OPTIMISING DETECTION AND MANAGEMENT METHODS FOR SOIL BORNE PEST INCURSIONS



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A final report on project DPI 05/02

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ABSTRACT

Grapevine phylloxera *Daktulosphaira vitifoliae* (Fitch) is clearly a major threat to the sustainability of all viticultural regions in Australia, and the early detection of this pest is essential in minimising the impact of outbreaks and ensuring the continued success of quarantine protocols and the development of targeted management strategies. Given that 85% of Australian vineyard plantings are on susceptible ungrafted *Vitis vinifera*, and there is evidence for associations of particular phylloxera genotypes with rootstock hybrids, phylloxera will remain a major threat to the long-term viability of the Australian viticultural industry in the foreseeable future. To improve the phylloxera detection and management processes the insects associations with soil were investigated using electromagnetic induction soil sensing, soil analyses, conventional trapping techniques and spectral remote sensing. Textural and chemical soil characteristics along with phylloxera genotype were shown to influence the population dynamics, dispersal behaviour and establishment of the insect. Ultimately these associations will influence the level of sensitivity and effectiveness of early detection and management processes.

EXECUTIVE SUMMARY

Early detection of quarantine pests which predominate in a soil environment, such as grapevine phylloxera, is critical for the profitability of the grape industry due to costs and restrictions in grape movement and market access when an infestation is detected. While 85% of vines in Australia are still susceptible to phylloxera, and the National Phylloxera Management Protocols restrict the movement of grapes and grape products from phylloxera infested zones (PIZ's) to phylloxera exclusion zones (PEZ's), the costs associated with replanting vines with phylloxera-resistant rootstocks, which can be up to four fold greater than ungrafted vines, are estimated at least $20,000 to $25,000 per hectare. A combination of ground and aerial survey techniques are currently used in Australia to detect and monitor phylloxera infestations. These methods rely on visual vine stress symptoms typically seen 2-3 years into a phylloxera infestation making it (i) a relatively 'late' detection system and (ii) 'stress-specific' rather than 'phylloxera-specific'. In some cases vines infested with less-virulent phylloxera genetic strains do not even exhibit foliar decline indicative of phylloxera. Therefore while aerial surveys are good for screening large numbers of vineyards and pinpointing 'weak spots in the vineyard' caused by abundant phylloxera populations; the inherent problem lies with the follow-up ground validation and detecting low levels of abundance. Ground surveys can only be conducted in a limited 2-3 month period when phylloxera populations are active on the roots and are reliant on trained personnel observing phylloxera on the root system. Ground surveys do not survey whole vineyard blocks and all individual vines due the high labour requirement. Infestations therefore can remain undetected, especially in cases where galls are less evident or phylloxera population levels are low. Furthermore a 2-3 year delay in the initial detection of phylloxera in vineyards can allow for widespread movement of the insect throughout the vineyard, imposing an even greater risk of spread to neighbouring vineyards and breakdown in quarantine through movement on viticultural machinery and grape products.

Early detection and monitoring of the spread of this pest via ground assessment, complemented with remotely sensed information has been identified as an important activity in the control of its spread to uninfested regions of Australia. The National

Phylloxera Technical Reference Group hence strongly endorsed the use of such monitoring technologies for early detection, as part of its 2000-2005 Phylloxera Research and Development Plan.

This project examined the interactions between grapevine phylloxera genotype and soil characteristics using field and controlled environment based studies. By understanding phylloxera's interaction with its host plant below-ground this will aid in improving area-wide detection and surveillance systems. The use of a combination of electromagnetic induction soil sensors, remote spectral sensors, insect traps and soil characterisation improved our fundamental understanding of the effects of the soil environment on grapevine phylloxera's ability to establish, develop and disperse. Electrical conductivity, clay content, exchangeable aluminium capacity and potassium: magnesium ratio were factors which appeared to influence phylloxera population abundance and spatial distribution in infested vineyards. This was evident from the two most widespread genetically phylloxera genotypes present in Australia. The relationship between these factors was less apparent for a less virulent and less common genotype. Preliminary findings in this study have highlighted which soil factors may be important in the risk of phylloxera establishment. However, because of the genetic diversity of grapevine phylloxera in Australia, further investigations need to be undertaken to ensure that a robust targeted early detection system can be developed. Research activities and outputs were communicated throughout the project in a variety of extension formats.

INTRODUCTION

Project DPI 05/02*:* Optimising Detection and Management Methods for Soil Borne Pest Incursions

This is a report on phylloxera research, development and extension activities carried out from October 2005 to September 2007. The work was funded by the Grape and Wine Research and Development Corporation (GWRDC Project DPI 05/02) and the Department of Primary Industries, Victoria (DPI).

Project Objectives

The key project objectives were to:

- Map risk areas for phylloxera genotype incursions based on soil type and climate.
- Develop and map scenarios to account for phylloxera genotype incursion.
- Predict rate of phylloxera genotype spread in given soil types.
- Optimise and validate systems for detection and monitoring spread rate of soil borne pests and diseases using phylloxera as a model organism.
- Optimise incursion plans based on scientifically-validated data

Overall Aim and Objectives

The project's overall aim was to improve our understanding of the interactions between grapevine phylloxera with the soil environment, and by doing this assess and improve the effectiveness of 'early' detection techniques through targeting priority areas or hot spots where phylloxera may be more likely to establish.

These objectives were met through:

Utilising electromagnetic induction (EM38) soil survey techniques to measure and subsequently map, underground soil conductivity in the vicinity of the grapevine root

zone. Electrical conductivity (ECa) of soil is related to soil texture and chemistry. Chemical and textural analysis of soils would add value to the ECa data collected and assist in interpretation of phylloxera genotype establishment on specific soil types. EM38 maps of infested sites were linked with remotely sensed spectral data and phylloxera dispersal information, collected using trapping techniques, from infested sites. It was planned that the outcome of such analyses would point to the subsequent use of soil maps and aerial imagery to predict underground population migration in phylloxera-infested vineyards. This objective of the project is also relevant to the development of detection methods for other potential soil-borne pests of grapevines.

The main aim of the project was to significantly improve the predictability and sampling strategy required to eventually optimise novel detection methods such as trapping, soil molecular probes, chemical fingerprints or spectro-optical detection techniques.

Clearly quantifying risks of phylloxera establishment and dispersal for given soil types and improving detection efficiency will lead to:

Increased productivity: by detecting phylloxera early crop losses will be reduced and rootstock replanting will be implemented earlier,

Informed planting decisions: by quantifying the degree of risk of phylloxera establishment for specific soil types,

Targeted survey techniques: Survey procedures may be modified based on the degree of risk allowing earlier achievement of phylloxera free status. Leading to fewer restrictions on market access.

PROJECT AIMS AND OUTPUTS

The project objectives and outputs achieved are summarised as follows:

Objectives	**Outputs**
Map phylloxera risk areas	Vineyards mapped by electromagnetic induction (EM38) methods in the King Valley, Yarra Valley, Murchison and Upton Phylloxera Infested regions.
Link between soil variation and spectral imagery quantified	Comparisons made between EM 38 and aerial and 'Greenseeker' collected NDVI spectral imagery.
Knowledge transfer via National Phylloxera Workshops	Presentations made at six Phylloxera Workshops. Presentations made at the Australian Wine Industry Technical Conference, Adelaide. Presentations made at the Fourth International Phylloxera Symposium, Keselthy, Hungary. Presentations made at the Australian Entomology Society meeting Beechworth, Victoria. Industry journal article in Australian Viticulture. Scientific journal article published in Acta Horticulturae.
Phylloxera population mapped and dispersal rate assessed in three soil types	Abundance of the two main phylloxera genotypes, G1 and G4 monitored at sites in the King Valley, Yarra Valley, Murchison and Upton Phylloxera Infested regions.
Information on phylloxera genotypes in selected soil types	Risk of phylloxera genotypes G1 and G4 establishment and population development determined under controlled environment conditions for three soil types. Influence of soil chemistry on phylloxera dispersal quantified under field conditions.

CHAPTER ONE

Soil Sensing in Phylloxera-infested Vineyards

Objective: Mapping of phylloxera risk areas based on soil variation

SUMMARY

Two primary sites, and three secondary sites, were selected in phylloxera-infested commercial vineyards to characterise soil variation across phylloxera-infested blocks of ungrafted *Vitis vinifera* L. with a view to linking phylloxera population dynamics with potential 'hot spots' (Chapter 2) and soil chemical characteristics (Chapter 4). Significant subsoil spatial variation occurs in vineyard blocks and this variation is likely to be a key determinant in phylloxera establishment, development and dispersal and ultimately influence sensitivity of detection methods; because the insect spends most of its life-cycle in a subterranean environment feeding on the grapevine root system. All selected sites were surveyed using electromagnetic induction sensors (EM38) to generate maps to combine with phylloxera distribution and population dynamics and compare with spectral imagery (Chapter 3). Primary sites were surveyed twice, whilst secondary sites were surveyed once because they were detected midway though the project period. The soil imagery obtained was used to determine phylloxera trap placement (Chapter 2) and soil core collection points (Chapter 4).

INTRODUCTION

Grapevine phylloxera-soil interactions were highlighted in a recent review of priority areas for phylloxera research (NVHSC, 2005). Soil structure has long been regarded as a key driver of phylloxera establishment, rate of spread and risk of dispersal; cracked clay soils are deemed to facilitate underground population migration whereas sandy-loam soils seemingly do not. However, these assumptions are primarily based on anecdotal data and could lead to the misleading assumption that vineyards on sandy-

loam are not at risk from phylloxera infestation. There is some limited overseas field data regarding the influence of soil texture on phylloxera presence. De Klerk (1974), in studies conducted in South Africa, showed that there was a higher risk of phylloxera in soils with less than 65% sand content. In Canada (Stevenson, 1963) phylloxera infestations were lower on loam and sandy-loam soils than clay soils; yet in all the vineyards with predominantly sandy soils phylloxera was still detected. However, in both studies there were limitations in survey protocols used in their assessments (i) they were primarily conducted on grafted vines, which naturally support lower levels of phylloxera on roots than ungrafted vines (ii) the examination was conducted to a soil depth of only 30cm for all soil types (depth of phylloxera and root system could vary between soil types) and (iii) sample number was limited. No detailed studies have been conducted in Australia to quantify the risks of soil type in phylloxera establishment and subsequent rate of spread. Recent observations in infested vineyard blocks has shown distribution of phylloxera and rate of spread do differ both spatially and temporally (Edwards *et al*., 2004) in different infested blocks within the same vineyard. Preliminary studies indicate this may be due to differences in soil characteristics (Powell pers. observ.).

Electromagnetic (EM-38) soil survey techniques measure and subsequently map, underground soil conductivity in the vicinity of grapevine root zone. Soil conductivity is also related to soil texture. Mapping of infested sites and linking spectral data and phylloxera dispersal information from infested sites should improve current detection methods and possibly allow the use of soil maps and aerial imagery to predict underground population migration in phylloxera infested vineyards. This also has the potential to be utilised in detection of other soil-borne pests of grapevines.

The data obtained from such an integrated detection approach would reduce reliance on (i) historical quarantine status (ii) perceived soil risk based on anecdotal evidence and (iii) relative proximity to existing phylloxera infested zones (PIZs; Department of Primary Industries, 2005).

Electromagnetic induction sensors measure bulk electrical conductivity which is determined by relative proportions of clay, salinity and moisture in the soil (Profitt *et al*., 2006). The EM38 sensor collects data from 0-1.5m reflecting the typical depth of grapevine roots and the known depths at which grapevine phylloxera is found on the root system (Buchanan, 1990). In the vertical dipole position 65% of its response comes from the 25-150cm soil depth; the data from which is collected by two passes.

Typically in the vineyard environment EM38 data is collected by mounting the equipment on a rubber sled and pulling via an all-terrain vehicle (ATV) through the centre of the vine mid-row. To accurately map a vineyard area a differential global positioning system (DGPS) is used in conjunction with EM38.

METHODS

Equipment

Measurement of apparent soil electrical conductivity was completed using a Geonics® EM38RT unit (Geonics Ontario, Canada) operated in vertical dipole mode. Prior to conducting each set of measurements throughout the course of the experiment, the instrument was switched on and warmed up for a period of 15 minutes, and zeroed following the standard Geonics® protocol. Immediately following the zeroing procedure, the unit was placed on the ground at a pre-designated calibration point and the EC_a measured and checked to ensure it was repeatable to within 5%.

Two-dimensional surveys were completed by towing the EM38 unit behind an ATV on a rubber sled (Fig. 1) at a speed of 10km per hr, with particular attention paid to ensuring the sled did not deviate more than 10cm either side of the track, thereby minimizing trellis-induced artefacts in the readings (Lamb *et al*., 2005). The EM38 unit itself was placed in a thermoplastic case with styrofoam insulation on either side and a cover over the top to reduce case temperature fluctuations (Brevik *et al*., 2004). The distance between the unit and the surface of the ground, as dictated by the bottom of the case and thickness of the rubber sled was approximately 15 mm. Other studies on the impact of small variations in case-ground distance on a similar EM unit demonstrated no significant impact for the distance described here (Brevik *et al*., 2003). The continuous output data stream from the EM38 unit was fed into a Trimble TSCe® datalogger along with the DGPS location information, every second, from a Trimble DGPS (Trimble, Sunnyvale California, USA).

EM38 surveys

Surveys were undertaken in three phases as follows:

1) Phase 1 – In December 2005 surveys were undertaken at the Upton, King Valley and Buckland Valley sites (site details as described in Chapter 2).
2) Phase 2 - In August 2006 the Upton, King Valley and Buckland Valley sites were re-surveyed.
3) Phase 3 - In May 2007 surveys were undertaken at the Yarra Valley and Murchison infestations detected in 2006 (site details as described in Chapter 2).

All equipment used during the surveys was disinfested according to standard procedures described in the National Phylloxera Management Protocols (National Vine Health Steering Committee, 2003).



Fig. 1. EM38 equipment set up for surveying at the King Valley site, August 2006 showing EM38 transmitter on a sled, and DGPS attached to an ATV.

Primary study site 1 – Upton

At the Upton site two EM38 surveys were conducted and this covered 4 vineyard blocks labelled Block 17 (1.34ha), Block 18 (1.34ha), Block 20 (3.28ha) and Block 21 (2.1ha). Phylloxera ground surveys conducted prior to the EM38 surveys indicated

that Blocks 17, 18 and 21 were phylloxera infested with widespread distribution in Block 17. No phylloxera was detected in Block 20. The uninfested block was surveyed first to ensure no phylloxera transfer between blocks.

Primary study site 2 – King Valley

At the King Valley site two EM38 surveys were conducted and this covered a single vineyard block (2ha). Phylloxera ground surveys, conducted prior to the EM38 surveys, indicated that phylloxera infestation within the block was widespread.

Secondary study site 3 – Murchison

At the Murchison site one EM38 survey was conducted which covered a single vineyard block (1ha). Phylloxera ground surveys conducted prior to the EM38 survey indicated that phylloxera infestation within the block was widespread.

Secondary study site 4 – Yarra Valley

At the Yarra Valley site one EM38 survey was conducted and this covered a single vineyard block (6ha). Phylloxera ground surveys conducted prior to the EM38 survey indicated that phylloxera infestation was predominantly located near the centre of the block.

Secondary study site 5 – Buckland Valley

At the Buckland Valley site two EM38 surveys were conducted and this covered a single vineyard block (3ha). Phylloxera ground surveys conducted prior to the EM38 surveys indicated that phylloxera infestation was isolated within a relatively small region (around 50 vines) within the block.

RESULTS

Primary study site 1 – Upton

EM38 imagery from the Upton site in December 2005 (Fig. 2) shows an ECa range of 5.5-45mS/m which is only slightly lower, within the range 0-32mS/m, in August 2006 (Fig. 3). These relatively low values are consistent with the high sand content evident in the soil 'A' horizon at Upton (Chapter 4).

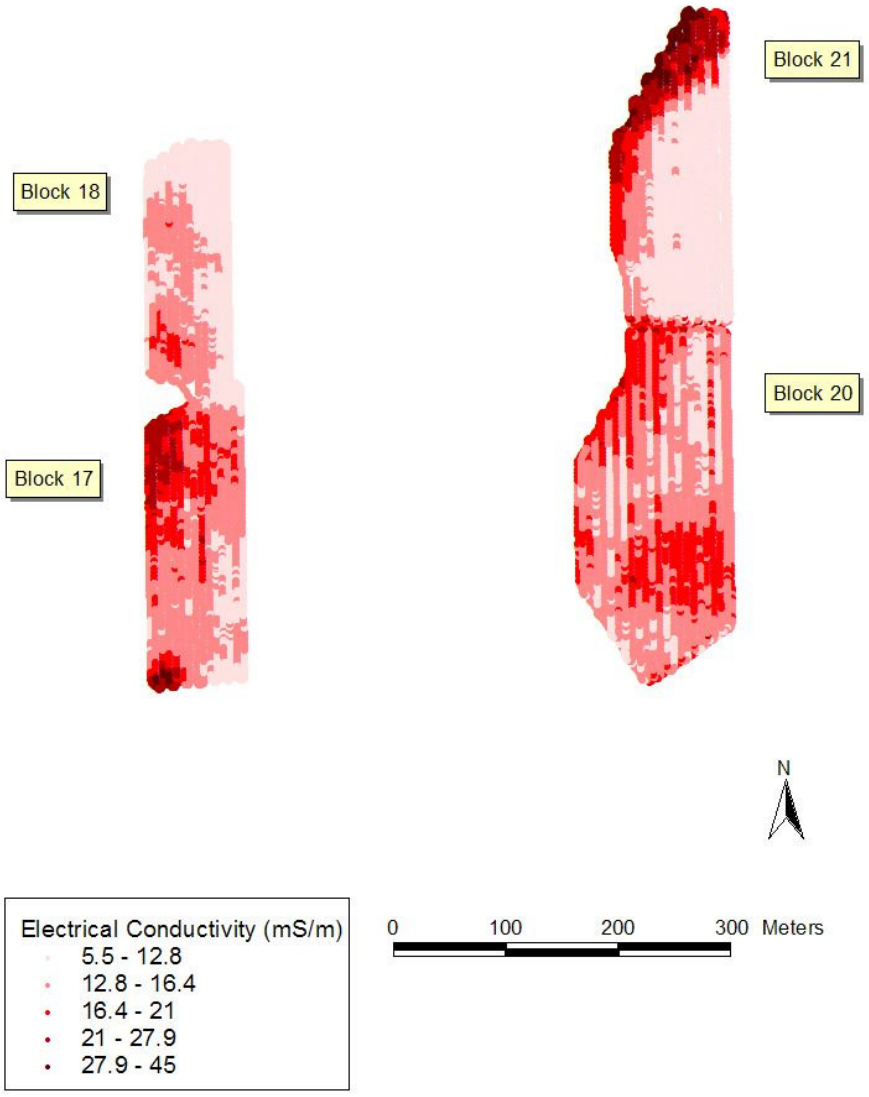


Fig. 2. An electromagnetic induction (EM38) map, collected December 2005, in phylloxera-infested *Vitis vinifera* blocks (17, 18 and 21) and an uninfested block (20) in an Upton vineyard.

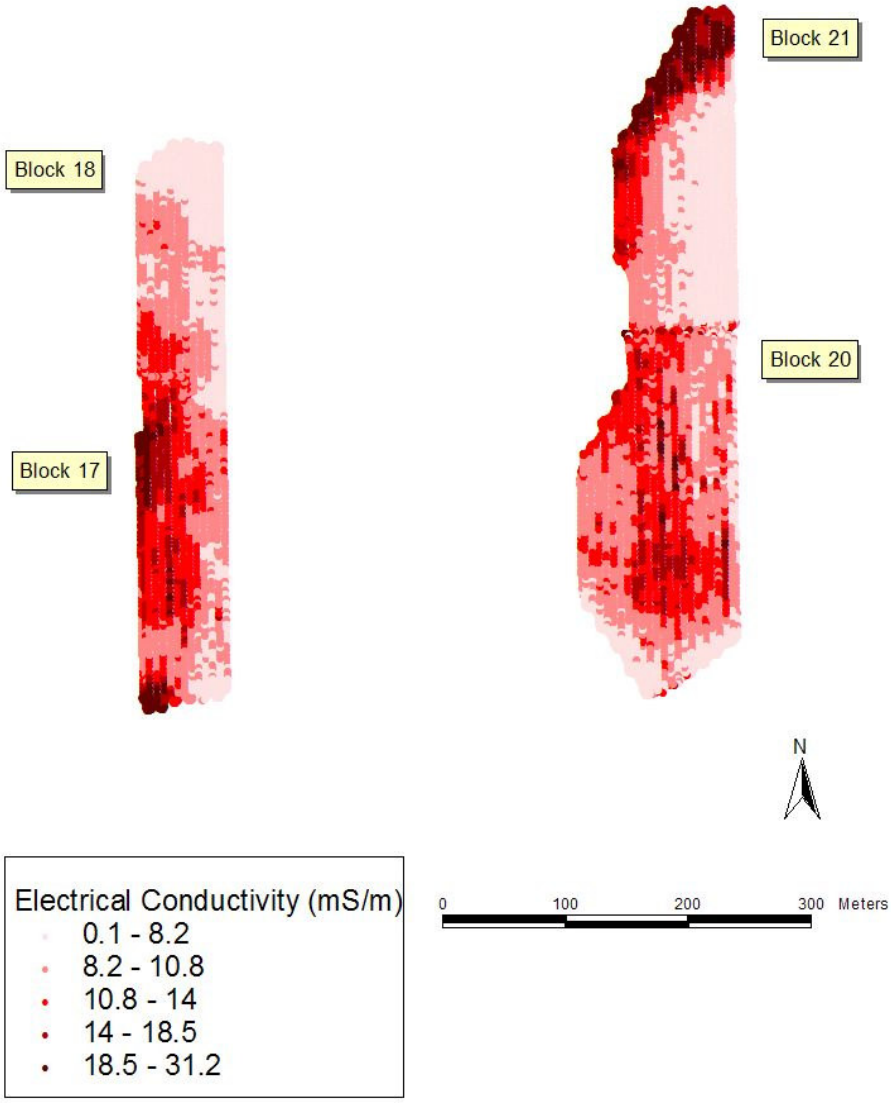


Fig. 3. An electromagnetic induction (EM38) map, collected August 2006, in phylloxera-infested *Vitis vinifera* blocks (17, 18 and 21) and uninfested block (20) in an Upton vineyard.

Primary study site 2 – King Valley

EM38 imagery from the King Valley site indicates and ECa range of 0-20.9mS/m in December 2005 (Fig. 4) and higher ECa values, up to 189.2mS/m, in August 2006 (Fig. 5). This reflects much wetter soil conditions for the August 2006 survey as there had been a rainfall event two days prior to the August data collection.

Secondary study site 3- Murchison

An EM38 survey map at the Murchison site (Fig. 6), shows an ECa range of 0-31 mS/m, with higher ECa values observed in the centre of the study block from which vines had been removed due to high phylloxera populations.

Secondary study site 4 - Yarra Valley

An EM38 survey map at the Yarra Valley site (Fig. 7), shows an ECa range of 0-70 mS/m within a relatively high EC range (31-70mS/m) in around 50% of the block.

Secondary study site 5 - Buckland Valley

EM38 imagery from the Buckland Valley site indicates and ECa range of 0-11.8mS/m in December 2005 (Fig. 8) and higher EC values up to 55.6mS/m in August 2006 (Fig. 9). Wetter soil conditions for the August 2006 survey would have contributed to these higher values.

Data from all sites was used to optimise phylloxera population sampling to determine abundance and distribution (Chapter 2).

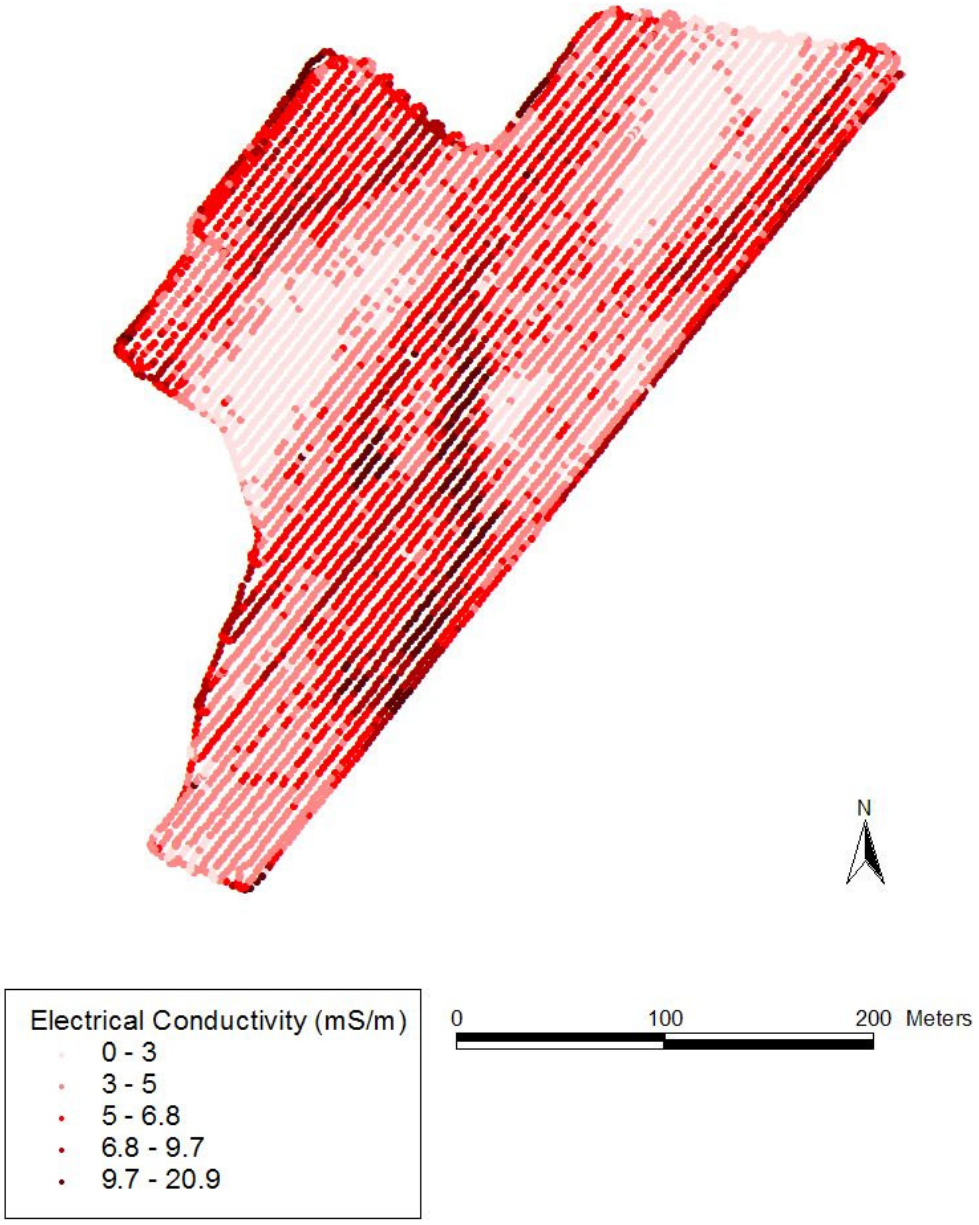


Fig. 4. An electromagnetic induction (EM38) map, collected December 2005, in a phylloxera-infested *Vitis vinifera* block in a King Valley vineyard.

