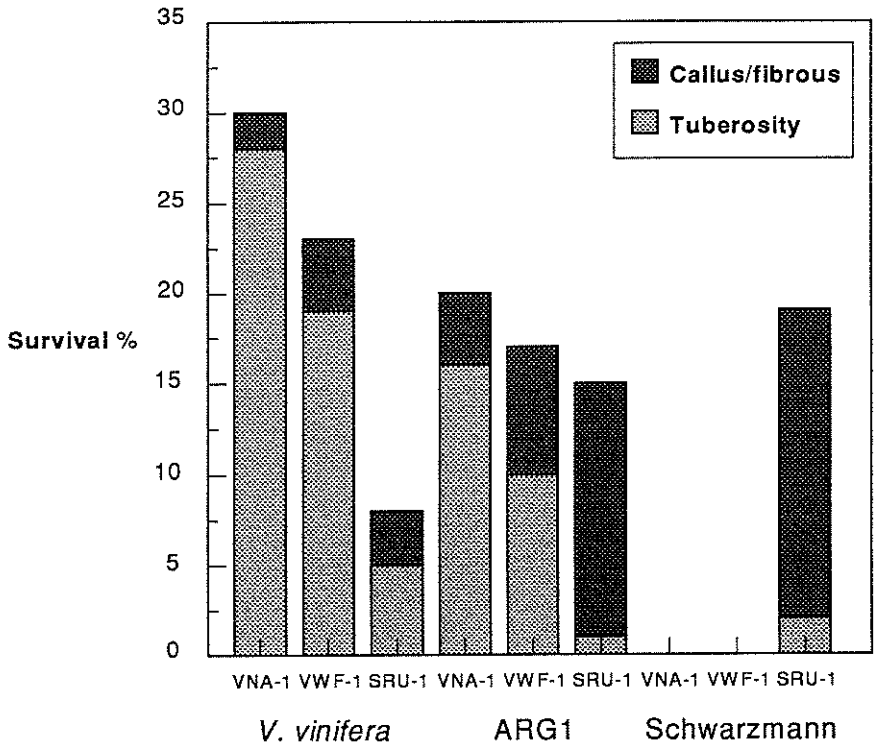


Figure 2. The survival of phylloxera strains VNA-1, VWF-1 and SRU-1 on callous tissue (and fibrous root) or tuberosities of *V.vinifera*, ARG1, and Schwarzmann roots.

Phylloxera galls on fibrous roots are not considered a significant cause of vine decline, because the roots are renewed each year. However, the development of phylloxera on fibrous roots may facilitate rapid increase of phylloxera populations during spring and summer. This could be important in spread of phylloxera; also there is the possibility that survival on fibrous roots of rootstocks could allow the evolution of strains that overcome resistance of rootstocks.

The behaviour of SRU-1 in settling on roots and callus tissue of "resistant" rootstocks is similar to that reported by Granett et.al. 1996 for phylloxera populations from different parts of north America. It seems that in north America, distinct phylloxera populations may have quite different biological characteristics that represent adaptations to different *Vitis* species and/or different environments. Knowledge of the biological characteristics of different phylloxera strains is important when considering strategies for quarantine and control of phylloxera.

Recommendations

1. The awareness of phylloxera and quarantine procedures to minimise its spread should be maintained by continued publicity projects, managed by the appropriate industry organisations in the various States. Coordination between States is necessary, and in the short term should be managed by the Phylloxera and Grape Industry Board of South Australia.

2. Annual phylloxera workshops should be continued on a full cost recovery basis. The workshops should be run by Agriculture Victoria in collaboration with the Phylloxera and Grape Industry Board of South Australia.

3. Surveys of vineyards in and near phylloxera infested districts should be conducted annually by Victoria and New South Wales, and reported to the appropriate peak national body (preferably a National Phylloxera Council).

4. The tristate protocols should be further refined for the 1998 vintage, and implemented. The recommendations in the discussion paper should be acted upon during 1998.

5. Protocols for the movement of grape material or machinery should be based on formal pest risk analyses, supported by further studies of phylloxera populations on roots and canopies of grapevines.

6. Further research should be initiated to determine the aggressiveness to *V.vinifera* and rootstocks of phylloxera strains, and to understand other biological characteristics that could affect quarantine of phylloxera.

7. DNA typing of Australian populations of phylloxera should continue, preferably using more efficient techniques (eg, microsatellites), and the Australian strains should be compared with overseas strains.

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Appendices

Appendix 1. Published paper: Corrie, A.M., G.A. Buchanan and R Van Heeswijck (1997). DNA typing of populations of phylloxera (Daktulosphaira vitifoliae (Fitch)) from Australian vineyards. Aust. J. Grape and Wine Research, **3**, 50-56.

Appendix 2. Draft paper: Corrie, A.M., G.A. Buchanan, and R. Van Heeswijck. Australian strains of phylloxera (*Daktulosphaira vitifolii)* differ in their survival on susceptible (V. *vinifera*) and resistant (*V. rupestris x V. riparia)* grapevines.

Appendix 3. Potential disinfestation techniques for grape phylloxera, and the survival of phylloxera in grape juice and must and juice.

Appendix 4. Vineyard surveys and aerial photography.

Appendix 5. Tristate quarantine protocols for containment of phylloxera.

Appendix 6. Screening rootstocks for resistance to phylloxera.

Appendix 7. The influence of nutrition and irrigation on phylloxera damage to grapevines.

Appendix 8. Chemical control of phylloxera on ungrafted vines.

Appendix 9. Increased awareness of phylloxera through improved communication and training.

Appendix 1 :

Published paper: Corrie, A.M., G.A. Buchanan and R. Van Heeswijck (1997). DNA typing of populations of phylloxera (*Daktulosphaira vitifoliae* (Fitch)) from Australian vineyards.

Aust. J. Grape and Wine Research, 3, 50-56.

Australian Journal of Grape and Wine Research 3, 50–56, 1997

DNA typing of populations of phylloxera (*Daktulosphaira vitifoliae* (Fitch)) from Australian vineyards

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Keywords: *Grape phylloxera, Daktulosphaira vitifoliae, biotype, dual tissue culture, RAPD*

Abstract

A comparative analysis of six populations of *Daktulosphaira vitifoliae*, the insect grape phylloxera, from Australia was made using the Random Amplified Polymorphic DNA typing technique. The populations analysed fell into three distinct groups with similarity between groups ranging from 48% to 86%. The significance of these groupings is discussed with reference to the history of phylloxera in Australia, recommendations on rootstock use and quarantine.

Introduction

Grape phylloxera (*Daktulosphaira vitifoliae* (Fitch)) is a native of the eastern parts of North America. The insect was first identified as a major pest of *Vitis vinifera* L. during the 1860s in France (Ordish 1972). Since then it has been found in most of the world's grape-producing areas, including Australia.

Phylloxera grows on both the leaves and roots of grapevines, although the relative abundance and effects of the infestations vary between different *Vitis* spp. and their hybrids. On *V. vinifera*, phylloxera induces swellings (galls) on fibrous and woody roots. The eventual decay of the root galls leads to decline in grapevine growth and yield. On many grapevine species native to America the phylloxera populations develop mainly in leaf galls, and root galls, when present, are confined to fibrous roots. These American species, or hybrids of them, are resistant to and/or tolerant of root infestation by phylloxera, and have been successfully used as rootstocks for *V. vinifera* scions to enable continued grape production in phylloxera-infested vineyards.

The insect was first found on grapevines in Australia near Geelong (Victoria) in 1877. It was thought that phylloxera was introduced into Australia from the United Kingdom and spread through infested nursery material (Adcock 1914). The Geelong infestations were quarantined, and eradication of the insect was attempted through removal of all infested vineyards. However, additional phylloxera infestations were discovered during the 1890s at several locations in Victoria and New South Wales. These remained restricted to discrete regions of south-east Australia (Figures 1 and 2), presumably because of quarantine restrictions and the relative isolation of the infested viticultural regions.

Since the early 1900s it has been proposed that the species *Daktulosphaira vitifoliae* (Fitch) consists of a number of different strains, where each strain is a genetically uniform population derived clonally from a single source. This is based on observed differences in morphology and galling ability of different phylloxera populations on leaves or roots (Boerner 1914, Boerner and Heinze 1957 as cited by King and Rilling 1985, Rilling 1968, Stevenson 1970, Rilling 1975, de Klerk 1979, King and Rilling 1985, Granett et al. 1985, Song and Granett 1990, Hawthorne and Via 1994, Granett et al. 1996). In many of these studies, differences in aggressiveness or virulence of phylloxera on different grapevine genotypes has also been observed. Measurable differences in rates of development, survival and fecundity on different vine genotypes has subsequently been used to group phylloxera strains into different groups termed 'biotypes' (Granett et al. 1985). More recently, the criterion of demonstrated field decline of particular vine types has been included as a requirement for definition of a phylloxera biotype (Fong et al. 1995, Granett et al. 1996). Whilst of practical value, use of this criterion fails to identify the full extent of biological variation that exists in phylloxera, which may reach increased significance in the future.

The identification of so-called biotype B phylloxera by Granett et al. (1985) provided the first clear evidence that the increased virulence and/or aggressiveness of phylloxera strains could lead to the breakdown of resistance of a rootstock, in this case ARG1 (a *V. vinifera* × *V. rupestris* hybrid). This

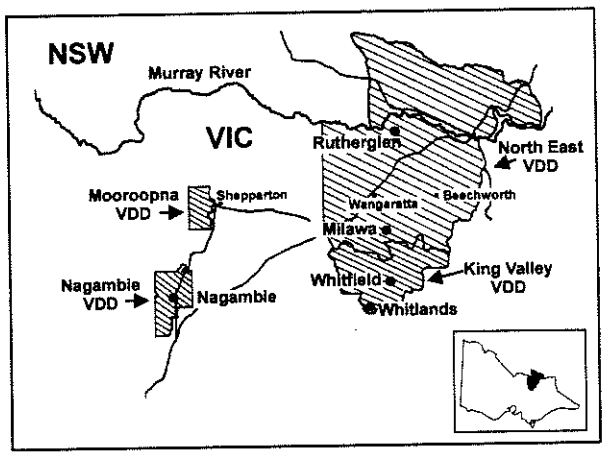


Figure 1. Defined phylloxera areas and phylloxera collection sites in Victoria, Australia. The four areas with known phylloxera infestations ('Vine Disease Districts', VDD, indicated by shading) are Nagambie, Mooroopna, North-East and King Valley. Phylloxera populations (codes in brackets) were collected at Nagambie (VNA-1), North East (VRU-1, VML-1) and King Valley (VWL-1, VWF-1).

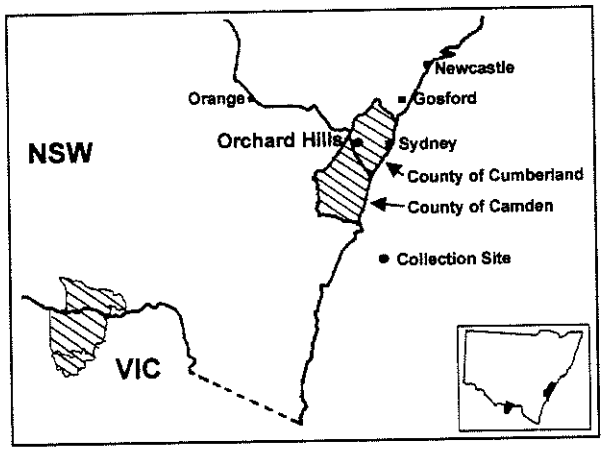


Figure 2. Recorded phylloxera areas and phylloxera collection sites in New South Wales (NSW), Australia. Shading indicates the two areas with known phylloxera infestations in NSW, Corowa and Sydney and, to show their proximity, two of the Victorian Vine Disease Districts. Phylloxera was collected in the Sydney area near Orchard Hills (population code VOH-1).

corroborated earlier reports of the insufficient field resistance of this rootstock to phylloxera in a number of locations around the world (reviewed by Boubals 1994). A recent report indicates that phylloxera collected from declining vineyards planted with vines on the rootstocks 5C, 5BB and 125AA (all *V. riparia* × *V. berlandieri* hybrids) may be more aggressive on rootstocks of this genetic background (Granett et al. 1996). The full implications of this enhanced aggressiveness in terms of the long-term durability of rootstock resistance remains unknown.

The research presented in this paper is the first step in elucidating the nature and diversity of phylloxera populations present in Australian vineyards. Before conducting this research it was generally thought that genetically different populations present in Australia may be small in number, or even non-existent, reflecting the belief that only a limited number of introductions of infested grapevine material had occurred. Additionally, any generation of new genetic diversity within or between these populations was thought to be minimal due firstly to the rarity of the sexual cycle of phylloxera in commercial vineyards under Australian conditions (Coombe 1963, Buchanan and Hardie 1978), and secondly to the limited possibility for recombination between populations because of physical distance and the imposition of rigid quarantine laws regarding the movement of grapevine material.

Predictions on the level of diversity of phylloxera populations have also been made in California where phylloxera is not a native insect but rather an introduced pest in commercial vineyards. There, the level of genetic diversity that was found was much higher than had been expected, considering that phylloxera was supposed to have been introduced into that State over a century ago and that all phylloxera populations were believed to be strictly parthenogenetic, excluding any possibility of generating diversity through sexual recombination (Fong et al. 1995). The source of the genetic diversity revealed remains unknown, but it has prompted investigation into the possibility of multiple introductions and the role of sexual recombination and somatic mutation on phylloxera variability (Fong et al. 1995, Granett et al. 1996).

Determining the range of different phylloxera populations in Australia is important to the future management of this pest in Australian viticulture. Current rootstock recommendations for phylloxera resistance are based on a combination of overseas data, field trials conducted in one or more discrete geographical locations in Australia and trials with container-grown vines using phylloxera derived from a single vineyard near Nagambie, Victoria (Whiting et al. 1987, Buchanan 1990, May 1994,). If it were found that phylloxera does vary geographically, the relevance of currently available results from field- and pot-trials to all viticultural regions would need to be established before future rootstock recommendations could be made. Quarantine boundaries may also need to be reexamined. The spread of different strains of phylloxera, even within present quarantine boundaries, may be undesirable depending on the rootstocks used in a particular area.

Materials and methods

Nomenclature

We propose that a standard nomenclature be established for phylloxera isolates and/or populations.The nomenclature used here is based on the work of Fong et al. (1995) and Granett et al. (1996), and defines the vine species and region from which a phylloxera population is derived, for example, for VRU-1: V = *V. vinifera*, RU = Rutherglen, 1 = population sample number.

Dual tissue culture of phylloxera and grapevines

Hardwood cuttings of the *V. vinifera* L. cvs Cabernet Sauvignon clone G9V3 and Shiraz clones PT10 and PT23 were obtained from the Victorian and Murray Valley Vine Improvement Association (VAMVVIA),

Irymple, Victoria. Cuttings were rooted, planted in containers and maintained in a glasshouse according to standard techniques (Neldner et al. 1983). Sterile shoot cultures were established from nodal segments (Chee et al. 1984) placed onto solid medium no.1 [half strength MS salts (Murashige and Skoog 1962), 2% (m/v) sucrose, MS vitamins (Sigma) and 7 g/L of agar (Sigma) with the pH adjusted to 5.7 with NaOH]. Culture conditions were a 12-hour photoperiod with light emitted at 50 $\mu E/m^2 \cdot s$ by cool fluorescent lights at 23°±1°C. When the explants had produced 3–4 nodes and 2–3 roots, they were placed onto medium no. 2 [half-strength MS salts, MS vitamins, 2% (m/v) sucrose, 5 g/L agar and 5 g perlite per 40 mL of medium, pH 5.7]. Once roots had become established, the tissue-cultured grapevines were infested with surface-sterilised eggs of phylloxera.

Collection of phylloxera populations and establishment in dual tissue culture

Phylloxera populations were collected from the roots of grapevines from several vineyards (Table 1), chosen in order to represent the major phylloxerated districts of Australia.

The populations were collected from 3–4 vines in vineyard areas of approximately 30 m^2 during late summer and autumn, and processed at the Institute for Integrated Agricultural Development (IIAD), Rutherglen, Victoria, which is within a Vine Disease District (Figure 1). Movement of phylloxerated material between Vine Disease Districts in the two States of Victoria and New South Wales was carried out under permits issued under the Vegetation and Vine Diseases Act 1958 (Victoria), and the Plant Diseases Act 1924 No. 38 (New South Wales). Phylloxera eggs were collected from the vine roots and surface-sterilised by placing them onto a 50 µm sieve, washing them with 70% ethanol for 30 s and then rinsing them three times with sterile water. A sterile paint brush (washed with 70% ethanol and then rinsed with sterile water) was used to move the eggs from the sieve onto the roots of established tissue-cultured grapevines.

Random amplified polymorphic DNA (RAPD) analysis

Using the grinding method of Lin and Walker (1996), DNA was extracted from adult females of phylloxera reared in dual tissue culture as described above. The DNA was stored in TE buffer (10 mM Tris-HCl, pH 7.5, and 0.1 mM EDTA) and stored frozen at –20°C. Amplification of phylloxera DNA sequences was performed using 10-mer oligonucleotide primers OPA3, OPA4, OPA7, OPA9, OPA11, OPA13, OPA18, OPB5, OPB13 (Operon Technologies, Alameda, CA, USA) as described by Williams et al. (1990). 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 template DNA. The polymerase chain reaction (PCR) was performed in a Perkin Elmer 480 DNA thermal cycler with the following amplification conditions: 4 min at 92°C, 35 cycles of 1 min at 92°C, 1 min at 35°C, 30 s at 45°C, and 2 min at 72°C, followed by a final extension cycle of 3 min at 72°C. Amplification products were analysed by electrophoresis in 1.5% (m/v) agarose gels in TAE buffer (40 mM Tris-acetate and 2 mM Na_2EDTA). The gels were then stained with ethidium bromide, and the PCR products photographed under UV light. The bands were scored as present (=1) or absent (=0) and similarity coefficients were determined according to Sneath and Sokal (1973).

Table 1. Source of populations of phylloxera (*Daktulosphaira vitifoliae* (Fitch)).

Population†	Location‡	Vine source
VRU-1	Rutherglen, Victoria	Cabernet Sauvignon
VML-1	Milawa, Victoria	Cabernet Sauvignon
VWL-1	Whitlands, Victoria	Pinot Noir
VWF-1	Whitfield, Victoria	Pinot Noir
VNA-1	Nagambie, Victoria	Semillon
VOH-1	Orchard Hills, NSW	Black Muscat

†For nomenclature see 'Materials and methods'.
‡Refer to Figures 1 and 2 for locations.

Results

Genetic differences between phylloxera populations were readily apparent from the different sizes of DNA fragments obtained with the RAPD technique (Figures 3 and 4). Thirty oligonucleotide primers from the Operon random primer kits A and B were initially tested, from which nine were selected for further use, based on the criterion that they demonstrated polymorphism between two or more of the populations in this study. Only bands that could be reproduced at least twice in independent assays were used to calculate similarity coefficients according to the method of Sneath and Sokal (1973). A total of 71 RAPD bands were scored, of which 47 bands were polymorphic between one or more populations.

The phylloxera populations analysed fell into three distinct groups based on their RAPD band patterns. Calculation of the similarity coefficients (Table 2) confirmed these groupings and further indicated that whilst two of these groups were similar, the third was quite distinct. The populations from Nagambie (VNA-1) and Orchard Hills (V0H-1)

Table 2. Similarity coefficients for the six populations† of phylloxera (*Daktulosphaira vitifoliae* (Fitch)), calculated by using the method of Sneath and Sokal (1973).

	VRU-1	VML-1	VWF-1	VWL-1	VOH-1	VNA-1
VRU-1	1.00					
VML-1	0.51	1.00				
VWF-1	0.51	1.00	1.00			
VWL-1	0.51	1.00	1.00	1.00		
VOH-1	0.48	0.86	0.86	0.86	1.00	
VNA-1	0.48	0.86	0.86	0.86	1.00	1.00

†For description of populations see Table 1.

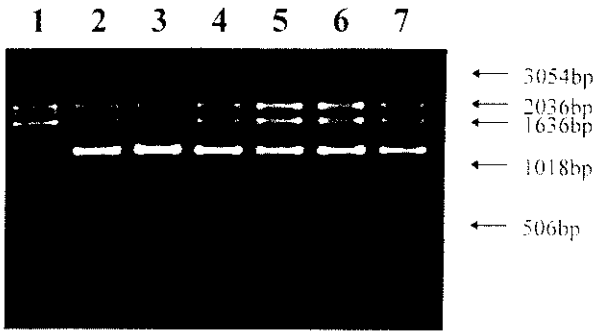


Figure 3. RAPD–PCR amplified phylloxera DNA using primer OPA4. Lane identification: 1 = VRU-1, 2 = VML-1, 3 = VWF-1, 4 = VWL-1, 5 = VOH-1, 6 = VNA-1, 7 = VNA-2. Fragment sizes were estimated from the molecular weight marker, 1 Kb ladder (Gibco-BRL) (not shown). bp = base pairs.