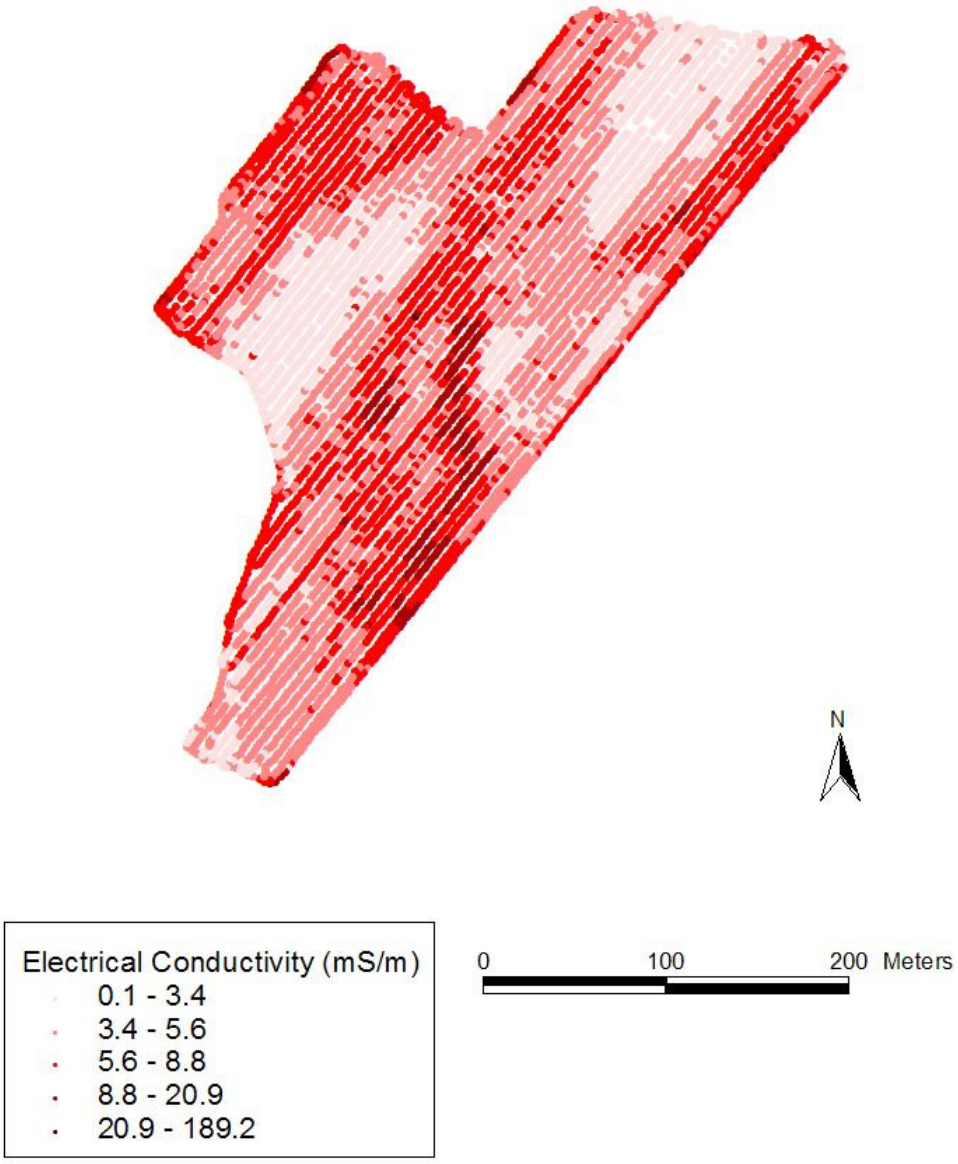


Fig. 5. An electromagnetic induction (EM38) map, collected August 2006, in a phylloxera-infested *Vitis vinifera* block in a King Valley vineyard.

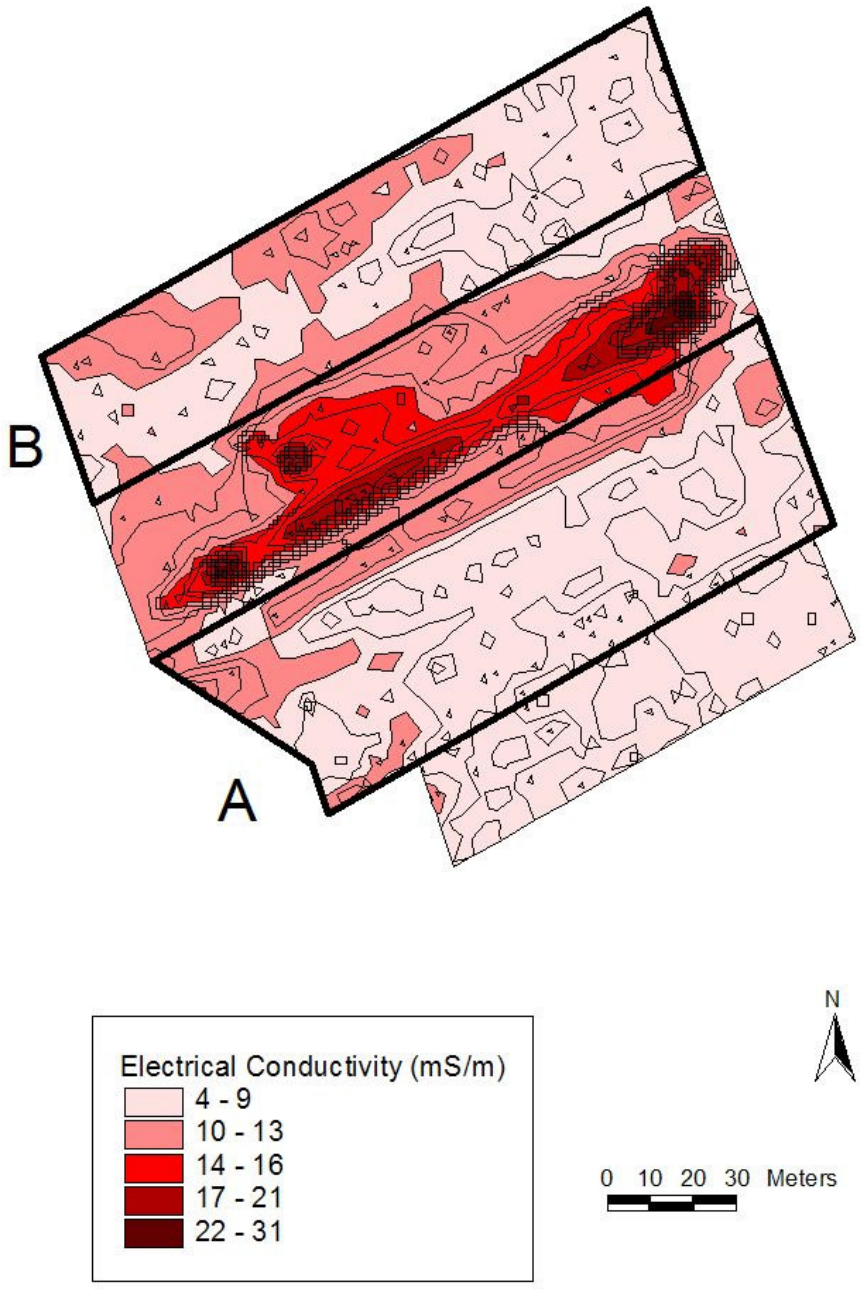


Fig. 6. An electromagnetic induction (EM38) map, collected May 2007, in a phylloxera-infested *Vitis vinifera* block in a Murchison vineyard. Boundaries indicate blocks (A and B) where phylloxera was monitored.

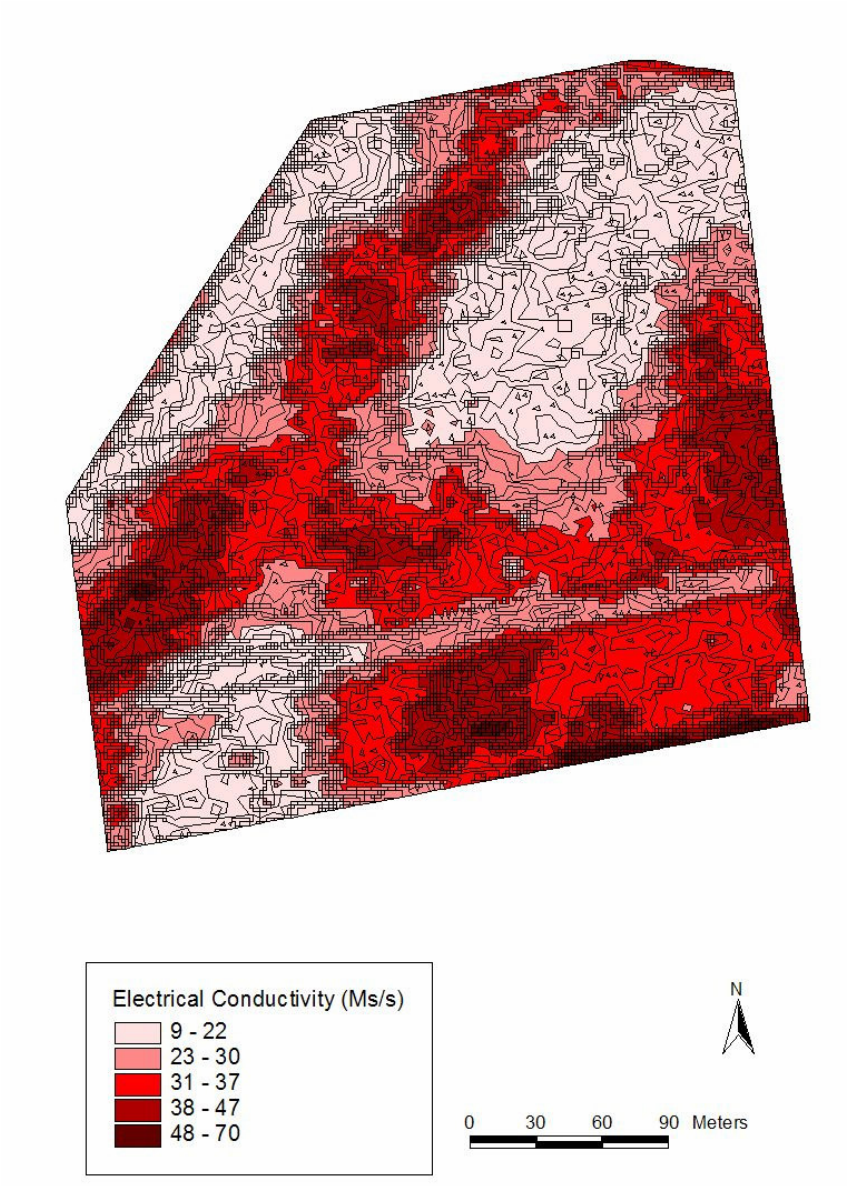


Fig. 7. An electromagnetic induction (EM38) map, collected May 2007, in a phylloxera-infested *Vitis vinifera* block in a Yarra Valley vineyard.

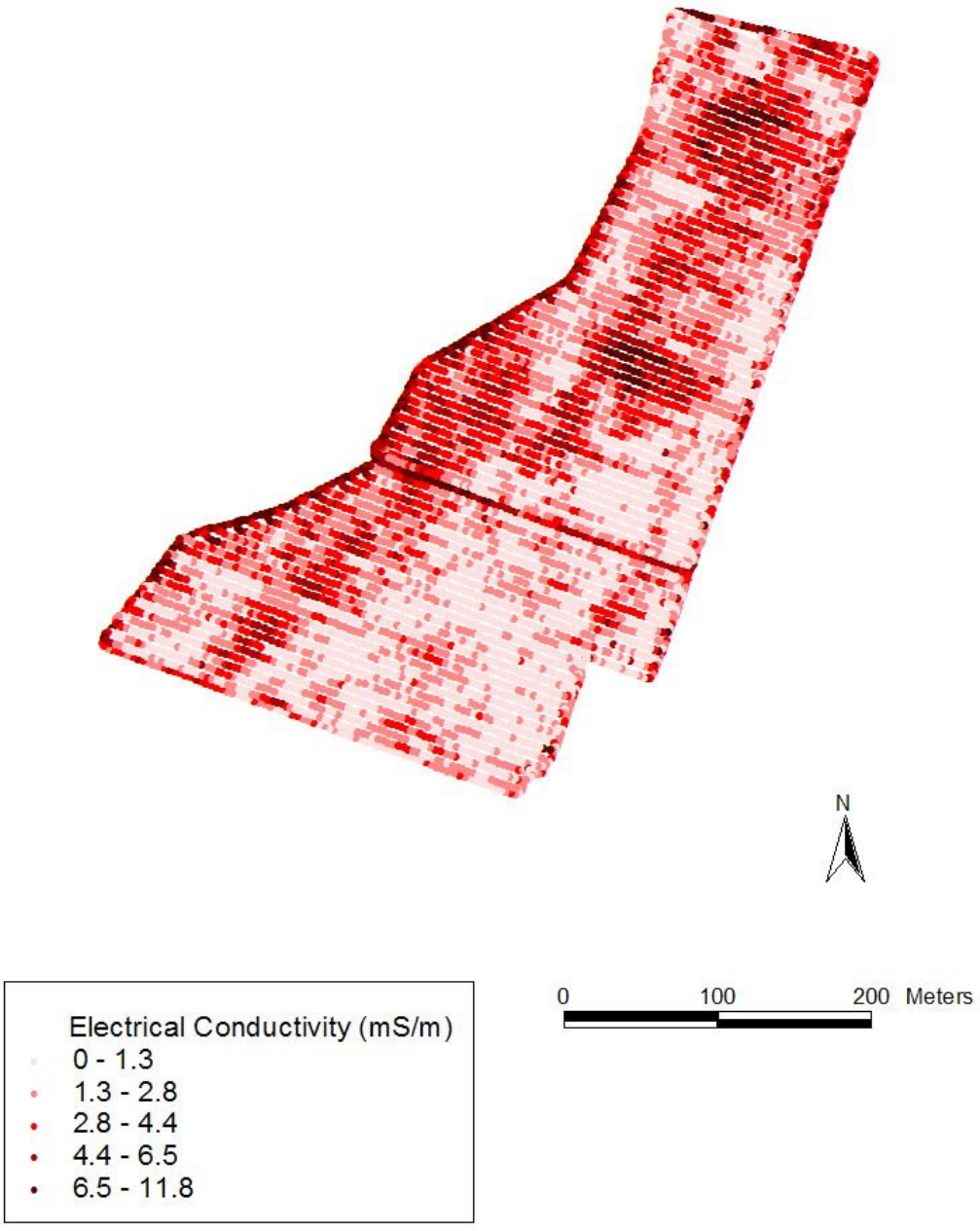


Fig. 8. An electromagnetic induction (EM38) map, collected December 2005, in a phylloxera-infested *Vitis vinifera* block in a Buckland Valley vineyard.

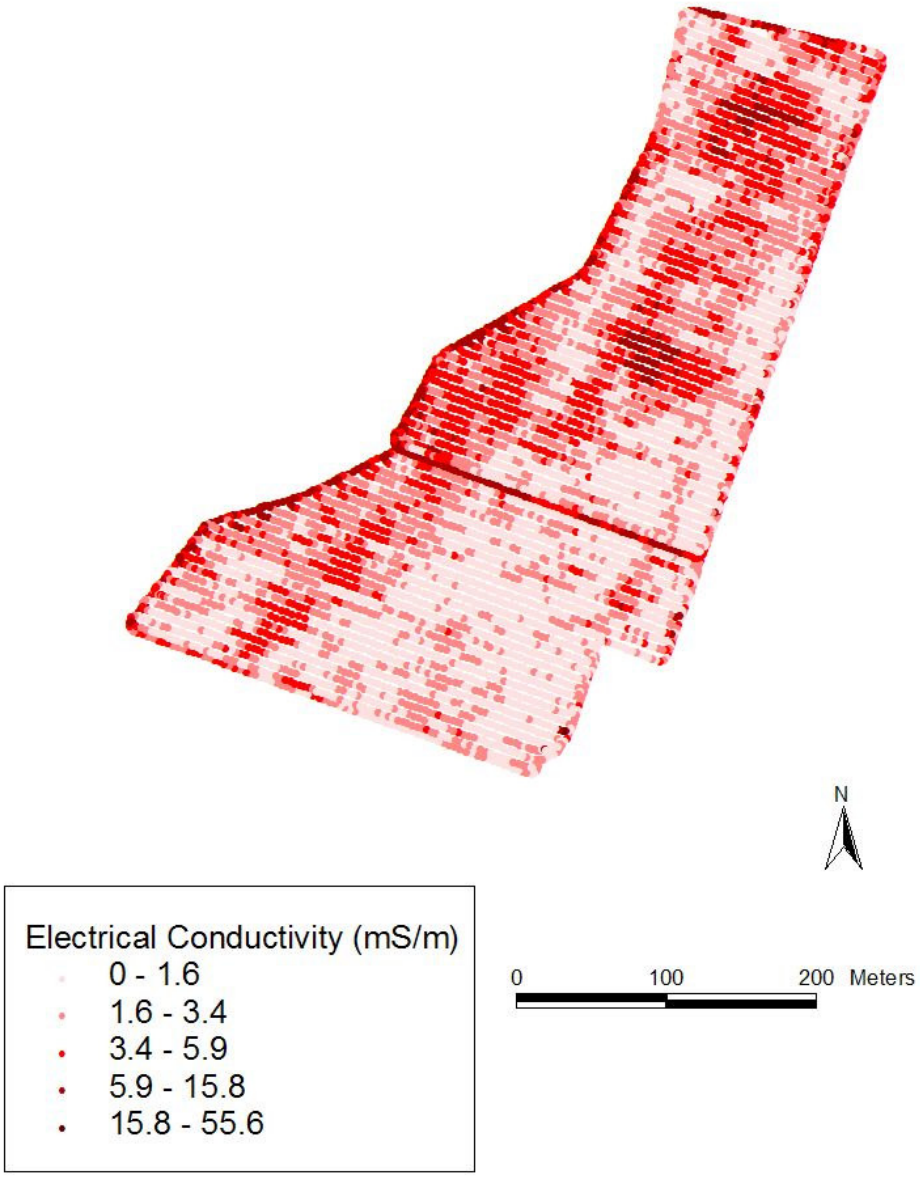


Fig. 9. An electromagnetic induction (EM38) map, collected August 2006, in a phylloxera-infested *Vitis vinifera* block in a Buckland Valley vineyard.

DISCUSSION AND RECOMMENDATIONS

The EM38 sensor measures the bulk electrical conductivity of the soil (apparent electrical conductivity – abbreviated to ECa). When used in vertical dipole mode as for these surveys, the EM38 sensor is less sensitive near the surface, and approximately 65% of the response comes from a soil depth of 25-150cm (McNeill, 1980, op. cit. in Proffitt *et al*., 2006). According to Proffitt *et al.* (2006), the optimum time to conduct an EM survey is when soil moisture is at or close to field capacity. Variation in electrical conductivity values was observed both spatially and temporarily at all sites. The ECa values for the August 2006 surveys were generally higher than those of the December 2005 surveys for the King Valley and Buckland Valley data sets, indicating the moisture content of the soil was higher during the latter surveys. In three of the sites studied, phylloxera was initially discovered in the higher ECa regions where stress symptoms in the canopy appeared more visible (Powell pers. observ.). To determine what influence the ECa variation may have on phylloxera distribution and abundance ECa ranges were divided into low, medium and high categories and used to determine placement of traps for phylloxera monitoring (Chapter 2). The ECa is determined by relative amounts and type of clay, salts, rock and moisture. Anecdotal reports suggest clay content and moisture are important in phylloxera establishment and could therefore also affect population dynamics and dispersal. This aspect is investigated later in this report (Chapter 2-5).

Phylloxera is known to occur in around 60 commercial vineyards in Australia yet limited phylloxera related data is available from these sites. Population studies to date in Australia, although detailed, have been sporadic and restricted to a few sites in the King Valley (3 sites), Yarra Valley (1 site), Nagambie (2 sites), Upton (1 site), Murchison (1 site), Glenrowan (1 site) and Rutherglen (2 sites) – representing about 20% of the known phylloxera-infested vineyards. Due to limitations in resources and geographical distribution of the sites studied EM38 surveys have only been conducted on five vineyards representing about 7% of the known infested vineyards. Before recommendations on targeted surveys can be made more geographically extensive EM38 surveys, soil core analysis and remote sensing are recommended at a range of phylloxera-infested sites.

CHAPTER TWO

Monitoring of Phylloxera Infestations and Linking Population Dynamics to Soil Sensing

Objective: Mapping of risk areas for phylloxera incursions based on soil type

SUMMARY

Two primary sites were selected in phylloxera-infested commercial vineyards to monitor grape phylloxera populations on ungrafted *Vitis vinifera* L. with a view to linking phylloxera establishment 'hot spots' and dispersal activities to soil properties as assessed by using EM38 imagery (Chapter 1) and soil chemical analysis (Chapter 4). Primary sites were monitored over two successive seasons and selected on the basis of single phylloxera genotype presence. In the Upton region, the phylloxera genotype G1 was monitored and in the King Valley region phylloxera genotype G4 was monitored. Both genotypes are regarded as 'superclones' as they are the most widely distributed and virulent of the 83 genotypes known to occur in Australia (Umina *et al*., 2007).

In the second year of the project further outbreaks of phylloxera occurred in the Yarra Valley and Murchison regions. After determination that the genotype at both sites was G1 these two sites were also monitored for a single season, to add value to the project, with the support of DPI-Victoria's Graduate program. In addition data on population dynamics collected in 2004 from a secondary vineyard site in the Buckland Valley (Powell *et al*., 2006) is also presented to show a comparison with a less virulent and less geographically widespread phylloxera.

The abundance of phylloxera dispersive life-stage emergence above-ground varied between sites, seasons and genotypes but followed similar trends observed in previous studies (Powell *et al*., 2003) emergence peaks occurred and which genotype provided higher populations. The implications of these results for phylloxera management, risk of transfer and detection are discussed.

INTRODUCTION

Vitis vinifera L. has been screened for relative susceptibility to specific phylloxera clonal lineages in laboratory, glasshouse and field studies and is known to be highly susceptible to the phylloxera genotypes G1 and G4 and moderately susceptible to genotype G20 (Viduka *et al*., 2003; Korosi *et al*., 2007). The genotype G1 has been detected in all phylloxera infested zones (PIZs) in Australia (Powell *et al*., 2006; Umina *et al*., 2007) with the exception of the King Valley PIZ. Whereas G4 has been detected in the North East PIZ (Powell *et al*., 2006) and the King Valley PIZ (Corrie *et al.*, 2002) but not to-date in the Victorian PIZs of Upton, Nagambie or Maroondah or in the NSW PIZs. G20 phylloxera has limited distribution, having only been detected to date in Milawa and the Buckland Valley regions of the NorthEast Victoria PIZ.

Phylloxera was first detected in the King Valley region near Whitlands in 1991 and near Cheshunt in 1994. All detections to-date in the King Valley PIZ have been the same genotype, G4. Of the phylloxera outbreaks since 2000 which have occurred outside existing PIZs most, with the exception of the Buckland Valley detection (which was genotype G20), were identified as genotype G1 including those at Upton (2000), Murchison (July 2006) and the Yarra Valley (December 2006). The field sites selected for research therefore focused primarily on these two phylloxera genotypes which have been described as 'superclones' (Umina *et al*., 2007). In addition data from a previous study involving a less virulent genotype G20 detected in 2003 in the Buckland Valley was included for comparison purposes.

At all selected sites phylloxera were monitored by root surveys and emergence traps and soil properties were characterised using core analysis (Chapter 4) and electromagnetic induction techniques (Chapter 1). Selected sites were also examined using spectral imagery (Chapter 3).

METHODS

Site details and experimental design

Population data was collected at phylloxera-infested commercial vineyards, in study sites located in the Upton (Site 1), King Valley (Site 2), Nagambie (Site 3), Maroondah (Site 4) and the Northeast (Site 5) PIZs. All studies were conducted on phylloxera-infested ungrafted *Vitis vinifera* L. grapevines.

Primary study site 1 - Upton

Phylloxera was detected at the Upton site in April 2000 (Powell *et al*., 2003). The soil was classified on the basis of texture and chemistry as a dystrophic brown kandosol (Isbell, 1996), which has developed on a granitic terrain. The soil is duplex, typified by having a sandy 'A' horizon overlaying a clay-rich 'B' horizon. The phylloxera at the site was DNA typed using microsatellite markers (Umina *et al*., 2007) and identified as genotype G1, a relatively virulent genotype. However, despite this the phylloxera has spread slowly over a 7-year period since detection within one of the selected trial blocks (Block 21), up to a maximum of only 60 metres away from the initial discovery point (K. Powell pers. observ.). All sample vines were drip-irrigated, however. Limited water availability in February and March 2007 resulted in the vines showing the effects of moderate water stress at this time. Two study blocks were selected at the Upton site; one containing ungrafted *V. vinifera* cv. Cabernet Franc onto which had been grafted Pinot Gris (Block 18) and one containing ungrafted *V. vinifera* cv. Cabernet Sauvignon (Block 21) with a row spacing of 2.9m and vine spacing of 1.4m. An EM38 surveys was conducted in December 2005 and another in August 2006 (Chapter 1) and soil cores collected in November 2006 (Chapter 4).

Primary study site 2 – King Valley

Phylloxera was first detected at the King Valley site near Cheshunt in 1998. Soil at the King Valley site consists of a brown silt alluvial soil, developed on the margins of the King River. The soil is relatively uniform down the profile, with only weak development of differentiated horizons. The soil contains scattered, rounded cobbles and pebbles of acid volcanics. The phylloxera genotype at the site was DNA typed as G4. A preliminary root survey indicated that phylloxera was widespread throughout the study block which was also evident from the appearance of characteristic patches of dead vines. As a result of marked yield decline from phylloxera damage and low water availability due to drought in 2006, the vineyard owner excised all grapevines at the base of the trunk, using a chainsaw just above the dripper line, in the second season of monitoring, between 30th November 2006 and 20th December 2006. This was after the first two emergence trap collections had been made, however the trap monitoring continued until 28th March 2007, to ascertain the effects of this form of vine management on phylloxera population abundance and dispersal. Collection of

traps scheduled for 14th December 2006 was postponed for six days due to road closures resulting from bushfires occurring in close proximity to the site.

One study block was selected at the site which was planted to a mixture of ungrafted *V. vinifera* cv. Chardonnay and ungrafted *V. vinifera* cv. Pinot Noir with a row spacing of 2.5 metres and vine spacing of 1.5m. All vines were planted in 1989. Two EM38 surveys were conducted in December 2005 and August 2006 (Chapter 1) and soil cores collected in November 2006 (Chapter 4).

Supplementary study site 3 – Murchison

Phylloxera was first detected at the Murchison study site in July 2006 when overwintering phylloxera were detected on an infested lignified root sample (K. Powell; pers. observ.). Soil at the site consists of a sandy loam and brown clay loam. The phylloxera genotype at the site was DNA typed and confirmed as G1. Two study blocks were selected at the site and were located on both sides of the area, where phylloxera was first detected, as the vines in the originally detected area had been removed and replanted with rootstocks by the grower in an effort to remove the source of infestation. Block A consisted of 12 rows of ungrafted *V. Vinifera* cv. Semillon. Block B consisted of 10 rows of ungrafted *V. vinifera* cv. Riesling grapes. Row spacing was 2.5 metres and vine spacing 1.8m. Emergence traps were positioned in alternate rows in each block with two traps per block. All vines were planted in the mid-1980's. A single EM38 survey was conducted in May 2007 (Chapter 1).

Supplementary study site 4 – Yarra Valley

Phylloxera was first detected in a block of 7-year old ungrafted *V. vinifera* cv. Cabernet Sauvignon at the Yarra Valley study site in early December 2006. The phylloxera genotype at the site was characterised as G1. The study block was selected following a root survey and visual canopy assessment selecting 27 rows as the initial study block with the central row showing a high degree of canopy decline ie a noticeable 'weak' spot. All vines were planted in 2000. A single EM38 survey was conducted in May 2007 (Chapter 1).

Supplementary study site 5 – Buckland Valley

The study site was located in a commercial vineyard near Porepunkah, located close to the Buckland River and within 20 kilometres of the boundaries of the North East and King Valley PIZs. Phylloxera was first detected in the vineyard in December 2003. The phylloxera genotype within the study block was characterised as G20, which is one of the least common genotypes found in Australia (Umina *et al*., 2007) but is one of the six clonal lineages recommended for further studies (Hoffman, 2003). A soil survey was conducted at the study site (Powell *et al*., 2006) and the soil classified as an acidic mesotrophic red dermosol (Isbell, 1996). Two EM38 surveys were conducted in December 2005 and August 2006 (Chapter 1) and soil cores collected in November 2006 (Chapter 4).

Emergence traps

At all sites phylloxera populations were monitored with emergence traps. These consist of 3.25 litre translucent plastic containers, inverted and secured adjacent to vine trunks with 3 tent pegs (Powell *et al*., 2000; Fig. 1). A Differential Global Positioning System (DGPS) was used to determine accurate trap locations. Initial field positions were collected with a Trimble GeoXT GPS, followed by post-processing using Land Victoria GPSnet data, for sub-metre accuracy. Traps were rinsed with tap water prior to placement resulting in a film of condensation forming, which effectively trap emerging phylloxera dispersive life-stages. Trapped insects were collected each fortnight by washing into plastic vials, with 80% ethanol. Trap samples were transported under quarantine conditions to the DPI-Rutherglen quarantine laboratory and examined using a low power binocular microscope. Emergent phylloxera life-stage numbers, consisting of phylloxera (first instar nymphs) and alates (winged adults) were recorded.



Fig. 1. Phylloxera emergence trap placed adjacent to grapevine trunk and secured with tent pegs

Emergence trap positioning

Trap positions within the primary trial sites, Upton and King Valley, were selected on the basis of pre-placement root surveys and EM38 surveys conducted on the 14th and 15th December 2005 respectively (Chapter 1). EM38 imagery of the vineyards was used to classify the study blocks into high, medium and low ECa categories.

As phylloxera was detected in late 2006 and no EM38 data was available prior to trap placement at the Murchison and Yarra Valley sites trap positioning was based on a root survey and visual symptoms of vine decline in the canopy. EM38 data was collected at the Buckland Valley site on 13th December 2005, but further emergence sampling could not be conducted as the grower removed the ungrafted grapevines and replanted the block to phylloxera-resistant rootstocks.

Primary study site 1 – Upton

At the Upton site in the 2005-2006 season two study blocks were selected which were identified as Block 18 and Block 21 by the vineyard manager (Fig. 2). In block 21 blocks three regions were selected based on EC category being 'low' (5.5-16.4mS/m) 'medium' (16.4-27.9mS/m) and 'high' (27.9-45mS/m). In block 18, as no high EC regions were detected, two EC regions were selected as 'low' (5.5-16.4mS/m) and 'medium' (16.4-27.9 mS/m). Each of these EC regions (blocks) was 50m x 50m.

Within each EC category block 5 emergence traps were randomly positioned adjacent to the grapevine trunk in locations where phylloxera had been detected following

surveys. Traps were positioned at the site on 27th January 2006 and monitored fortnightly until 24th March 2006.

In the 2006-2007 season, due to relatively low numbers the previous season in Block 18, emergence monitoring was only continued in Block 21. Trap number was increased, to improve replication for statistical analysis, with a total of 50 traps distributed across 'low' (0-10.8mS/m), 'medium' (10.8-18.5mS/m) and 'high' (18.5-31.2mS/m) EC categories (Fig. 3). Traps were positioned earlier than the previous season commencing 31st October 2006 and monitored fortnightly until 20th April 2007. Phylloxera abundance on lignified roots of each 'trap' vine was assessed (as described in Powell *et al*., 2003) on 21st February 2007.

Primary study site 2 – King Valley

At the King Valley site in the 2005-2006 season two blocks per EC category ie: 'low' (0-5mS/m), 'medium' (5-9.7mS/m) and 'high' (9.7-20.9mS/m) were selected (Fig. 4). Each block was 100m long by 50m wide. Within each block five emergence traps were randomly positioned adjacent to the grapevine trunk. For each EC category there were therefore ten emergence traps. Traps were positioned at the site on 23rd January 2006 and monitored fortnightly until 22nd March 2006.

In the 2006-2007 season, to improve replication across the site, three blocks per EC category designated as 'low' (0-5.6mS/m), 'medium' (5.6-8.8mS/m) and 'high' (8.8-189.2mS/m) were selected (Fig. 5). Each block was 100m long by 50m wide. Within each block five emergence traps were randomly positioned adjacent to the grapevine trunk. For each EC category there were 15 emergence traps. Traps were positioned earlier than the previous season commencing 3rd November 2006 and monitored fortnightly until 28th March 2007. Phylloxera abundance on lignified roots of 'trap' vines was also assessed on 20th February 2007.

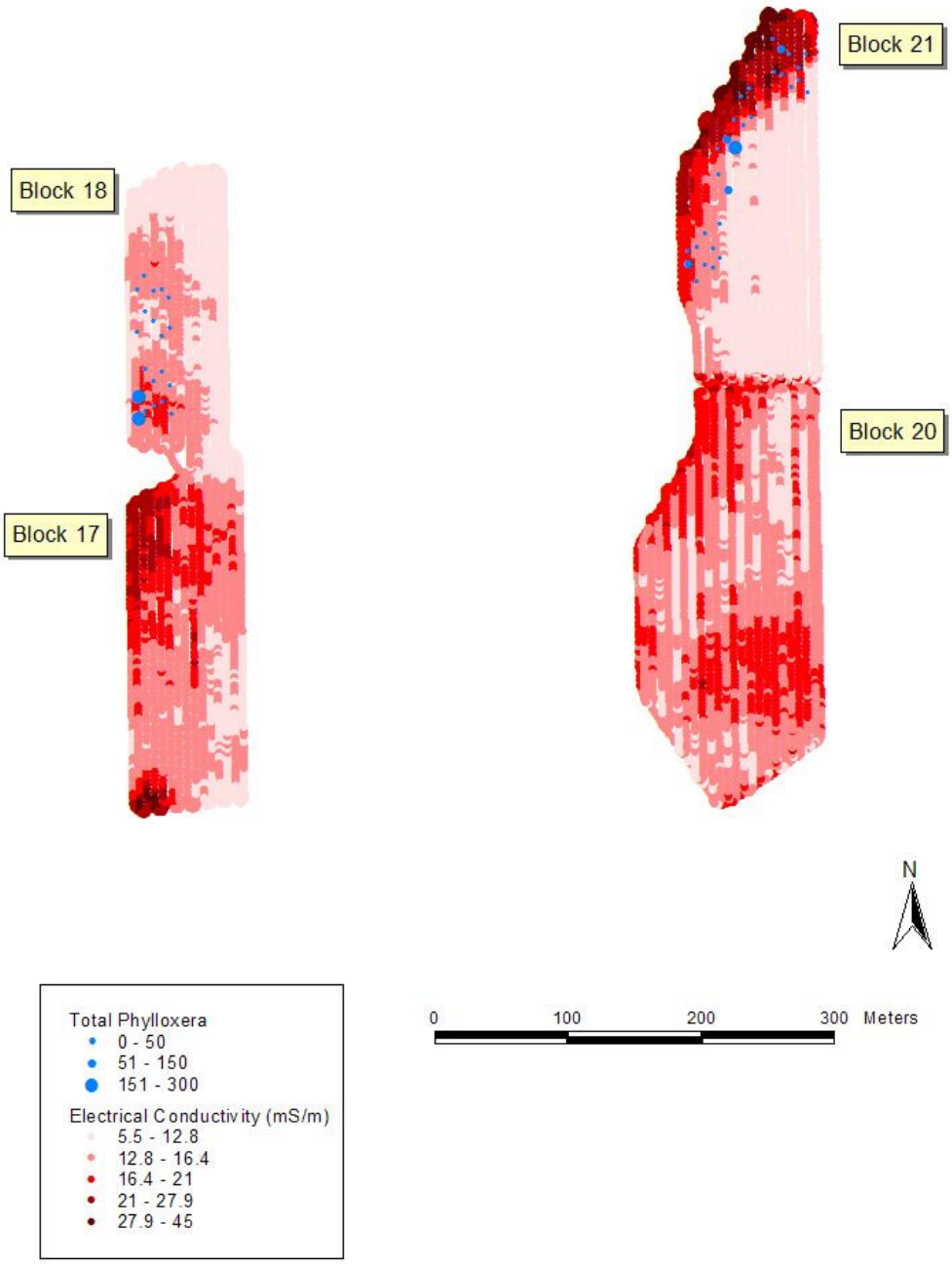


Fig. 2. EM38 imagery, collected 14th December 2005, showing relative total abundance of phylloxera in traps located in blocks 18 and 21 for the 2005-2006 season at the Upton site.

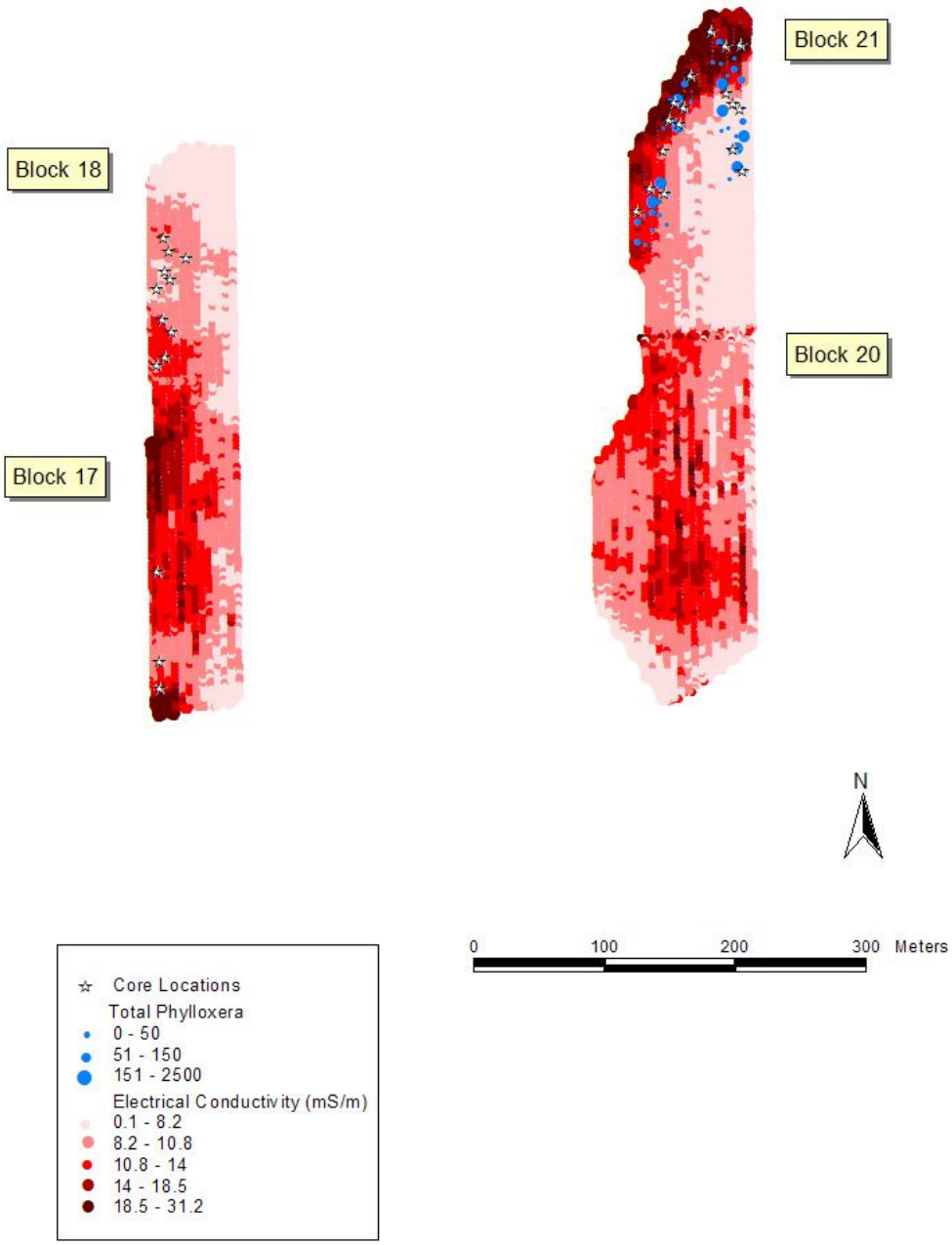


Fig. 3. EM38 images, collected August 2006 showing relative total abundance of phylloxera in the traps for the 2006-2007 season and location of soil core sampling points at the Upton site.

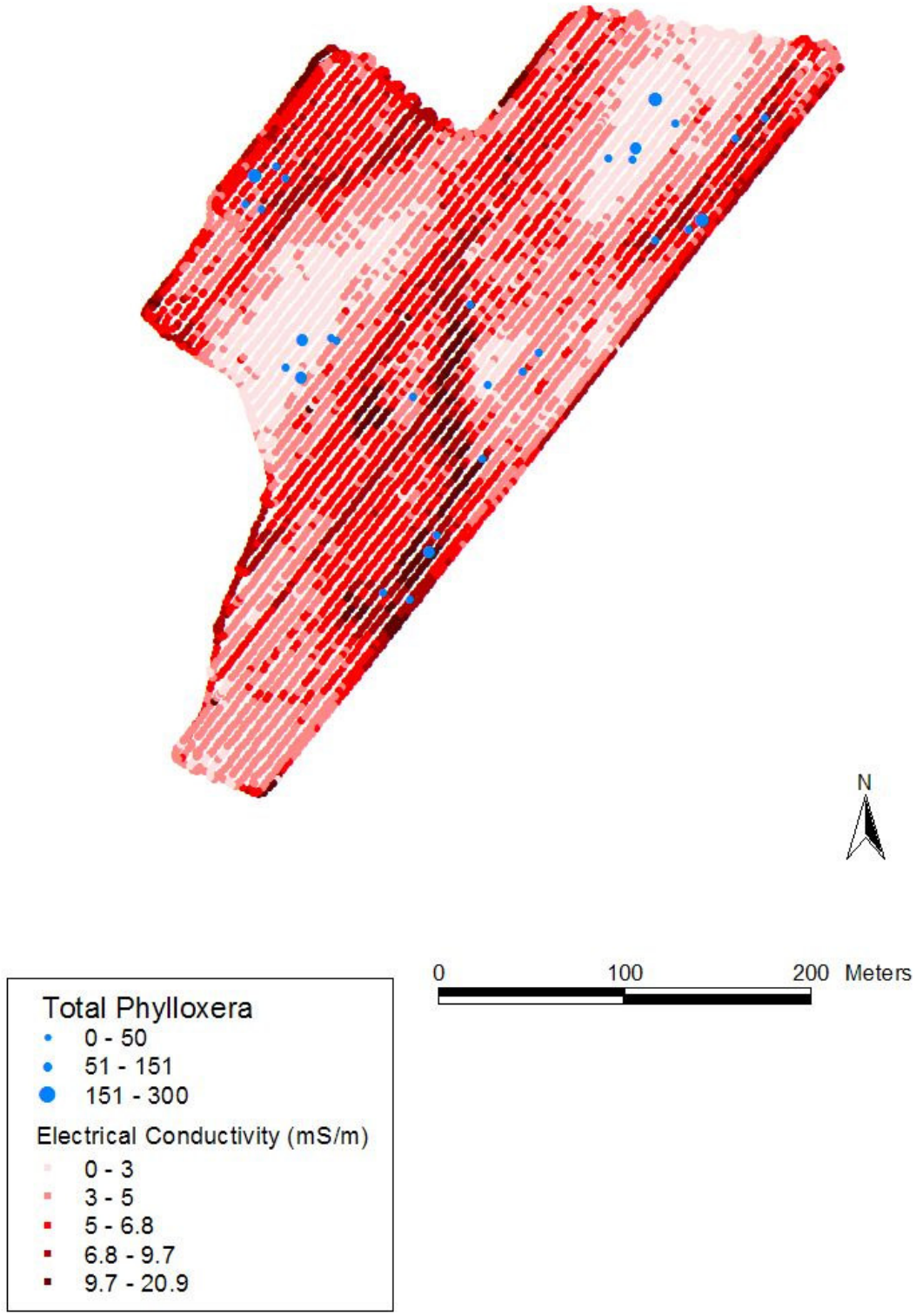


Fig. 4. EM38 images, collected 15th December 2005 showing relative total abundance of phylloxera in traps for the 2005-2006 season at the King Valley site.

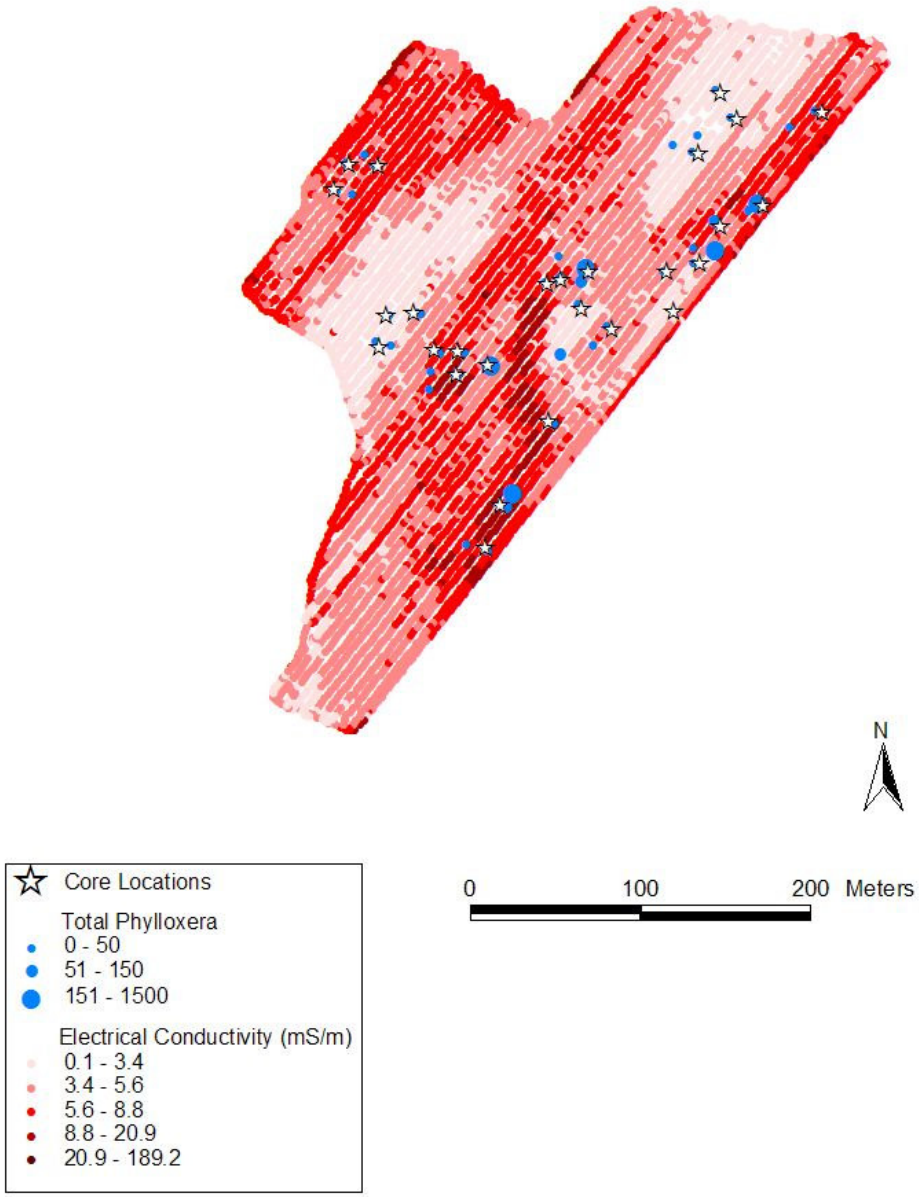


Fig. 5. EM38 images, collected August 2006, showing location of soil cores sampling points and relative total abundance of phylloxera in emergence traps for the 2006-2007 season at the King Valley site.

Supplementary study site 3 – Murchison

Emergence traps were randomly positioned predominantly in low EC (0-9mS/m) regions in alternate rows in each of two blocks A and B with two traps per row (Fig. 6). The two blocks were positioned either side of a region of relatively high EC values which had been replanted onto rootstocks, following phylloxera detection at the site in July 2006. Total trap number was ten per block. Traps were positioned at the site on 3rd November 2006 and monitored fortnightly until 5th April 2007.

Supplementary study site 4 – Yarra Valley

Emergence traps were positioned in every third row in 11 rows within the infested block. Traps were positioned at every tenth vine with seven traps per row (Fig. 7). Traps were positioned at the site on 21st December 2006 and monitored fortnightly until 19th April 2007.

Supplementary study site 5 – Buckland Valley

In each of five parallel rows there were five 'monitor vines', therefore a total of 25 infested vines, in parallel panels, selected to monitor above-ground phylloxera dispersal and phylloxera population dynamics (Fig. 8). Traps were positioned at the site on 20th October 2004 and monitored fortnightly until 9th May 2005 (using data sourced from Powell *et al*., 2006).

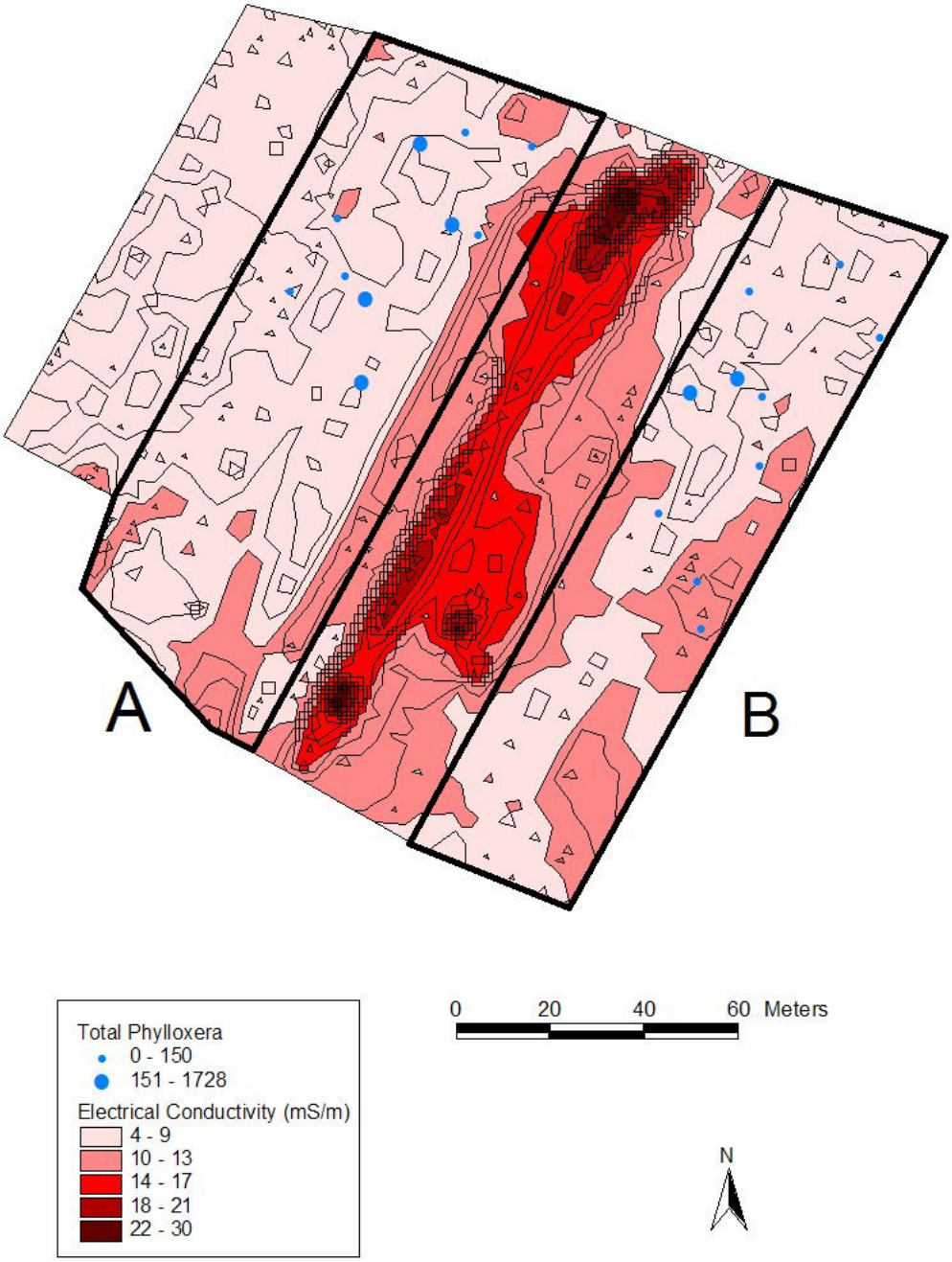


Fig. 6. EM38 imagery, collected May 2007 showing relative total abundance of phylloxera in traps for the 2006-2007 season and location of trial blocks A and B at the Murchison site.