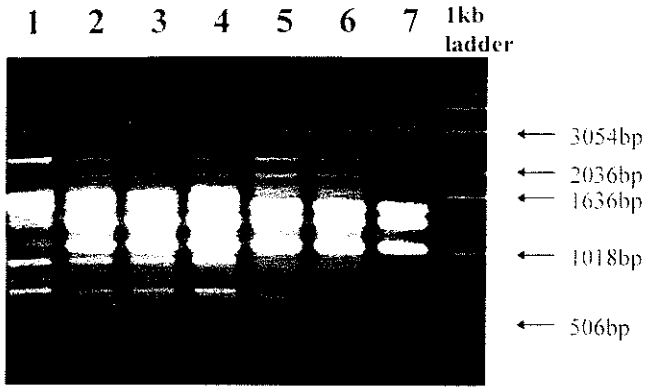


Figure 4. RAPD–PCR amplified phylloxera DNA using primer OPA11. Lane identification: 1 = VRU-1, 2 = VML-1, 3 = VWF-1, 4 = VWL-1, 5 = VOH-1, 6 = VNA-1, 7 = VNA-2. Fragment sizes were estimated from the molecular weight marker, 1 Kb ladder (Gibco-BRL). bp=base pairs.

demonstrated no differences. Likewise the populations collected from Milawa (VMW-1), Whitfield (VWF-1) and Whitlands (VWL-1) demonstrated 100% similarity over the range of primers used. The population collected from Rutherglen demonstrated only 48% and 51% similarity with the other two groups. The three population groups are designated VRU-1 (collected at Rutherglen), VMW-1 (Milawa) and VNA-1 (Nagambie), based on the type populations collected in these three locations. Population groups VMW-1 and VNA-1 appear to be closely related, with a similarity coefficient of 0.86. Three of the primers could not distinguish between these groups, as demonstrated in Figure 3 with primer OPA4. Other primers clearly distinguished between these two groups, as demonstrated in Figure 4 with primer OPA11.

Discussion

The results from this study constitute the first real evidence that phylloxera in Australia does not consist of one homogeneous population. The results of the RAPD analyses clearly indicate that there are at least three genetically distinct population groups. Because of the limited number of samples collected, this survey cannot be regarded as presenting a complete picture of the genetic diversity of phylloxera within or between all infested regions of Australia.

The identification of only a few genetically distinct populations in this study can be compared with the results obtained for the genetic analysis of phylloxera isolated from California and Oregon by Fong et al. (1995), using a combination of the RAPD technique and the biotype assessment of Granett et al. (1985). From a total of 13 isolates, Fong et al. (1995) appear to have identified at least 12 distinct strains. This can be compared with our results in which 6 populations fall into 3 distinct groups. Two additional populations collected from Rutherglen (VRU-2) and Nagambie (VNA-2), analysed using a smaller number of primers, could also be shown to belong to two of these three groups: the banding pattern of VRU-2 was identical to that of VRU-1, while that of VNA-2 was identical to VNA-1 (cf. e.g. lanes 6 and 7, Figures 3 and 4). It appears, therefore, that there may be a smaller number of genetically distinct strains present in Australia than in California. It is not possible to directly compare the results of this study and that of Fong et al. (1995). Nevertheless, it is interesting to note that the degree of genetic variation between two of the Australian groups (for example VNA-1 and VRU-1, with a calculated similarity coefficient of 0.48, Table 1) is much greater than that between any of the Californian populations (where similarity coefficients between all strains are 0.74 or greater). The significance of this remains unknown.

The greater number of distinct genotypes found in Californian phylloxera might simply reflect the introduction of a greater number of different strains due to repeated introductions into that region. Alternatively it could indicate high levels of sexual recombination or somatic mutation within the introduced populations. It is important to note that the isolates used in the study of Fong et al. (1995) were obtained from at least six different grapevine genotypes, namely two *V. vinifera* and four rootstock cultivars bred from American species, whilst the Australian populations in the present study were sourced entirely from *V. vinifera* grapevines. The increased level of genetic variation in the Californian study may therefore also reflect an influence of host-vine genotype on phylloxera diversity through selection or adaptation of specific strains.

When phylloxera will be sourced from a range of grapevine genotypes in Australia, it may indeed be discovered that there is a greater amount of variation amongst its phylloxera strains than determined here. Preliminary results from RAPD analysis of a phylloxera population derived from leaf galls of the rootstock Schwarzmann in Rutherglen (SRU-1), collected from the same vineyard as VRU-1, indeed indicated minor genome differences between the two (results not shown). More extensive sampling is under way so that an increased range of phylloxera populations can be established in dual tissue culture

for subsequent DNA typing.

In assessing the reliability and significance of these results it is important to discuss some aspects of the experimental procedure. Each original collection of phylloxera was sourced from a number of grapevines in one location, and consisted of more than one individual. It is therefore conceivable that each population contained a mixture of strains. The genetic variation observed between different population groups might therefore simply reflect the variation in number and type of strains present in any one location. At present, this possibility cannot be refuted. However, we believe it to be unlikely for three reasons.

Firstly, using a limited number of primers, no significant differences were found between the RAPD band-patterns obtained for the phylloxera populations described above and those obtained for single adult insects obtained from these same populations. In the case of the latter, DNA was extracted according to the method of Lin and Walker (1996). Unfortunately, in our hands, it was difficult to obtain reliable RAPD analyses from the DNA extracted from single adult insects, otherwise this would have been the preferred method of analysis for all populations studied. The lack of reliability probably stemmed from our inability to quantify, and therefore standardise, the very small amount of template DNA isolated from single insects. It is well documented that the reproduciblity of RAPD analyses is affected by a number of variables including the amount of template DNA (MacPherson et al. 1993, Rafalski et al. 1994).

Secondly, comparisons of the results of RAPD analyses of an additional population from *V. vinifera* with that of the original population at Rutherglen (VRU-2 *versus* VRU-1) and at Nagambie (VNA-2 *versus* VNA-1) failed to show any differences between the two in each case. This indicates that there is conservation of genetic types within geographic regions. If these genetic types were to be made up of mixtures of strains, the number and type of strains would also need to be tightly conserved throughout these regions.

Thirdly, there was no change in RAPD band-pattern from DNA extractions taken from dual tissue cultures of the same population over a period of 12 months despite repeated subculturing, indicating that if these genetic types were to be made up of mixtures of strains, the relative ratios of different strains would need to be maintained throughout the dual tissue culturing procedures.

The origin of the three distinct population groupings of phylloxera identified in this study is unclear. They may have originated through separate introductions into Australia of infested vine material, occurring on several occasions. The initial introduction in the 1870s was believed to have come from an English nursery (Adcock 1914). Quarantines were imposed both within and between States after the discovery of phylloxera in the Geelong region in 1877, but it was not until the early 1890s that grapevine imports from overseas countries were prohibited (Hopton 1891, Quinn 1924).

In the period 1907 to 1915, grafted rootlings were imported directly from France to overcome shortages of replanting material. These imports were probably responsible for the introduction of downy mildew (*Plasmopara viticola*) to Australia (de Castella and Brittlebank 1917), and could also have carried phylloxera. New infestations could therefore have been from an overseas source and not from Geelong, as is commonly assumed. Clarification of any genetic similarities and differences between the original Geelong phylloxera population and the populations found in other parts of Australia is not possible since phylloxera can no longer be found in Geelong (Buchanan 1987).

Phylloxera was first found in Central Victoria in the Bendigo region, in 1893. It was subsequently detected at Nagambie in 1897, and at Rutherglen in 1899 (Dubois 1902). Systematic inspections of vineyards were not carried out from 1902 till 1978. It is believed that at least one vineyard in Milawa was infested between 1902 and 1920, but the year of initial detection cannot be verified (Buchanan 1987). These four locations, Bendigo, Nagambie, Rutherglen and Milawa, were all part of the same quarantine zone, the North Central Vine Disease District, for over 70 years, until 1986. Then, as the result of a new survey of phylloxera infestations carried out between 1978 and 1982 (Buchanan 1987), the total size of the quarantined area was reduced, while a number of smaller Vine Disease Districts was declared (Figure 1), including the Central (Nagambie) and North East Districts (Victorian Government 1986).

A single introduction of phylloxera into North Central Victoria could have spread across this region and been responsible for infestations at all these locations. This is not supported by the results reported in this paper however, where the phylloxera population-group VRU-1 was shown to be quite distinct from VML-1 and VNA-1. This indicates that there were at least two introductions of phylloxera into Victoria. The present indications are that the spread of phylloxera even within a quarantine district was quite limited and that consequently the mixing of these strains did not occur. This will have to be tested further, by more extensive sampling in individual vineyards and by examining vineyards at some distance from those providing the samples of this study, e.g. at Glenrowan, situated approximately 40 km south-west of Rutherglen and 20 km west of Milawa.

The finding of 100% similarity between phylloxera populations VNA-1 and VOH-1 indicates that these two populations have come from the same source. Further, the results indicate that the populations have maintained their clonal state and/or have been subjected to similar selective pressures during the period of separation. Orchard Hills was identified as being infested in 1969, presumably from other Sydney/Camden phylloxerated vineyards. The introduction of phylloxera into NSW, as reviewed by

Preedy (1896), was believed to have been due to movement of planting material from Victoria into Camden in 1884. There is no information on whether this planting material was from the Nagambie district.

The detection of phylloxera in Whitlands and Whitfield was very recent, 1991 and 1995 respectively. There are no detectable differences between the phylloxera populations VWL-1 and VWF-1, based on the RAPD band patterns obtained in this study. The close proximity (ca. 5 km) of the vineyards from which these phylloxera populations were sourced indicates that phylloxera may have spread by natural means from one vineyard to the other. However, previous dispersal studies of phylloxera in Australia led to the conclusion that the maximum rate of natural spread was 100 m per year (Buchanan 1990). This study was undertaken in Nagambie, an area with a different climate and topography to Whitfield and Whitlands, and it is feasible that the natural spread of phylloxera could be more extensive in the latter regions. The apparent similarity between VWL-1, VWF-1 and VML-1 indicates that the original source of the Whitlands/Whitfield infestations was Milawa. The considerably greater distance (approximately 40km) between these districts further indicates that the spread of phylloxera was associated with the movement of machinery or people rather than natural dispersal.

Whilst our results provide evidence that there were at least two distinct introductions of phylloxera into Australia, and that this might explain some if not all of the genetic variation that we observed, the possibility still remains that there has been some evolution and/or selection of phylloxera since arriving in Australia. The sexual life-stages of phylloxera have not yet been seen in the field in Australia, and confirmation of the presence or absence of the sexual cycle is required (Buchanan and Hardie 1978). Consequently, it is unknown whether sexual recombination has contributed to the generation of genetic diversity of phylloxera in Australia and this problem awaits investigation.

Sexual recombination is not the only method by which genetic diversity can be generated. Somatic mutation is also considered to be a significant driving force in aphid evolution (Dixon 1985). Somatic mutations are highly likely to result in varied offspring due to the combination of parthenogenetic reproduction from a single cell within the mother and high rates of reproduction (Dixon 1985).

Elucidation of the full extent of genetic variation, and its origins, requires more comprehensive sampling of phylloxera both within and between vineyards, and over time. In order to make this feasible, alternative methods of DNA typing are being examined whereby reliable results can be generated using DNA prepared from a single phylloxera egg sourced directly from the vineyard. A technique using sequence-tagged microsatellite sites (Estoup et al. 1993) offers some potential, and preliminary work in our laboratories has already detected microsatellites, or short regions of repetitive DNA, within the genome of phylloxera (Winnall 1996).

The detection of at least three population groups of phylloxera in Australia has potential implications for the future management of this insect pest with regard to both rootstock recommendations and quarantine procedures. These issues become even more critical if the genetic differences that we have identified correlate with differences in virulence on different *Vitis* genotypes.

The existence of different biotypes in different locations has been suspected for some time based on field observations of the performance of different rootstocks, ARG1 in particular (de Castella 1937, Whiting et al. 1987). If different biotypes are present, then recommendations for use of particular rootstocks for phylloxera control will need to take into account the particular population or biotype present within the site to be planted with grapevines. Results of rootstock field trials from overseas, or from one Australian region, may not be immediately applicable to another Australian region. Trials with container-grown vines which have been used more recently for rapid screening of the resistance of new rootstocks to phylloxera may also need to be modified to provide for assessment against more than one phylloxera strain.

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Appendix 2 :

Draft paper: Corrie, A.M., G.A. Buchanan and R. Van Heeswijck. Australian strains of phylloxera (*Daktulosphaira vitifolii*) differ in their survival on susceptible (*V. vinifera*) and resistant (*V. rupestris x V. riparia*) grapevines.

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Draft paper

Australian strains of phylloxera (*Daktulosphaira vitifolii*) differ in their survival on susceptible (V. *vinifera*) and resistant (*V. riparia x V. rupestris*) grapevines.

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Keywords: *Grape phylloxera, Daktulosphaira vitifolii, Biotype, Dual tissue culture, RAPD, Vitis*

Abstract:

Quarantine and the use of resistant rootstocks are the methods by which phylloxera is managed in Australia. Rootstocks differ in their resistance to phylloxera, but the mechanisms involved are not well understood. Until recently (Corrie et al 1997), there had been no satisfactory way to characterise phylloxera strains and subsequently to determine their virulence to grapevines. This paper reports testing of three strains of phylloxera using a bioassay, and shows that the strains differ in their survival on *Vitis vinifera* and a resistant rootstock (*V. riparia x V. rupestris*).

All three strains survived and developed on the rootstock ARG1 (*V. vinifera* x *V.rupestris*). One strain, SRU-1, is less aggressive on grapevines of European parentage (*V. vinifera*) but can establish and reproduce on callus tissue and fibrous roots of the rootstock Schwarzmann (*V riparia* x *V. rupestris*). The other strains did not survive on Schwarzmann, but survived and developed well on *V. vinifera*. The results of this study indicate that populations of phylloxera on the roots of *V. vinifera* or rootstocks are likely to differ according to the strain(s) of phylloxera present in the vineyard. This has implications for the effects of phylloxera on ungrafted grapevines, and could explain variation in measurements of phylloxera resistance between trials in Australia and overseas.

INTRODUCTION:

The practice of grafting commercial varieties of grapes onto resistant rootstocks is the only successful management option for controlling phylloxera infestations. The rootstocks are derived from hybrids or selections of American vines including, in some cases, *V. vinifera* parents. Consequently, rootstocks have a diverse genetic background and vary in their level of tolerance and/or resistance to phylloxera. The resistance of rootstocks has been determined in different ways, usually by measurement of phylloxera populations and/or growth of the infested rootstock in comparison to an uninfested rootstock or infested *V.vinifera*. Ratings of resistance have varied in different countries (Viala and Ravaz 1901, Boubals 1966, King and Rilling 1985, Granett et al. 1987, Whiting et al. 1987).

The variation in rootstock assessments between field trials have been attributed to differences in soils or other environmental factors, and sometimes to misidentification of cultivars.

Another explanation could be that different strains of phylloxera exist, and they differ in their virulence to grapevines. It was the identification of biotype B by Granett et al. (1985) that provided the first evidence that the increased virulence/aggressiveness of phylloxera strains could explain the breakdown of resistance of a rootstock, in this case ARG1 (*V. vinifera x V. rupestris*), which was observed during the 1980's in California (Granett et al. 1985).

In Australia current rootstock recommendations for phylloxera resistance are based on a combination of overseas data, field trials conducted in one or more parts of Australia, and container grown vines using phylloxera derived from a single vineyard near Nagambie, Victoria (Whiting et al. 1989, Buchanan 1990, May 1994). Inconsistencies noted between trials at Nagambie and Rutherglen were attributed to environmental effects (Whiting et al. 1987, Buchanan 1990).

The demonstration that different genetic strains of phylloxera are present in Australia (Corrie et al. 1997), together with the Californian experience of biotypes that are virulent against ARG1 rootstock, provides reasons to investigate the biological attributes of phylloxera in Australia. Survival of phylloxera has been found previously to be the most important parameter used to differentiate phylloxera biotypes (Granett et al.1985, Song and Granett 1990), and can be used as an indicator of the level of plant resistance. This paper reports the survival and development of three different biotypes of phylloxera on susceptible vines (*V. vinifera*), a "tolerant" rootstock (ARG1), and a "resistant" rootstock (Schwarzmann).

MATERIAL AND METHODS:

Phylloxera source

A representative population of each of the three strains (SRU-1, VNA-1 and VWF-1) of phylloxera identified previously by DNA typing were used in the bioassays (Table 1). All populations were raised in dual tissue culture, according to techniques described in Corrie et al. (1997), and maintained on the vine type from which they were collected.

Table 1. Source of populations of phylloxera

Population[#]	Location	Vine Source
SRU-1	Rutherglen, Victoria	Schwarzmann (*V. riparia* x *V. rupestris)*
VWF-1	Whitfield, Victoria	Pinot Noir (*V. vinifera)*
VNA-1	Nagambie, Victoria	Semillon (*V. vinifera)*

Nomenclature based on the method of Corrie et al (1997), whereby VWF-1: V= *Vitis vinifera*, WF = Whitfield, 1 = population sample number.

25 Day Bioassays

The 25 day bioassays were performed on excised root pieces based on the method of Granett et al. (1987). Root pieces (secondary roots) were collected from a rootstock trial during March, and taken back to the laboratory in sealed moist plastic bags. The success of the bioassays (level of establishment) was highly dependent upon the condition of the root pieces; therefore only those roots that exhibited no obvious signs of pathogens were chosen for the study.

The roots, of 2-5 mm in diameter, were cut into 4cm long sections. They were then washed with sterile distilled water and dipped into 300mg/l chlorothalonil solution (Bravo^{TM}), blotted dry, wrapped on the proximal end with cotton wool (moistened with sterile water) and placed into filter paper lined plastic petri dishes. Four root pieces were placed into each petri dish. The petri dish lid had a hole (diameter 1 cm) covered with 40µm cloth material (Sigma), to prevent

high humidity and the consequent build up of fungi and bacteria. The petri dishes were sealed with parafilm and stored in plastic containers at 23°C± 1°C, in the dark.

The experiments were initiated by placing 10 two to five day old eggs from a dual tissue culture population of the appropriate phylloxera strain (VNA-1, VWF-1, or SRU-1) on a root piece. The three vine types used were Muscadelle (*V. vinifera*), ARG1 (*V. vinifera* x *V. rupestris*), and Schwarzmann (*V. rupestris* x *V. riparia*). There were 3 replicates of each phylloxera strain *vinetype treatment, where a replicate consisted of a petri dish containing four excised root pieces (each with 10 eggs).

The numbers of surviving phylloxera, classified according to development stage and feeding site, were recorded after 25 days. Survival of phylloxera was expressed as the number of live phylloxera on the vine roots. The development of phylloxera was expressed as mean estimated instar (MES)= Σ [N.*x* I] /N where N is the number of surviving phylloxera and I is a number corresponding to the instar stage (e.g., 1st instar=1, adult=5).

Statistical Analysis

The bioassay results were analysed by Analysis of Variance using Genstat.

RESULTS:

Significant differences were found in the survival of phylloxera between the different phylloxera strains on the different rootstocks (Table 2). The main difference was that phylloxera strain SRU-1 survived and developed on Schwarzmann, whereas the other phylloxera strains did not. In addition, strain VNA-1 survived significantly better on *V. vinifera* than strain SRU-1. Strain SRU-1 developed faster on ARG1 than strains VNA-1 or VWF-1.

Table 2. Survival and development of three phylloxera strains on susceptible (*V.vinifera*), tolerant (ARG1), and resistant (Schwarzmann) rootstocks in a bioassay test.

	Strain of phylloxera		
	VNA-1	**VWF-1**	**SRU-1**
Survival			
V.vinifera	12.00	9.00	3.33
ARG1	8.00	6.67	6.00
Schwarzmann	0.00	0.00	7.67
LSD (p=0.05)	5.98		
Development (MES) [1]			
V.vinifera	3.56	3.30	3.64
ARG1	3.30	3.06	4.93
Schwarzmann	0.00	0.00	4.63
LSD (p=0.05)	1.52		

[1] Mean estimated instar =Σ [N.*x* I] /N where N is the number of surviving phylloxera and I is a number corresponding to the instar stage (e.g., 1st instar=1, adult=5).

The populations VNA-1 and VWF-1 behaved similarly in respect to survival on *V. vinifera* and ARG1, the majority of the phylloxera settling on the main root, forming tuberosities (Figure 1). In contrast, most of the SRU-1 individuals were feeding on the callus tissue or fibrous roots of both ARG1 and Schwarzmann.