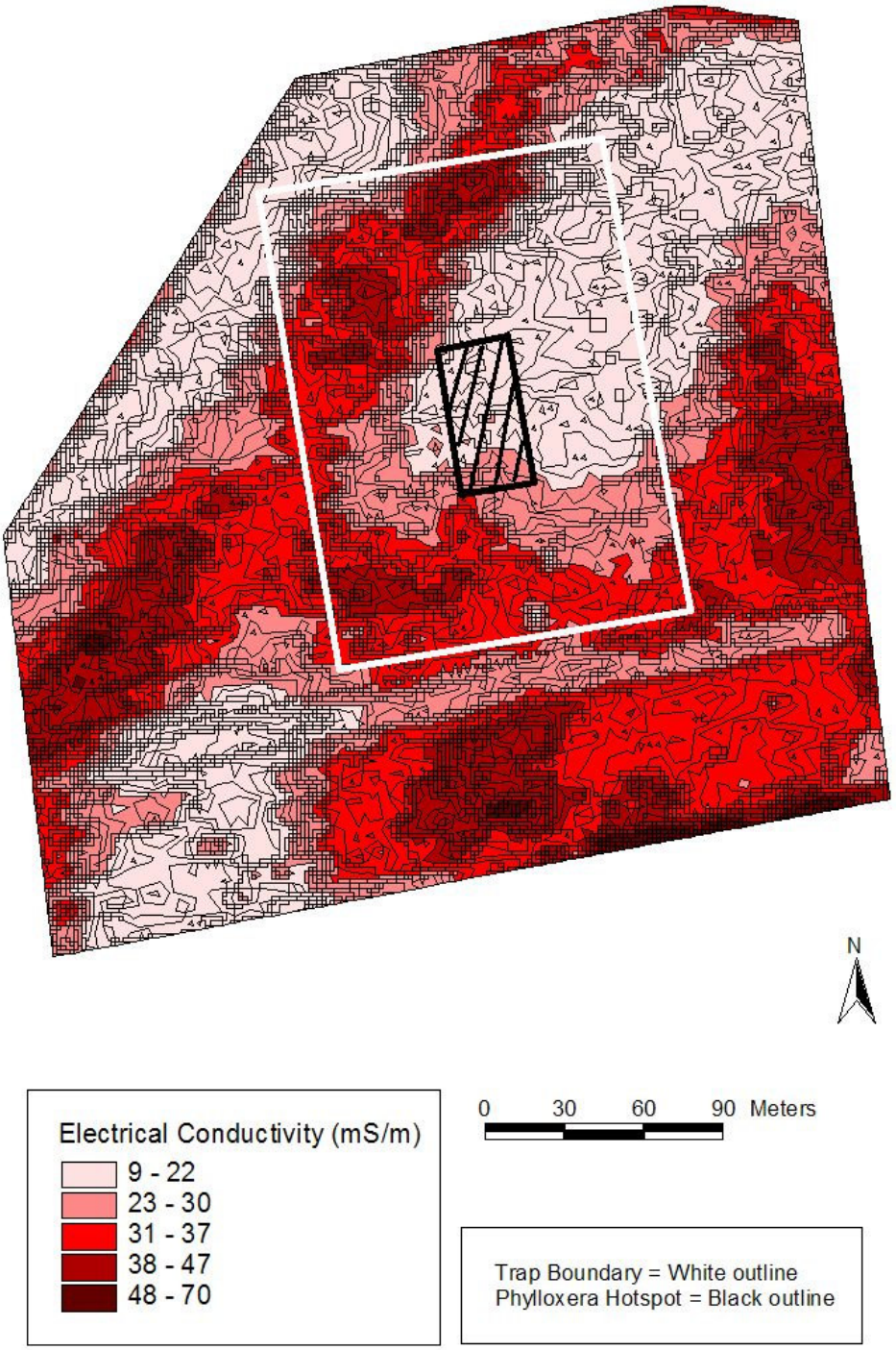


Fig. 7. EM38 images, collected May 2007 showing location of emergence trapping boundary and phylloxera 'hot spot' region at the Yarra Valley site.

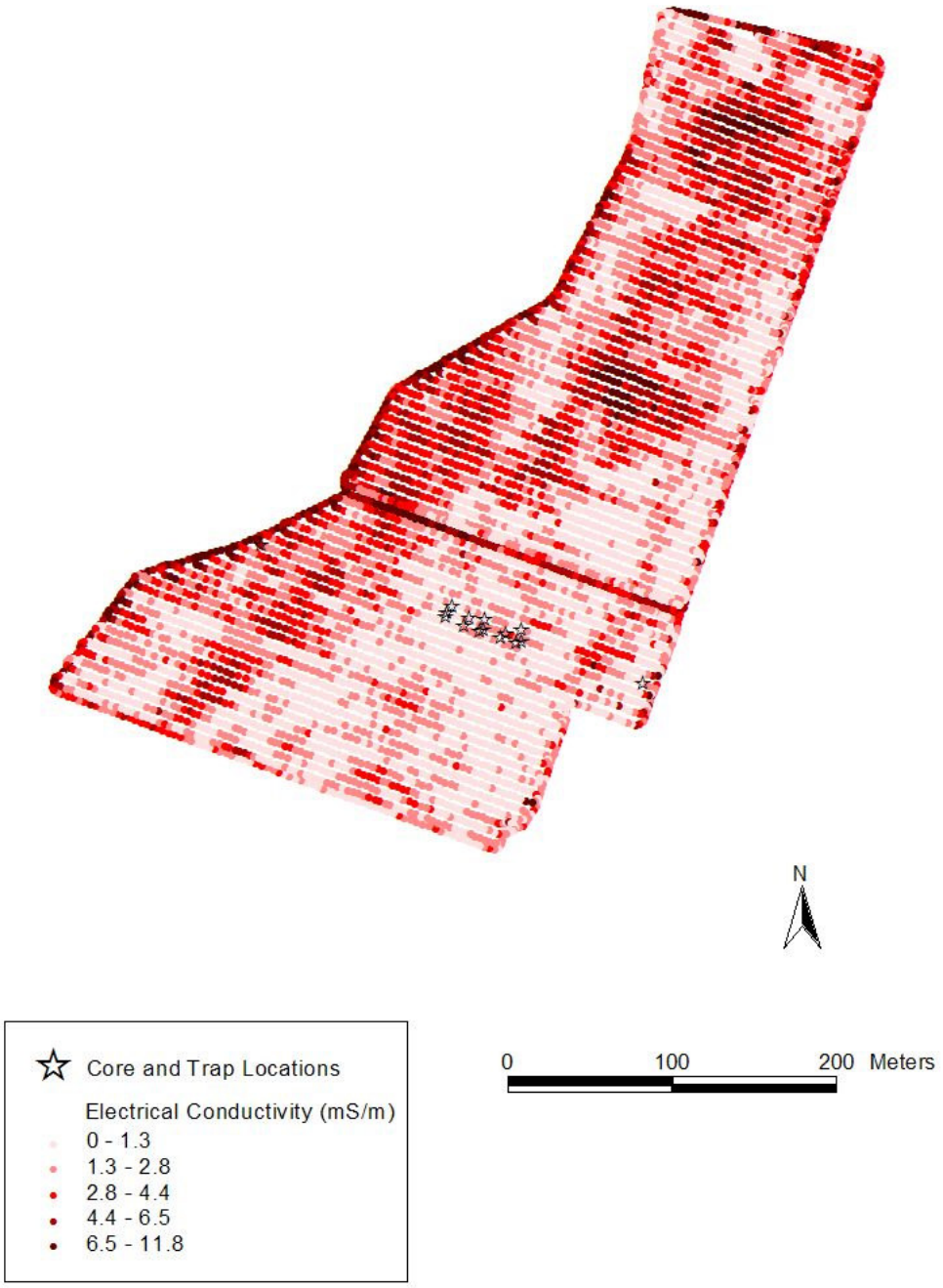


Fig. 8. EM38, images collected December 2005 showing location of 2004-2005 emergence traps and 2006 soil core sampling points at the Buckland Valley site.

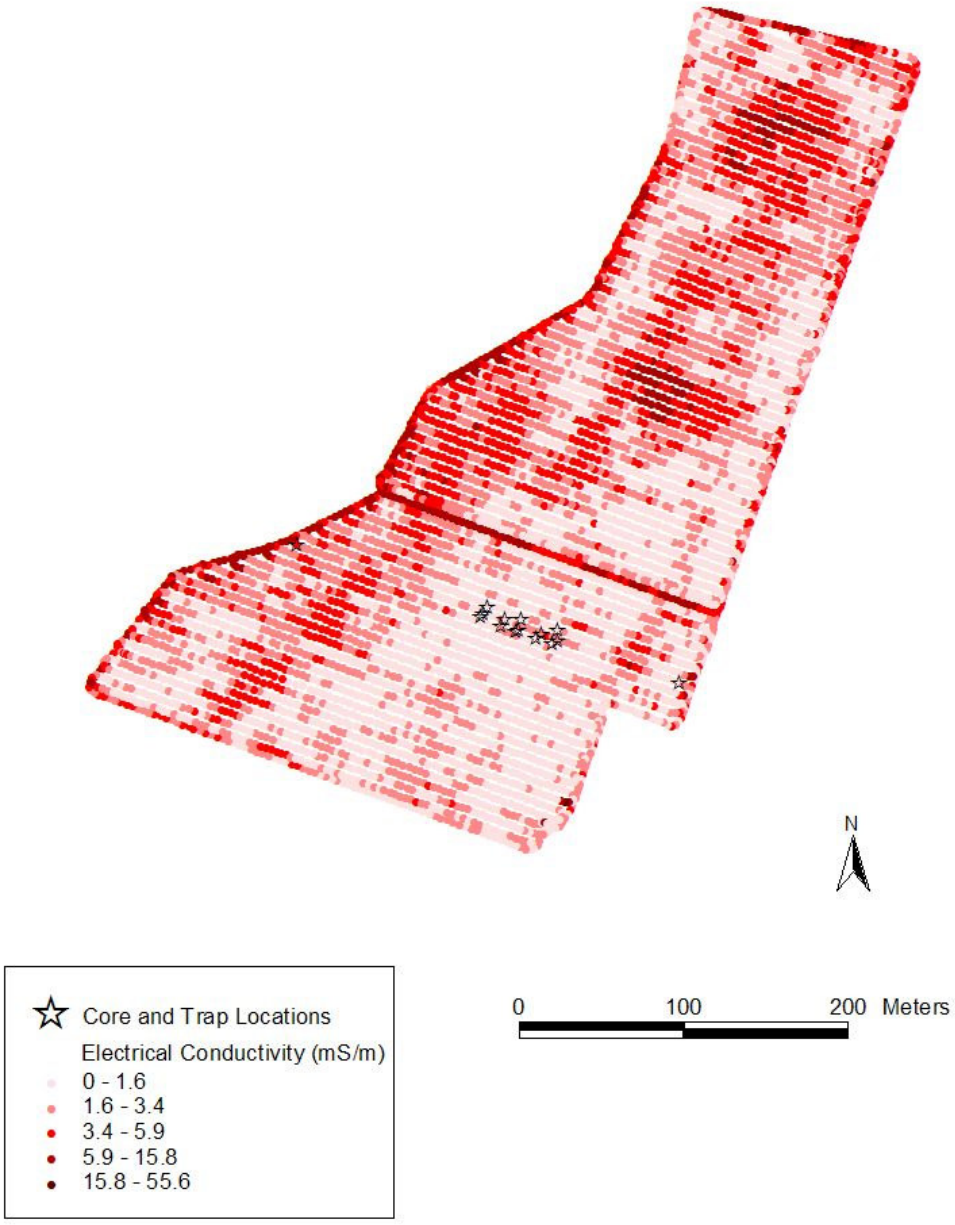


Fig. 9. EM38 images, collected August 2006, showing location of emergence traps and location of soil core sampling points at the Buckland Valley site.

Root sampling

At all phylloxera emergence trap locations from the two primary sites monitored, root samples were taken on the opposite side to the trap, thereby avoiding disturbance to the root system directly below the trap, to assess phylloxera populations on roots. Collection was in February 2007, timed to coincide with relatively high phylloxera abundance as determined by previous studies (Powell *et al*., 2003a). Three root pieces, approximately 10cm in length and 2-4cm diameter, were collected from different roots to a depth of between 10-30cm. The samples were placed in sealed plastic bags and transported in a sealed, ice-filled, insulated container under quarantine conditions to the DPI-Rutherglen quarantine laboratory. In the laboratory root pieces were washed and gently brushed with a soft toothbrush into a 53µm aperture sieve to collect phylloxera. Samples were then examined under binocular microscope, and the number of each phylloxera life-stage was recorded. Categories used were eggs, crawlers, intermediate instars, wingless adults and winged adults. The roots were oven-dried at 50°C for 48 hours and weighed to determine dry weight.

Statistical analysis

The raw population data for individual emergence traps showed a high degree of variability, which was not evident in the means. Numbers ranged from 0 to 3053 phylloxera per trap, and there were a substantial number of data points with zero phylloxera, indicating a highly skewed distribution. Conventional analysis of variance (ANOVA) relies on the assumption of normality, which in this case was unsatisfactory. Consequently, a more conservative, nonparametric approach was adopted. The method used was Kruskal-Wallis one-way ANOVA (Payne *et al*., 2005). The null hypothesis states that the groups are equally distributed (equal population means). The alternative hypothesis states that the group is not equally distributed (at least one group mean is not equal). The test statistic is:

$$H = \frac{12}{n(n+1)} \sum_{i=1}^{k} \frac{R_i^2}{n_i} - 3(n+1)$$

Where n_i represents the sample size for each of the k groups in the data. The combined sample is ranked (R_i = the sum of ranks for group i). This statistic approximates a chi-square distribution with k-1 degrees of freedom if the null hypothesis of equal population is true. The null hypothesis of equal population means is rejected if the test statistic H is greater than the 2 (α, k-1) where α is typically set to 0.05.

RESULTS AND CONCLUSIONS

Phylloxera Emergence – Primary site 1 (Upton)

Total and mean phylloxera emergence numbers for Block 18 at the Upton field site in 2005-6, are shown in Figures 2 and 10 respectively. Phylloxera levels were low in this block overall, with only 10% of traps having total insect number within the 151-300 range (Fig. 2). No phylloxera was detected in traps in the medium EC category and levels in the low EC category were low, reaching a maximum mean of 25 phylloxera per trap in early March 2006 (Fig. 10), with individual trap numbers ranging from 0 to 155 phylloxera. Monitoring in this particular block was therefore not continued for a second season.

In Block 21 in 2005-6 phylloxera emergence levels were again low (Figs. 2 and 11) and the low EC category peaked at 25 phylloxera per trap in early March and 35 phylloxera per trap in early February 2006 (Fig. 11) for the medium EC category with individual trap numbers ranging from 0 to 76 phylloxera.

In the following season monitoring commenced earlier and was restricted to Block 21 (Fig. 3) and total phylloxera abundance was relatively high compared with the previous season (Fig. 2) reaching up to 2500 in some traps. In the 2006-07 season a strong peak in the Low EC category was observed in early January 2007 (Fig. 12) reaching a mean maximum of 145 phylloxera per trap with a smaller secondary peak in late March.

Kruskal-Wallis data analysis (Table 1) indicates that for all the sample dates in 2006-07, with the exception of 8th February 2006 the emergence levels in traps located in the low EC category consistently rank the highest. This can be viewed as a broad trend, however, as an examination of the P values indicates no significance at P=0.05. Using a less rigorous P=0.1, which is probably appropriate here given the nature of the experiment, phylloxera emergence levels in the low EC category on 9th January

and 22nd March 2007 were significantly higher than both the high EC and medium EC categories, with P values of 0.06 and 0.08 respectively (Table 1).

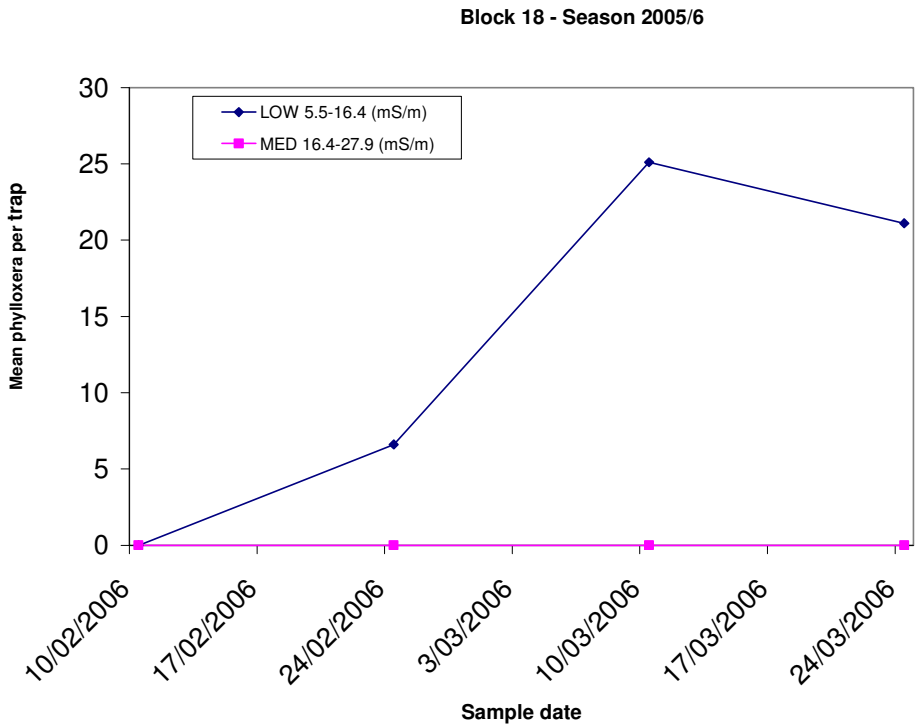


Fig. 10. Phylloxera dispersive stages collected in emergence traps positioned at the trunk base of *V. vinifera* cv. Pinot Gris grapevines (Block 18), at the Upton field site, over the 2005-2006 growing season, n = 20 traps. Traps were located in low (5.5-16.4mS/m) and medium (16.4-27.9mS/m) EC categories as determined by EM38 surveys conducted in December 2005.

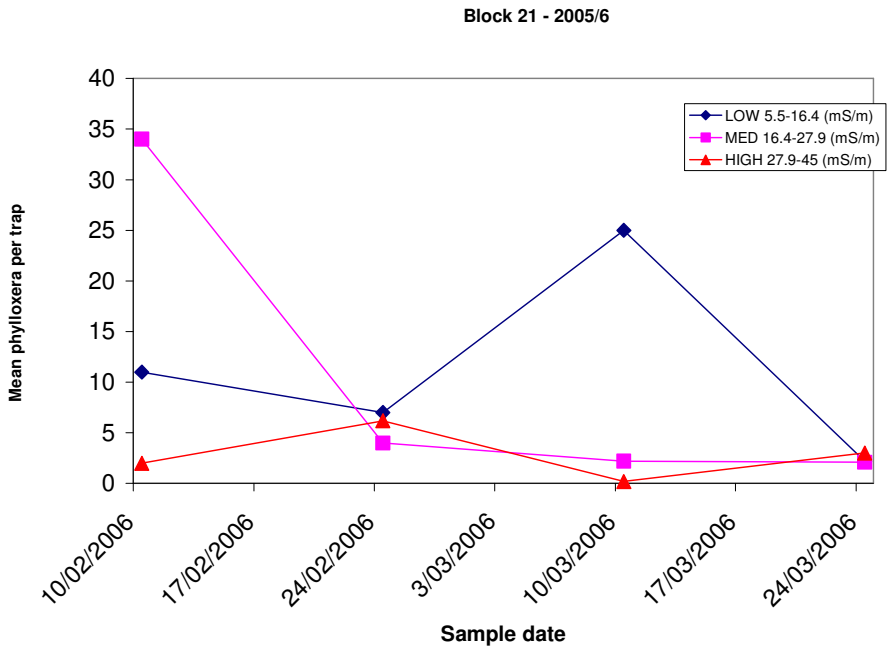


Fig. 11. Phylloxera collected in emergence traps positioned at the trunk base of *V. vinifera* cv. Cabernet Sauvignon grapevines (Block 21), at the Upton field site, over the 2005-2006 growing season, where n = 30 traps. Traps were located in low (5.5-16.4mS/m), medium (16.4-27.9mS/m) or high (27.9-45mS/m) EC category zones as determined by EM38 surveys conducted in December 2005.

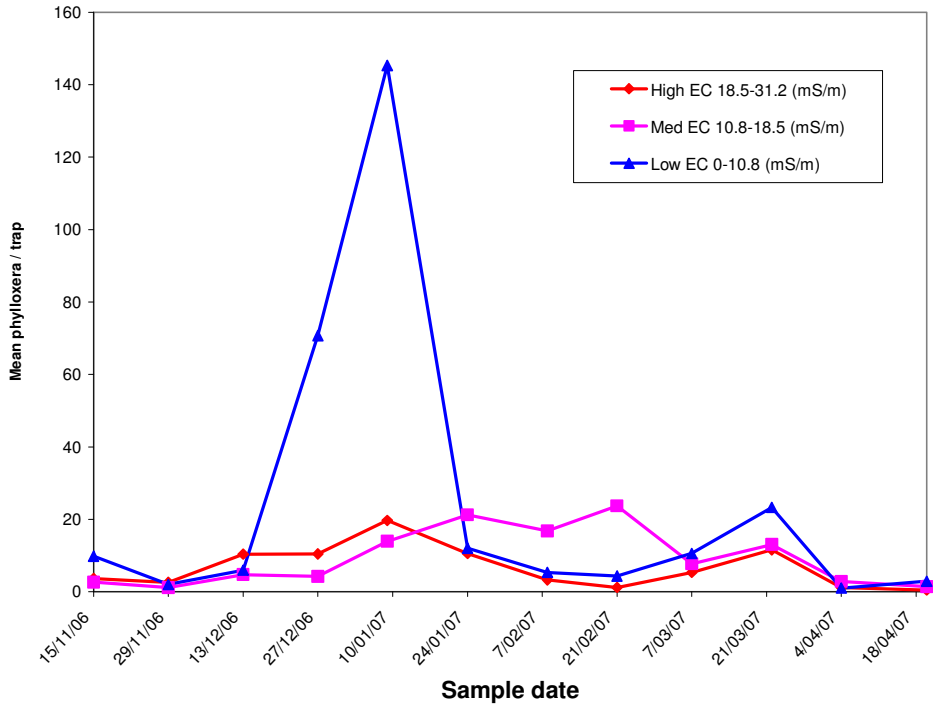


Fig. 12. Phylloxera collected in emergence traps positioned at the trunk base of *V. vinifera* cv. Cabernet Sauvignon grapevines (Block 21), at the Upton field site, over the 2006-2007 growing season, where n = 50 traps. Traps were located in low (0-10.8mS/m), medium (10.8-18.5mS/m) or high (18.5-31.2mS/m) EC categories as determined by EM38 surveys conducted in August 2006.

Table 1. Summary of the Kruskal-Wallis one-way ANOVA for EC category and total phylloxera abundance for Upton site (Block 21) in the 2006-07 season.

Sample date	High EC	Medium EC	Low EC	H	H*	Df	P value[1]
15/11/2006	23.70	22.93	30.47	2.51	3.41	2	0.18
29/11/2006	25.00	23.07	28.60	1.12	1.58	2	0.45
13/12/2006	25.25	22.73	28.60	1.23	1.49	2	0.48
27/12/2006	24.52	21.6	30.70	3.07	3.41	2	0.18
09/01/2007	23.57	21.00	32.57	5.30	5.52	2	0.06*
24/01/2007	23.75	25.93	27.40	0.56	0.59	2	0.75
08/02/2007	23.48	30.37	23.33	2.39	2.84	2	0.24
21/02/2007	23.23	23.87	30.17	2.21	3.00	2	0.22
07/03/2007	21.93	26.97	28.80	2.12	2.48	2	0.29
22/03/2007	20.20	27.87	30.20	4.60	5.12	2	0.08*
04/04/2007	22.27	27.07	28.23	1.68	2.68	2	0.26
20/04/2007	22.05	27.10	28.50	1.94	2.56	2	0.28

H = Test statistic, H* = Test statistic adjusted for ties, [1] Chi-squared probability

Phylloxera emergence - Primary site 2 (King Valley)

Total and mean phylloxera emergence numbers for the King Valley site over the 2005-2006 growing season was recorded. Overall total abundance was relatively low reaching a maximum total of up to 300 over the entire growing season for some traps (Fig. 4). A peak in phylloxera emergence was observed on 6th March 2006 for all EC categories (Fig. 13) with the highest mean phylloxera abundance on the low EC category region and individual phylloxera number per trap ranging from 0 to 177 phylloxera. The low EC category had the highest rank in all four sample dates. On the 6th February (1st sample date) there was a significant ($P<0.05$) difference between the EC categories in terms of phylloxera number (Table 2).

The total phylloxera abundance was much higher in the 2006-2007 growing season with 5-fold higher total phylloxera number in some traps reaching up to 1500 insects (Fig. 5). The peak in phylloxera emergence was observed much later in this season (Fig. 14). The earlier phylloxera emergence peak in December 2006 was particularly evident in the medium EC category regions (Fig. 14) and may have been induced by a change in grapevine management practices. The vineyard manager excised the

grapevine trunks with a chainsaw, which may have influenced the phylloxera dispersive stages to emerge earlier and disperse away from the root system.

To observe a peak in phylloxera emergence so early in the season is unusual when compared with earlier studies (Powell *et al*., 2000; 2003a) which would indicate the emergence levels were indeed influenced by a change in the root physiology as a consequence of trunk excision. The subsequent sample collected on 3rd January 2007 had reasonable phylloxera numbers, but in later samples populations were extremely low.

Individual trap numbers were highly variable prior to the post vine excision decline in numbers in January 2007, ranging between 0 and 1438 phylloxera per trap. The Kruskal-Wallis rankings for 2006-07 show no consistent pattern overall, but noticeably in the low EC category had the highest rank initially, but subsequently became the lowest rank (Table 3).

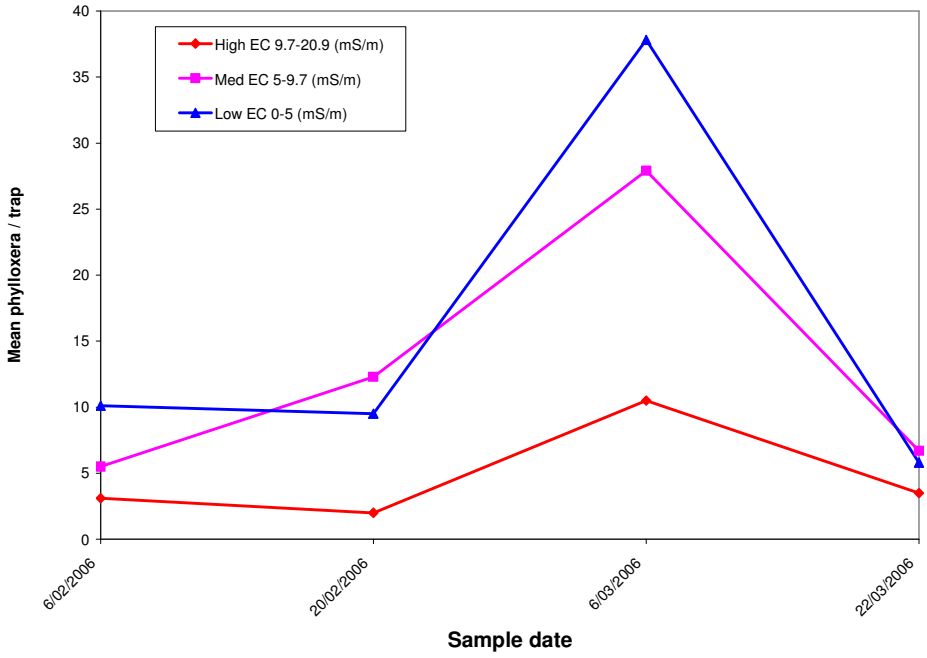


Fig. 13. Phylloxera collected in emergence traps positioned at the trunk base of *V. vinifera* cv. Chardonnay and cv. Pinot Noir grapevines, at the King Valley field site, over the 2005-2006 growing season, where n = 30 traps. Traps were located in low (0-5 mS/m), medium (5-9.7 mS/m) or high (9.7-20.9 mS/m) EC categories as determined by EM38 surveys conducted in December 2005.