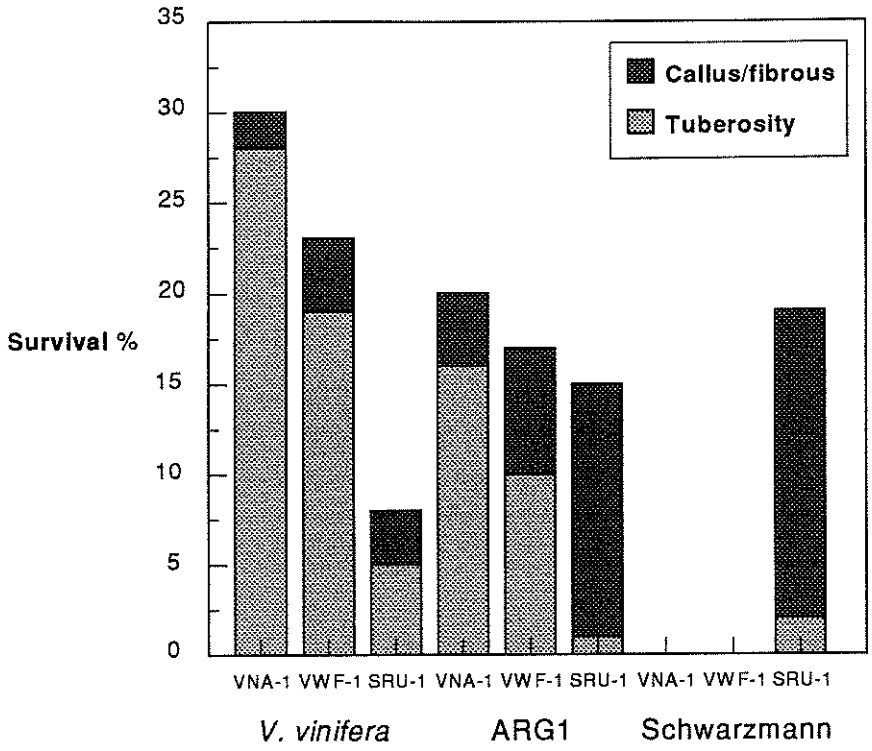


Figure 1. The survival of phylloxera strains VNA-1, VWF-1 and SRU-1 on callus tissue (or fibrous root), or tuberosities of *V.vinifera*, ARG1, and Schwarzmann roots.

DISCUSSION:

The bioassays have demonstrated that strains of phylloxera differ in their ability to colonise and develop on *V. vinifera*, ARG1, and Schwarzmann grapevines, which are rated as susceptible, tolerant and resistant, respectively, by Boubals (1966). The phylloxera strains VWF-1 and VNA-1 are similar biological types in that they survive and develop well on *V.vinifera* and ARG1. The other strain, SRU-1, has a capacity to grow on the young fibrous roots, or callus tissue of the rootstocks ARG1 and Schwarzmann. Results from DNA typing of the phylloxera strains are consistent with the biological relationship, in that VWF-1 and VNA-1 are closely related genetically, but SRU-1 is distinct (Corrie et. al. 1997).

The differences observed in the bioassays are consistent with field observations in the regions from which the phylloxera was sourced. Strain VNA-1 was sourced from a vineyard at Nagambie where phylloxera devastated ungrafted vines. Sampling of a rootstock trial in the same vineyard showed high populations of phylloxera on ungrafted vines and ARG1, but no phylloxera were recovered from rootstocks including Schwarzmann (Buchanan 1990). Strain VWF-1 is genetically identical to phylloxera at Milawa, Victoria, where sampling of rootstocks showed high populations on ungrafted *V.vinifera* and significantly lower numbers on the roots of Schwarzmann (ARG1 was not included in this trial). A rootstock trial at Rutherglen, where strain SRU-1 was present, had similar numbers of phylloxera on the roots of ungrafted vines, Schwarzmann, and ARG1.

The biological differences between phylloxera strains are likely to be reflected in the effects of phylloxera on the vigour and yield of ungrafted *V.vinifera* vines. Boubals (1966) noted that the presence of phylloxera galls (tuberosities) on secondary roots was the most important indicator of phylloxera damage. Most individuals of phylloxera strains VNA-1 and VWF-1 were feeding on tuberosities on *V.vinifera* and ARG1, whereas SRU-1 individuals were relatively less abundant and were on both nodosities and tuberosities of *V vinifera* (Figure 1). Further work is necessary to define the virulence of phylloxera strains to ungrafted vines and to various rootstocks.

Phylloxera galls on fibrous roots are not considered a significant cause of vine decline, because the roots are renewed each year, but they may facilitate rapid increase of phylloxera populations during spring and summer. The observation that strain SRU-1 developed faster on ARG1 than the other phylloxera strains could be a result of its feeding site on fibrous roots or callous tissue; further analyses are required to confirm this.

Phylloxera populations in California from rootstocks, including Harmony, Dog Ridge, Teleki 5C, demonstrate behaviour similar to SRU-1, in that they had a tendency to establish on the fibrous roots of the rootstocks (Granett et al 1996). Fergusson-Kolmes and Dennehy (1993) demonstrated that a population derived from wild grapes in New York State had a lower attachment rate on *V. vinifera* than phylloxera obtained from the commercial vineyards in California. The capacity of phylloxera strains to survive on fibrous roots may be linked to other biological characteristics, including the ability to form leaf galls, which is important in natural dispersal of populations. Also, there is the possibility that survival on fibrous roots of rootstocks could allow the evolution of strains that overcome resistance of rootstocks.

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Appendix 3 :

Potential disinfestation techniques for grape phylloxera, and the survival of phylloxera in grape juice and must and juice.

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Appendix 3. Potential disinfestation techniques for grape phylloxera, and the survival of phylloxera in grape juice and must and juice.

Introduction

Australia is one of the few countries in the world that does not have grape phylloxera endemic throughout its grapegrowing regions. Vineyards infested by phylloxera are contained within quarantine areas, and movement of grapevine material from the quarantine areas is restricted. In the past, the restrictions applied to any part of the grapevine, including fruit harvested for fresh markets, wine or drying. However, the restrictions were not applied to crushed grapes (juice or must), or to wine.

King and Buchanan (1986) showed that phylloxera crawlers (first instar nymphs) were present on the canopy of grapevines, and associated them with spread of phylloxera in or between vineyards. This finding resulted in the development of cleaning and disinfestation protocols for mechanical harvesters and other vineyard equipment and implements. It also raised the prospect that crawlers could be present on harvested grapes, and in the resulting must or juice, or discarded residues, after processing.

Modern viticultural and winemaking technology involves mechanical harvesting of grapes and rapid transport to processing facilities that may be hundreds of kilometres from the source vineyards. Thus there is an increased risk of spreading phylloxera on transport equipment, in the harvested grapes, or perhaps even in crushed grapes. Consequently, it is necessary to understand the factors that influence survival of phylloxera that may be present on harvested grape bunches, and to further investigate potential disinfestation techniques. The objectives of the work described in this document were to:

1. Determine the effectiveness of currently used or potential new disinfestation techniques in killing the relevant stages of grape phylloxera.
2. Investigate the survival of phylloxera in grape juice and must in order to understand the risks associated with transport of processed grapes.

Methods and results

Phylloxera was cultured and experiments were carried out at the Institute for Integrated Agricultural Development, Rutherglen, which is within the North East Vine Disease District of Victoria (a phylloxera quarantine zone). Phylloxera was cultured in a glasshouse on the roots and leaves of *V. vinifera*, usually Shiraz, Sultana, or Cabernet Sauvignon. The phylloxera culture was sourced from leaf galls on Schwarzmann rootstocks in a vineyard at Rutherglen, Victoria. Phylloxera for the different experiments were collected on either root galls or leaf galls.

Eight different experiments were carried out to determine the effects of different treatments on survival of various stages of phylloxera. These are described in chronological sequence. As the methods used for each experiment were similar, they are only described in detail for the first experiment and later descriptions refer to variations in the methods.

Experiment 1. The tolerance of active stages of phylloxera to washing (cold water, detergent) and disinfestation treatments (immersion in hot water, 70% ethyl alcohol, or 1% sodium hypochlorite solutions). (December 1994).

Objective: To determine the survival of active stages of phylloxera when immersed in hot and cold water, detergent, 70% ethyl alcohol, and 1% sodium hypochlorite solutions.

Methods:

Individual root galls (nodosities), which carried all stages of phylloxera, were cut from roots of potted grapevines. At least 10 galls, bearing 50-100 active stages (ie., nymphs or adults) of phylloxera were placed in a polycarbonate plastic vial which had stainless steel mesh (mesh openings of 50 microns) on each end. These vials contained all stages of phylloxera while allowing entry of the various liquids being tested.

The experiment was designed as a randomised block, with a single vial of insects randomly allocated to each of ten experimental treatments and five replicates. The control consisted of vials kept at 25^{0}C and a relative humidity of 95% within a dessicator. The other treatments consisted of immersion in various liquids for specified times (Table 1), after which the vials were drained, and held in a dessicator at 25^{0}C and 95% relative humidity prior to assessment of insect viability.

The numbers of active stages of phylloxera were counted and classified as live or dead using a dissecting microscope. Active stages of phylloxera were scored as live if they moved, if necessary after prodding with a hair of a brush, or dead if no movement could be elicited. Results are expressed as % live phylloxera.

Results:

The results (Table 1) show that active stages of phylloxera are tolerant of immersion in water at 25^{0}C for up to 1 hour. Survival was reduced significantly by immersion in water at 52^{0}C for 1 minute, and 70% alcohol for 1 or 5 minutes. No phylloxera survived immersion in water at 52^{0}C for 5 minutes, or 1% sodium hypochlorite solution for 1 minute.

Table 1. The survival of active stages of phylloxera after immersion in water at 25^{0}C and 52^{0}C, 1% detergent, 70% ethyl alcohol, or 1% sodium hypochlorite.

Treatment	Time	% live active stages of phylloxera	No. insects tested
Control (air)	5 min	66.2	288
Water 25^{0}C	1 min	54.2	305
Water 25^{0}C	5 min	51.8	277
Water 25^{0}C	1 h	55.4	333
Water 52^{0}C	1 min	5.8	296
Water 52^{0}C	5 min	0.0	333
1% detergent	1 min	58.9	251
70% ethyl alcohol	1 min	0.8	303
70% ethyl alcohol	5 min	2.9	287
1% hypochlorite	1 min	0.0	324
LSD (p=0.05)		28.9	

Experiment 2. The tolerance of grape phylloxera to immersion in hot water, 70% ethyl alcohol, Sultana must, and Sultana must containing various levels of potassium bisulphite. (February 1995).

Objective: To further investigate the effectiveness of hot water and 70% ethyl alcohol as disinfestation treatments, and to determine the tolerance of active stages of phylloxera to immersion in Sultana must, with or without potassium metabisulphite.

Methods:

Individual root galls (nodosities), which carried all stages of phylloxera, were cut from roots of potted grapevines, and placed in polycarbonate vials, as described in Experiment 1. Sultana must was prepared by macerating fresh Sultana grapes with a laboratory blender, and then removing bunch and berry stalks from the "must" by straining with a coarse sieve.

The experiment was designed as a randomised block, with a single vial of insects randomly allocated to each of ten experimental treatments and five replicates (Table 2). The control consisted of vials kept at 25^0C and a relative humidity of 95% within a dessicator for 15 hours. The other treatments consisted of immersion in various liquids for specified times (Table 2), after which the vials were drained, and held at 25^0C and 95% relative humidity prior to assessment of insect viability. The numbers of active stages of phylloxera were counted and classified as live or dead using a dissecting microscope, as described in Experiment 1.

Results:

The results (Table 2) confirm the efficacy of hot water as a disinfestation treatment for phylloxera, but also show that immersion in 70% alcohol for 1 minute is not completely effective as a disinfestation treatment. Survival of phylloxera in must is reduced in comparison to that in water, but the addition of metabisulphite has no significant effect at the rate (50ppm) normally used in winemaking.

Table 2. The survival of active stages of phylloxera after immersion in 70% ethyl alcohol, hot water, or Sultana must with or without sodium metabisulphite.

Treatment	Time	% live phylloxera	No. insects tested
Control (air)	15h	48.7	269
70% ethyl alcohol	1 min	3.2	181
water 52^0C	1 min	0.0	267
water 52^0C	5 min	0.0	348
water 25^0C	15h	14.7	224
Sultana must	15h	0.6	158
Sultana must +50ppm NaS_2O_5	15h	2.3	205
Sultana must + 100ppm NaS_2O_5	15h	2.2	227
Sultana must + 200ppm NaS_2O_5	15h	3.4	187
Sultana must + 400ppm NaS_2O_5	15h	0.0	191
LSD (p=0.05)		11.6	

Experiment 3. The tolerance of grape phylloxera to immersion water, metabisulphite solutions, 20% sucrose solution, and clear grape juice. (March 1995).

Objective: To investigate the tolerance of active stages and eggs of phylloxera to prolonged immersion in water, metabisulphite solutions, grape juice, and a sucrose solution.

Methods:

Individual root galls (nodosities), which carried all stages of phylloxera, were cut from roots of potted grapevines, and placed in polycarbonate vials, as described in Experiment 1. Clarified white grape juice was obtained from a commercial winery.

The experiment was designed as a randomised block, with a single vial of insects randomly allocated to each of eleven experimental treatments and five replicates (Table 3). The control consisted of vials kept at 25^0C and a relative humidity of 95% within a dessicator for 24 hours. The other treatments consisted of immersion in various liquids for specified times (Table 3), after which the vials were drained, and held at 25^0C and 95% relative humidity prior to assessment of insect viability. The numbers of active stages of phylloxera were counted and classified as live or dead using a dissecting microscope, as described in Experiment 1. Eggs were removed from each vial, placed in a petri dish covered with polythene film, and kept for 8 days before counting live crawlers, and unhatched eggs.

Results:

This experiment confirmed that immersion in water, or metabisulphite solutions, for 24 h has little effect on survival of active stages or eggs of phylloxera (Table 3). However, survival of active stages was significantly reduced following immersion in 20% sucrose and in grape juice. The survival of eggs was also reduced after immersion in sucrose or grape juice, but the results were not significant due to the small number tested.

Table 3. The survival of phylloxera after immersion in water, metabisulphite or sucrose solutions, and grape juice.

Treatment	Time	% live active stages	No. insects tested	% hatched eggs	Total no. eggs
Control (air)	24 h	36.2	306	17.0	52
water	5 min	38.8	282	17.0	57
water	1 h	15.6	261	12.8	36
water	4.5 h	18.3	290	8.0	51
water	9 h	52.2	272	51.3	68
water	24 h	17.1	333	57.9	59
water + 50ppm NaS_2O_5	24 h	39.7	311	27.5	34
water + 250ppm NaS_2O_5	24 h	85.9	302	20.8	46
water+500ppm NaS_2O_5	24 h	41.4	274	16.8	57
20% sucrose	24 h	8.6	295	3.6	39
grape juice	24 h	0.6	267	0	41
LSD (p=0.05)		24.8		29.0	

Experiment 4. The tolerance of phylloxera to immersion in water and fermenting Merlot must. (April 1996).

Objective: To determine the survival of phylloxera in water and fermenting must.

Methods:

Individual root galls (nodosities), which carried all stages of phylloxera, were cut from roots of potted grapevines, and placed in polycarbonate vials, as described in Experiment 1. The experiment was designed as a randomised block, with a single vial of insects randomly allocated to each of 12 treatments (three treatments*4 time periods), replicated 5 times (Table 4). Treatments consisted of immersion in fresh Merlot must (with yeast added to initiate fermentation) obtained from a commercial winery, or water, and controls which were vials kept in air at 95% relative humidity.

The vials were immersed in the water and must treatments for 16, 36, 72, or 120 hours, drained, and held at 25^{0}C and 95% relative humidity prior to assessment of insect viability. The numbers of active stages of phylloxera were counted and classified as live or dead using a dissecting microscope, as described in Experiment 1. Eggs were removed from each vial, placed in a petri dish covered with polythene film, and kept for 5-9 days before counting the number of live crawlers and unhatched eggs.

Results:

The active stages of phylloxera did not survive in fermenting Merlot must for 16 hours, but a few eggs survived immersion for up to 36 hours (Table 4). Survival of both eggs and active stages was reduced in water, with no survivors after 72 hours immersion.

Table 4. The survival of active stages, and eggs, of phylloxera in fermenting Merlot must or water.

Treatment	Time	% live active stages	No. insects	% live crawlers	No. eggs and crawlers counted
Control (air)	16 h	89.9	378	3.5	54
	36 h	85.4	288	12.2	95
	72 h	90.3	451	45.0	82
	120 h	83.9	377	58.9	254
water	16 h	4.7	277	0.0	42
	36 h	0.3	360	6.8	105
	72 h	0.0	338	0.0	120
	120 h	0.0	366	0.0	101
Merlot must	16 h	0.0	293	0.0	61
	36 h	0.0	326	2.2	80
	72 h	0.0	267	0.0	74
	120 h	0.0	340	0.0	85
LSD (p=0.05)		5.7		19.2	

Experiment 5. The survival of phylloxera eggs in Malbec must, clarified grape juice, and water.

Objective: To further investigate the survival of phylloxera eggs in grape juice and must.

Methods:

Eggs collected from leaf galls of phylloxera were sorted to select young (pre red-eye stage) eggs. Fifty eggs were placed into each of 60 vials, or 20 petri dishes (controls), and randomly assigned to each treatment and replicate. The eggs were kept for two days in air at 95% relative humidity before treatments were imposed. The experiment was a randomised block design, with 5 replicates of 16 treatments (4 treatments*4 time periods). The treatments were immersion in red grape must, clarified juice, or water for 3,6,12 or 24 hours; the controls were kept in air at 95% relative humidity. After the specified time periods, vials were removed from the treatments and drained, then transferred to petri dishes. The number of hatched and unhatched eggs was determined after 7 days.

Results:

The only treatment to have a significant effect on survival of phylloxera over a 24 h period was immersion in red (Malbec) must.

Table 5. Survival of phylloxera eggs after 24 hours immersion in water, red must, or water.

Treatment	Time	% hatched eggs
Air (control)	3 h	99
	6 h	96
	12 h	97
	24 h	98
Malbec must	3 h	100
	6 h	99
	12 h	38
	24 h	33
Grape juice	3 h	99
	6 h	97
	12 h	97
	24 h	91
Water	3 h	96
	6 h	100
	12 h	97
	24 h	96

Experiment 6. Tolerance of phylloxera crawlers to red must, grape juice, and water.

Objective: To determine survival of phylloxera crawlers in grape juice and must.

Methods:

Eggs collected from leaf galls of phylloxera were placed into each of 60 vials, or 20 petri dishes (controls), and kept at 25^{0}C and 95% relative humidity for 4 days, to allow hatch of crawlers before treatments were applied. The experiment was designed as a randomised block, with 5 replicates of 16 treatments (4 treatments*4 time periods). The treatments were red grape must, clarified juice, or water for 3,6,12 or 24 hours; the control was air. After the specified time periods immersed in the treatments, vials were removed and drained, and the numbers of live or dead crawlers were counted.

Results:

Survival of crawlers in red must declined rapidly during the experiment, whereas in grape juice they survived up to 24 hours.

Table 6. Survival of phylloxera crawlers after 24 hours immersion in water, red must, or water.

Treatment	Time	% live crawlers
Air	3 h	42
	6 h	39
	12 h	40
	24 h	39
Malbec must	3 h	61
	6 h	43
	12 h	6
	24 h	2
Grape juice	3 h	53
	6 h	53
	12 h	52
	24 h	13
Water	3 h	63
	6 h	44
	12 h	39
	24 h	36

Experiment 7. The tolerance of phylloxera eggs and crawlers to red must, clarified grape juice, and water.

Objective: To compare the survival of phylloxera eggs and crawlers in grape juice and must over 48 hours.

Methods:

Seventy eggs collected from leaf galls of phylloxera were placed into each of 60 vials, and kept at 25^{0}C and 95% relative humidity for 4 days, to allow hatch of crawlers before treatments were applied. Another 20 eggs were added to each vial one day before treatments were applied, to ensure a good mix of eggs and crawlers.

The experiment was designed as a randomised block, with 5 replicates of 12 treatments (3 treatments*4 time periods). The treatments were immersion in red grape must, clarified juice, or water for 12, 24, 36, or 48 hours. After the specified time periods in the treatments, vials were removed and drained, and the numbers of hatched or unhatched eggs, and live or dead crawlers were counted.

Results:

The results (Table 7) show that eggs are more tolerant of red must or grape juice than crawlers, and that both stages of phylloxera are more tolerant of white juice than red must.

Table 7. Survival of phylloxera crawlers and eggs after immersion in red must, white juice or water for different times.

Treatment	Time	% live crawlers	% hatched eggs
Red must	12 h	20	64
	24 h	0	17
	36 h	0	2
	48 h	0	5
White juice	12 h	58	93
	24 h	23	70
	36 h	2	15
	48 h	4	19
Water	12 h	44	96
	24 h	43	93
	36 h	29	83
	48 h	39	88

Experiment 8. Cold storage as a disinfestation treatment for phylloxera.

Objective: To determine the suitability of freezing as a disinfestation method.

Methods:

Approximately 30 adults, and eggs, of phylloxera on individual root galls (nodosities) were placed in a polycarbonate plastic vials, which had stainless steel mesh (mesh openings of 50 microns) on each end. The vials were then placed directly into a laboratory freezer at -16°C, or immersed in liquid nitrogen (-196 °C) for 2-3 minutes before placement in the laboratory freezer, or kept in air at 23 °C and 95% relative humidity. The experiment was a randomised block with three treatments as described, four periods (5 minutes, or 12, 24 or 36 hours)in the laboratory freezer, and with three replicates.

After removal from the freezer and defrosting for 30 minutes, the number of live or dead adults and crawlers were counted, and approximately 30 eggs from each vial placed in a petri dish sealed with Parafilm. The eggs were kept at 23 °C for 7-11 days, after which crawlers and unhatched eggs were counted.

Results:

No insects survived immersion in liquid nitrogen, but all stages survived -16 °C for 5 minutes, and a single egg hatched after 12 hours at -16 °C (Table 8).

Table 8. The survival of phylloxera adults, eggs, and crawlers after exposure to low temperatures.

		% live insects		
Treatment	Time	adults	crawlers	eggs
Liquid N2 & (-16 °C)	5 min	0	0	0
	12 h	0	0	0
	24h	0	0	0
	36h	0	0	0
Freezer (-16 °C)	5 min	64	63	45
	12 h	0	0	1
	24h	0	0	0
	36h	0	0	0
Air (23 °C)	5 min	99	100	100
	12 h	96	98	98
	24h	99	99	97
	36h	97	100	98

Discussion

Disinfestation treatments

The results showed that the following disinfestation techniques were sound, ie.:

- Hot water (52 ^{0}C for 5 minutes) killed all eggs and active stages of phylloxera (Tables 1 and 2).
- A 1% solution of sodium hypochlorite killed active stages of phylloxera after immersion for 1 minute (Table 1).
- Phylloxera adults, crawlers, and eggs did not survive freezing at -16^{0}C for 24 hours, or liquid nitrogen (-196^{0}C) for 5 minutes.(Table 8).

Hot water is used to disinfest grapevine cuttings and rootlings, and these data show that provided the desired temperature are reached under the bark of the grapevine material, the treatments will be effective in killing phylloxera. Similarly, heat (45^{0}C for 2 hours) has been used to disinfest vineyard implements, based on the work of Buchanan (1990).

Freezing is an option where dead but biologically preserved phylloxera insects need to be studied or processed for scientific experiments, and the use of liquid nitrogen followed by storage in a freezer as the basis for the transport of frozen specimens has now been established.

A small proportion of active stages of phylloxera survived immersion in 70% ethyl alcohol for 5 minutes. Both hypochlorite and alcohol solutions have no effect on eggs immersed for short periods (ie., 1 minute), and have been used for surface sterilisation of eggs used to initiate phylloxera populations on tissue cultured vines. Thus while useful as disinfestation agents, hypochlorite and alcohol should be used in conjunction with other techniques, particularly washing, to reduce risks of transferring phylloxera.

Detergent (Table 1) and metabisulphite solutions (Tables 2 and 3) had little effect on survival of phylloxera. Grape phylloxera is known to be susceptible to sulphur dioxide (Buchanan 1990), but obviously the addition of metabisulphite to grapes has no potential for phylloxera disinfestation, at least at rates (50ppm) that are used in winemaking.

Survival of phylloxera in water, grape juice or must

The series of experiments on survival of various life stages of phylloxera in water, grape juice, and grape must demonstrated that phylloxera is tolerant of submersion in water, but less tolerant of juice and must. The reasons for the differences in tolerance were not determined, but the observation that 20% sucrose also reduced survival (Table 3) suggests that osmotic pressure may be involved. In any event, it is clear that the survival of any phylloxera present on harvested grapes will be reduced by prolonged immersion in grape juice or must.

Phylloxera crawlers developed from root populations are the life stage most likely to be found on the canopy of grapevines (Buchanan 1990, King and Buchanan 1986). Experiments 1-4 on survival of active stages of phylloxera taken from root galls, of which crawlers are the predominant stage, demonstrated survival of a few individuals after immersion for 36 hours in water, 24 hours in grape juice, and 15 hours in grape must. Survival of phylloxera in Sultana must (Table 2) and fermenting Merlot must (Table 4) was reduced in comparison with phylloxera in the control (air) or water treatments. Eggs from root populations would not be expected on the canopy, but as with the active stages, survival was reduced by prolonged immersion in must (Table 4).

Leaf galls of phylloxera are not common in commercial vineyards in Australia, but are occasionally found on rootstock mother vines or on rootstock suckers. Where they do occur, large populations of eggs and crawlers can be present on the vine canopy. Experiments 5-7 showed that survival of both eggs and crawlers from leaf gall populations was reduced by immersion in grape juice or must, but that some eggs hatched and a few crawlers survived after 48 hours immersed in juice or must (Figures 1, 2).

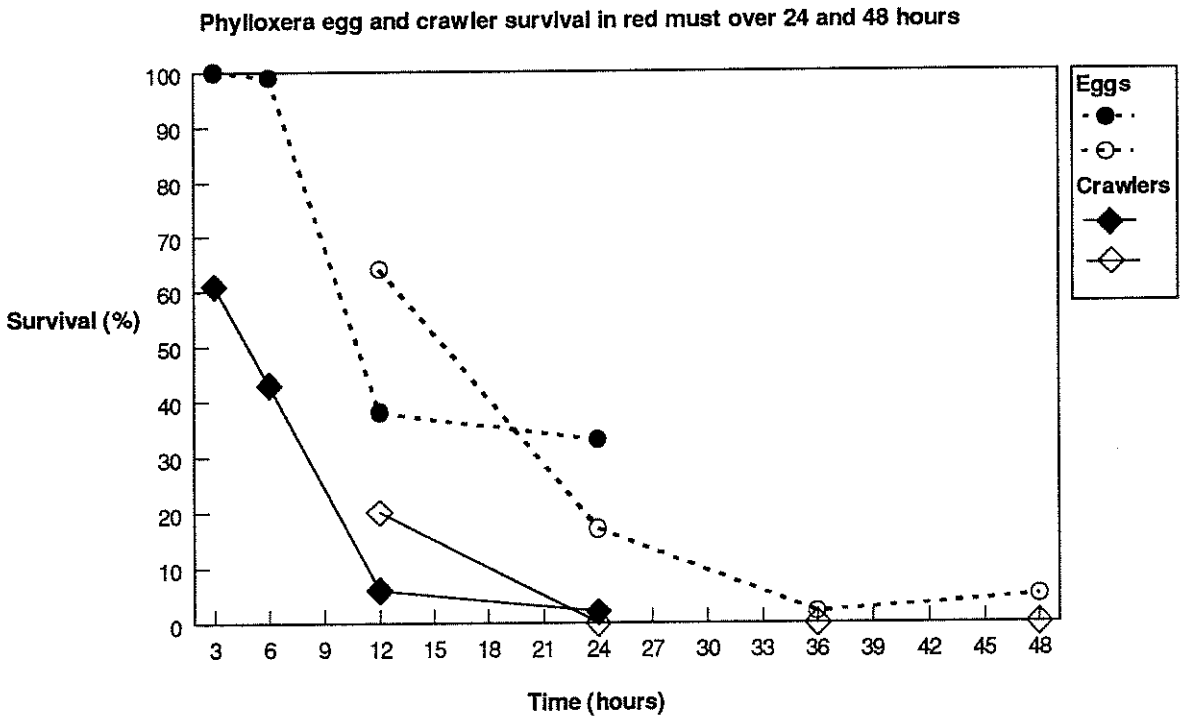


Figure 1. Survival of phylloxera eggs and crawlers from leaf galls in grape must (data from Exps 5,6,7)

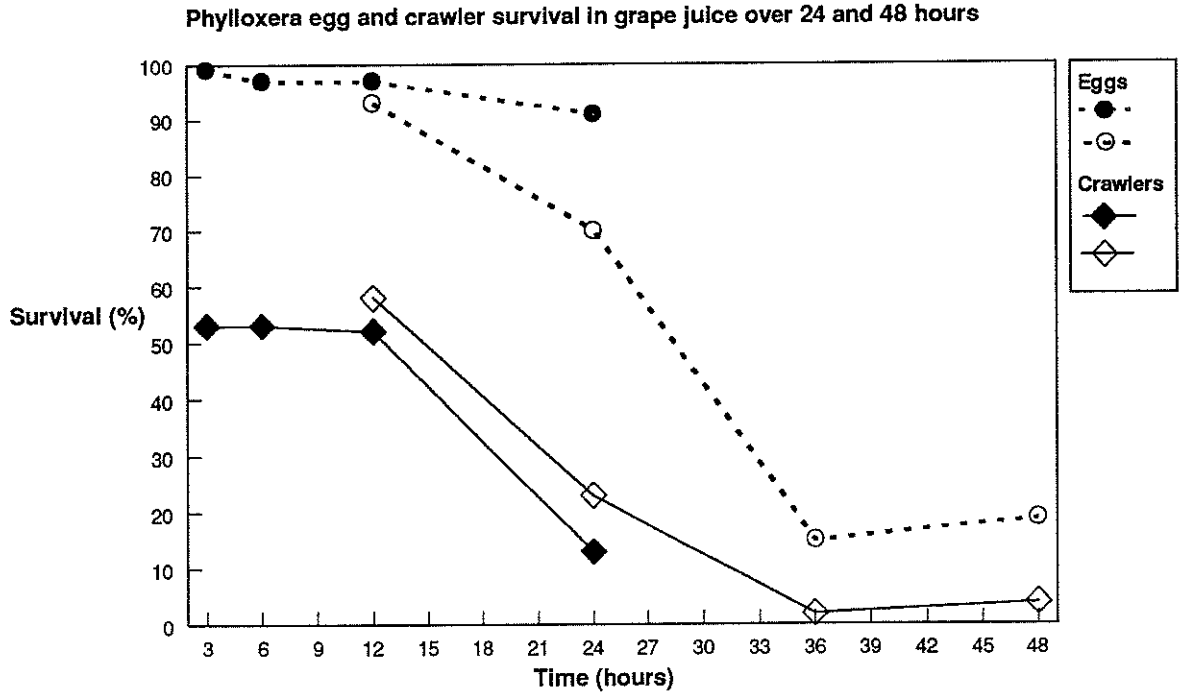


Figure 2. Survival of phylloxera eggs and crawlers from leaf galls in grape juice (data from Exps 5,6,7)

In commercial vineyards, it is uncommon to find phylloxera on canopies of grapevines. The data obtained in these experiments shows that phylloxera present on harvested grapes are likely to survive in humid air for up to 120 hours, (Table 4), or in water for 48 hours (Table 7). If the phylloxera are crushed with the grapes and are present in grape juice or must, most crawlers will die within 24 hours in must or within 48 hours in juice. Fermentation of the must further reduces the chances of survival. In the unlikely event that phylloxera eggs wind up in juice or must, most will die in must within 48 hours and in juice sometime after 48 hours. However, it is impossible on the basis of these data to conclude that there is no risk of phylloxera surviving the processing of grapes into juice or must.

These data should be used by individuals and companies to develop protocols for disinfestation of equipment used in vineyards, and to determine the potential risks in transfer of grape juice or must for winemaking purposes. A systematic approach of Pest Risk Assessment is being used to develop policies for Governments and industry bodies, that will subsequently translate into quarantine legislation and industry recommendations.

Appendix 4 :

Vineyard surveys and aerial photography.

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Appendix 4: Vineyard Surveys and Aerial Photography

Introduction

At present, a vineyard survey with direct inspections of roots is the only method available to detect phylloxera. Surveys of vineyards in Victoria over the past 15 years focused on determining the extent of spread of phylloxera, and the results were used to redefine quarantine boundaries. The surveys have also been used to confirm the apparent freedom of those districts not known to be infested by phylloxera, along with other criteria. Ungrafted vines need to be targeted, as they generally show above ground symptoms of vine decline and support high numbers of phylloxera on the roots. Grapevines grafted to resistant rootstocks may support low numbers of phylloxera on the root system, but vine vigour is not affected.

Increased awareness and training of industry people in diagnosis of phylloxera has had positive benefits. Most of the recent phylloxera detections have been made by grape growers. In the King Valley in particular there was heightened awareness of phylloxera after several infestations were disclosed. This news encouraged growers to check out weak spots in their vineyards and phylloxera was confirmed in several instances.

The quarantine officers of State Governments have a role in checking uninfested areas around quarantine zones as well as systematic surveys in newly declared quarantine zones. To support this process aerial photography was trialed in the 1996 season and expanded in the 1997 season. The aim of the photography was to detect weak patches that may have been missed by grower observation from the ground and to locate weak patches for the survey teams to inspect.

Methods

The members in the survey team were trained in recognising phylloxera at the workshops at Rutherglen. Further field training was undertaken in infested vineyards to explain what vine symptoms to look for. Each team consisted of 3 to 5 members and an experienced DNRE Plant Standards officer accompanied the team. A survey form was prepared so that consistent information could be obtained from the landholder. This information was entered onto a database as records that could be sorted and extracted for later use. Links with other vineyards, eg sharing of vineyard equipment, were recorded.

The system used for sampling was based on a stratified sample method where approximately every fifth row was sampled and the roots of a vine in every fifth panel down the row inspected. In addition any weak spots identified by the grower were also sampled. The surveys were generally conducted between February and May. One small survey was conducted in November but several vineyards where no phylloxera were found at that time were later (February) found infested by phylloxera. Surveys could be conducted quite late into the season providing the autumn break had not occurred. Once conditions became cold and wet it became very difficult to detect phylloxera.

During the inspection program, all staff undertook a thorough cleaning of boots, shovels and clothing after inspecting vineyards. Each vehicle was equipped with disinfestation gear comprising methyl alcohol or chlorine along with appropriate scrubbing gear. Uninfested vineyards were sampled before ones that were thought to be infested.

The aerial surveys have been performed by the Resource Information Group of the Department of Environment and Natural Resources, South Australia. The first year survey, 1996, was undertaken as a pilot study in conjunction with another survey along the Murray Valley. Photographs were taken at 10,000 feet with false infrared colour and provided as 23cm × 23cm prints. Various options are available to print off blown up copies but the price of about $100

each was prohibitive for use on a regular basis. Only a limited number of vineyards were selected for the pilot study and a sequence of three photographs of each vineyard enabled them to be viewed in 3D.

The second survey in 1997 was expanded to include most vineyards in the King Valley as well as some areas near to quarantine zones. Other areas of interest to survey, eg. Yarra Valley, were selected because fruit had been taken to that area from the King Valley just prior to the King Valley Vine Disease District being proclaimed. Most areas were photographed from 3000m (10,000 feet) apart from the Yarra Valley which was photographed from 6000m (20,000 feet).

Results and discussion

Surveys

1994

In the 1994 season most of the vineyards surveyed were in areas outside quarantine zones. The Ovens Valley region is a rapidly expanding area for viticulture and at present the quarantine boundary cuts through part of the region. Growers in the area were keen to have the boundary changed so the region could operate under uniform protocols for the movement of fruit. Thus vineyards in the Ovens Valley/Whoroughly area were surveyed to assess the situation for a possible change of boundary. Of the 16 vineyards inspected none were found to have phylloxera (Table 1). It is recognised that several years of survey will be required to determine if the area is free of phylloxera.

TABLE 1: SUMMARY OF VINEYARD INSPECTIONS FOR PHYLLOXERA

Season	District	No Vineyards inspected	No Vineyards infested	Total ha inspected	% inspected area on rootstock	% infested area on rootstocks
1994	Ovens/ Whoroughly	16	0	129	42	-
	Heathcote/ Graytown	24	0	139	<1	-
	Central Vic	9	3	65	22	11
	Macedon	2	0	9	0	
Total		51	3	342	-	-
1995	King Valley	37	4	461.5	39	10
	Central Vic	2	0	13	62	-
Total		39	4	474.5	-	-
1996	King Valley	5	4	86.5	12	13
1997	King Valley	7	3	204.5	36	10
	Ovens/ Whoroughly	4	0	41	41	-
Total		11	3	245.5	-	-

Another large survey was conducted in the Graytown/Heathcote area. There are several old vineyards at Graytown which is approximately 15 kms from the nearest infested area at Nagambie. Over the years with extensive tourist traffic it may have been expected some transfer of phylloxera could have occurred. Also the Heathcote vineyards are approximately 40 kms from Nagambie and again on a direct tourist path. Of the 24 vineyards surveyed in the

area none were found with phylloxera. In addition two vineyards in the Macedon region that had reported restricted vine growth problems were also inspected but no phylloxera was found.

A number of vineyards in and around the Nagambie Vine Disease District were also surveyed. Three vineyards were found to have phylloxera but none were new discoveries.

1995

Surveying focused on the King Valley because in the 1994/95 season phylloxera was suspected in several vineyards and confirmed by DNRE inspection. A comprehensive survey (461 ha) of the King Valley was undertaken and four vineyards were confirmed to have phylloxera. These confirmations when added to the three previously known infestations and brought the total number of phylloxera infested vineyards in the King Valley to seven. These detections resulted in the declaration of the King Valley Vine Disease District. The survey confirmed that all outbreaks were contained within the boundaries of the new Vine Disease District.

1996

Surveys were limited to a pilot study of the usefulness of aerial photography. A selected number of properties with and without phylloxera were photographed and then surveyed to confirm where phylloxera was present within vineyards. No additional vineyards were found to have phylloxera but phylloxera within vineyards was found to have spread beyond the areas delineated in the 1995 survey.

1997

Resources to conduct surveys were restricted by the suspected fireblight outbreak and the transfer of staff to undertake field surveys of orchards. A further series of aerial photographs were taken in this season and some survey work undertaken based on the appearance of weak vines in the photographs. This survey detected one new infested vineyard within the King Valley Vine Disease District taking the total in that area to eight. Inspection of several other vineyards showing weak spots in the photographs did not reveal phylloxera. Weak vines were generally associated with soil levelling, missing vines or heavy weed growth.

Several vineyards in the Ovens/Whoroughly area that were inconclusive from earlier inspections were re-surveyed and no phylloxera was detected.

Summary

Surveys of vine root systems are required to confirm the presence of phylloxera. New outbreaks have predominantly been reported by growers who have investigated weak growth of their vines. Department surveys have generally focused on monitoring in and around phylloxera areas with the aim of ensuring the existing boundaries meet industry and quarantine needs. Variations have occurred in the ability of government to commit resources to regular surveying for phylloxera when other quarantinable issues develop, eg. fireblight. About 40% of the vineyards in the King, Ovens, and Whoroughly valleys are planted with resistant rootstocks.

Aerial photography to assist detection of phylloxera infestations

1996

Infrared photographs of a sample of infested and uninfested vineyards in the King Valley were taken in March 1996. This timing was selected to maximise differences in vine growth due to stress. Colour photographs from a light aeroplane have been inspected in the past but were not particularly useful due to different heights, angles and perspectives on the vineyards. The

infrared photographs obtained for this project were taken at 3000m (10,000ft) directly above the vineyard. This perspective combined with the sun at an oblique angle to the rows of vines produced some startling and clear phylloxera spots. Not all weak spots were attributed to phylloxera with soil levelling, missing vines and heavy weed growth also contributing to weak growth. Figure 1 shows a comparison of two vineyard blocks. The block in the upper photograph had a several weak spots, one of which was phylloxera, the others were not. The lower photograph also showed weak spots but this block was planted with rootstock and the weak areas were associated with soil levelling. **(Note: Due to several stages of scanning and copying these photographs from the originals, these Figures do not have the resolution of the initial photographs.)**

Most vineyards were photographed with a series of three photographs which were used to view the vineyards in 3 dimensions through a stereoscope. Whilst this provided a better perspective of the vineyards it did not improve the visual determination of weak areas of vines. The other useful feature of the photographs was the ability to more accurately define the boundaries of the vineyards and to transfer this information to Arcview maps (Figure 2). This will enable more accurate measurements of distances between vineyards for example when comparing the spatial separation of infested vineyards. Maps can be printed out and the files created can be used to overlay any other map prepared in Arcview.

In all, 22 photographs were taken, covering 14 vineyards. The cost of this pilot survey per unit was $77 per photograph or $121 per vineyard.

1997

Based on the successful demonstration in 1996 of the ability of aerial infrared photographs to reveal phylloxera spots, more extensive photographs were taken in 1997. Only one photograph was taken of each vineyard but in some cases several vineyards were present in the one photograph. During this survey, the areas photographed at 3000m (10000ft) were Graytown, Nagambie, Seymour, King Valley and Glenrowan; the Yarra Valley was photographed at 6000m (20,000ft). A total of 79 photographs were taken and 154 vineyards were covered. The average cost per photograph was $154 which was greater than 1996 due to the greater geographic spread of the survey. However the greater number of vineyards reduced the average cost per vineyard $80 compared to $121 in 1996. The photographs at 6000m costed $42 per vineyard and at 3000m, $114 per vineyard. The disadvantage of the photographs at the higher altitude is a loss of resolution.

Due to the transfer of resources to surveying for fireblight, large scale ground follow up surveying was not conducted in 1997. However, the photographs will be available to ground truth in the early 1998 survey program. Ground truthing was restricted to a number of suspicious vineyards in the King Valley. This limited survey detected one new infested vineyard that had shown a weak spot on the photograph. The weak spot had been noticed by the vineyard proprietor for many years as it was an area where a tree had been removed and the vines had always grown weakly. The owner had not thought to survey this area on a regular basis. This situation shows that even though a weak spot may initially be free of phylloxera and explainable for some reason it should continue to be checked for phylloxera.