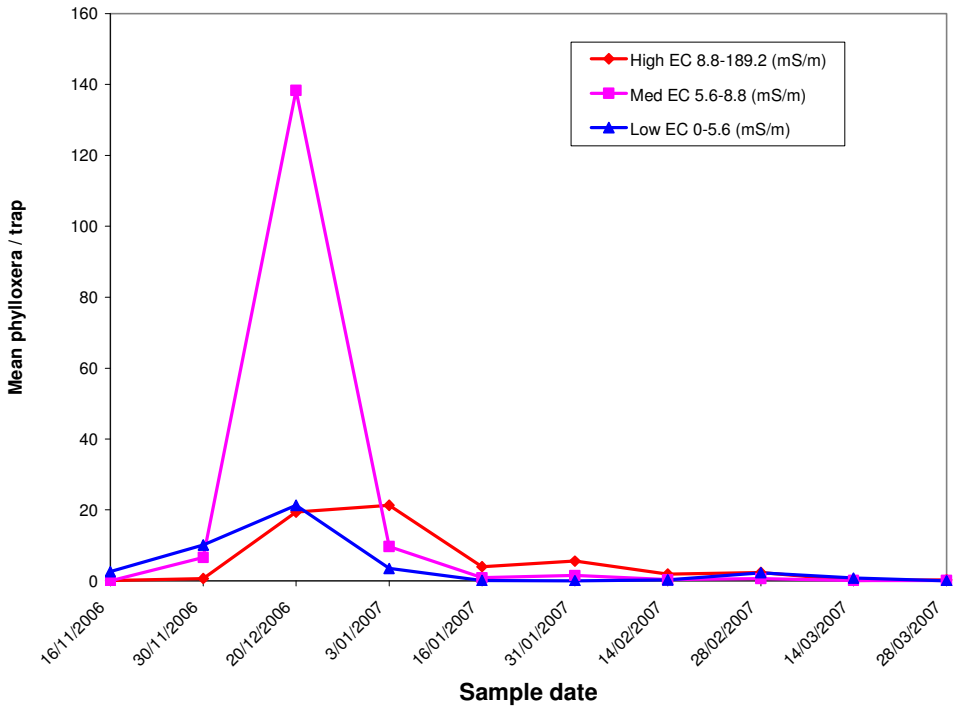


Fig. 14. Phylloxera collected in emergence traps positioned at the trunk base of *V. vinifera* cv. Chardonnay and Pinot Noir grapevines, at the King Valley field site, over the 2006-2007 growing season, where n = 30 traps. Traps were located in low (0-5.6 mS/m), medium (5.6-8.8 mS/m) or high (8.8-189.2 mS/m) EC categories as determined by EM38 surveys conducted in August 2006.

Table 2. Summary of the Kruskal-Wallis one-way ANOVA for EC category and total phylloxera abundance for the King Valley site in the 2005-06 season.

Date	High EC	Medium EC	Low EC	H	H*	df	P value[1]
06/02/2006	10.20	15.65	20.65	7.05	7.54	2	0.023*
20/02/2006	14.15	14.80	17.55	0.84	0.96	2	0.618
06/03/2006	16.10	12.25	18.15	2.32	0.24	2	0.306
22/03/2006	15.00	13.80	17.70	1.03	1.08	2	0.583

H = Test Statistic, H* = Test Statistic adjusted for ties, [1] Chi-squared probability

Table 3. Summary of the Kruskal-Wallis one-way ANOVA for EC category and total phylloxera abundance for the King Valley site in the 2006-07 season.

Date	High EC	Medium EC	Low EC	H	H*	df	P value[1]
16/11/2006	22.00	20.50	26.50	1.70	5.70	2	0.058*
30/11/2006	21.60	25.03	22.37	0.57	0.77	2	0.679
20/12/2006	23.03	25.07	20.90	0.76	0.78	2	0.677
03/01/2007	26.33	24.10	18.57	2.78	2.89	2	0.236
16/01/2007	27.03	24.07	17.90	3.78	5.38	2	0.068*
31/01/2007	26.83	23.40	18.77	2.85	5.02	2	0.081*
14/02/2007	27.23	22.50	19.27	2.79	4.18	2	0.124
28/02/2007	26.67	21.73	20.60	1.81	3.19	2	0.203
14/03/2007	22.53	23.87	22.60	0.10	0.40	2	0.818
28/03/2007	25.50	22.50	21.00	0.91	2.32	2	0.314

H = Test statistic, H* = Test statistic adjusted for ties, [1] Chi-squared probability

Phylloxera emergence – Supplementary site 3 (Murchison)

In the 2006-07 season phylloxera emergence at the Murchison site showed a trimodal peak (Fig. 15) with the primary peak occurring in early February 2007 and two smaller peaks in late December 2006 and early March 2007. Phylloxera emergence levels were relatively low, compared to other observations of G1 field populations at other sites in the Nagambie PIZ (Powell *et al.*, 2006), with an average of 57 phylloxera per trap in early February and individual trap numbers ranging from 0 to 607 phylloxera.

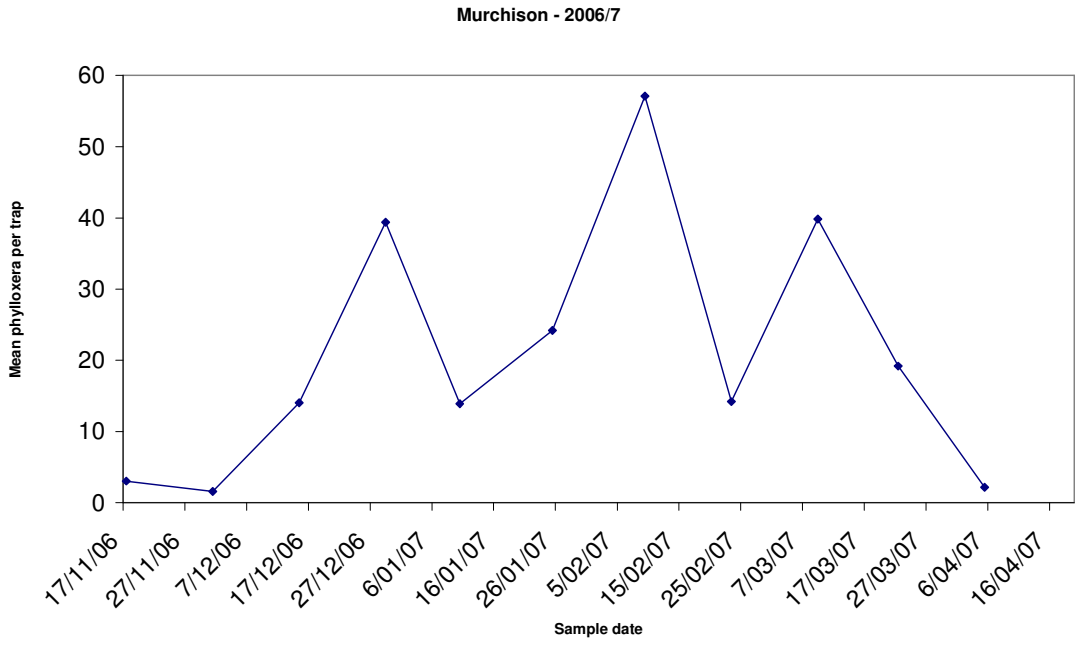


Fig. 15. Phylloxera collected in emergence traps at the base of *V. vinifera* cv. Semillon and cv. Riesling grapevines, at the Murchison field site, over the 2006-2007 growing season, where n = 20 vines.

Phylloxera emergence – Supplementary site 4 (Yarra Valley)

In the 2006-07 season phylloxera emergence at the Yarra Valley site showed a bimodal peak (Fig. 16) with the primary peak occurring in late January 2007 and a smaller peak in early March. Phylloxera emergence levels were relatively high, compared to other observations on G1 observed at Upton and Murchison during the same season with an average of 250 phylloxera per trap in late January and individual trap numbers ranging from 0 to 3053 phylloxera. Even though herbicide was applied to the site in March and grapevine trunks were cut, phylloxera was still emerging above-ground in mid-April.

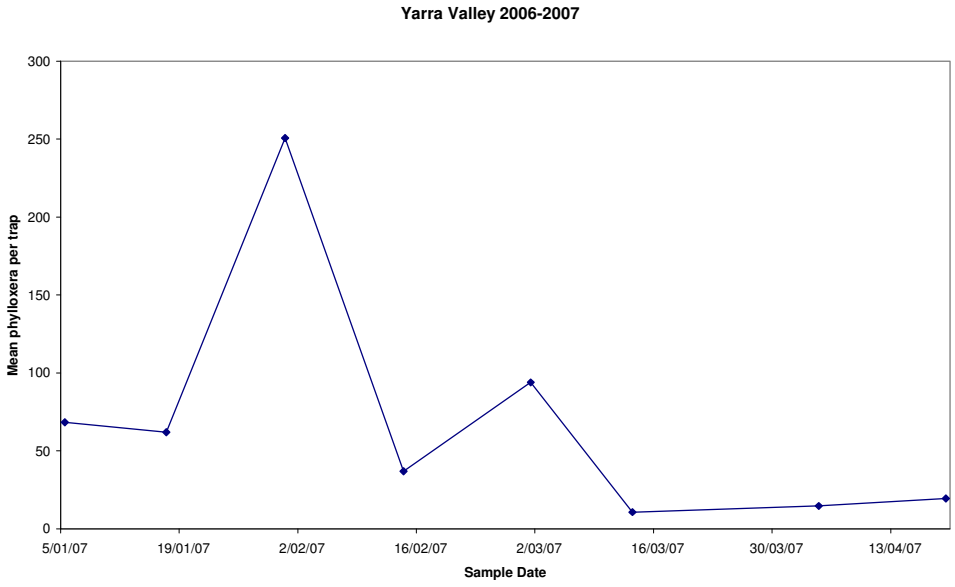


Fig. 16. Phylloxera collected in emergence traps at the base of *V. vinifera* cv. Cabernet Sauvignon grapevines, at the Yarra Valley field site, over the 2006-2007 growing season, where n = 40 vines.

Phylloxera emergence – Supplementary site 5 (Buckland Valley)

During the 2004-2005 season (Fig. 17), phylloxera were first detected in emergence traps in mid-December, peaked in abundance in mid-January with a mean of over 100 phylloxera per trap and were still detectable in late April. At harvest (March) although still present above-ground mean phylloxera numbers were relatively low (<20 phylloxera per trap) some individual vines still had over 100 phylloxera per trap during harvest.

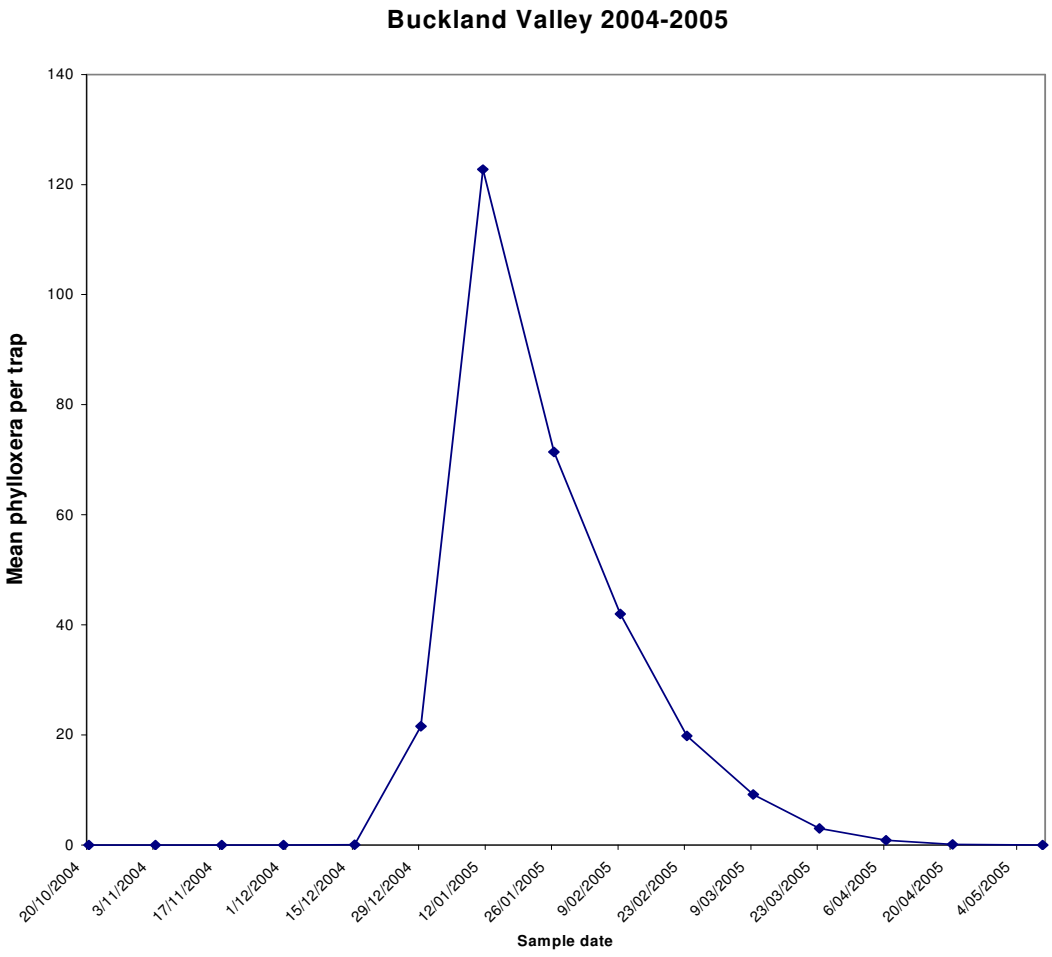


Fig. 17. Phylloxera collected in emergence traps at the base of *V. vinifera* cv. Pinot Noir grapevines, at the Buckland Valley field site, over the 2004-2005 growing season, where n = 25 traps.

Root samples

Root sample phylloxera data collected in February 2007 were compared with the emergence trap data for the Upton (R^2 = 0.23) and King Valley (R^2 = 0.38) site. The best correlation was seen for the King Valley site. In general, it is assumed that vines with higher root populations would have higher numbers of emergent phylloxera, due to population pressure. However, this was not evident in the data. The root samples were taken from the opposite side of the vine to the emergence trap which may have been a factor, and they only represent a single sample point compared with 10-12 for

the traps. Therefore the trap data is considered more reliable and consequently may prove a more reliable indicator for phylloxera detection. When mean phylloxera numbers from the root samples are considered for the three 3 EC categories for Upton and the King Valley a clearer picture emerged. The Low EC and Medium EC categories from Upton and King Valley show higher root populations, which mirrored the emergence trap data in Figures 3 and 5.

A significant feature of the phylloxera from the King Valley root samples was the high percentage of phylloxera with a blackened appearance (visually estimated at 60-70%) which is indicative of premature death prior to collection of the root samples. Presumably the vastly reduced capacity of the vines following the removal of vine trunks by chainsaw resulted in a reduced ability to support phylloxera populations. The rapid increase in numbers in the emergence traps immediately after the vines were cut, followed by a big decline in phylloxera numbers as described above, supports this contention. The rapid increase in emergent crawlers following the cutting of the vines has implication for phylloxera management, indicating that cutting the vines trunks may initially hasten the spread of phylloxera but reduces the viable population remaining on the root system at least in the short-term. However, fresh shoots appeared on some of the vine stumps in the weeks following excision, particularly near the King River boundary of the vineyard, where moisture content of the soil was higher, but very dry soil conditions due to drought mitigated against fresh shoot growth in most vines away from the river boundary.

CONCLUSIONS AND RECOMMENDATIONS

Post-monitoring of phylloxera emergence has shown that population abundance differs between sites and seasons. At the two primary sites we were able to assess sets of seasonal data and compare population dynamics of the two major, in terms of distribution and relative virulence, phylloxera genotypes present in Australia. At the Upton site, where phylloxera genotype G1 is present, in the 2005-2006 season phylloxera levels were relatively low, reaching only up to a maximum total of 300 insects per trap. Higher phylloxera levels were primarily recorded in the lower EC ranges (5.5-16.4 (mS/m)) within the infested blocks. In the following season levels overall were much higher reaching a maximum of 2500 per trap; with most detected in the low and medium EC value range. When phylloxera was first detected in this

vineyard in April 2000 it was discovered in the area now mapped as a High EC value range and its is likely that dispersal of the insect during the 5 years has resulted in a decline in populations in the high EC range due to depleted nutrient supply from damaged root systems. The increase in phylloxera abundance during 2006-2007 season is likely to be due to climate variation (i.e. drought conditions) as this has been observed at the same site in a previous study (Powell *et al*., 2003).

At the King Valley site where the phylloxera genotype G4 is present relatively low populations were recorded in the 2005-2006 season phylloxera levels reaching only up to a maximum total of 300 insects per trap and again higher phylloxera levels were primarily recorded in the lower (0-5mS/m)) and medium (5-9.7mS/m) EC ranges. In the following season levels overall were much higher reaching a maximum of 1500 per trap; with most detected in the low and medium EC value range. When phylloxera was first detected in this vineyard in 1998 it was discovered in the area now mapped as a High EC value range and it is likely that dispersal of the insect during the 7-9 years has resulted in a decline in populations in the high EC range due to depleted nutrient supply from damaged root systems.

At some of the sites where phylloxera was only monitored for a single season the relationship is less clear. At the Murchison site phylloxera (G1) was first detected in the higher EC range region of the infested block but had spread, after vine removal, outwards into low EC areas but population levels were fairly low. Whereas at the Yarra Valley field site the phylloxera population (G1) were relatively abundant reaching a maximum in January on one vine of 3053 crawlers and the highest numbers of phylloxera were evident in the lower EC range. At the Buckland Valley site where a low virulence genotype G20 is present phylloxera had not spread far enough to determine if EC had any relationship with dispersal or establishment, and vines were replanted to rootstocks in the season following detection.

Overall these results do indicate that phylloxera may prefer high EC levels and disperse away from these regions at varying rates dependening on the length of time since infestation, phylloxera genotype virulence level and soil characteristics (as discussed in Chapters 4 and 5). Therefore high EC areas could be targeted as potential ‘hot spots’ when surveying. It is important however, to have the opportunity to monitor new incursions for at least two seasons post-detection as variability in population abundance is clearly apparent.

CHAPTER THREE

Remote Sensing of Phylloxera-infested Vineyards

Objective: Validation and utilisation of spectral imagery to improve targeted detection and monitoring of grapevine phylloxera

SUMMARY

Remotely sensed aerial imagery was obtained for three phylloxera-infested field sites. This data along with soil sensing data, obtained using EM38 sensors, was utilised to assist in placement of traps to monitor phylloxera abundance and dispersal. Effectiveness of aerial imagery as a tool to improve targeted detection varied between sites and no consistent relationships were evident.

INTRODUCTION

Reflectance data imagery was obtained from a number of sources, to ascertain its effectiveness for targeted early detection of phylloxera and to allow comparison with phylloxera abundance data as determined by root surveys and trap data (Chapter 2) and to provide an additional data layer to compare with soil sensing imagery (Chapter 1) and soil characterisation data (Chapter 4). Aerial remotely-sensed data was acquired by aircraft for the Phylloxera and Grape Industry Board of South Australia and the Victorian Department of Primary Industries and grounded-based imagery collected using a 'Greenseeker' sensor provided by Wine Network Consulting. The parameters used for the images are essentially measures of the vegetation density, and hence the vigour of the vines. Phylloxera associated damage to ungrafted *Vitis vinifera* grapevines can result in markedly reduced vine vigour which varies according to the level of virulence of the phylloxera genotype present on the root system. Weak spots evident on the images are obvious sites to check for phylloxera infestation.

METHODS

Remotely sensed aerial imagery

Remote imagery was obtained for phylloxera-infested grapevine blocks in three vineyards, located at Upton (Primary study site 1), King Valley (Primary study site 2) and Buckland Valley (Supplementary study site 5), referred to elsewhere in this report Chapters 1, 2 and 4). All data was collected in February 2005 unless specified differently in the text.

Four types of images were produced for each of the study sites:

1) True-colour image: information from the blue, green and red wavebands is projected into blue, red and green of the screen or printer, generating an image similar to a colour photograph (Proffitt *et al*., 2006).

2) False-colour image: green, red and near infrared wavebands are projected into the blue, green and red of the screen/printer (Proffitt *et al*., 2006). Regions of greater photosynthetically active biomass strongly reflect near infrared radiation, which is highlighted in this type of image.

3) Normalised Difference Vegetation Index (NDVI): the index quantifies the relative difference between the near infrared reflectance peak and the red reflectance trough from the spectral reflectance profile (Proffitt *et al*., 2006) and is calculated from the formula:

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

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

4) Plant Cell Density (PCD): is similar to the NDVI and is calculated from the formula:

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

Values are high for areas of high photosynthetically active biomass (Proffitt *et al*., 2006).

Additional historical data was available from the King Valley site from imagery collected in March 1995 by the Victorian Department Primary Industries (formerly Department of Natural Resources and Environment).

Remotely sensed ground-based imagery

The side-imaging 'Greenseeker' reflectance sensor (N Tech Inc., USA), was trialled at the Upton site on the 27th February 2007 to allow comparison with NDVI imagery collected using airborne sensors. The 'Greenseeker' system was mounted on an ATV, and alternate rows were measured from both sides of the canopy. The system measures NVDI (Proffitt *et al*., 2006). Light-emitting diodes emit infrared and red wavelengths, and the reflected signal is recorded by a pair of photo-detectors.

RESULTS

Primary study site 1 – Upton

Imagery obtained for the Upton field site focused primarily on Block 21, although imagery was also collected for other infested blocks, as this was the block where phylloxera was first detected by ground surveys in April 2000 and detailed ground surveys have been conducted at this site for successive seasons during 2001-2003 (Powell *et al*., 2003b) and are also detailed in this report for the 2005-2007 period (Chapter 2). From the airborne imagery collected in 2005 four basic images: true colour, NDVI, PCD and false colour were produced (Figs. 1-4). No obvious characteristic 'weak' spots associated with phylloxera presence, as determined by ground surveys or traps, were evident from this imagery. In addition 'Greenseeker'

imagery was obtained. Of the five sets of imagery the 'Greenseeker' NDVI image (Fig. 5) showed clearer canopy differences and matched better with the distribution of phylloxera in the block and aligned well with EM38 survey imagery. This imagery was obtained using a ground-based sensor mounted on an ATV and took side-views of the canopy which was particularly useful in the survey block where a less vigorous, tightly-controlled, canopy was present compared with other sites.

Primary study site 2 – King Valley

Imagery obtained for the King Valley field site focused on an ungrafted *V. vinifera* block where phylloxera was first detected in 1998 and detailed ground surveys and trapping was conducted during 2005-2007 (Chapter 2). From the airborne imagery collected in 1995 weak canopy areas are clearly evident (Fig. 6). At this time phylloxera was not known to be present as ground surveys conducted by Plant Standards Victoria did not detect the insect at that time. This suggests that the initial weakness in the canopy was due to soil variation and moisture issues within the block. Airborne imagery collected in February 2005 allowed the production of four basic images; true colour, NDVI, PCD and false colour (Figs. 7-10). When comparing these figures with imagery taken ten years earlier it is clear that the weak vigour areas have increased in size, and this is particularly evident in the PCD (Fig. 9) and false colour imagery (Fig. 10). This observation is consistent canopy with decline caused by the spread of phylloxera as assessed by trapping (Chapter 2). The 2005 aerial imagery also matches well with the EM38 imagery obtained in 2006 (Fig. 11) with generally high EC regions aligning with areas of reduced grapevine canopy.

Supplementary study site 5 – Buckland Valley

From the airborne imagery collected in 2005 four basic images; true colour, NDVI, PCD and false colour, were produced (Figs. 12-15). A weak spot corresponding with an area of low vigour due to phylloxera presence was evident near the centre of the western block in the true colour (Fig. 12) and false colour images (Fig. 15), but no obvious relationship with EM38 imagery could be determined (Fig. 16).

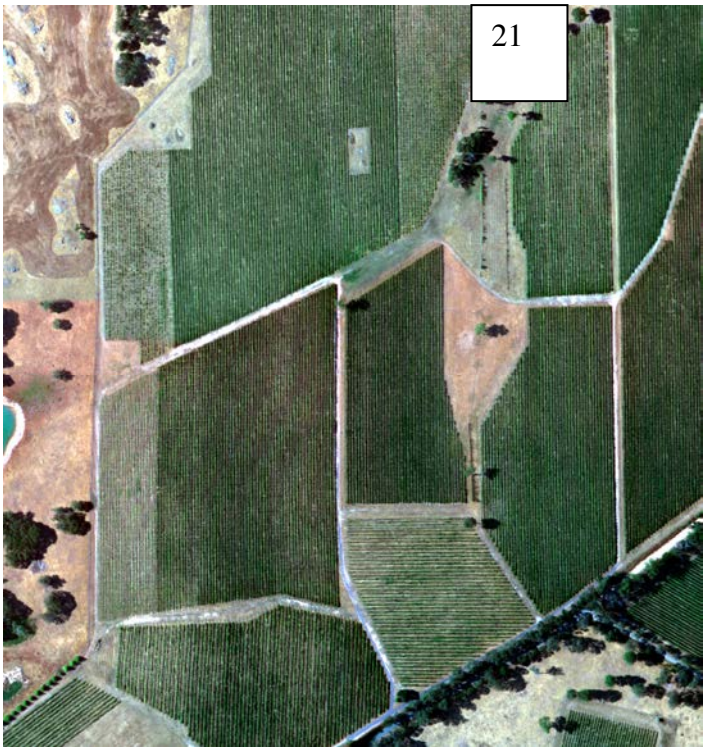


Fig. 1. True-colour image of Upton site, showing phylloxera-infested Block 21.

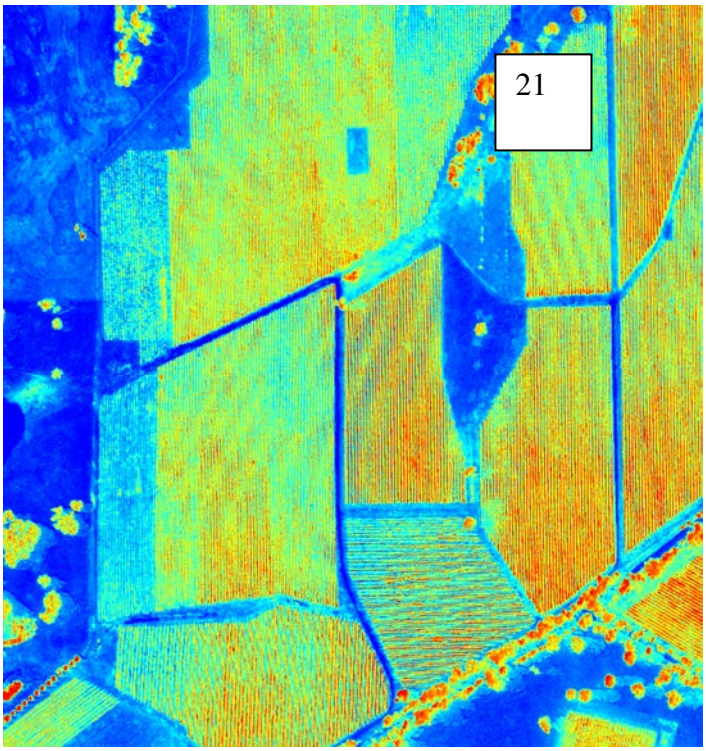


Fig. 2. Normalised Difference Vegetation Index (NVDI) image of Upton field site, showing phylloxera-infested Block 21.

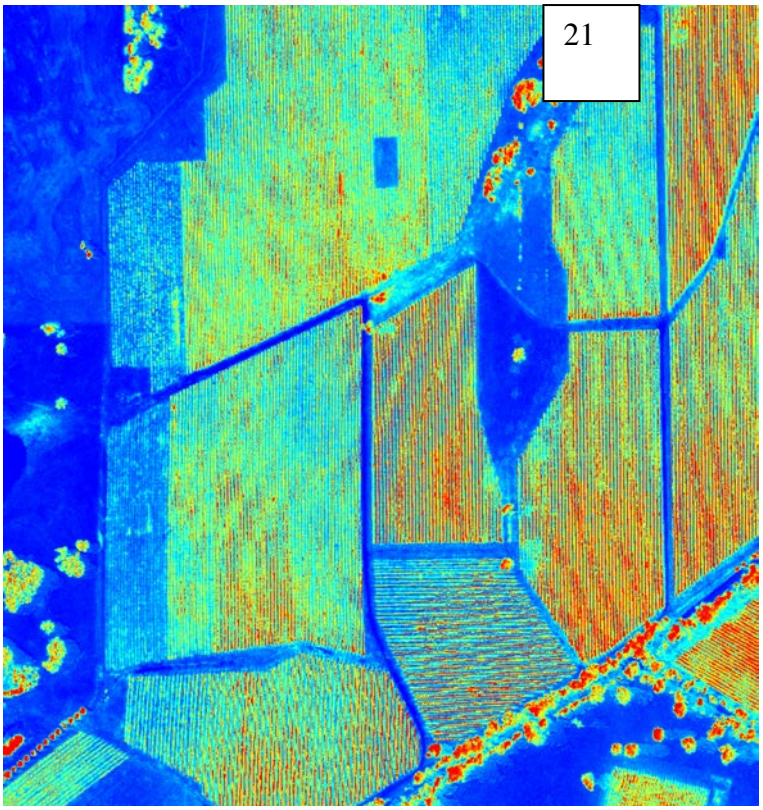


Fig. 3. Plant Cell Density (PCD) image of Upton field site, showing phylloxera-infested Block 21.

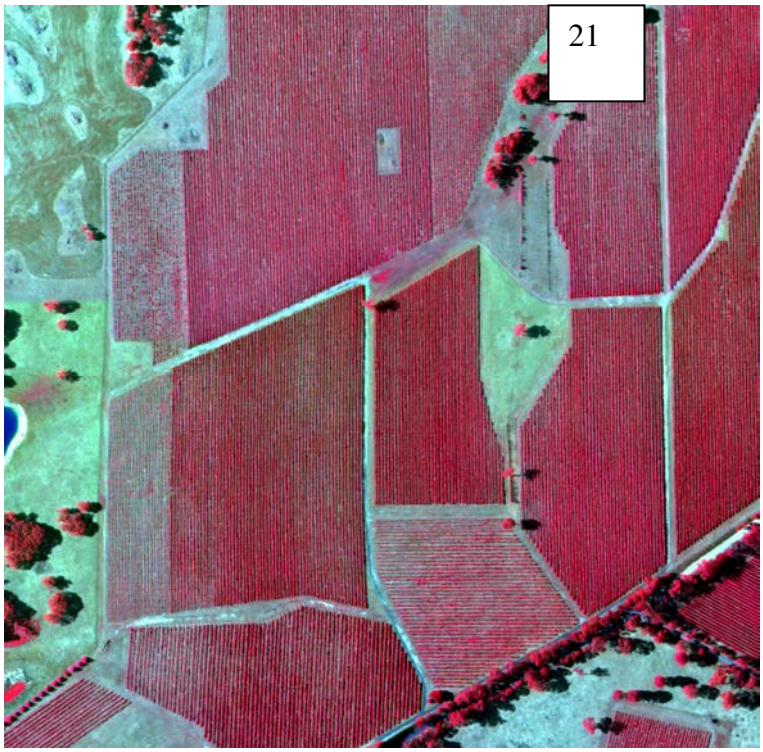


Fig. 4. False-colour image of Upton site, showing phylloxera-infested Block 21.

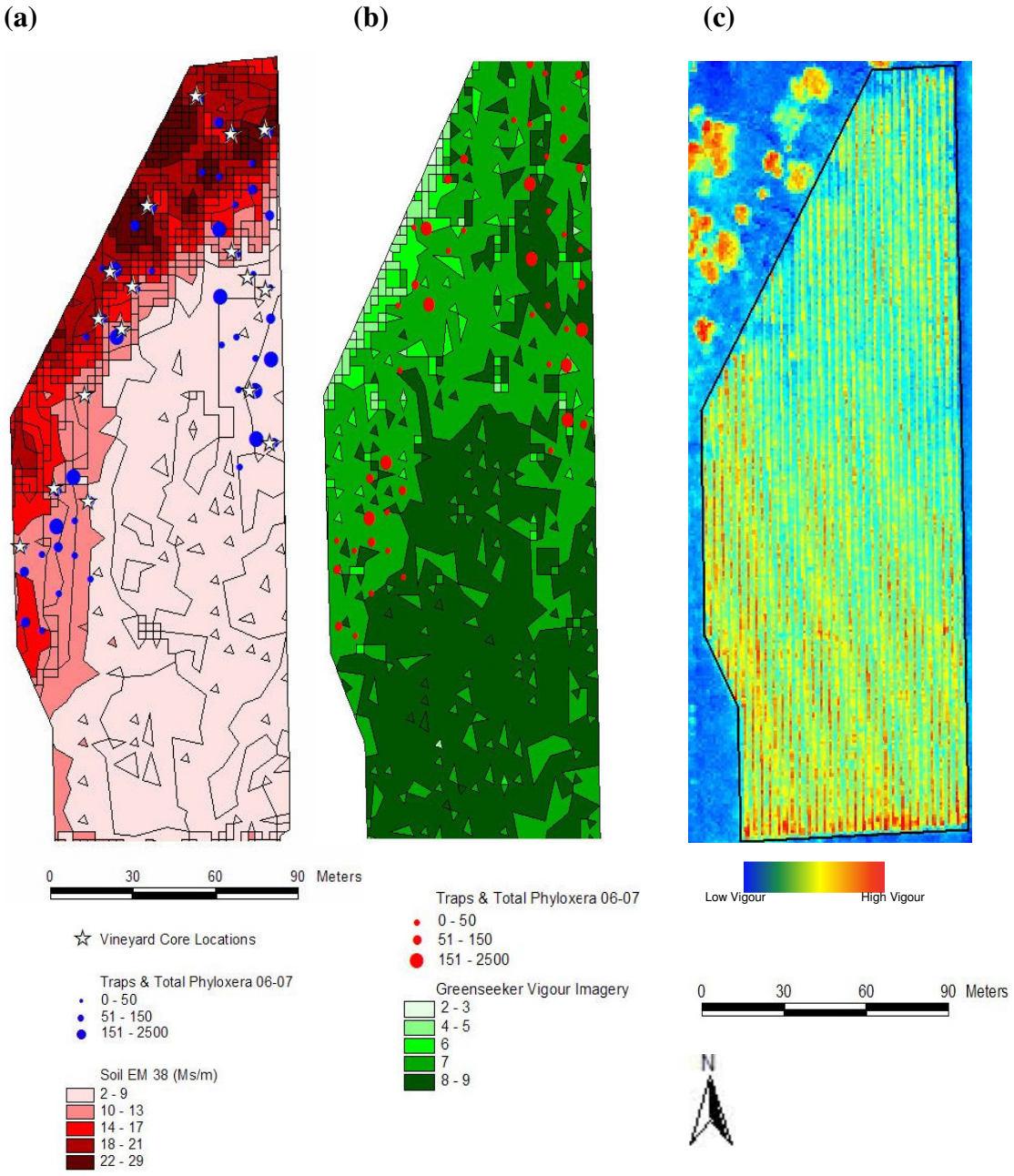


Fig. 5. **Maps showing (a) EM38, (b) 'Greenseeker' and (c) NDVI aerial imagery collected in August 2006, February 2007 and February 2005 respectively in Block 21 of ungrafted *Vitis vinifera* 'Cabernet Sauvignon' at the Upton site. Emergence trap locations with total emergent phylloxera and soil core locations for the 2006/2007 season are indicated.**



Fig. 6. True-colour image of phylloxera-infested vineyard block at the King Valley field site collected in 1995.



Fig. 7. True-colour image of phylloxera-infested vineyard block at the King Valley field site collected in 2005.

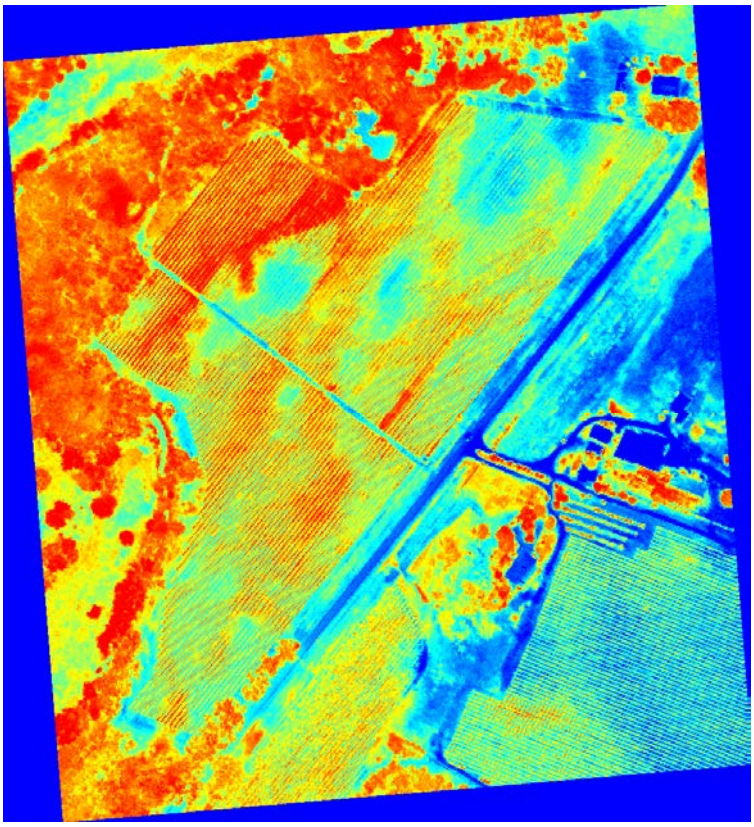


Fig. 8. NVDI image of phylloxera-infested vineyard block at the King Valley field site collected in 2005.

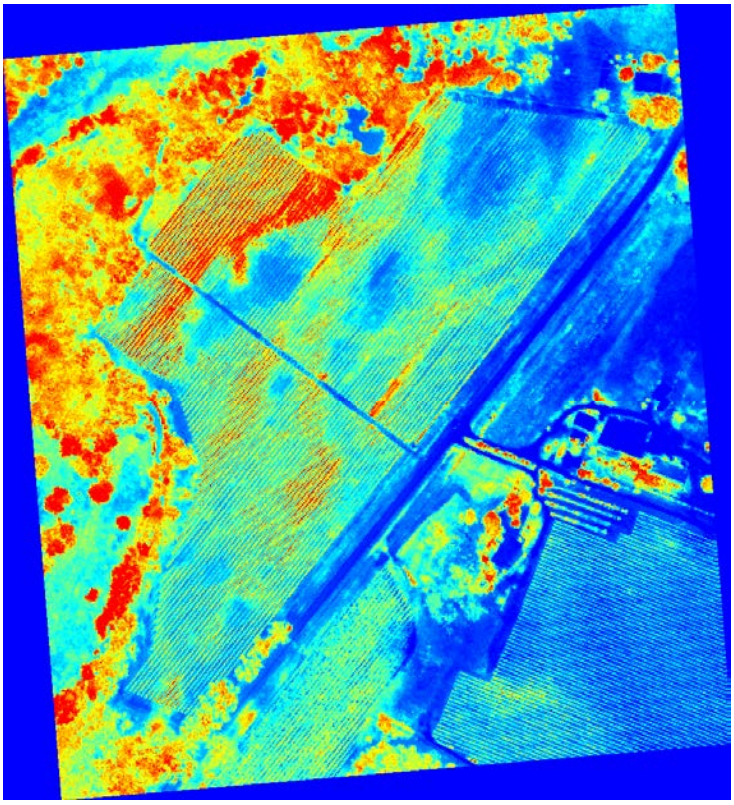


Fig. 9. PCD image of phylloxera-infested vineyard block at the King Valley field site collected in 2005.

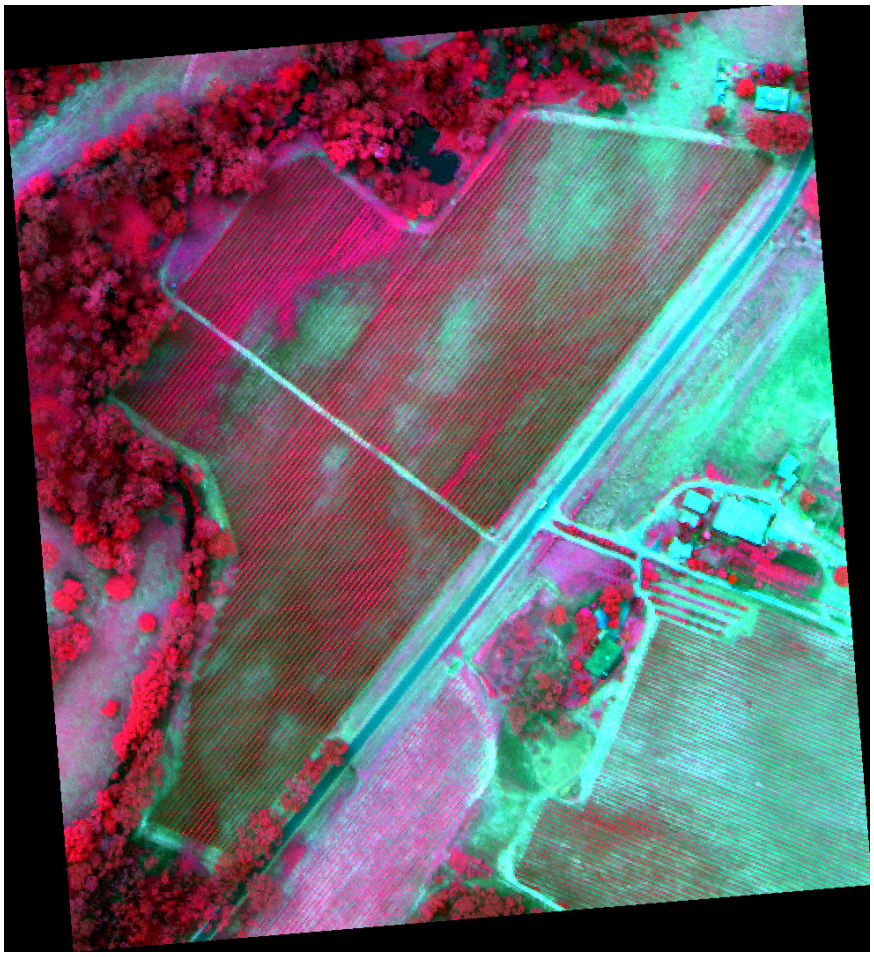


Fig. 10. False-colour image of phylloxera-infested vineyard block King Valley field site collected in 2005.

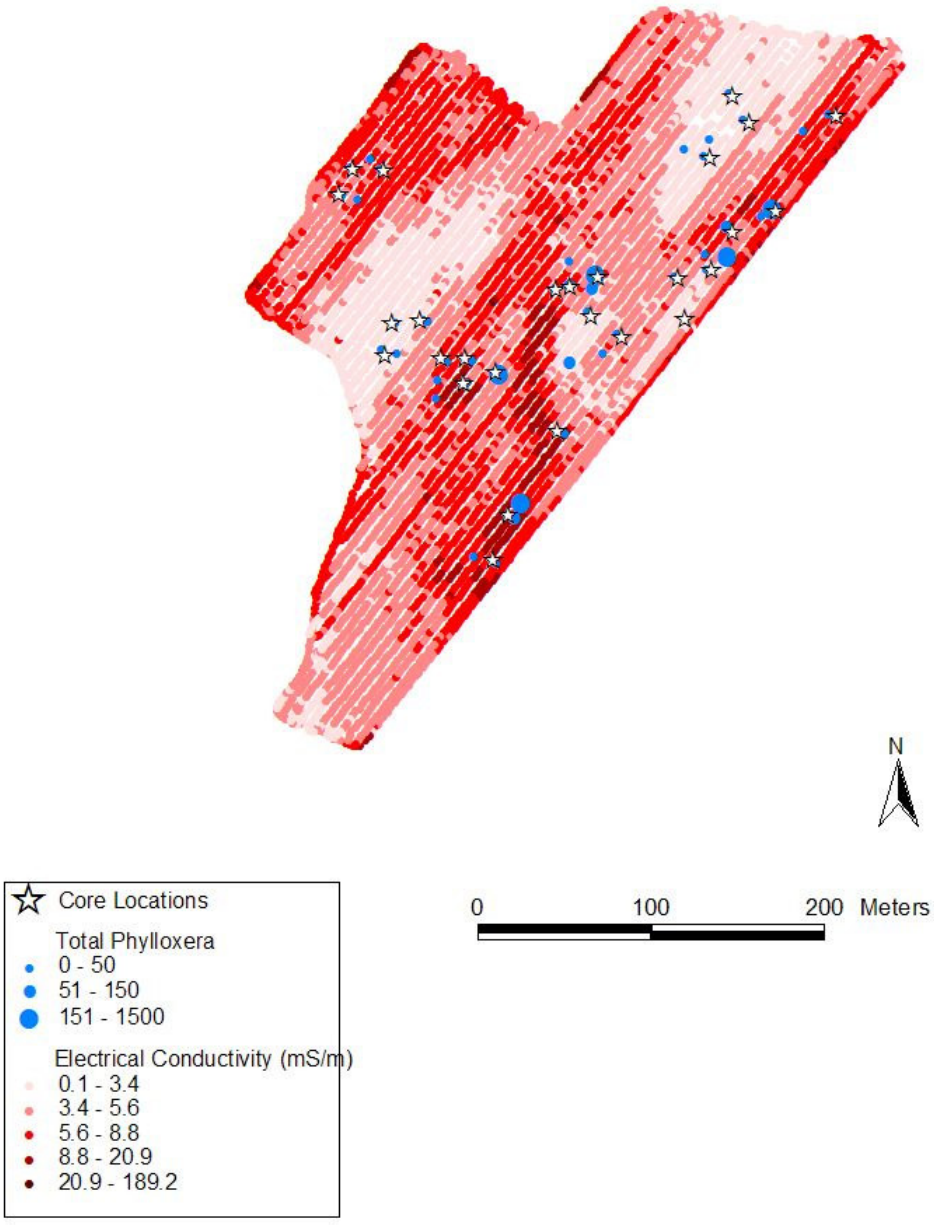


Fig. 11. EM38 images collected August 2006 showing location of soil core sampling points and relative total abundance of phylloxera in emergence traps for the 2006-2007 season at the King Valley site.

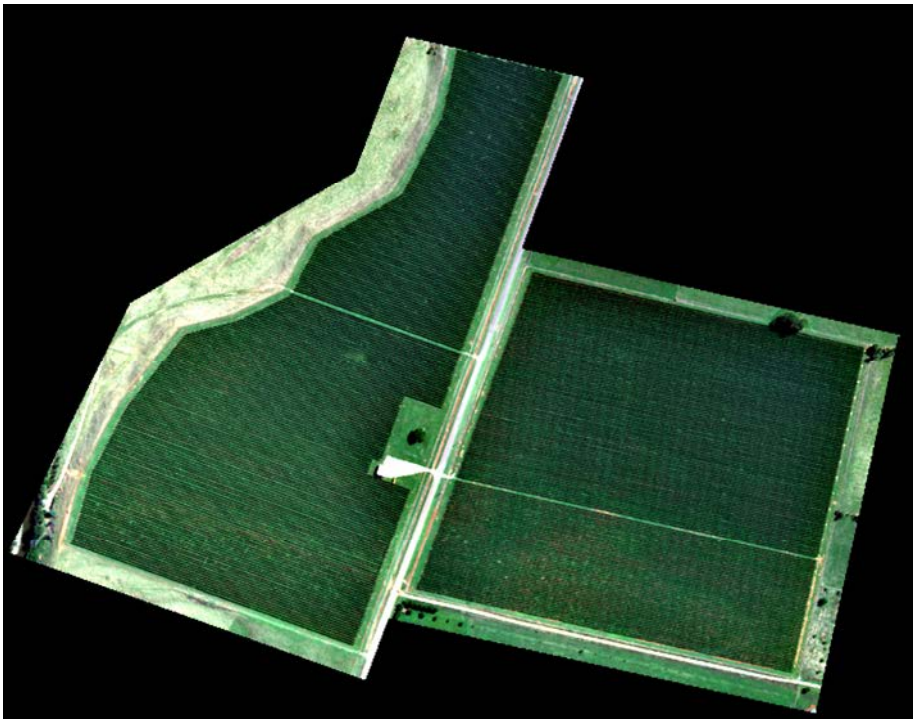


Fig. 12. True-colour image phylloxera-infested vineyard block at the Buckland Valley field site collected in 2005.

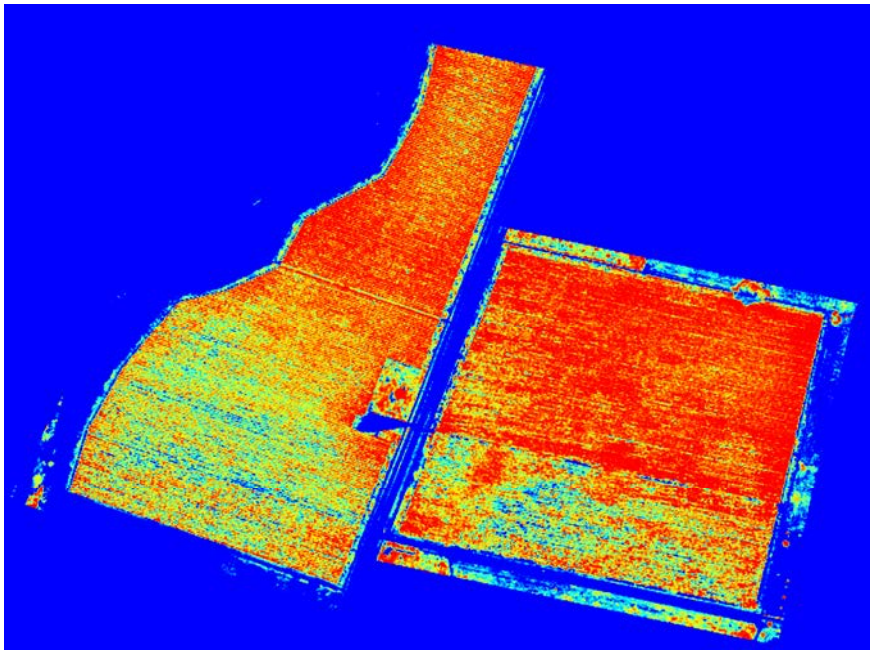


Fig. 13. NVDI image of phylloxera-infested vineyard block at the Buckland Valley field site collected in 2005.

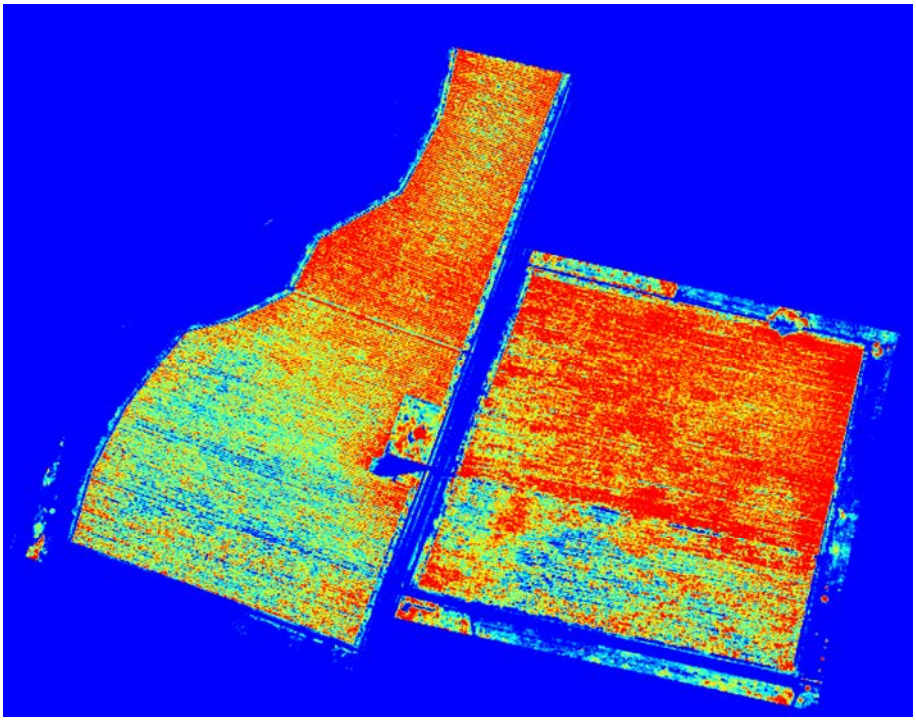


Fig. 14. PCD image of phylloxera-infested vineyard block at the Buckland Valley field site collected in 2005.

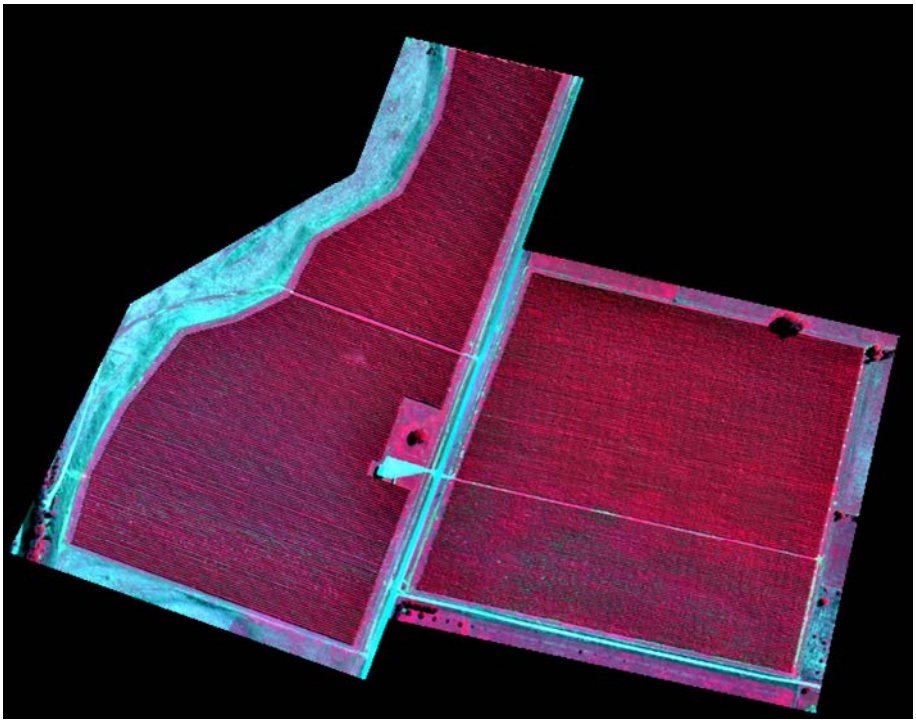


Fig. 15. False-colour image of phylloxera-infested vineyard block at the Buckland Valley field site collected in 2005.