Sequential photographs of the same vineyard block have been useful in monitoring the spread of phylloxera. Figure 3 shows the same infested block of vines over two consecutive years. In 1996 two phylloxera spots were detected and in 1997 more spots were located. The average rate of spread over the two years has been around 100m per year, which matches the rate of spread previously reported from studies at Nagambie. In this particular vineyard no vines were removed although the grower took some precautions to minimise spread.

A phylloxera outbreak in another vineyard was treated differently. In this case affected vines and an area some 10m around that were removed and precautions against further spread were

taken. Figure 4 shows the vineyard in 1996 where one small patch of vines were removed, and again in 1997 when more vines were removed. Removing vines has not eliminated the phylloxera but has contained the spread to a small zone around the initial infestation and no satellite infestations have appeared at this stage.

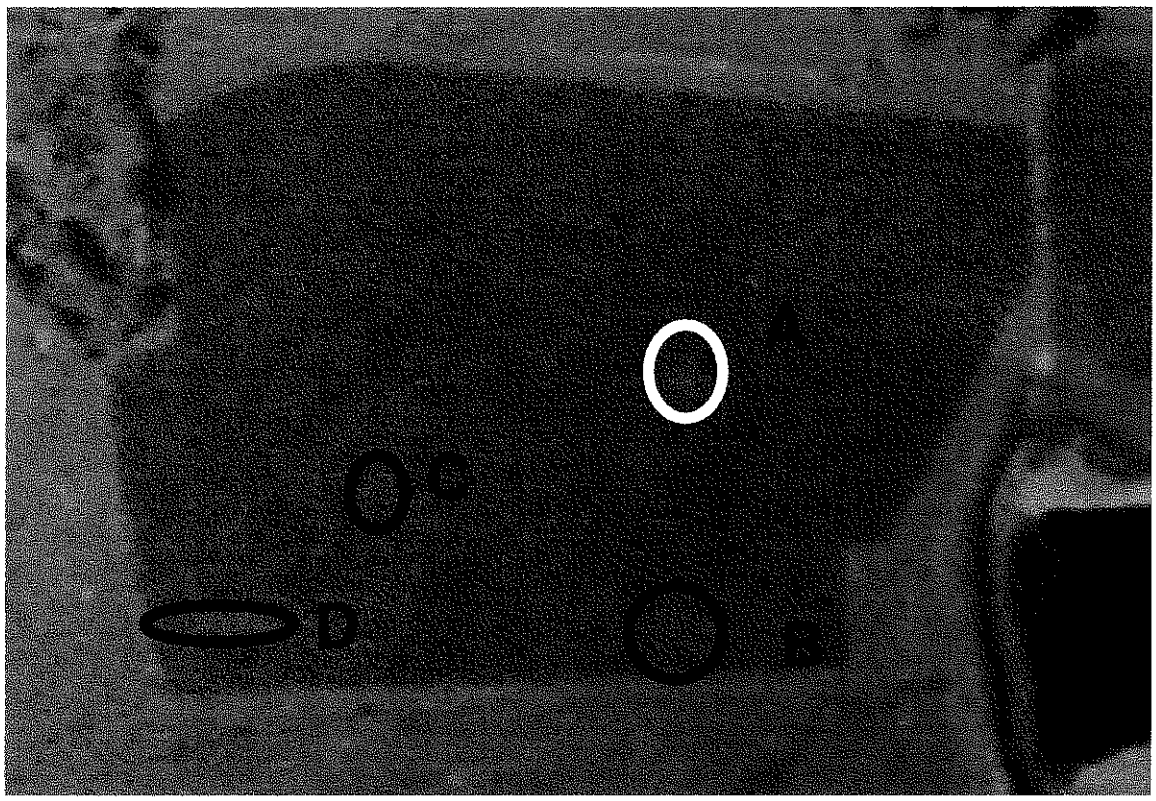
Summary

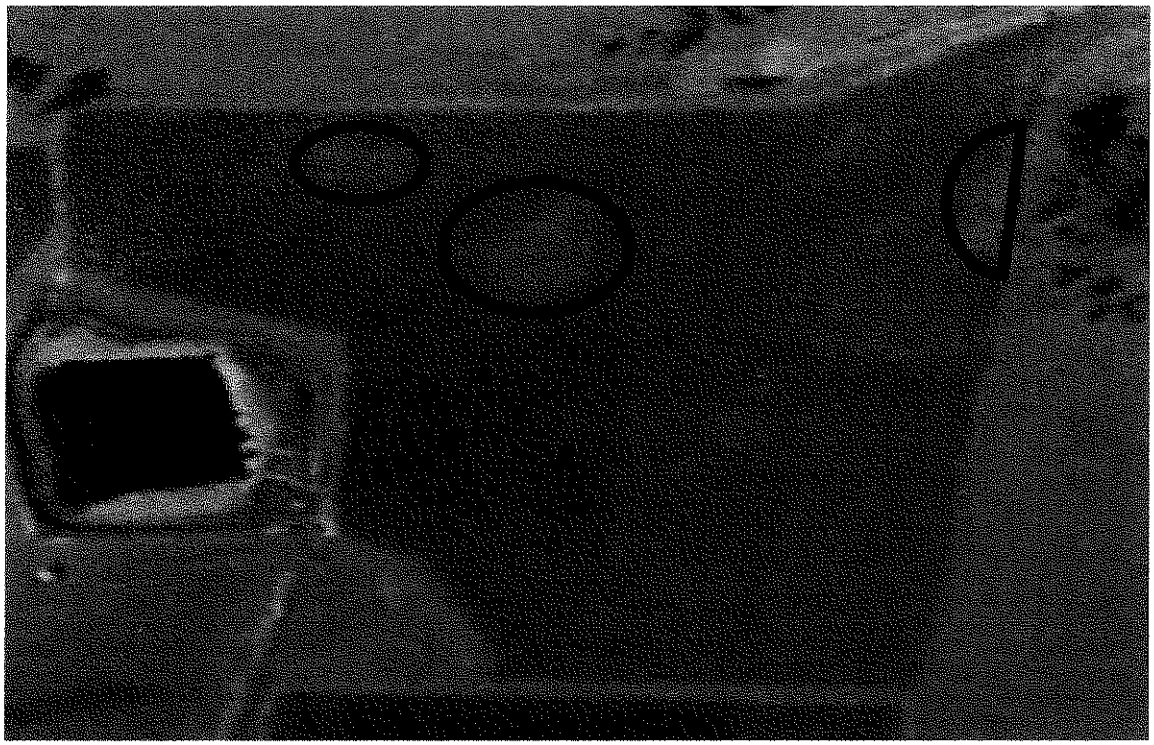
Aerial infrared photography can be effective in detecting weak areas of vines in vineyards. The conditions under which the photographs are taken needs to be well specified in order to accentuate differences in growth between vines, ie. take photographs in March, have the sun at oblique angle to direction of vine row, fly at 3000m to retain resolution and provide precise coordinates for the aircraft. The identification of weak areas in vineyards allows survey teams to focus on those sections rather than walk through areas of healthy vines, and thus reducing survey time and costs. The photographs also allow the survey team to be familiar with the vineyard layout prior to arrival as well as producing more accurate maps to assist with monitoring of spread of phylloxera.

Image enhancement

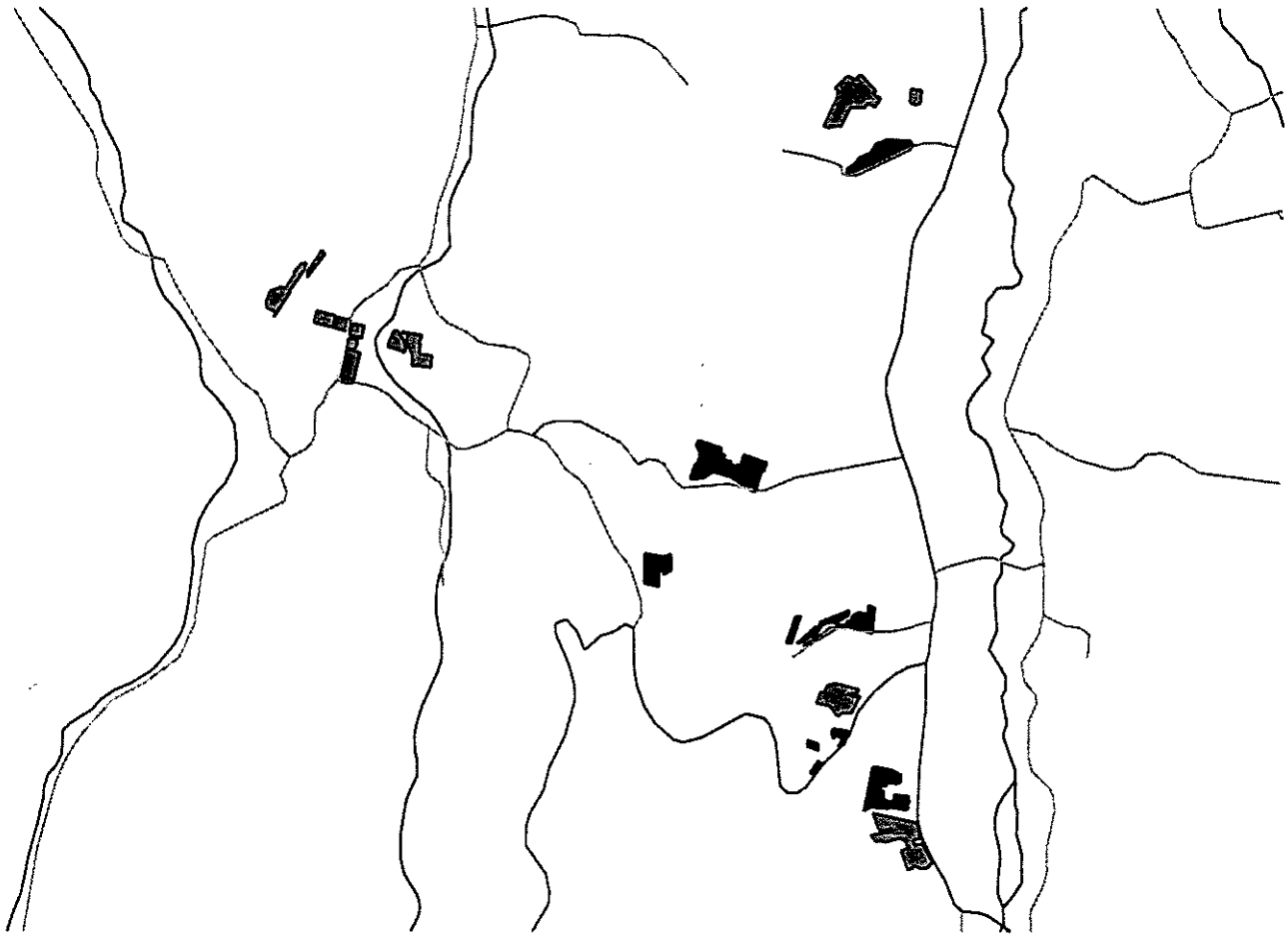
Digitising the aerial photographs will enable image enhancement processes to be used. Image enhancement will select certain infrared and colour spectrums from one part of the photograph and match the same spectrum across the full photograph. These particular spectrums can be highlighted in a false colour to make them stand out above the remaining colours. The GIS group at Tatura spent a limited amount of time on this process. Weak spots in the photographs displayed a different colour spectrum to healthy vines. However the enhancement of these weak areas across the full photograph was not particularly revealing and of little assistance in defining weak areas. The usefulness of image enhancement needs some more intensive analysis before it should be dismissed.

Figure 1. Upper photograph. Infrared aerial photograph of an infested vineyard in the King Valley. A = phylloxera infested weak spot; B = weak spot, no phylloxera detected, soil had been levelled; C = weak spot, no phylloxera detected, heavy and poorly drained patch of soil; D = weak spot, no phylloxera detected, low lying gully with poor drainage.





Lower photograph. Infrared aerial photograph of a vineyard in the King Valley. Circled areas are weak vines. However this patch is all on rootstock and weak vines were the result of the removal of topsoil during levelling operations.



0 5 10 Kilometers

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Minor_rd.shp
River.shp
Creek.shp
Phot_unl.shp
Phot_inl.shp

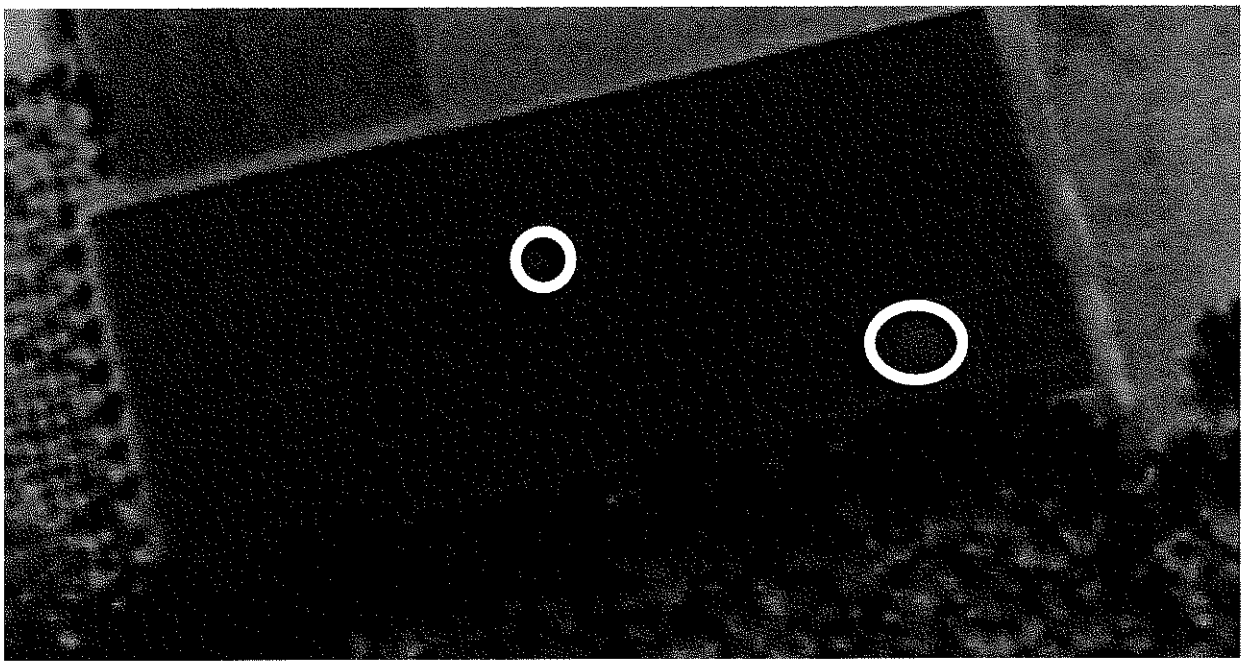
Infested & Uninfested Vineyards

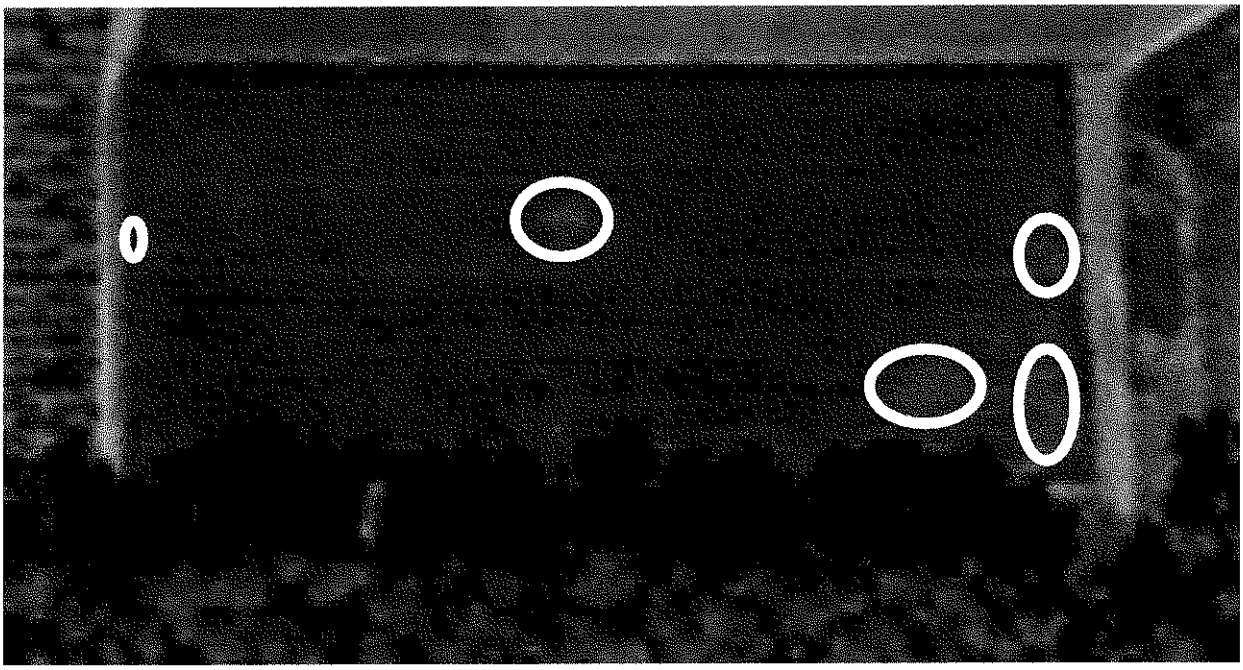
Prepared by Shelley Lanyon
ISIA Tatura

N

Figure 2

Figure 3. Upper photograph. Aerial infrared photograph in 1996 of a phylloxera infested vineyard. The initial weak spot in 1995 was to the right in the photograph. In 1996 the weak spot to the left was apparent in the photograph and inspection revealed it was infested with phylloxera. No phylloxera was detected at the left hand end of the vineyard.

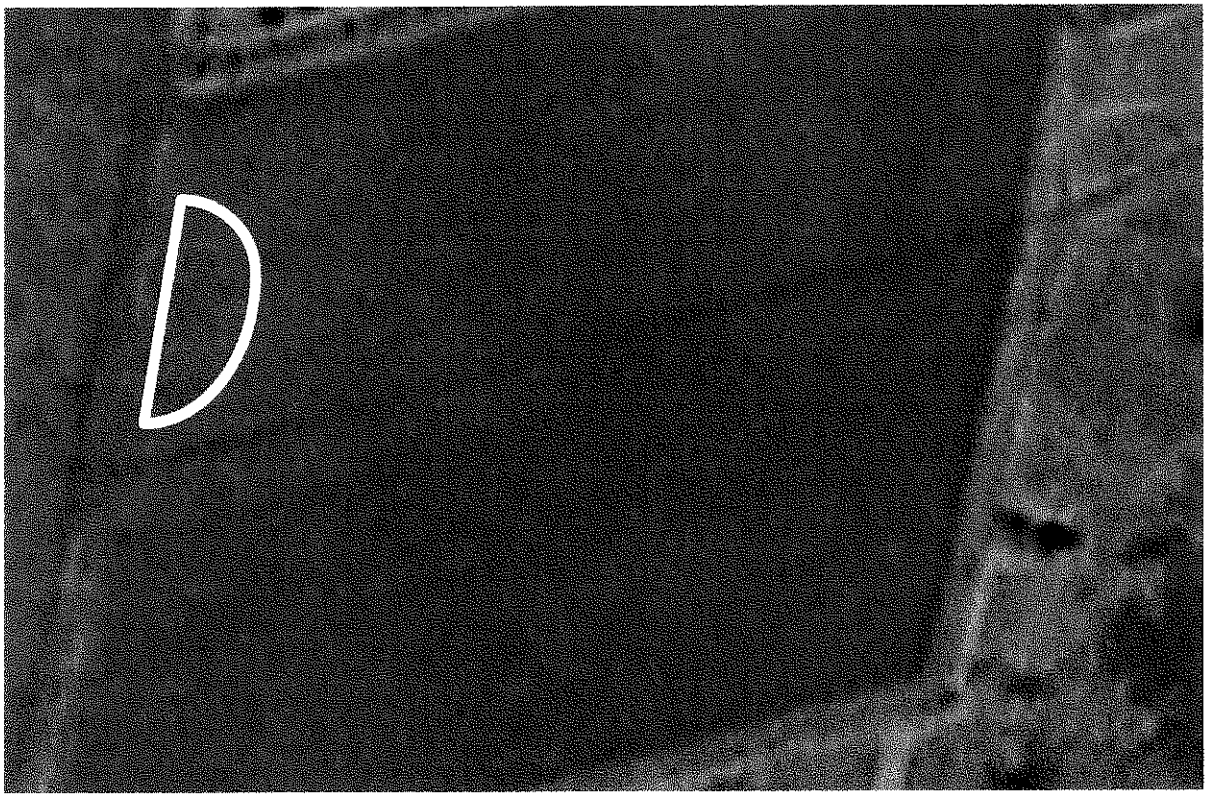




Lower photograph. Same vineyard block as above taken in 1997. Weak spots had enlarged, and more weak spots present that inspection revealed were infested with phylloxera. Phylloxera had spread to the left end of the block, approximately 200m from the initial phylloxera detection in 1995.