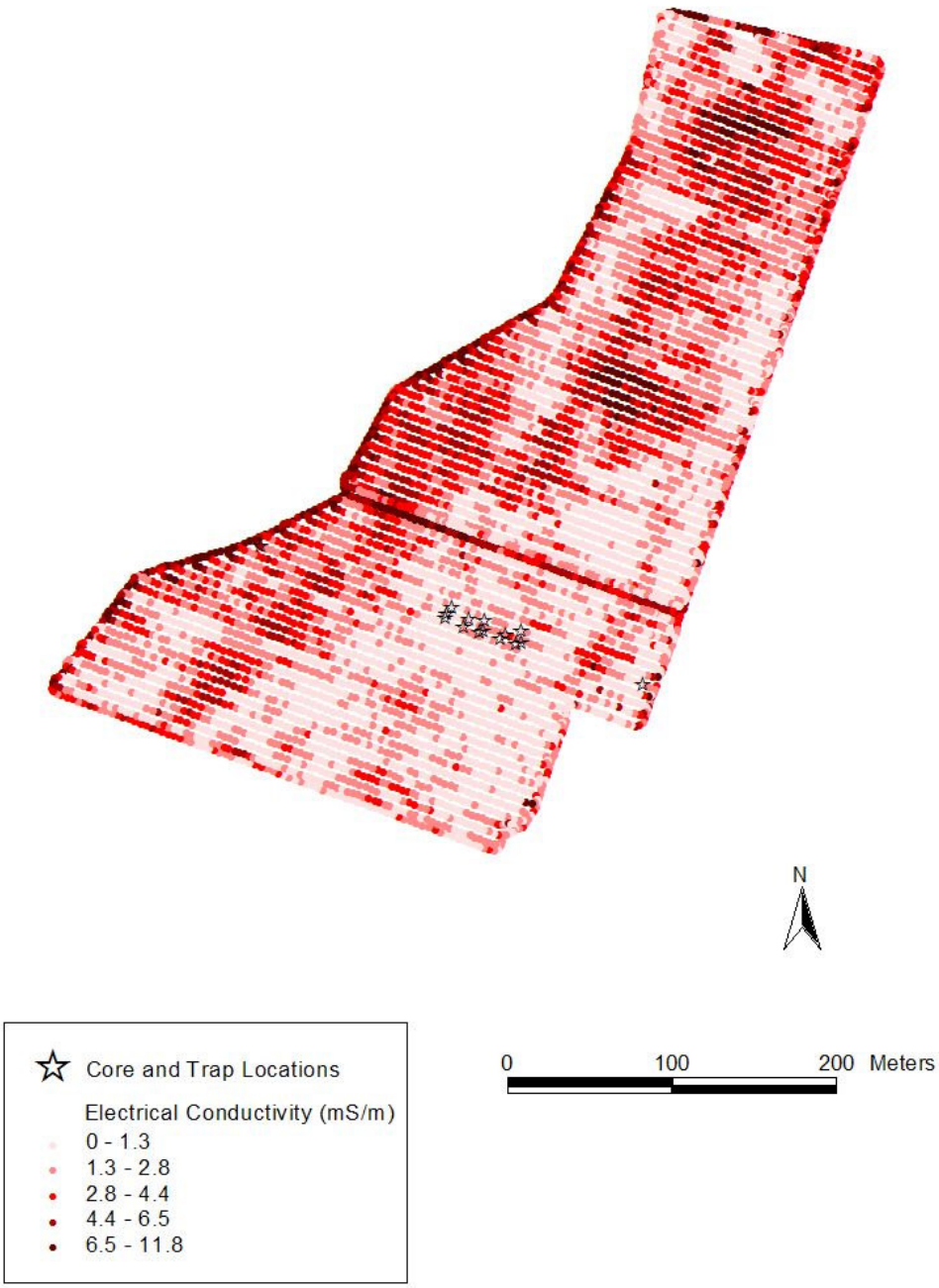


Fig. 16. EM38 images collected December 2005 showing location of 2004-2005 emergence traps and 2006 soil core sampling points at the Buckland Valley site.

DISCUSSION AND RECOMMENDATIONS

Area-wide detection, large scale population dynamics predictions and efficacy of post detection control of insect pests is potentially feasible using either satellite imagery (Luther *et al*., 1997; Brewster *et al*., 1999; Fleischer *et al*., 1999; Seelan *et al*., 2003) or airborne imagery (Carter *et al*., 2001) but most of the work to-date has focused on above-ground pests.

Multispectral imagery collected from airborne sensors, for either targeted detection of phylloxera or temporal monitoring of its spread rate, focuses on identifying oval-shaped areas of low canopy vigour which then need to be followed up by ground surveys and visual inspection for visual symptoms of phylloxera presence or associated gall induction on the root system. This method was pioneered in California (Wildman *et al*., 1983; Johnson *et al*., 1996) and has since been used in Australia in the states of Victoria (Frasier *et al*., 2004) and South Australia (Edwards *et al*., 2004).

This method of phylloxera 'detection' relies fundamentally on the (grapevine) host plant response to phylloxera feeding on the root system. There are several problems with relying on a host plant response such as reduced canopy vigour. This symptom alone can be due to many factors such as herbicide drift, drainage problems, nutritional issues, other pests and diseases or water related problems. Even if a weak spot is due to phylloxera it is very probable that the phylloxera has been present in the weak spot area for several seasons and due to reduced root system has moved on to vines which appear vigorous in comparison.

The 'weak spot' system of detection does not take into account genetic variability of either the host plant or the pest. For example it cannot be used in a rootstock planted vineyard because phylloxera does not cause sufficient damage to these vines for weak spots to be evident. This system cannot be used successfully for low virulence phylloxera genotypes which may be present in relatively low abundance levels on ungrafted *V. vinifera* roots and take decades for any expression of symptoms in the foliage. It also does not consider the soil environment and the influence this may have on both phylloxera interactions and related vine damage. For improved detection of grape phylloxera further population dynamics data from a wide range of infested sites is required before spatial and temporal models can be developed to predict phylloxera interactions on an area-wide scale and potential establishment 'hotspots'

which may or may not be ‘visible’ by remote sensing. In addition improved methods of spectral sensing such as hyperspectral sensors are likely to be more sensitive if developed further than multispectral sensing for phylloxera detection (Renzullo *et al*., 2004; 2006; 2007).

CHAPTER FOUR

Characteristics of Soils From Phylloxera-infested Vineyards

Objective: To investigate relationships between phylloxera abundance and soil chemistry and texture

SUMMARY

In this study chemical and textural characteristics of soils were examined to determine their influence on phylloxera abundance in three phylloxera-infested vineyards in Victoria. Some factors such as exchangeable aluminium, potassium, magnesium and clay content influenced phylloxera abundance levels at some sites. Other factors appeared to have little or no influence on phylloxera abundance. Variation was evident between sites and phylloxera genotype and its interaction with the host plant under different soil conditions is likely to be significant. The importance of these findings for phylloxera detection, dispersion and monitoring is discussed.

INTRODUCTION

Grape phylloxera in infested Australian vineyards is dominated by asexual genotypes of the insect with a root-galling habitat (Umina *et al*., 2007). Despite the fact that phylloxera is reliant on a conducive soil environment for establishment and development on the grapevine root system there have only been very limited studies investigating the impact that soil physical and textural characteristics on insect establishment, development and ultimately dispersal characteristics. This study focused on the influence of soil characteristics on phylloxera abundance in known phylloxera-infested vineyards. Following mapping of phylloxera-infested grapevine blocks using electromagnetic induction surveys (Chapter 1) three sites were selected for strategic sampling to investigate the influence of physicochemical soil characteristics on phylloxera abundance and dispersal.

METHOD

Soil sampling

A small, track-mounted hydraulic/percussion soil corer (Geoprobe 54 LT) was used to collect soil samples (Fig. 1) to a depth of 1m. After extraction from the profile the soil core was inserted intact by the Geoprobe into 44mm plastic tubes. Cores were taken in the centre of grapevine mid-rows, adjacent to emergence trap locations. A total of 69 soil cores were collected between the 15th and 17th November, at the King Valley, Upton and Buckland Valley vineyard sites.



Fig. 1. Geoprobe 54LT soil corer collecting 1 metre soil cores in the mid-row at a commercial vineyard in the King Valley.

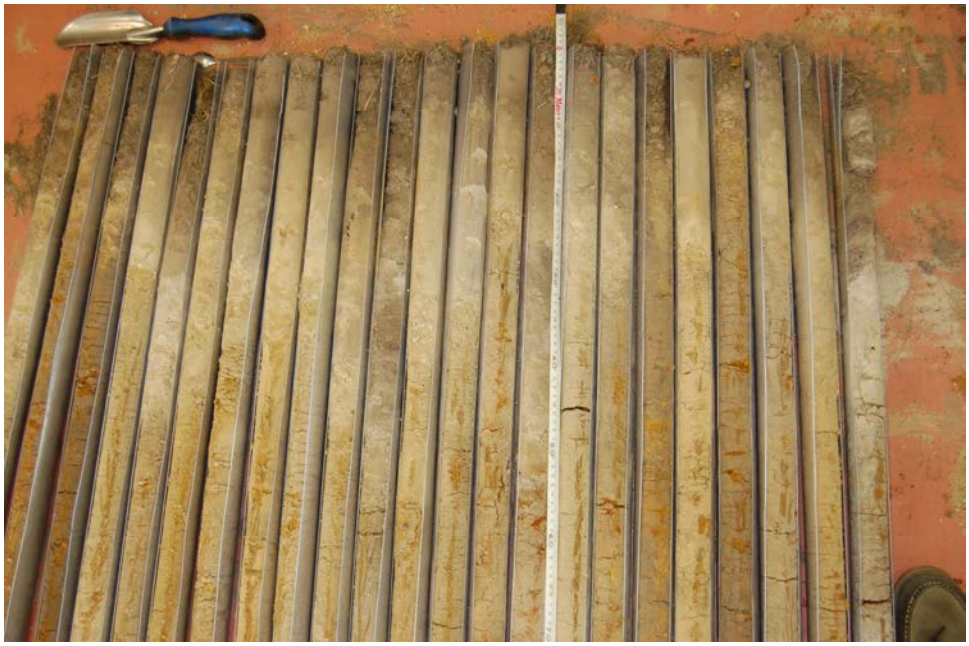


Fig. 2. Representative sample of soil cores collected from the Upton field site.



Fig. 3. Representative sample of soil cores collected from the King Valley field site.



Fig. 4. Representative sample of soil cores collected from the Buckland Valley field site.



Fig. 5. Comparative samples of soil cores collected from the King Valley, Buckland Valley and Upton field sites.

Soil characterisation and analysis

At DPI Rutherglen, core tubes were cut open enabling the 1m profile to be logged and compared for each site (Figs. 2-5), using similar nomenclature to that used previously to describe soil pits at these locations (Powell *et al*., 2006). Core locations were recorded with a Garmin76S GPS to enable their position to be mapped on EM38 imagery (Chapter 2). Cores were classified into horizons, each of which was then processed as separate samples, using the following protocol:

1) Oven-dried at 45°C for 24 hours.
2) Tumbled in a soil rumbler to breakdown aggregates, and screened to 2mm.
3) Bagged and sent under quarantine to the State Chemistry Laboratory, Werribee, for chemical and textural analysis.

All 'A' horizon samples were analysed for a standard suite of cations (exchangeable Aluminium (Al), calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na)), pH (both H_2O and $CaCl_2$), organic carbon (OC), organic matter (OM), electrical conductivity (EC) and nitrogen (N). Selected samples also underwent textural analysis. The 'B' horizon samples were retained for future analysis. Results were compared with phylloxera emergence trap data from three field sites located in the King Valley, Upton and Buckland Valley (Chapter 2) to ascertain any correlations. The soil core data for the Upton and King Valley sites was also utilised in a glasshouse trial (Chapter 5) where 'A' horizon soils from these sites were used as soil treatments.

Statistical analysis

The most critical aspect arising from the soil core analyses was to establish if there were any trends or correlations between soil chemistry and phylloxera abundance as assessed from emergence traps (Chapter 1). Two statistical treatments were used to analyse the data. Firstly multivariate analysis was undertaken for the cations (exchangeable Al, Ca, K, Mg, and Na), OC, N, OM and pH ($CaCl_2$) and compared with phylloxera emergence trap data. The K/Mg ratio was included, since a previous study by Reisenzein *et al.* (2007) indicated a strong relationship between this ratio and phylloxera abundance in Austrian soils. The data compared was the individual trap

data with the adjoining soil core. The individual trap data shows high variability. To smooth out this variability, a second multivariate analysis was undertaken using average trap numbers for the area surrounding the core site, using the assumption that the soils in the immediate vicinity of the core site would exhibit minimal variation. In the case of the Upton site, a 15m radius circle was drawn around the core site, and phylloxera emergence totals from any traps falling within the circle were averaged. For the King Valley site, the traps were originally set up in discrete sub-blocks, of five traps, based on similar EC category (Chapter 2). The phylloxera numbers for the five traps were totalled, and compared with the soil cores falling within the sub-block. For the Buckland Valley site emergence trap data was used from the 2004 season and cores were located in the mid-row between two traps so trap numbers were pooled and averaged for comparison. All analyses were undertaken using Genstat software (Payne *et al*, 2005).

RESULTS

Primary study site 1 - Upton

For the Upton site in the 2006-2007 growing season, analysis of soil A horizon data with individual phylloxera trap data showed a positive relationship between phylloxera abundance and exchangeable Al, Mg and a negative correlation with organic matter and K:Mg ratio (correlation coefficients of 0.488, 0.459, -0.337 and -0.432 respectively – Table 1). The averaged pooled trap data shows a stronger positive relationship between phylloxera abundance and Mg concentration (0.609), but somewhat lower positive relationship with exchangeable Al (0.323), and a negative correlation with K/Mg (-0.338). The correlation between phylloxera and exchangeable aluminium is supported by previous work at a vineyard in Rutherglen, where the soil has exchangeable Al below detection levels (<10 mg/kg) (Powell *et al*., 2003) and very low phylloxera numbers were recorded in emergence traps for three successive seasons. The Upton soil was included in a glasshouse trial discussed in Chapter 5, where the soil is described.

Primary study site 2 – King Valley

The King Valley data in general showed poorer correlations than the Upton sites, particularly for individual trap data (Table 3), with the best being K, Ca, pH with correlation coefficients of 0.259, 0.214 and 0.229 respectively). The sub-block analysis correlations (Table 4) are somewhat better for K (0.315), exchangeable Al (0.226), and Na (-0.309). The K:Mg correlation is very weak, but as with Upton is also negative (-0.164).

The King Valley site has been infested with phylloxera for a longer period of time than the Upton site and is infested by a more virulent phylloxera genotype which may have masked some relationships between soil characteristics and phylloxera abundance at this site.

Supplementary study site 5 – Buckland Valley

The Buckland Valley data showed a poor negative relationship between phylloxera abundance and K and Mg with coefficients of -0.416 and -0.298 respectively (Table 4). The Buckland Valley site has been infested with a low virulent phylloxera strain for a relatively short period of time compared to the Upton and King Valley sites which may have masked some relationships between soil characteristics and phylloxera abundance at this site.

Table 1. **Correlation matrix for Upton soil analyses and individual total phylloxera numbers from individual emergence traps in the 2006-2007 growing season.**

Ca	-0.173								
K	0.176	0.741							
K:Mg	0.094	0.168	0.592						
Mg	0.357	0.411	0.264	-0.515					
Na	-0.282	-0.110	-0.195	-0.471	0.161				
N	0.002	0.283	0.233	0.344	-0.087	-0.248			
OM	-0.239	0.573	0.654	0.333	0.127	-0.357	0.224		
pH	-0.604	0.488	0.064	-0.173	0.119	0.100	0.089	0.276	
Phylloxera	0.488	0.161	-0.125	-0.432	0.459	0.009	-0.013	-0.337	0.132
	Al	Ca	K	K:Mg	Mg	Na	N	OM	pH

Table 2. **Correlation matrix for Upton soil analyses and average total phylloxera numbers from emergence traps, analysed in the 2006-2007 growing season, for pooled traps falling within 15m radius of core sites.**

Ca	-0.173								
K	0.176	0.741							
K:Mg	0.094	0.168	0.592						
Mg	0.357	0.411	0.264	-0.515					
Na	-0.282	-0.110	-0.195	-0.471	0.161				
N	0.002	0.283	0.233	0.344	-0.087	-0.248			
OM	-0.239	0.573	0.654	0.333	0.127	-0.357	0.224		
pH	-0.604	0.488	0.064	-0.173	0.119	0.100	0.089	0.276	
Phylloxera	0.323	0.026	-0.105	-0.338	0.609	-0.219	0.037	-0.112	0.059
	Al	Ca	K	K:Mg	Mg	Na	N	OM	pH

Table 3. **Correlation matrix for King Valley soil analyses and individual emergence trap phylloxera numbers from the 2006-07 growing season.**

Ca	-0.583								
K	-0.203	0.346							
K-Mg	0.194	-0.264	0.758						
Mg	-0.407	0.552	-0.082	-0.559					
Na	-0.050	-0.031	-0.510	-0.580	0.699				
N	-0.111	0.097	-0.230	-0.327	0.021	-0.008			
OM	-0.476	0.454	0.480	0.282	-0.040	-0.448	-0.130		
pH	-0.716	0.781	0.319	-0.152	0.448	-0.175	-0.039	0.516	
Phylloxera	-0.125	0.214	0.259	0.069	0.086	-0.176	-0.080	0.099	0.229
	Al	Ca	K	K:Mg	Mg	Na	N	OM	pH

Table 4. **Correlation matrix for King Valley soil data, 2006-07 phylloxera trap numbers totalled for each sub-block of 5 traps.**

Ca	-0.583								
K	-0.203	0.346							
K:Mg	0.194	-0.264	0.758						
Mg	-0.407	0.552	-0.082	-0.559					
Na	-0.050	-0.031	-0.510	-0.580	0.699				
N	-0.111	0.097	-0.230	-0.327	0.021	-0.008			
OM	-0.476	0.454	0.480	0.282	-0.040	-0.448	-0.130		
pH	-0.716	0.781	0.319	-0.152	0.448	-0.175	-0.039	0.516	
Phylloxera	0.226	0.086	0.315	0.196	-0.045	-0.309	-0.056	0.147	-0.011
	Al	Ca	K	K:Mg	Mg	Na	N	OM	pH

Table 5. **Correlation matrix for Buckland Valley soil analyses and average total phylloxera numbers from emergence traps, analysed in the 2006-2007 growing season, for two pooled traps falling either side of core site.**

Ca	-0.914								
K	-0.633	0.439							
K:Mg	0.204	0.006	-0.788						
Mg	-0.702	0.616	0.876	-0.771					
N	-0.455	0.354	0.401	-0.570	0.679				
Na	-0.490	0.420	0.578	-0.462	0.620	0.237			
OM	-0.497	0.336	0.468	-0.641	0.721	0.843	0.415		
pH	-0.776	0.879	0.279	0.003	0.541	0.525	0.429	0.356	
Phylloxera	0.195	-0.157	-0.416	0.212	-0.298	-0.214	0.155	-0.041	-0.065
	Al	Ca	K	K:Mg	Mg	N	Na	OM	pH

General analysis

Analysis for all sites shows a strong negative correlation between pH and exchangeable (i.e. available) Al, and strong positive correlation between pH and calcium. Both are typical soil phenomena, and provide support for the integrity of the analyses. According to Slattery *et al*. (1999), exchangeable Al increases markedly as soil pH ($CaCl_2$) falls below 4.8, and can become toxic to plant growth, affecting roots by causing lateral and tip root thickening, greatly reducing the development of fine branching roots and root hairs (Menzies *et. al.* 1994, cited in Slattery *et. al.* 1999) and excluding the uptake of other cations (Foy, 1984; Ritchie, 1989, cited in Slattery *et. al.* 1999). The relationship between pH and exchangeable Al below pH 4.8 is clearly illustrated for the King Valley data (Fig. 6).

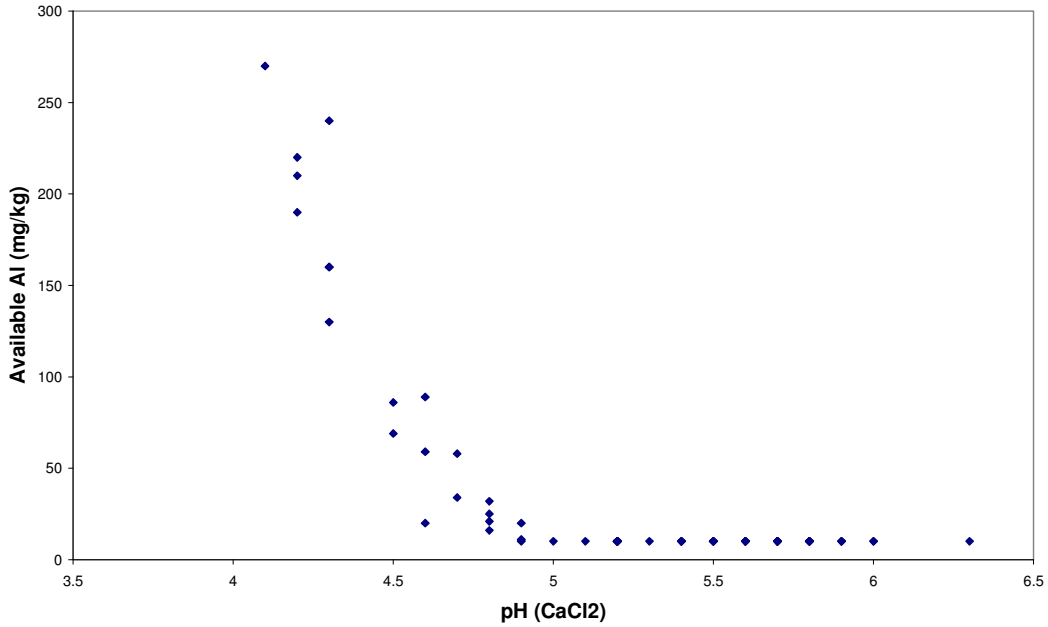


Fig. 6. Scatter plot showing strong negative relationship between available Al and pH for King Valley soils.

Linear regression analysis was also undertaken for some of the key factors noted above. The best relationship found was for available Al and phylloxera at Upton, using combined data from both 2005-06 and 2006-07 seasons (Fig. 7).

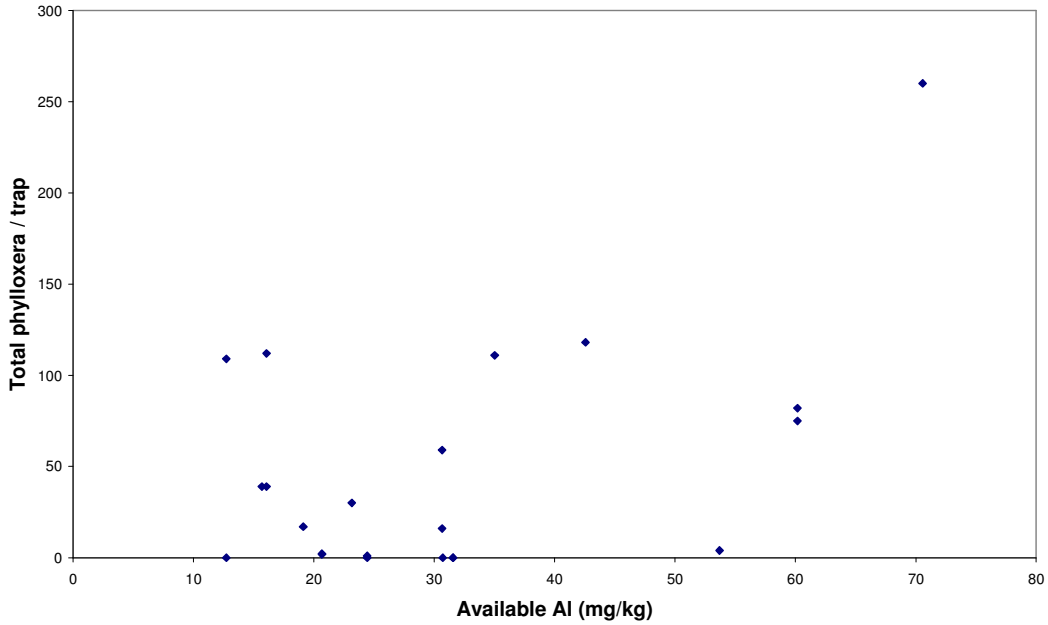


Fig. 7. Scatter plot of available Al and emergence trap phylloxera populations from Upton for 2005-06 and 2006-07 seasons (r^2 = 0.51).

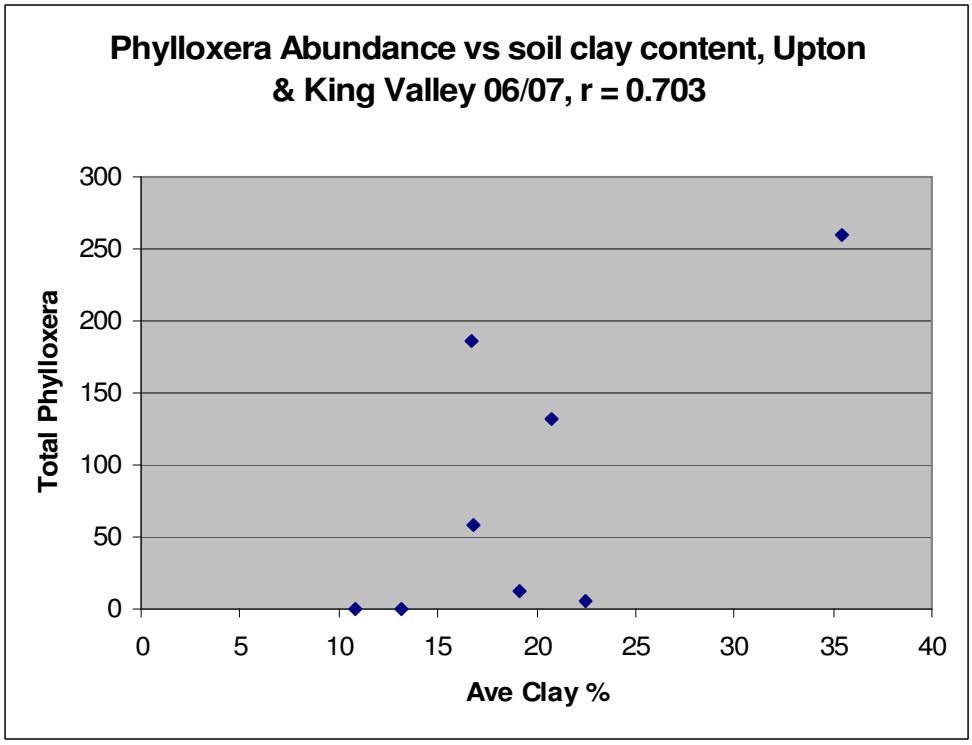


Fig. 8. Scatter plot of average clay content and emergence trap phylloxera populations from Upton and the King Valley sites combined 2006/7 data sets (r^2 = 0.703).

Particle size analysis

The clay content at the Upton site, as assessed by particle size analysis, was in the 16.8-35.4% range, whereas at the King Valley site it ranged from 9.5-24.5%. When the relationship between clay content and phylloxera abundance at the two sites combined was examined a positive correlation was observed (Fig. 8).