Figure 4. Upper photograph. Photograph taken in 1996 of a vineyard where vines were removed after phylloxera was discovered in 1995. White semi-circle delineates the area of infested vines removed plus an area some 10m beyond that. The black circle delineates an example of apparent weak vines which upon inspection were missing vines.

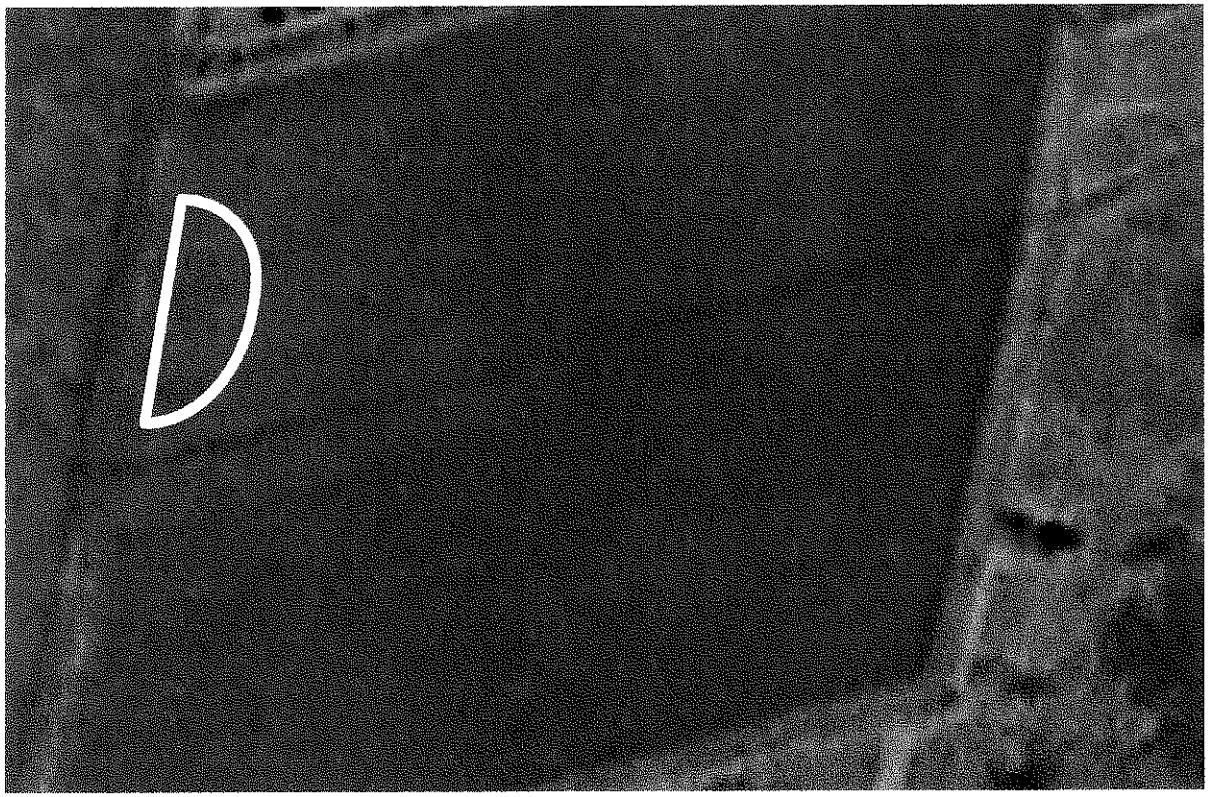




Lower photograph. Same vineyard as above taken in 1997. The white semi-circle outlines where further vines have been removed due to slow spread of phylloxera. No other satellite infestations have been detected in this block.

Figure 4. Upper photograph. Photograph taken in 1996 of a vineyard where vines were removed after phylloxera was discovered in 1995. White semi-circle delineates the area of infested vines removed plus an area some 10m beyond that. The black circle delineates an example of apparent weak vines which upon inspection were missing vines.





Lower photograph. Same vineyard as above taken in 1997. The white semi-circle outlines where further vines have been removed due to slow spread of phylloxera. No other satellite infestations have been detected in this block.

Appendix 5 :

Tristate quarantine protocols for containment of phylloxera.

COORDINATING QUARANTINE STRATEGIES FOR PHYLLOXERA

INTERIM WORKING DOCUMENT FOR 1997

INCLUDING
INTERIM PROTOCOLS FOR 1997

Prepared by the TRI STATE PHYLLOXERA WORKING GROUP

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1. The Review Process

The Interstate Plant Health Regulation Working Group has been established as a sub-committee under the direction of the Plant Health Committee (PHC) of the Standing Committee on Agricultural Resource Management (SCARM). Its objective is to review regulations relating to plant health issues between States with a view to harmonisation of regulations across Australia.

The Victorian "Draft Code of Practice - Phylloxera" associated with this paper has been written as part of the project "**Development of a National Management Strategy for Grape Phylloxera**" funded by the Grape and Wine Research and Development Corporation. This paper is intended to stimulate discussion and informed debate that will lead to an agreed national approach to the control of phylloxera in Australia. It aims to:

- assess risks of infestation of grape growing regions by phylloxera.
- further develop appropriate quarantine regulations and actions required to minimise risks.
- support development of action plans to detect, contain and control any new infestations of phylloxera.
- achieve support of Australia's viticultural industries.

The first draft of this discussion paper was circulated to state and national viticultural associations in October 1995, with a request for feedback by November 1995. A second draft was circulated in January and was subject to discussion at a Victorian Wine Industry Association meeting in February. *This draft was then discussed at an industry meeting on August 20, 1996. A working group chaired by Peter Hayes with representatives from each State's grape growers, winemakers and State Chief Plant Health Regulatory Quarantine Officers , as well as the original working group, has modified the draft to become the interim working document for 1997*. The main changes include:

- Use of three zones;
  1. PIZ (Phylloxera Infested Zone)
  2. PEZ (Phylloxera Exclusion Zone)
  3. PRZ (Phylloxera Risk Zone)
- PIZs will include those areas infested with phylloxera which have already been gazetted. This paper recommends that the boundaries of a PIZ *should be at least 15 km, with opportunity for regional variation to a minimum of 5 km and a maximum of 40 km (subject to national agreement),* from the boundaries of an infested vineyard.
- *It is proposed that a National Phylloxera Board will be established and will be comprised of members representative of State (eg State Phylloxera/Grape Industry Boards) and National (eg WFA, WGCA) peak bodies, together with relevant Department of Agriculture technical personnel.*
- *In the interim a National Phylloxera Reference Group, representative of state and national interests, will be the interim committee for the proposed national board .*
- *The final determinate of PIZ boundaries, based on risk analysis of a particular regions situation, will be the national board. The Phylloxera Reference Group will be responsible for development, and endorsement, of all protocols and be the receiver and arbiter on technical issues on the board's behalf.*
- PEZs will include areas with existing regulations to control movement of grapevine material and equipment. This will include Western Australia, Northern Territory, South Australia, Tasmania, the North West Vine Protected Area in Victoria, and the Murrumbidgee Irrigation Area, Hunter Valley, Mudgee, Cowra and South West area in New South Wales.
- PRZs will include all remaining areas (ie not included as a PIZ or PEZ). These PRZ areas may begin implementing actions for declaration as PEZ once the legislative and regional protocol criteria determined are satisfied.
- Emphasis on industry-based protocols for operators in Phylloxera Infested Zones *and for operators in PRZs and PEZs who are interacting with PIZs.*

2. Introduction

Phylloxera is an aphid, native to North America, that attacks the root system and foliage of grape vines. Its effects on the root system of the European grapevine species, *Vitis vinifera*, which is the planting stock of most Australian commercial vineyards, are devastating. Its arrival in Europe, in the second half of the 19th century, destroyed large tracts of vineyards, necessitating their replanting with vines grafted on phylloxera resistant rootstocks. Phylloxera later spread to most other grape growing countries, including Australia.

Since the initial outbreaks of 1875-1900 in central and north eastern Victoria and, around Sydney, phylloxera has largely been confined to these areas. It appears to have been eradicated from Geelong, Bendigo, and a small area near Brisbane (now Brisbane airport). So far most of Australia's commercial grape production areas have escaped invasion by the pest. This has provided the Australian wine industry with scope for continued grape production and the opportunity to avoid the need to replant vineyard areas with more costly grafted vines. However, the use of ungrafted vines has placed all the Australian grape industries at risk, should phylloxera escape into uninfested production areas.

State government quarantine regulations aimed at containing phylloxera, the activities of the Phylloxera and Grape Industry Board of South Australia, and industry awareness, have all played a role in preventing the spread of phylloxera. However, the recent outbreaks in the King Valley region, adjacent to the North East Vine Disease District in Victoria, have given a forceful reminder of the potential threat of phylloxera to the viticultural industries of Australia. The *initial* outbreak of phylloxera in the King Valley *in 1991* may have occurred through the movement of infested planting material or vineyard equipment. There had been no commercial movement of grapes into the King Valley and the distance from known infested vineyards *(50 km) was* too great for natural spread. *Subsequent outbreaks appear to be related to natural or people assisted spread.*

The risk of spread has greatly increased in recent years as wine companies develop their operations on a national scale, with the attendant increased movement of grapes, propagation material, grape must, juice and wine and vineyard equipment between both regions and States. In addition there has been a proliferation of new vineyards, new growers and new regions throughout Australia, all adding to the risk of spread of phylloxera. Grapegrowers now repeatedly rate phylloxera, and the risk of its spread to their vineyards, as a major concern and prevention of its spread as a high priority issue.

Thus there is a need to redefine the phylloxera regulations, which are proclaimed and administered by State governments. To be effective the phylloxera regulations in the different States need to complement each other and to operate at a regional, state and national level. They should be owned and supported by industry and reinforced by a public awareness campaign.

The purpose of this paper, generated by the Phylloxera Tri-State Working Party (Victoria, New South Wales and South Australia), is to work through the above issues and develop, in consultation with industry, policies on phylloxera for the benefit of future grapegrowing activities in Australia.

3. The spread of phylloxera and risk factors associated with movement of grapevine material and vineyard equipment.

Natural spread

Grape phylloxera is an aphid which lives on both roots and leaves of grapevines. It has no alternative hosts and hence needs grapevines to survive. The natural dispersive stages are the first instar "crawler", which hatches from eggs laid on roots or in leaf galls, and a winged form which develops from root populations.

Wind may assist the natural spread of crawlers. The winged form of phylloxera is the precursor to the sexual and leaf gall stages of the life cycle. The leaf gall stage does not occur on *V. vinifera* vines in the field, but is observed regularly on rootstock vines and suckers of grafted vines in Northeast Victoria. Observations in Victoria suggest that the spread of phylloxera by the winged form is only likely to occur where leaves of rootstock vines are available to develop leaf galls in the spring. The major avenue of natural spread of phylloxera is through the movement of crawlers.

Spread by man

Man is by far the most effective vector of phylloxera, having successfully transported it from North Eastern America via Europe to Australia. Hence, viticultural activities are the most likely way the pest will be spread further to non-infested vineyards and regions. There are many ways, with different risk levels, in which phylloxera may be inadvertently moved. These and suggested disinfestation treatments are listed in Table 1.

Table 1. Potential hazards for transfer of phylloxera from infested vineyards and zones

Operation or Material	Form transported	Degree of risk **	Disinfestation Treatment
dormant cuttings	winter eggs	*medium*	hot water dip[1]
dormant rootlings	dormant crawlers	*very* high	hot water dip[1]
active rootlings	all stages	*very* high	na
grapes	crawlers	medium	*sulphur pads*[4]
Vinifera leaves	crawler	medium	na
rootstock leaves	leaf galls	*very* high	na
pre ferment must			
- red and white	crawlers	*medium*	*proper waste disposal*[4]
juice	crawlers	*medium*	fermentation *completed*
wine	-	*low*	*na*
Pre ferment waste	*crawlers*	*medium*	*proper waste disposal*[4]
Post ferment waste	*crawlers*	*low*	*proper waste disposal*[4]
soil and roots	all stages	*very* high	na
mechanical harvester	crawlers	*high*	clean, heat[2]
picking bins	crawlers	*high*	clean, hot water[3]
tractors/other machinery	crawlers	*high*	clean, heat[2]
transports	crawlers	*medium*	*cleaning procedure*[4]
vineyard workers	*crawlers*	*high*	*hygiene procedure*[4]
vineyard visitors	*crawlers*	*high*	*hygiene procedure*[4]
tourists	*crawlers*	*high*	*strong discouragement or hygiene procedure*[4]
wind	crawlers & winged insects	*high*	distance from source
water	all stages	*medium*	na

[1]	hot water bath, 55°C for 5 minutes (see Appendix 2)
[2]	45°C air temperature for 2 hours (see Appendix 2)
[3]	hot water bath, 70°C for 2 minutes
[4]	See code of practice Appendix 2
na	Not available
must	includes juice, skin and seeds

** *Risk levels are defined as follows;*

low	*no expectation of problems with the operation / material*
medium	*problems may or may not occur*
high	*expectation that phylloxera will be transferred*
very high	*certainty that phylloxera will be transferred*

Natural spread of phylloxera can expand an existing infestation within a vineyard, and will be important in areas of contiguous vineyards. However, in Australia the main concern is preventing spread of phylloxera between vineyards through viticultural activities. Spread by man can be prevented by:

- prohibition or severe restriction of the movement of vines and vineyard equipment **out of** known Phylloxera Infested Zones.
- regulation of the movement of vines and vineyard equipment **into** phylloxera free vineyard areas/zones.
- establishment of protocols to minimise risks from people moving between infested and uninfested vineyards.

There is need to geographically define Phylloxera Infested Zones and Phylloxera Exclusion Zones. There is much debate as to the distances necessary to prevent natural spread and spread by man. In the Goulburn Valley phylloxera infestations have been recorded spreading 27-100m per year. From this and other observations it may be deduced that natural spread is limited to a maximum of 1 km per year. However, in view of the *recently* observed spread (*1995,* greater than *4* km) in the King Valley and the fact that new infestations may not be detected for 5 years, a minimum 5 km buffer zone, *within a quarantine zone (ie PIZ),* against natural spread is more realistic.

Thus, while movement of material from an infested vineyard poses a great risk of spreading phylloxera, there is still a risk in movement of material from a nearby, apparently non-infested, vineyard that may be harbouring an undetected infestation. Not only is it important to prevent the spread of phylloxera from an infested zone, but it is critical to prevent its further spread within that zone.

The movement of high risk material from all vineyards in Phylloxera Infested Zones to Phylloxera Exclusion Zones must be prohibited. Further, the movement of low or medium risk material requires stringent disinfestation procedures and/or controlled handling.

The current NSW regulation assumes 40 km to be sufficient distance from known infested vineyards for the safe movement of vine material and equipment into non-infested zones in NSW. Although the 40 km distance has so far not been found to be wanting, it has no factual basis.

Peter May in his 1994 review, *Using Grapevine Rootstocks, the Australian Perspective*, says that only slightly more than 1% of Australia's vineyard area is infested with phylloxera. "Remarkably and in contrast to almost all other countries, the majority of the Australian vineyards, mostly planted after the arrival of phylloxera, have escaped infestation, be it because of quarantine laws, good management, conditions unfavourable to the spread of the disease or even because of good luck."

The only way to eliminate phylloxera is to destroy all infested vineyards, which is not practical. It is not commercially acceptable to impose a total ban on movement of vine material, equipment and personnel from infested areas, which is the only way to eliminate the risk of spread of phylloxera. Hence, our regulations aim to minimise the risk of spread of phylloxera, and in this paper we outline the processes that will keep the risks at low levels.

In the past, industry has accepted bans and/or strict controls on the movement of planting material. Apart from individuals doing their own propagation in the Phylloxera Infested Zones, there should be no encouragement for vines to be propagated within Phylloxera Infested Zones. The risk from vines commercially propagated within a Phylloxera Infested Zone being inadvertently or deliberately moved outside the zone is high, relative to the commercial benefits or convenience gained by the propagators.

4. Definition of Phylloxera Management Zones

Three types of zones are proposed for phylloxera risk management;

a) *Phylloxera Infested Zone* *(PIZ)*
b) *Phylloxera Exclusion Zone* *(PEZ)*
c) *Phylloxera Risk Zone* *(PRZ)*

a) Phylloxera Infested Zone (PIZ)

A PIZ contains vineyards known to be infested with phylloxera or known to have been infested with phylloxera (and not subsequently revoked by the relevant State government). Its boundary incorporates a minimum buffer zone of 5 km, (and a maximum of 40 km), from the edges of the nearest infested vineyards. The boundaries of the PIZ may be determined by regional industry groups, dependent on the approval of the relevant State Chief Plant Health Regulatory Officer. The boundary of the PIZ may be defined (by the individual States) by local government boundaries or other landmarks, provided they are no closer than 5 km from the edges of infested vineyards. *The final determinate of PIZ boundaries, based on risk analysis of a particular regions situation, will be the National Board. The Phylloxera Reference Group will be responsible for development and endorsement, of protocols and be the arbiter on technical issues on the Boards behalf.* All currently gazetted Phylloxera Infested Zones are listed in Appendix 1.

b) Phylloxera Exclusion Zone (PEZ)

A PEZ is governed by appropriate regulations to control the movement of grapevine material and vineyard equipment into the area. A PEZ is established by historical information and a survey program as not being infested by phylloxera. Areas with existing PEZ status include;

- Western Australia, South Australia, Northern Territory and Tasmania
- The Vine Protected Area in North Western Victoria
- The Hunter-Mudgee-Central Tablelands and MIA-Sunraysia-Mid Murray areas of New South Wales

WA, SA, NT and Tasmania have existing PEZ status. It is envisaged that regional committees will manage areas which will include all the main existing plantings, with appropriate declaration of boundaries and development of regional protocols. Although the remaining (large) areas of these States will not be actively managed by regional committee protocols they must be treated as PEZ areas rather than reduce to PRZ status, assuming retention of existing and appropriate State legislation controlling movement of grapevine material and vineyard equipment.

c) Phylloxera Risk Zone (PRZ)

A PRZ is not known to contain phylloxera infested vineyards, but its status has not been confirmed by surveys. The boundaries of and regulations applying to a PRZ are determined by neighbouring Phylloxera Infested Zones and Phylloxera Exclusion Zones. A PRZ may become a PEZ, provided;

- *appropriate surveys are completed.*
- *the necessary quarantine and hygiene procedures to prevent introduction of phylloxera are in place, at State legislative and regional management committee level, for 5 years unless substantive evidence of minimal risk exposure can be established. The final arbiter will be the proposed National Phylloxera Board.*
- *the zone satisfies criteria determined by the National Phylloxera Reference Group.*

5. Restrictions on movements between Phylloxera Zones

This section to be read in conjunction with Appendices 2 and 3a, 3b, 3c.

a) Phylloxera Infested Zone (PIZ)

The movement of grapevine material and vineyard machinery from the PIZ and infested vineyards within the PIZ is heavily restricted. In addition vineyard operators within a PIZ must follow a code of practice on the movement of personnel and vehicles between vineyards within the PIZ. Protocols should be developed by regional industry groups in consultation with the State Chief Plant Health Regulatory Officer.

Movement from a PIZ

Movements of the following from a PIZ <u>are prohibited</u> with exceptions in brackets, that must be subject to permit from the State Chief Plant Health Regulatory Officer;

- grapevine planting material in any form (except germplasm under permit)
- grape leaves or shoots (except dried samples, and diagnostic samples under protocol to specified laboratories)
- grape roots (except under permit for research or diagnostic purposes to specified laboratories)
- soil from a vineyard (except under permit as diagnostic samples)
- unprocessed grapes (except packed table grapes with a sulphur dioxide pad, diagnostic samples under permit, or grapes for processing in another PIZ as per Appendix 2)
- vineyard machinery (except certified cleaned and heat treated)

Subject to specific conditions the following <u>may</u> be moved from a PIZ;

- germplasm (with permit and disinfestation)
- samples of soil, fresh grapes, leaves, roots or shoots for diagnosis (as per protocols in Appendix 2 to approved laboratories under permit or under an audited certification assurance scheme)
- grapes for processing in another PIZ (subject to permit)
- wine, grape juice and dried grapes
- dried samples of vine leaves or shoots (for diagnostic purposes)
- viticultural machinery, including harvesters (cleaned, heat treated and certified by an independant inspection authority before leaving PIZ or in accordance with an audited certification assurance scheme)
- table grapes packed for market (with sulphur dioxide pad in package)
- *must containing skins and seeds (grapes processed through a crusher / destemmer prior to movement, controlled disposal of pre and post fermentation waste at least 5 km from the nearest vines or through a distillation processing plant eg. Tarac)*
- transports (subject to cleaning)

b) *Phylloxera Exclusion Zone (PEZ)*

Movement of the following **into a PEZ** from the specified zones of origin are **permitted** subject to permit from the State Chief Plant Health Regulatory Officer and any codes of practice endorsed by the regional management committee:

- grapevine planting material in any form from a PEZ (subject to import approval).
- cuttings from a PRZ (subject to hot water treatment and permit)
- grapevine material from a PEZ.
- diagnostic samples of grapevine material from a PEZ, PRZ; and a PIZ (only under special permit and code of practice)
- soil from a PEZ
- unprocessed grapes from a PEZ or PRZ.
- packed table grapes with sulphur dioxide pads *from a PIZ*
- wine and dried grapes
- grape juice, free of stems, skins and seeds
- must containing skins and seeds from a PEZ or PRZ; and from a PIZ (subject to disposal of marc at least *5 km* from the nearest vines, *as per code of practice)*
- vineyard machinery, transports and harvesters from a PEZ or PRZ subject to cleaning.
- vineyard machinery and harvesters from a PIZ, subject to cleaning and heat treatment and certification
- transports from a PIZ (subject to cleaning)

c) *Phylloxera Risk Zone (PRZ)*

As a PRZ has no boundaries of its own (other than those of neighbouring PIZ and PEZs) nor an internal regulatory process, the only restrictions on movement of grapevine material and vineyard machinery into a PRZ are those imposed on PIZs and by PEZs. However, a PRZ seeking PEZ status will have to impose PEZ restrictions immediately upon application *to the National Phylloxera Reference Group*. If there are significant vineyard areas that maintain PRZ status, then existing State quarantine restrictions will continue to apply to such areas.

6. Funding and Management of Phylloxera Zones

The activities described in this paper will require funding support in order to be universally and efficiently implemented. An annual budget in excess of $500,000 is envisaged for the three States. It is anticipated this should be funded by an industry levy, struck on a per hectare basis. Opportunities to match industry funds with Commonwealth Government funding will be investigated.

Key activities include:

Regional committees

Regional committees will be required to administer, finance and operate awareness campaigns and surveys.

National Phylloxera Reference Group

It is anticipated that a National Phylloxera Board will be established with representation from State Grape Industry/Phylloxera Boards and National peak bodies (eg WFA, WGCA), together with relevant Department of Agriculture technical personnel, to ensure coordination, and implementation, of Phylloxera policy on a national basis.

In the interim a National Phylloxera Reference Group will be the interim committee for the yet to be established Phylloxera Board with members nominated to represent ;

- *State industry interests*
- *State legislative/regulatory interests in plant health.*

This group is to act in policy development and review with implementation of the outcomes to be at a regional level.

Phylloxera surveys

May involve aerial photography but must also include root examination in a proportion of vineyards in each Phylloxera Exclusion Zone and in and near Phylloxera Infested Zones. Phylloxera Risk Zones will require systematic surveys before they may be upgraded to a Phylloxera Exclusion Zone.

The surveys should be conducted annually, between December and March.

Awareness

Awareness campaigns have been successful in South Australia through activities of the Phylloxera and Grape Industry Board and through the GWRDC supported Phylloxera research programs. Further application of these approaches are essential for both industry and public awareness.

The law and Phylloxera

- Vineyards infested with phylloxera are currently quarantined in "Vine Disease Districts" of Victoria, or "Phylloxera Infected Areas" of New South Wales. The phylloxera infested vineyards are in central (Nagambie, Mooroopna) and northeast Victoria (North East, King Valley), in southeast New South Wales (Corowa), and in the Counties of Camden and Cumberland near Sydney.
- No vine cuttings, rootlings or unprocessed grapes can be removed from a quarantine district.
- No viticultural equipment including mechanical harvesters can be removed from a quarantine district without washing, steam cleaning or heat treatment, and inspection.

APPENDIX 2. OUTLINE OF PROTOCOLS FOR PHYLLOXERA RISK MANAGEMENT

PROPOSED 1997 INTERIM PROTOCOLS

The following protocols provide an indication of the level of detail which will be required for definition of legislative and regional protocols for phylloxera risk management. Further work will be required to ensure that all risk aspects are covered and that protocols are written in standard operating procedure format. *It is recommended that these protocols be adopted as the interim protocols for 1997 pending establishment of the national Phylloxera Reference Group which will oversight further refinement of the protocols.*

As these protocols are based on an expectation of compliance it will be essential to ensure a process for random audits to be implemented.

a) Hot water treatment of propagation material
b) Cleaning and heat treatment of machinery and equipment
c) Protocol for packaging and handling of diagnostic samples
d) Protocols for movement of unprocessed grapes, must, and juice.
  (i) PIZ
  (ii) PEZ
  (iii) PRZ
e) Protocol for hygiene for personnel and vehicles working in or visiting phylloxera infested vineyards
f) AVIA accreditation documentation

a) Hot Water Treatment of Propagation Material

The following hot water dipping treatment options satisfy the certification requirements for phylloxera disinfestation. The first treatment is currently used for cuttings and rootlings prior to distribution from nurseries. The second is prescribed in the National Vine Accreditation Scheme for treatment of cuttings.

Cuttings and bare rooted rootlings	55°C for 5 minutes
Rootstock and vinifera cuttings	50°C for 30 minutes

The material should be fully dormant and pre-soaked in clean water for 12 hours before dipping to minimise bud damage. Immediately after the prescribed treatment period the material should be removed from the hot water dipping tank and plunged into clean water at ambient temperature for 20-30 minutes. *Documentation of this process will be required to accompany the material to the receiving destination.*

b) Cleaning and Heat Treatment of Machinery and Equipment

Equipment moving out of a PIZ or into a PEZ must be cleaned under supervision of an independant authority or Department of Agriculture inspector. Alternatively equipment would be cleaned, treated and moved in accordance with an audited Certification Assurance scheme.

It is important that cleaning is thorough. All surfaces must be washed with a pressure hose or steam cleaning equipment to remove all plant and soil matter. This may require removal of parts of the equipment to gain access to all remote and protected parts. A detailed cleaning protocol along the lines of the draft prepared by Agriculture Victoria must be followed. This detail will be further developed and included in the Phylloxera Code of Practice

Basically, all soil and plant material must be removed from the equipment and machinery before movement from a phylloxera infested vineyard. Before moving out of a PIZ, the equipment and machinery must be heat treated. The machinery must be held in a dry heat (at least 45°C) atmosphere for at least two hours, beginning when all parts of the equipment have

been brought up to a temperature of at least 45°C. Alternatively grape bins and other suitable equipment may be treated by dipping in hot water at 70°C for a minimum of 2 minutes. *Grape bins used in a side or tilt situation on transports may be steam cleaned in situ for a minimum of 2 minutes.*

c) *Protocol For Packaging And Handling Of Diagnostic Samples*

(WARNING !!!! Dispatch of grapevine foliage, fruit or root samples, or vineyard soil, from a PIZ without a permit is prohibited.)

Samples for root or foliage pests or diseases from vineyards within a PIZ should be diagnosed at research institutes within a PIZ wherever possible (e.g., Rutherglen, Tatura, Camden). Where specialist facilities or staff are required for the diagnoses, the samples may be transported under permit and strict conditions to specific laboratories. The conditions should reflect the need to ensure that any phylloxera on or in the sample is killed before removal of the sample from a PIZ, and there is no risk of cross contamination of other grapevine material. The guidelines for different types of samples are:
Grape roots may only be removed from a PIZ under permit for research purposes. The roots must be frozen (at least -12^{0}C for 2 hours) before removal from the PIZ and transported in dry ice.
Leaf blade, petiole or shoot samples for nutrient analyses must be dried in an oven microwave.
Grape berries for chemical analyses must be frozen, or crushed and transported as juice.
Vineyard soil must be heated in an oven (45^{0}C for 2 hours).

Samples of live grapevine tissues for diagnoses by approved laboratories must not be sent until a permit is obtained, must be clearly labelled with details as to the origin of the material, and must be forwarded direct to the laboratory:

i) Sample material should be placed and sealed in an approved heavy duty plastic bag (supplied by the diagnostic laboratory), packaged in an approved external bag (also supplied by the diagnostic laboratory) and dispatched directly to the diagnostic laboratory.

ii) The sample should be clearly labelled with appropriate warnings and instructions according to the quarantine requirements.

iii) The protocols for handling samples at the receiving laboratory must be documented and approved by the State Chief Plant Health Regulatory Officer, who will be responsible for auditing these procedures.

vi) When examination of the samples is completed, all plant material and soil must be microwaved or autoclaved before disposal, or incinerated.

d) *Protocols for movement of unprocessed grapes, must, and juice*

(i) PIZ
(ii) PEZ
(iii) PRZ

Discussion to include pre- and post- fermentation material

Protocols for processing within a PIZ

Wineries and crushing or processing plants within a PIZ need to develop protocols to ensure that interactions between equipment associated with grapes from phylloxera infested vineyards and vineyards free of phylloxera are minimised. A system for logging receivals from phylloxera infested vineyards should ensure that wine bins etc are cleaned and heated treated as necessary. Receivals of grapes should be separated from areas where the processed products are despatched.

The following protocols detail procedures to be followed for grape material delivered to a PEZ (or PRZ) from a PIZ

1. *Unprocessed grapes:*

   Unprocessed grapes may, with a permit from the State Chief Plant Health Regulatory Officer, be transported from one PIZ to another PIZ for processing. The grapes must be in a container that will not allow spillage and transported directly to the processing facility, i.e. without entering any vineyard in a PEZ or PRZ en route. Similarly, grapes from one PRZ or PEZ may be transported under permit through a PIZ to another PRZ or PEZ, provided they do not enter any vineyard within the PIZ. The conditions applying to movement of unprocessed grapes are:

   i) Each bin or the complete load must be covered to prevent loss of grapes or other material during transport. The covers need to be heavy duty plastic or tarpaulin and must remain secure during the trip.

   ii) The trucks must use designated roadways that avoid passing other vineyards. The grapes must be conveyed directly to the crushing plant in the destination PIZ and must not visit any vineyards outside the PIZs.

   iii) A log of all deliveries is to be maintained by the dispatching grower and the receiving winemaker. Any spillages are to be reported to the State Chief Plant Health Regulatory Officer immediately. *Protocols for all clean up procedures will need to be developed for each region under the direction of the national Phylloxera Reference Group.* All grower and winery records are to be made available for auditing by state authorities.

   iv) All bins used for the transport of grapes must be thoroughly cleaned after the grapes are removed for crushing and before the bins are sent to any other vineyards (see protocol for treating machinery and equipment).

   All winegrapes to be removed from a Phylloxera Infested Zone and not destined for another PIZ must be processed through a crusher/destemmer or press within the PIZ.

2. *Pre fermentation must (white and red)*

   Transport of grape must from a PIZ is strongly discouraged*. This is due to the possibility of survival of phylloxera in must and the consequent risk of phylloxera in the untreated skins after pressing. If must has to be removed from a PIZ then it should be transported as described below.*

   - *Winegrapes grown within a Phylloxera Infested Zone must be processed through a crusher/destemmer before being removed from a PIZ.*
   - *The must has to be transported in sealed tankers direct to the destination winery. Any spillages must be cleaned up to ensure no contamination of adjacent vineyards. At the winery all of the load must be pumped directly into the fermentation tanks and fermented in a closed system process. The wine tanker must be cleaned prior to moving from the winery or taking another load (see protocol on cleaning machinery and equipment).*
   - *Any spillages and hosing down effluent must be flushed into the winery effluent disposal system and not run off into a vineyard area.*

3. *Grape juice (red or white grapes)*

   Grape juice obtained by crushing/destemming and/or pressing may be removed from a PIZ for subsequent fermentation. Grape juice that is not for fermentation *must* be filtered or centrifuged prior to leaving the PIZ.

   Transporting conditions as outlined for *"must"* must apply. At the receiving winery the

juice must go directly into the fermentation tank and be fermented in a closed system. Any residues after fermentation is completed may be disposed of as prescribed.

4. *Disposal of pre-fermentation residues grapes originating from a PIZ*

Where partially fermented juice is pressed off in less than 4 days of fermentation then the skins should be handled as follows;

i) All skins from the load from a PIZ must be separated from other winery wastes. The press must be thoroughly cleaned out prior to using it for another batch.

ii) The skins must be transferred to an area at least 5 km from any grapevines and composted for a minimum of 6 months prior to application in any vineyard.

iii) The pre-ferment skin residues must not be sent directly to another secondary processing plant, eg Tarac.

iv) These procedures must be logged by the winery and made available to authorised persons upon request.

5. *Disposal of post-fermentation residues from grapes originating from a PIZ*

Any marc remaining after fermentation to near dryness may be disposed of in the prescribed manner (ie no less than 5 km from vines or through a distillation processing plant. Post-fermentation residues can be sent out of a PIZ for further processing, eg. Tarac, provided they are segregated from pre-fermentation residues and other vine material.

6. *Table grapes:*

Table grapes from a PIZ may be marketed in a PRZ or PEZ, subject to certification of fumigation with sulphur dioxide during storage, or when a sulphur dioxide pad is contained within the packed grapes. The Table grapes must be packed to the relevant State standards.

e) *Protocol for Hygiene for Personnel and Vehicles Working in Or Visiting Phylloxera Infested Vineyards*

People who work in or visit infested vineyards are potentially capable of transferring phylloxera, or other pests and diseases, from one vineyard to another. The main risks are during the summer and autumn, when phylloxera crawlers may be present on clothing etc of people in contact with vine foliage or fruit. In addition, vineyard soil on vehicles, boots, or implements could contain vine rootlets and phylloxera. Simple hygiene precautions based on commonsense will minimise the risk of transfer of phylloxera. The guidelines suggested are:

1. Plan vineyard visits so that infested vineyards are visited *after visits to non-infested vineyards*, at the end of the day, even if it means extra travel or time. This makes it easier to ensure an overnight stop, shower and fresh clothes before visiting uninfested vineyards on the following day.

2. On infested properties, visit uninfested sections of the vineyard and infested sections last.

3. Carry at least two sets of external clothing and footwear to be used separately for infested and uninfested vineyard. Protective clothing such as light weight plastic lined overalls used with spraying are suggested as they have no pockets to catch leaves or insects and can be disposed of readily. Plastic overshoes can also be used.

4. As far as practical, implements and machinery that are used in infested vineyards should not be used in uninfested vineyards or uninfested sections of vineyards. This would include implements such as sprayers, hedgers, foliage lifters and any cultivation equipment. Where possible separate vehicles should be used when driving in infested vineyards.

5. Always park vehicles outside the vineyard, preferably in the sun, so that heat (>45°C) will effectively disinfest items of personal equipment left in the vehicle. Where possible utilise gravelled loading areas set aside by growers for parking. If leaving a Phylloxera Infested Zone or infested vineyard, either disinfest your vehicle through heat or spray the interior with a household aerial spray (eg. pyrethrins). Commercial car wash facilities that wash underneath the vehicle should be used if leaving a Phylloxera Infested Zone in a vehicle that has picked up mud in a vineyard.

6. Carry a bucket, brush, and either 70% alcohol or 1% (active ingredient) sodium hypochlorite solution. Clean, disinfest, rinse and dry footwear and instruments (eg. secateurs, picking knives, shovels etc.) before leaving each infested vineyard.

7. Pickers who move between properties in Phylloxera Infested Zones should receive instructions pertaining to quarantine protocols from vineyard managers before commencing work in a vineyard. Discrete areas should be provided for parking vehicles, cleaning down equipment and changing clothes.

f) AVIA accreditation documentation

The AVIA accreditation documents are intended to work in a coordinated fashion with these protocols. The processes indicated in the AVIA national accreditation program should therefore be an addendum to this interim working document.

APPENDIX 3a. ENTRY INTO PEZ

PROHIBITED	ENTRY CONDITIONS	NO CONDITIONS

MATERIAL	from PIZ	from PRZ	from PEZ
CUTTINGS		Permit	Permit
GERMPLASM	Permit	Permit	Permit
DORMANT ROOTLINGS			Permit
ROOTLINGS			Permit
LEAVES			
PETIOLES	Permit and protocol (Appendix 2)	Protocol (Appendix 2)	
DIAGNOSTIC	Permit and protocol (Appendix 2)	Protocol (Appendix 2)	
SOIL			
GRAPES		Permit	
TABLE GRAPES	Sulphur dioxide pad in package		
WINE, JUICE AND DRIED GRAPES			
MUST	Marc disposal 5 km from vines, or distillation processed		
HARVESTERS AND VITICULTURAL MACHINERY	Cleaned, heat treated and certified before leaving zone	Cleaned before leaving zone	
TRANSPORTS	Cleaned		

APPENDIX 3b. ENTRY INTO PRZ

PROHIBITED	ENTRY CONDITIONS	NO CONDITIONS

MATERIAL	from PIZ	from PRZ	from PEZ
CUTTINGS			
GERMPLASM	Permit		Permit
DORMANT ROOTLINGS		Permit	Permit
ROOTLINGS		Permit	Permit
LEAVES			
PETIOLES	Dried before dispatch (protocol, Appendix 2)		
DIAGNOSTIC	Permit and protocol (Appendix 2)		
SOIL			
GRAPES			
TABLE GRAPE	SO_2 pad in package		
WINE, JUICE AND DRIED GRAPES			
MUST	Marc disposed of 5 km from nearest vines, or through distillation processing plant		
HARVESTERS AND VITICULTURAL MACHINERY	Cleaned, heat treated & certified before leaving zone	Cleaned before leaving zone	
TRANSPORTS	Cleaned		

APPENDIX 3c. ENTRY INTO PIZ

PROHIBITED | ENTRY CONDITIONS | NO CONDITIONS

MATERIAL	from PIZ	from PRZ	from PEZ
CUTTINGS	Permit		
GERMPLASM	Permit		
DORMANT ROOTLINGS	Hot water treatment		
ROOTLINGS	Permit		
LEAVES	Permit		
PETIOLES	Dried before dispatch		
DIAGNOSTIC	Standard protocol followed (Appendix 2)		
SOIL			
GRAPES	Permit and protocol (Appendix 2)		
GRAPE PACKS	SO_2 pad in package		
WINE, JUICE AND DRIED GRAPES			
MUST	*Marc disposal within the PIZ according to regional protocols.*		
HARVESTERS AND VITICULTURAL MACHINERY	Cleaned, heat treated & certified before leaving zone	Cleaned before leaving zone	
TRANSPORTS	Cleaned		

Written responses to tristate paper:

1995

15/8/95	Lorenzo Cossignani, Ovens ValleyWine Grape Growers Association
15/9/95	Bryan Coombe, Waite Institute
21/9/95	Chris Green, NSW Agriculture
3/10/95	Kym Ludvigsen, Southcorp Wines
5/10/95	Malcolm Campbell, Campbell's Wines
9/10/95	Sam Plunkett, Plunkett Wines
9/10/95	Nigel Scott, CSIRO Horticulture
10/10/95	Peter Murphy, Australian Vine Improvement Association
11/10/95	Ovens Valley Grapegrowers Association
11/10/95	Ian Tinkler, W Drayton & Sons P/L
12/10/95	Ian Thorn, ADFA
12/10/95	G Behncken, Queensland DPI
13/10/95	Russell Johnstone, Orlando Wyndham
13/10/95	Jim Cullen, CSIRO Division of Entomology
13/10/95	Clare Grapegrowers Association
19/10/95	Linda Bowes, South Ausrtralia Wine & Brandy Corporation
19/10/95	Prue Henschke, Adelaide Hills Grapegrowers & Winemakers
23/10/95	G Darling, King Valley Grapegrowers Association
27/10/95	Stuart McGrath-Kerr, NSW Wine Industry Association
21/12/95	Rhett Marlowe, Winegrape Growers Council of Australia

1996

14/1/96	Alister Purbrick, Chateau Tahbilk
17/1/96	Anthony Couroupis, ADFA
31/1/96	Ross Johnstone, Shoalhaven Grapegrowers and Winemakers Association
2/2/96	Neville Bussell, Victorian Grafting Nurseries
12/2/96	Tan Nair, NSW Agriculture
19/2/96	Graeme Ray, VWIA
22/2/96	Bryan Coombe, Waite Institute
22/2/96	Graeme Ray, VWIA
26/2/96	Dennis Richards, Agriculture Victoria
26/2/96	Terry Lee, Australian Wine Research Institute
27/2/96	Anthony Couroupis, Australian Dried Fruits Association
28/5/96	Guy Darling, King Valley Grapegrowers Association
2/7/96	Mike Kinsella, Agriculture Victoria
15/7/96	Bob Taylor, Dried Fruits Research Council
23/7/96	P Brazil-Smith, Benwerren Vineyard
25/7/96	John Stocker, Pratt Industries
1/8/96	Duncan Farquar, Tasmania Dept Agriculture
2/8/96	Guy Darling, King Valley Grapegrowers Association
6/8/96	Aileen Norton, Robinvale Wine grape growers association
12/8/96	Kym Ludvigsen, Grampians Winemakers

Appendix 6 :

Screening rootstocks for resistance to phylloxera.

The information contained in this Appendix is intended for publication in research and industry journals. The material in the Appendix is not to be reproduced, photocopied, or published without the written permission of G.A.Buchanan, Sunraysia Horticultural Centre, P.O. Box 905, Mildura, Victoria 3502

Appendix 6: Screening rootstocks for phylloxera resistance.

Introduction

The resistance of rootstocks to phylloxera can be defined as the relative growth of the rootstock (or grafted grapevine) infested by phylloxera compared to uninfested vines. Testing procedures that are in accord with the definition have the advantage that they measure resistance without confounding factors such as adaptation to soils, water, or inherent vigour of the vines. The approach of comparing growth of infested and uninfested vines can be followed with vines grown in pots, or even parts of vines (eg., roots in tissue culture), by deliberately infesting vines and keeping the control vines free of phylloxera. It is potentially possible to test vines growing in the field, but a method of preventing the infestation of vines (eg., through chemicals) is lacking at present. The experiment described here was intended to measure phylloxera resistance by the growth of grafted vines grown in pots.

Methods

Cabernet Sauvignon cuttings were bench-grafted (October 1995) to the different rootstocks at Sunraysia Horticulture Centre, Mildura, propagated in a heat bed, and transferred to pots containing a standard potting mixture of coarse river sand, peat moss, and perlite adjusted to pH7. They were grown for a year in a shadehouse at Sunraysia Horticultural Centre, a site free of phylloxera. They were then taken (November 1996) to a shadehouse at the Institute for Integrated Agricultural Development Rutherglen (IIAD). The grafted vines were arranged in a randomised block design, with 5 replicates of 8 rootstock treatments. The plots were split into two infested or two uninfested vines, with infested and uninfested vines being kept on separate benches. The vines were watered by individual drippers in each pot.

The vines intended to be infested were inoculated on three separate occasions by inserting 5-10 root galls containing phylloxera into the pot. The phylloxera were taken from a glasshouse culture designated as population VRU-1, originally collected from Cabernet Sauvignon vines at Rutherglen, Victoria.

The growth of the vines and levels of phylloxera infestation was assessed in March 1997, and again in May 1997. On each occasion, one vine from each plot was destructively sampled. The shoots and leaves were cut into a paper bag, dried for 24 hours in an oven, and weighed. The roots were examined for phylloxera galls (nodosities), which were cut into a petri dish, counted, and the number and stage of phylloxera on the galls recorded by examination using a dissecting microscope. The roots were also oven-dried and weighed.

Data were analysed by Analysis of Variance using Genstat. Data on phylloxera numbers and number of galls was skewed, and transformed to Log10 (X+1) for analyses.

Results

Some of the "uninfested" grapevines did become infested, presumably due to dispersal of phylloxera crawlers in the shadehouse. However, there were significant differences in numbers of phylloxera, and in numbers of root galls, between infested and uninfested vines. There were also significant differences in phylloxera infestation between rootstocks. Higher numbers of phylloxera and galls were found in either or both assessment times on Cabernet Sauvignon, J17-48, J17-69, K51-32, K51-40 and SO4 than on ARG1, or Ramsey. Over all rootstocks tested, the infested vines had significantly lower root weights and shoot weights than uninfested vines (Table 1).

Table 1. The numbers of phylloxera, root galls, and root and shoot weight of Cabernet Sauvignon vines grafted to different rootstocks that were infested or not infested with phylloxera.

	Log (10) No. phylloxera		Log (10) No. galls		Root weight		Shoot weight	
Stock	infested	not infested	infested	not infested	infested	not infested	infested	not infested
March 1997								
Cab. Sauv.	1.911	0.000	1.512	0.000	7.19	12.30	7.85	11.66
ARG1	0.314	0.000	0.222	0.000	11.19	9.98	15.24	15.79
J17-48	1.391	0.000	1.101	0.000	9.04	12.08	9.00	12.17
J17-69	1.103	-0.063	0.921	-0.055	9.36	13.17	14.83	18.75
K51-42	1.289	0.020	1.805	0.015	9.90	12.84	12.35	12.02
K51-40	1.505	0.490	1.252	0.401	10.91	10.99	12.35	11.26
Ramsey	0.020	0.000	0.015	0.000	9.94	12.86	15.38	21.99
SO4	0.764	0.000	0.652	0.000	10.27	12.12	15.18	12.22
Mean					9.72	12.04	12.77	14.48
May 1997								
Cab. Sauv.	0.544	0.000	0.376	0.000	11.65	13.37	8.25	8.43
ARG1	0.000	0.000	0.000	0.000	10.46	11.58	13.88	14.05
J17-48	1.729	0.000	1.285	0.000	15.09	19.83	11.42	9.55
J17-69	-0.063	0.107	-0.055	0.088	10.14	15.77	5.85	12.52
K51-32	0.953	-0.306	0.732	-0.027	10.68	15.03	11.25	12.18
K51-40	1.567	0.670	1.144	0.476	11.71	15.89	7.48	9.38
Ramsey	0.544	0.000	0.244	0.000	10.15	12.51	13.26	16.90
SO4	1.206	0.000	0.865	0.000	13.16	22.67	14.80	19.67
Mean					11.63	15.38	10.77	12.84
LSD*	1.010		0.776		5.23		4.68	

* Least significant difference (LSD, p=0.05) for comparing individual means for rootstocks within or across rows for any one parameter.

Discussion

This experiment, infested with VRU-1 strain of phylloxera, had phylloxera infestations lower than desirable for a severe test of the rootstocks. The reasons for the modest infestation levels may relate to the density of the potting medium or watering regimes, or the inherent aggressiveness of the phylloxera strain. The levels of phylloxera on the control (Cabernet Sauvignon) vines suggest that a more virulent strain of phylloxera (eg. VNA-1) may have given different results. However, the experiment demonstrated that phylloxera populations can develop on some rootstocks, with debilitating effects on root and shoot weight of the infested vines.

The relatively high populations of phylloxera on the J and K series of rootstocks indicate that these rootstocks are not sufficiently resistant and should not be recommended where the VRU-1 strain of phylloxera exists. The performance of SO4 rootstock is also of concern, since this rootstock showed both high populations of phylloxera and significantly reduced root and shoot growth of infested vines relative to uninfested vines. Conversely, the relatively good performance of ARG1 is not a cause for optimism, given the susceptibility of this rootstock to phylloxera in both California and Australia.

Previous work (Corrie et al 1997) has shown that genetically distinct strains of phylloxera exist in Australia. It has been shown that the survival rate of phylloxera strains differs on different rootstocks in bioassays using excised root pieces (Appendix 2), and the biological characteristics of the strains correlate well with field observations (Buchanan 1990). The results from the current experiment with strain VRU-1 again indicate that some strains of phylloxera strain can survive and develop on several different rootstocks to a similar extent as on *V.vinifera*. Preliminary data from further DNA typing indicates that VRU-1 and SRU-1 are similar strains of phylloxera, which is not unexpected since they were sourced from adjacent vineyards.

The population growth of different strains of phylloxera and effects on vine performance needs further investigation to determine the appropriate methods for evaluating the phylloxera resistance of rootstocks. Ideally, the experiment described here should be repeated with the inclusion of the further factor of different phylloxera strains.

Appendix 7 :

The influence of nutrition and irrigation on phylloxera damage to grapevines.

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Appendix 7. The influence of irrigation and nutrition on phylloxera populations and damage to grapevines grown in pots.

Introduction

The interaction of vine health with the effects of phylloxera is poorly understood. Water stressed grapevines show above ground symptoms of phylloxera damage earlier and to a greater extent than well watered grapevines, suggesting that frequent, light watering may reduce the effects of phylloxera. Similarly, additional fertiliser is often applied to phylloxera affected vines to stimulate canopy growth.

This experiment aimed to determine the relative effects of additional irrigation and fertilisers on the growth of vines grafted to resistant (Kober 5BB), tolerant (ARG1), or susceptible (*V.vinifera*) rootstocks, either infested or not infested by phylloxera.

Methods

Cabernet Sauvignon cuttings were bench-grafted (October 1995) to the different rootstocks at Sunraysia Horticulture Centre, Mildura, propagated in a heat bed, and transferred to pots containing a standard potting mixture of coarse river sand, peat moss, and perlite adjusted to pH7. They were grown for a year in a shadehouse at Sunraysia Horticultural Centre, a site free of phylloxera, after which they were then taken (November 1996) to a shadehouse at the Institute for Integrated Agricultural Development Rutherglen (IIAD). The experiment was a factorial design, with three rootstocks (Cabernet Sauvignon, ARG1, Kober 5BB), infested or not infested by phylloxera (on separate benches), with two irrigation (wet or dry) and two fertiliser treatments (fertiliser, no fertiliser). The treatments were replicated three times.

The pots were watered by individual drippers, with the "wet" treatment set to deliver sufficient water to saturate the pot every second day, and the "dry" at half the amount. Fertilised pots received "Osmocote" slow release granules, and a liquid fertiliser ("Thrive") every two weeks. The vines intended to be infested were inoculated on three separate occasions by inserting 5-10 root galls containing phylloxera into the pot. The phylloxera were taken from a glasshouse culture designated as population VRU-1, originally collected from Cabernet Sauvignon vines at Rutherglen, Victoria.

The growth of the vines and levels of phylloxera infestation was assessed in May 1997 by destructive sampling. The shoots and leaves were cut into a paper bag, dried for 24 hours in an oven, and weighed. The roots were examined for phylloxera galls (nodosities), which were cut into a petri dish, counted, and the number and stage of phylloxera on the galls recorded by examination using a dissecting microscope. The roots were then oven-dried and weighed. Data on root and shoot weight were analysed by Analysis of Variance using Genstat. Data on phylloxera numbers and number of galls was not analysed, because of the large number of zeros in the infested treatments.

Results and Discussion

The level of infestation by phylloxera in this experiment was low, with only Cabernet Sauvignon vines infested (Table 1). Consequently, any interactions between phylloxera and water or irrigation could not be determined, even on the Cabernet Sauvignon vines.

Table 1. Effects of phylloxera infestation, fertilisers, and irrigation regime on Cabernet Sauvignon vines grafted to susceptible, tolerant or resistant rootstocks.

Rootstock			No. phylloxera	No. galls	Root weight	Shoot weight
Cab. Sauv.	infested	fert, dry	0	0	8.39	19.11
		fert, wet	0	0	10.73	23.52
		no fert, dry	23	38	6.90	0.96
		no fert, wet	58	16	9.61	2.76
	not infested	fert, dry	0	0	10.69	20.93
		fert, wet	0	0	13.51	29.95
		no fert, dry	0	0	12.73	5.65
		no fert, wet	0	0	13.22	6.66
ARG1	infested	fert, dry	0	0	7.86	30.28
		fert, wet	0	0	9.31	30.07
		no fert, dry	0	0	6.31	10.86
		no fert, wet	0	0	8.73	0.99
	not infested	fert, dry	0	0	7.33	30.09
		fert, wet	0	0	7.10	29.35
		no fert, dry	0	0	7.32	4.29
		no fert, wet	0	0	9.71	4.70
Kober 5BB	infested	fert, dry	0	0	9.32	33.85
		fert, wet	0	0	7.40	20.75
		no fert, dry	0	0	10.58	3.61
		no fert, wet	0	0	10.64	3.27
	not infested	fert, dry	0	0	11.80	19.49
		fert, wet	0	0	10.80	23.79
		no fert, dry	0	0	13.20	6.14
		no fert, wet	0	0	15.62	4.66
LSD*					4.01	11.36

* Least significant difference (p=0.05) for comparison of means in any one column.

There were highly significant effects of rootstock, and phylloxera infestation, on root weight. ARG1 vines had less roots (mean 7.96 gm dry weight) than Cabernet Sauvignon (10.72 gm) or Kober 5BB (11.17 gm). Infested vines had significantly less roots than uninfested vines; with infested Cabernet Sauvignon vines having a mean root mass of 8.91 gm compared to uninfested vines with 12.54 gm. It is possible that phylloxera infestations had been assessed too late in the season to obtain meaningful estimates of populations and root damage, as there were several

Cabernet Sauvignon vines with dead galls and decayed roots but with no live phylloxera or galls recorded. However, in the absence of clear relations between phylloxera numbers and root damage, the data must be treated with caution.

The application of fertilisers had highly significant effects ($p<0.001$) on shoot weight, with no interactions with other factors. Fertilised vines had shoots of 25.93 gm dry weight compared to unfertilised vines with 4.55 gm. The vines with extra water had increased root weights ($p=0.05$), but the irrigation treatment did not significantly affect shoot weight in this experiment.

This experiment failed to adequately demonstrate interactions of phylloxera, nutrition, and irrigation due to the low levels of phylloxera infestation.

Appendix 8 :

Chemical control of phylloxera on ungrafted vines.

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CHEMICAL CONTROL OF PHYLLOXERA ON UNGRAFTED VINES:

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Introduction

The grafting of grapevines of *V. vinifera* parentage to resistant rootstocks is currently the only method known to successfully control grape phylloxera. However 85-90% of vineyards in Australia are planted on their own roots, due to the cost, shortage of rootstock material, and extra cultural management required for grafted vines. When ungrafted vines become infested by phylloxera, the only long term solution is to replant the vineyard onto resistant rootstocks. Replanting involves re-establishment costs of approximately $25,000 per ha. Consequently, there is a need to develop methods for maintaining the vigour of phylloxera infested grapevines so that replanting costs can be avoided or deferred for as long as possible.

Chemicals have been tested for control of phylloxera but with unsatisfactory results (Buchanan and Godden 1989). The problems encountered with use of chemicals have been due to difficulty in reaching the target population, and with residues of persistent soil insecticides. Systemic chemicals or fumigants appear to have potential, but have yet to be developed as a practical control measure.

A new systemic chemical, Cinnacure™ (active ingredient denoted as CNMA), was tested in Victoria during November 1995 to March 1996, as part of collaborative research with Dr. Jeffrey Granett of University of California, Davis. This product had been tested on container grown vines with promising results. Subsequently, field trials were established in Australia and California. Limited details of the field trial established in Australia will be described here, due to a confidentiality agreement.

Methods

The experiment was conducted in a phylloxera infested Pinot Noir vineyard in the King Valley of Victoria. The vineyard was first found to be infested in 1995, but the infestation had spread

throughout the trial area. The trial was designed as a latin square, with 6 treatments replicated six times. Plots consisted of 16 vines (two rows of 8 adjacent vines). Within rows, there were four vine buffers between plots, and across rows, there were two buffer rows between treated rows. Treatments consisted of Cinnacure™ applied at different times (Table 1). Sprays were applied by a boom sprayer with hollow cone nozzles, with applications made to runoff.

Table 1: Treatment combinations Cinnacure applied to Pinot Noir vines. Prebloom spray was applied 16/11/95, Bloom spray applied 1/12/95, and the verasion spray applied 5/2/96.

Treatment no.	No. of sprays	Period of application of spray		
		PREBLOOM	BLOOM	VERASION
A.	3	Yes	Yes	Yes
B.	2	Yes	Yes	
C.	2		Yes	Yes
D.	1	Yes		
E.	1		Yes	
F.	1			Yes
G.	3	Control 1% wetting agent		

Phylloxera evaluations:

Phylloxera evaluations were made pre treatment (16/11/95), after the pre bloom spray (29/11/95), and before the verasion spray (2/2/96). Roots were scanned in the field and populations classed according to the criteria developed by Jeffrey Granett (Table 2).

Vine Vigour:

Vine vigour was estimated at two points during the trial, during prebloom (21/11/95) and at harvest (24/3/96). Two scales were used, the first (prebloom) involved estimation of the foliage density and determination of the no. of shoots that were greater than 25cm long. Vines were then classed into 4 categories; 1 = very poor, 2 = poor, 3 = moderate, 4 = vigorous. At harvest a 1-4 scale was also used but was based on mean shoot length for each vine, from less than 30cm (1 = very poor), to > 70cm (4 = vigorous).

Root health:

Roots from selected vines were evaluated for galling and fungal infections. Cross sections of roots were made using a razor blade and percent of the phloem that looked healthy, rotted or

galled was determined. From these estimations, percentages of roots which appeared to be active were determined.

Table 2: Classification criteria used to determine the size of a phylloxera population on the roots of grapevines.

Classification	Description of population classification
0	• No phylloxera seen
1	• Phylloxera seen on nodosities sometimes in large numbers but only individual phylloxera seen on tuberosities
2	• One to four reproducing phylloxera colonies seen on tuberosities
3	• Five or more reproducing colonies seen on tuberosities but there are no large patches of phylloxera
4	• Some large patches of phylloxera on roots making large sections appear yellow, and visible without a hand lens.

25 day Bioassays:

25 day Bioassays were undertaken on treated vine roots to evaluate ability of roots to support phylloxera according to the protocol of Granett et al.(1985). The secondary roots selected were cleaned of soil and existing phylloxera populations. Ten phylloxera eggs were then placed on each root piece, and stored in petri dishes at a constant temperature (23°C±1°C). Evaluation of the population survival and development involved determination of the population weighted by development at day 25, whereby weighted population = $\sum (n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_A)$ where n is the number of phylloxera per root and the subscript indicates the instar counted, with A = adult stage. Two set of bioassays were completed, during December 1995 and February 1996.

Results and discussion:

None of the treatments resulted in a substantial decrease in ratings of phylloxera populations, with the majority of replicates increasing their population rating from the pre treatment assessment. The population ratings averaged 2.31±0.89 pre treatment and 3.40±0.54 pre verasion, with no significant differences due to the Cinnacure treatment. Similarly, bioassays on root pieces showed no consistent differences between the treated or untreated vines. The ratings for vigour demonstrated the majority of the vines were poor to very poor in vigour

(rating 1-2), emphasising the severity of the phylloxera infestation in this vineyard. It was concluded that the Cinnacure treatments did not control the phylloxera populations, prevent root damage, or have beneficial effects on vine health or yields. Phytotoxicity effects on berries showed after the bloom sprays, which were attributed to the high (1%) concentration of wetting agent in the sprays.

Appendix 9 :

Increased awareness of phylloxera through improved communication and training.

Appendix 9. Increased awareness of phylloxera through improved communication and training.

Public awareness of phylloxera and its impact has been facilitated in two ways, firstly through direct dissemination of information to the scientific and grapegrowing industry and secondly, by providing the technical information and tools for public awareness and education activities undertaken by other groups or organisations.

Direct information/dissemination has included the phylloxera workshops, seminar presentations to growers, scientific and general audiences, radio interviews, TV interviews and distribution of phylloxera colour brochures and stickers. The colour brochure was updated and reprinted during 1996, and over 6000 have since been distributed to grower associations and Government agencies in all States. The stickers, produced by the Phylloxera and Grape Industry Board of South Australia, have been widely distributed and are a common sight on farm vehicles throughout Victoria and the rest of Australia. In addition staff from the program have presented information and results at forums including annual reviews of the Cooperative Research Centre for Viticulture, industry meetings, University of Adelaide and Latrobe University seminar series, grape growing association meetings and adult education classes (CAE).

A major presentation of phylloxera research was organised by program staff during September 1996, in conjunction with the Rutherglen Wine Show Society. Over seventy people attended from four Australian States. The theme "A century of phylloxera: Management for the future" focused on the history of phylloxera in Australia, and current approaches to its management. Four speakers presented topics on phylloxera including an international speaker, Dr Helen Fisher (Ontario, Canada), who spoke of the problems (or lack of problems) involved with producing wine in phylloxera's native habitat. Earlier in 1996 another overseas visitor, Dr Jeffrey Granett (University of California, Davis), also presented research results to industry in Wangaratta (Vic.) and Adelaide (SA), whilst undertaking collaborative field work in Australia with staff from this project.

The most important aspect of communication has been the flow on effect of providing information to other organisations. The successful IPM Research to Practice education module now includes a comprehensive section on phylloxera. Slides and accompanying notes for this workshop were supplied for this module, and to the Phylloxera and Grape Industry Board of SA for their publicity projects. Additional dissemination activities include:

- University of Adelaide, Melbourne and CSU all use phylloxera material in their undergraduate courses. In addition material has also been made available for websites at Universities. CSU has produced a CDRom that includes information on phylloxera.

- The Phylloxera and Grape Industry Board of SA has distributed material and information produced by the phylloxera project in SA.

- Victorian and Wine Industry Association and NSW Wine Industry Association have also conducted extensive phylloxera awareness campaigns in their respective states. Newsletters of these associations and commercial industry magazines regularly feature articles based on the phylloxera project and its activities.

- The phylloxera information display board and videos, produced from the previous HRDC project, continue to be widely used in extension activities.

BEAUFORT TREATED TIMBERS

GRAPE PHYLLOXERA



We're concerned too!

BEAUFORT TREATED TIMBERS

Company protocols regarding all transport vehicles leaving Beaufort Treated Timbers.

All vehicles MUST comply with the following:

- The complete prime mover and trailer unit is to be thoroughly washed. Wheels, mudflaps and mudguards to be free from soil, mud and vine material before leaving. (Wash pad facilities provided.)
- Beaufort Treated Timbers quality control officer to inspect and ensure that work has been carried out satisfactorily.
- Vehicles destined for vineyards shall not be permitted to leave Beaufort Treated Timbers without a signed Beaufort Treated Timbers Phylloxera Control Standard Certificate.
- All vehicles delivering into designated Phylloxera infected areas must wash down before leaving vineyards and receive a signed certificate from vineyard managers.

JW Lovell

Managing Director
Jeff Lovell