The clay content in the 'A' horizon of the Buckland Valley site was relatively high compared with both primary sites lying within the 30.5-40% range. However the population abundance at this site (Powell *et al*., 2006) is relatively low compared to the King Valley and Upton sites and no clear correlation was observed. Therefore caution is required when interpreting this relationship as other factors such as phylloxera genotype may be influence the relationship.

DISCUSSION AND RECOMMENDATIONS

Historically there have been a limited number of studies conducted which examined the effect of the soil characteristics on phylloxera populations on grapevine roots. Those published have tended to focus on permanent soil properties such as texture and structure, with only limited work done on potential nutrient or chemical effects. Other reports in the literature are either anecdotal or based on limited survey studies.

Soil textural properties

Distribution of phylloxera around the world has been determined predominantly from results of ground survey techniques of historical records. In South Africa phylloxera was first reported in 1886 and is reportedly widespread (De Klerk, 1972) but in some regions where red sandy soils occur it has not been detected. Ground surveys conducted in Canada relied on very limited sampling of 10-20 vines per vineyard taken at depths of 8-12 inches and repeated samples (up to 3) were required for detection even were phylloxera was relatively widespread. The survey showed higher infestation levels in clay and clay loam soils (84-99%) but phylloxera was still detected in 60% of sandy loam soils sampled even though galling was reduced.

It is commonly assumed that vine resistance to phylloxera improves proportionally with an increase in sand. Nougaret *et al*. (1928) first demonstrated this. They hypothesised that soils with greater than 60% silica content could be considered

immune to phylloxera attack. It was concluded from their study that moderate to heavy clays were more susceptible to phylloxera infestation due to their tendency to crack on drying, facilitating phylloxera movement. In contrast, sandy soils are physically looser, and lack the expansion and contraction properties of clay. Particle movement filling fissures in the soil matrix may restrict phylloxera mobility. Nougaret also recognised that phylloxera was prevalent on shallow-rooted vines growing in areas of compact and impervious soils with poor drainage. These vines also tended to decline rapidly once infested. Vines grown on sandy soils often have well established root systems as a result of good drainage, and the capillarity of sand allows water to percolate through the profile, affectively asphyxiating phylloxera on the roots (Nougaret *et al*., 1928).

A recent study by Reisenzein *et al.,* 2007 supports the findings of Nougaret *et al.* (1928). The authors found there were significant positive correlations between increasing clay and organic carbon content, and phylloxera infestation frequency. A study by De Klerk (1972) found that of the three major soil types in the study area, phylloxera was not found on the sandiest soil type. The study by Reisenzein *et al.* 2007 concluded that phylloxera preferred finely textured soils, however it is important to note that all eight soil types they studied did support phylloxera populations at a range of infestation frequency and intensities. It is also important to note that most of their studies were conducted on vineyards planted to the resistant rootstock 5BBKober which could have influenced phylloxera abundance. Similarly, Chitkowski *et al.* (2005) found that all soil types used in their glasshouse study supported phylloxera, most notably soil-less and sand/soil mixes. This contradicts presented anecdotal evidence that sand and soilless mixes would not support phylloxera populations.

Other evidence exists to indicate that damaging phylloxera populations cannot exist in sandy soil environments. Sand appears to inhibit phylloxera crawler dispersal from infested root systems to uninfested root systems, effectively minimising population growth and potential vine damage (Granett *et al.,* 2001). It has been proposed that there may be an ‘insecticidal’ effect of sandy soils on phylloxera (Nougaret *et al*., 1928). A study by Stevenson (1963) found that the percentage infestation of vineyards on sandy loam soils was significantly less than in vineyards on heavier soil types sampled. The same study found that root galling was less severe in lighter soils compared to heavier ones, and that these vineyards were likely to have a lower

percentage of infested vines. A trial conducted over two vineyard sites with a loam and clay profile, and a silty loam profile respectively, resulted in heavier infestations and severe vine decline of those growing in the heavier loam and clay profile compared to the vines growing in the silty loam profile (King and Buchanan, 1986). This supports the assumption that clay soils tend to support large phylloxera populations (Granett *et al.,* 2001). Our study was conducted at three sites where clay reached up to 40%. A positive correlation of increasing phylloxera abundance with increased clay content was observed at the King Valley and Upton sites. However, at the Buckland Valley site phylloxera levels were relatively low (Chapter 2) despite having consistently high clay contents. To obtain a definitive response between clay content and phylloxera abundance, controlled studies need to be conducted rather than field trials where several factors, such as phylloxera and grapevine genotype and other soil factors could influence the relationship.

Soil friability may also influence vine susceptibility. Friable soils that crumble on drying act similarly to sand by filling up surface cracks and fissures, creating an impermeable soil layer excluding phylloxera from the soil (Nougaret *et al*., 1928). Clay/sand mixes are often friable, and may therefore offer some protection against new phylloxera infestations and reduce the severity of infestation damage, particularly in situations where the subsoil has adequate drainage to allow a well-developed vine root system.

Soil physical properties

There are other soil factors that may affect phylloxera populations, separate from texture. Soil moisture influences soil structural properties and contributes to chemical and microbial activities within the soil, indirectly affecting phylloxera infestations and vine health. Helm *et al.* (1991) investigated the effect of drought conditions on phylloxera populations, and concluded that in well-managed, irrigated vineyards, phylloxera should not cause lost productivity. They stated that phylloxera is more likely to exacerbate the expression of water-stress rather than being the primary cause of vine decline. The same study speculated that populations were high on water-stressed vines due to a reduction in phloem quality and subsequent ease of root penetration by the phylloxera. Both the vineyards tested were on heavy clay loams, and phylloxera populations on vine roots were particularly obvious in ‘drought’ years. Population peaks are closely linked to root growth flushes (Powell *et al.,* 2000),

indicating a link between moisture content of the soil, root development and phylloxera incidence.

The effect of drought on phylloxera populations may be direct or indirect; dry conditions may result in degraded root quality and minimise fibrous root development, but may also result in root systems being inadequate to sustain large phylloxera populations. A study conducted by Powell *et al.* 2003 found that peak phylloxera emergence and root crawler abundance coincided with the driest months of the trial period. The highest root and above - ground populations were at the trial site with the lowest rainfall, and population peaks coincided with soil temperature peaks of 20-30°C and prolific root growth. Conversely, sites with soil temperatures lower than 18°C had the lowest phylloxera pressure and feeding initiation. Granett *et al*. (2001) also found that gall formation ceased to occur below 18°C.

While the conclusions drawn by Helm *et al.,* 1991 are valid, a simpler explanation may be that soil moisture affects soil structure. Soil moisture is closely linked to soil texture, as soil particle size and organic matter content, partially determine water availability. A small pore size and electrical charge associated with clay soils promotes a higher water holding capacity, and reduces aeration considerably. If the conclusions indicated by Helm *et al.,* 1991 are accurate, you would expect to see phylloxera in drier soil conditions, such as those of sand. This, however, is in contrast to Nougaret *et al*. (1928), who indicated the increased susceptibility of grapevines to phylloxera in clay soils rather than sandy soils. Powell *et al.* 2003, indicated that on clay soils phylloxera was more abundant in drier conditions. Therefore the moisture content of the soil may be more important than the texture for phylloxera infestation.

Soil chemical properties

Reisenzein *et al.* 2007 described relationships between phylloxera infestation intensity and frequency, and various nutrient factors in the soil. Soil pH was negatively correlated with phylloxera abundance in their study, which covered a pH range of 5-8, with carbonate free and acidic soils promoting phylloxera population growth. In our study pH had no significant affect on phylloxera abundance however our study was only undertaken on soils within the acidic or slightly acidic range as defined by Cass *et al*. (2002) within a smaller pH range of 4.1-6.1. This effect is likely to be indirect,

as acidic soil restricts root growth and nutrient uptake, and unbalances nutrient ratios, resulting in a weaker vine more susceptible to attack.

Reisenzein *et al.* (2007) also found relationships between phylloxera infestation and plant available nutrients. A significant negative association exists between phylloxera and potassium, phosphorous, copper and zinc; a significant positive relationship exists between phylloxera and magnesium; and a highly significant correlation exists between phylloxera infestation and magnesium/potassium ratio. Again it is likely that the effect of these nutritive factors on phylloxera populations is indirect, and that it is any subsequent effects on vine health that create either a favourable or inferior environment for phylloxera. In our study we did not examine copper, zinc or phosphorous but did quantify potassium and magnesium. At the Upton site there was a positive relationship between magnesium levels and phylloxera abundance and a negative relationship between K:Mg ratio and phylloxera abundance. At the King Valley site this relationship was not as evident.

The one consistent chemical factor across sites in our study was Aluminium exchange capacity which showed a positive relationship with phylloxera abundance. The levels observed in some cases were considered toxic and this toxicity can reduce plant vigour through reduced root elongation possibly weakening the vines and making them more prone to phylloxera attack. This supports previous evidence by Powell *et al*. (2003) who noted from data gathered from soil surveys conducted in four phylloxera infested zones in Australia that high phylloxera levels were generally observed where high aluminium levels were recorded. However, levels of abundance are also highly dependant on phylloxera genetic strain present within the vineyard as noted (Chapter 2).

Data collected thus far indicates that both chemical and textural soil characteristics play a role in phylloxera abundance, dispersal and risk of establishment. By fully understanding the interactions between these components we can improve management of susceptible soils and use this information to identify potential 'hot spots' or risk areas within the vineyard where targeted detection or surveillance should occur. In order to do this however we need to conduct controlled experiments where these and other variablesincluding grapevine type and phylloxera genotype can be manipulated. Preliminary work in this area is described in Chapter 5 of this report.

CHAPTER FIVE

Influence of Soil Characteristics on Phylloxera Genotype Establishment Under Controlled Conditions

Objective: To quantify the risk of phylloxera genotype establishment on different soil types

SUMMARY

The two most widely distributed phylloxera genotypes known to occur in Australia, G1 and G4, were screened over a 3-month period under controlled environment conditions to determine the influence of soil type on phylloxera establishment and vine damage. Both phylloxera genotypes caused root damage in the three soil types tested. Phylloxera soils from the King Valley region supported the establishment of populations of both phylloxera genotypes. Soils from the Rutherglen and Upton regions did not allow the establishment of either phylloxera genotype. Soil chemical and physical properties which may have influenced establishment are described. The relative risk of establishment, development and dispersal is discussed.

INTRODUCTION

Detection of phylloxera by ground surveys (i.e. visual examination of root damage and vine vigour), soil molecular probes, trapping systems or remote sensing are all influenced by a range of variables under field conditions including soil type, climate, vine genotype, vine management and phylloxera genotype. This makes development of a targeted method for phylloxera detection extremely difficult. The insect is most likely to be detected when considerable damage has been done to the ungrafted *Vitis vinifera* L. vine root system by highly virulent phylloxera genotypes, which can spread relatively rapidly. These highly virulent genotypes will have already spread within the vineyard and the risk of transfer and quarantine breakdown will be high as the insect is likely to have been present for several seasons and hence built up high populations prior to detection. Under some soil conditions phylloxera is found at

relatively low levels on ungrafted *V. vinifera* and subsequently spreads relatively slowly causing minimal damage to infested grapevines and taking longer to detect. To examine in more detail the interactions between soil type and phylloxera genotype under controlled conditions a glasshouse trial was established to determine the response of 2 highly virulent phylloxera genotypes to 3 different soil types from different phylloxera infested zones. Soil types and phylloxera genotypes were selected on the basis of known phylloxera abundance and published studies (Powell *et al*., 2003; Herbert *et al*., 2006; Umina *et al*., 2007; Chapter 2).

METHOD

Soil selection

Soils were selected from the A horizons of 3 phylloxera-infested vineyards located at Upton, the King Valley and Rutherglen. The soil type at the King Valley site is described earlier (Chapter 4) and the Rutherglen and Upton soils have been characterised on soil pit descriptions described previously (Powell *et al*., 2006; Fig. 1). At the Rutherglen site, 32 year-old ungrafted *V. vinifera* cv. Cabernet Sauvignon vines have co-existed with relatively low levels of phylloxera for decades without any apparent deleterious effects on the vine. The soil at the Rutherglen site is described as a subnatric brown sodosol, using the classification system of Isbell (1996). Previous studies have shown that phylloxera at the Rutherglen site is comprised of a mixture of genotypes (Corrie *et al*., 2002; Herbert *et al*., 2006) and population studies at the site have shown very low abundance levels (Powell *et al*., 2003a). At the Upton site the phylloxera genotype G1 has established and produces relatively high population levels but relatively slow dispersal (Chapter 2). At the King Valley soil site G4 phylloxera has spread rapidly and reached very high levels (Chapter 2).

Soil was co1lected from the 3 sites between 14th and 22nd March 2007. A shovel was used to collect the soil from a depth range of about 0-25cm, corresponding approximately with the A horizon as described in Chapter 3 and Powell *et al*. (2003b). Soil was taken from 10-12 sample points, within and around the margins of each vineyard block, and mixed to give a representative homogenous soil type. Soil was transported to DPI Rutherglen in sealed containers under quarantine regulations. The soil was disinfested in a steam soil steriliser at 60-70°C for a period of 2 hours to kill any phylloxera or fungal pathogens present.

a) **b)**



Fig. 1. Soil profiles at the (a) Rutherglen and (b) King Valley vineyard sites *Source: Powell et al, 2003b.*

Plant material

The planting material used was own-rooted *V. vinifera* cv. Cabernet Sauvignon (clone LC10) vines. The vines were sourced from a phylloxera exclusion zone in South Australia as 1-year old rootlings, and potted on 26th August 2005 into 4.5 litre plastic pots using 80% sterile potting mix and 20% Perlite. The vines were maintained in a shadehouse at DPI Rutherglen, until commencement of the trial. The vines were brought into a glasshouse on 5th March 2007 in order to acclimatise and prepare for infestation.

Insect material

Phylloxera genotypes for the trial were single-sourced from the Upton (G1 genotype) and King Valley vineyards (G4 genotype) at the field sites described in Chapter 2. Phylloxera populations were developed on excised root bioassay plates using a modified method of De Benedictis *et al.* (1993), to allow production of sufficient eggs for the trial.

Trial design

Potted vines were placed in a single glasshouse room on rows of metal crates. The vines were arranged in a randomised design. Nine combinations of soil type x phylloxera genotype were used as treatments, including uninfested vines as a control. Ten replicates of each treatment and control combination were used. Vines were watered for 2 minutes per day with an automatic dripper system. The glasshouse was illuminated with Son-T Agro 400 light sources for 13 hours (8am to 9pm), plus an additional hour boost at 1am. Temperature was maintained at 24°C during the day (9am to 9pm) and 21°C at night. Relative humidity was maintained at 50% using a Flipperdry™ dehumidifier. Predatory mites were regularly applied to the vines as a biological control measure against mite infestation.

Infestation procedure

Re-potting the grapevines with the three soil types was undertaken on the 30th March 2007. As a result of the vines being in pots for nearly 2 years, the majority were root-bound and required trimming of the root system. Vines were then replanted in pots which were numbered for identification.

Infestation of grapevine roots with the two phylloxera genotypes was undertaken on different days to avoid cross-contamination (G1 infested on 23rd April 2007 and G4 infested on 26th April 2007). The uninfested control grapevines were removed and repotted in the soil treatments on 24th April 2007. To infest grapevines twenty newly laid eggs were placed onto damp filter paper strips (1.5cm x 0.5cm), which were gently wrapped around a single attached lignified root piece (0.5-1cm). The root and eggs were enclosed along with a small quantity of treatment soil into a root enclosure made of 53µm nylon mesh, which was attached around the root with cable ties (Fig.

2). The ends and sides of the enclosure were also sealed with Tanglefoot™ to prevent active phylloxera escaping and cross-contamination between roots. Phylloxera was confined to the root enclosure to facilitate qualitative and quantitative analysis of population development and root damage at the end of the trial. Vines were then carefully re-potted, and pots were placed in 53µm nylon mesh bags sealed around the vine trunk with Tanglefoot™ and cable ties (Fig. 3). The latter measure was an additional protection used to prevent phylloxera spreading to surrounding grapevines should they 'escape' the root enclosure.



Fig. 2. Mesh root enclosure, surrounding an attached lignified grapevine root, used to confine phylloxera life-stages to a small volume of soil and area of root material.



Fig. 3. Mesh bag enclosing pot to prevent cross contamination of phylloxera genotypes

Sequential data collection

After twelve weeks incubation grapevines and soil were removed from the pots and sequential data collection took place based on period since infestation. Vines were removed from the pots and the root enclosure detached by cutting the enclosed root at both ends. The dry weight of whole root system, and above-ground plant parts, was obtained by washing roots to remove soil and cutting the base of the vine trunk at the soil surface. Then both samples were dried at 50°C for 48 hours, prior to weighing. Root enclosures were opened, and the contents washed into a 53µm sieve and root pieces brushed gently with a toothbrush, to collect any phylloxera life-stages present. The sieved contents were collected in water and examined under a low power binocular microscope, and phylloxera life-stages were counted and recorded. The root pieces inside and outside the root enclosure were also examined under the microscope for the presence of galls, in the form of either tuberosities on lignified roots or nodosities on non-lignified roots, which are indicative of phylloxera feeding sites.

Statistical analysis

Analysis was conducted using Genstat for Windows software (Payne *et al.*, 2005). Since the data was not normally distributed life-stage and tuberosity data was analysed using non parametric tests.

RESULTS AND DISCUSSION

Phylloxera establishment

Of the sixty vines infested with phylloxera 17% were found to have live phylloxera life-stages on the root system after 12 weeks. No phylloxera was detected on uninfested control vines. Phylloxera life-stages were only detected in grapevines treated with King Valley soil (Fig. 4). These included 60% infested with G1 and 40% infested with G4. The data indicate that the King Valley soil treatment clearly favoured establishment of both phylloxera genotypes on grapevines, which accords with field observations indicating that phylloxera G4 spreads quickly and caused greater vine damage in the King Valley region than other regions known to be infested in Australia (Chapter 2). To date G1 phylloxera has not been detected in the

King Valley region. However, the potential for its establishment in the soil types of this region is evidently high.

Although both G1 and G4 have been detected in the field in the Rutherglen region (Powell *et al*., 2006) their population levels have only been recorded at very low levels on ungrafted *V. Vinifera,* which suggests that the Rutherglen soils may have some suppressive properties. Interestingly, G1 phylloxera did not establish in vines planted to the Upton soil even though it has been detected in relatively high numbers in the field where the soil was collected. This may indicate that for establishment to occur a higher initial phylloxera inoculum level than twenty eggs may be required. Previous glasshouse studies have shown that the inoculum level used in this study allows good establishment of phylloxera in a potting mixture medium (Powell *et al*., 2006) but when using soils higher phylloxera inoculum levels may be required.

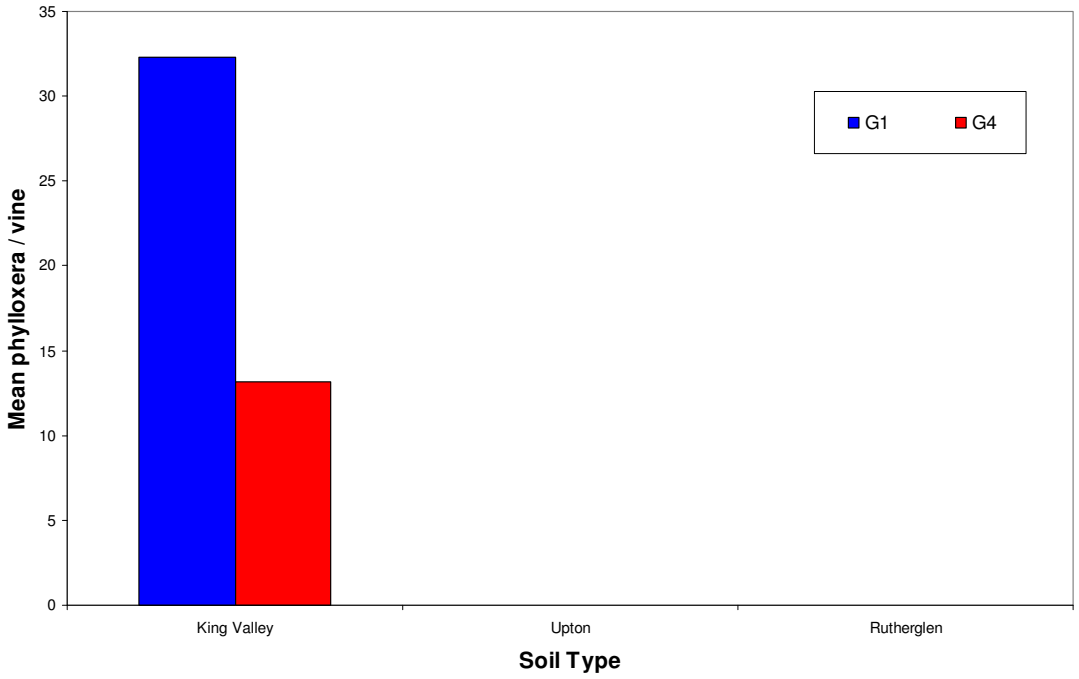


Fig. 4. Mean phylloxera abundance for two phylloxera genotypes, G1 and G4, and three soil types, King Valley, Upton and Rutherglen, after 12-weeks post-infestation on *Vitis vinifera* grapevine roots grown under glasshouse conditions.

Phylloxera associated root damage

Root damage in the form of tuberosities was observed on 81% of all grapevines infested with phylloxera, with the greatest degree of damage observed on soil collected from the Upton site (Fig. 5). However, there was no statistical difference between soil types and tuberosity levels recorded. The lack of phylloxera life-stages in the Upton and Rutherglen soils, despite the presence of tuberosities, suggests that the phylloxera eggs have hatched into crawlers, and commenced feeding, but have not established sufficient populations to survive the 12 week period. Yellow hook-shaped galls termed nodosities, which form in response to phylloxera feeding on non-lignified grapevine roots, were observed in 10% of the infested grapevines, predominantly in the King Valley soil treated vines but also in two of the infested Rutherglen soil grapevines, indicating that some of the root enclosures may have degraded during the trial allowing crawlers to move onto the main root system. The presence of nodosities in Rutherglen soils provide further evidence that phylloxera were initially present, but failed to fully establish colonies on lignified roots. No nodosities or tuberosities were detected in uninfested control grapevines.

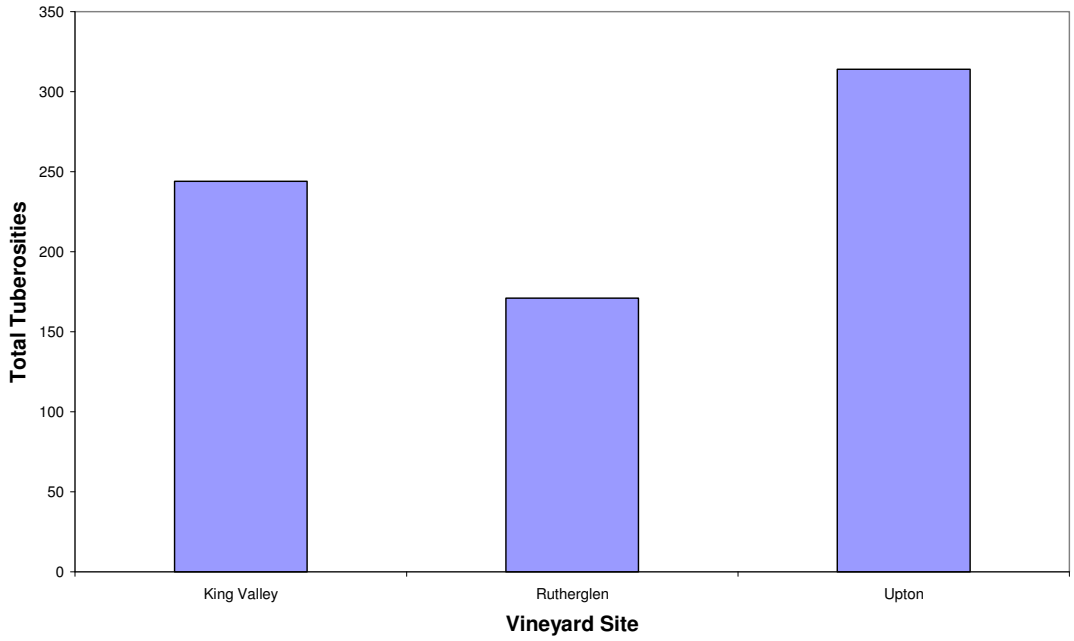


Fig. 5. Mean phylloxera-induced tuberosity development in three soil types, King Valley, Upton and Rutherglen, after 12-weeks post-infestation on *Vitis vinifera* L. grapevine roots grown under glasshouse conditions. Phylloxera genotype, G1 and G4, data combined.

Grapevine assessments

Above- (vine stem and foliage) and below-ground (root system) biomass was assessed after twelve weeks incubation (Figs. 6-7). No significant differences were observed in the dry weights (above- or below-ground) of control vines in the three different soil types. The above-ground dry weight, which includes foliage and stem combined, was significantly higher in the King Valley soils infested with phylloxera compared to infested grapevines in the Rutherglen and Upton soil treatments (Fig.7) and uninfested control vines. However, no significant difference was observed in root dry weights of infested or uninfested vines. This increase in vigour differs to that observed by Herbert (2005) who has shown a decrease in stem weight and leaf area on *V. vinifera* infested with G1 and G4 under glasshouse conditions. However, in Herbert's study, vigour was assessed after an 8 month post-inoculation period of a whole root system of grapevines which were planted in a potting mixture medium. In our trial, assessment was made after 3 months using a lower initial inoculum level, a different inoculation method and different soil conditions. This may explain the different response observed, but is arguably a more realistic comparison to field conditions.

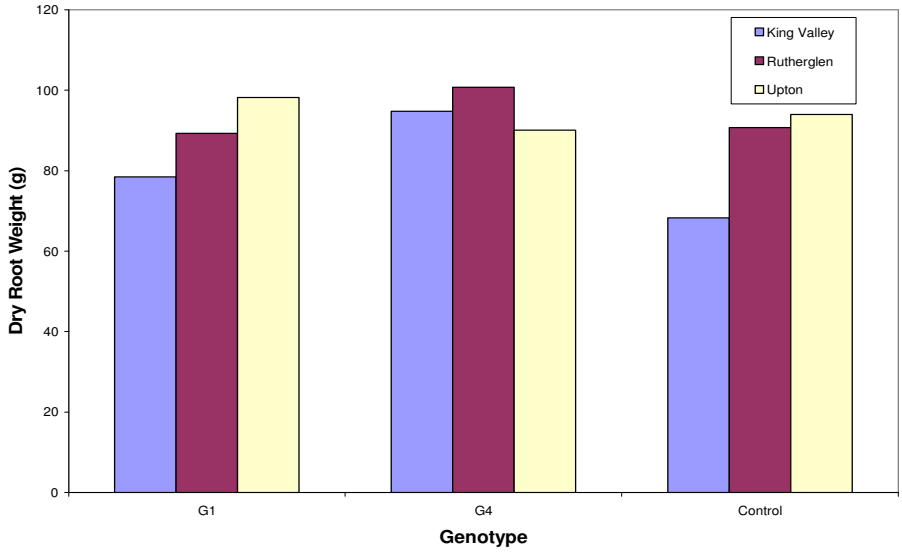


Fig. 6. Grapevine root dry weight after 12 weeks infestation with either G1 or G4 phylloxera genotypes under three different soil types (King Valley, Rutherglen and Upton). The control treatment represents vines not infested with phylloxera.

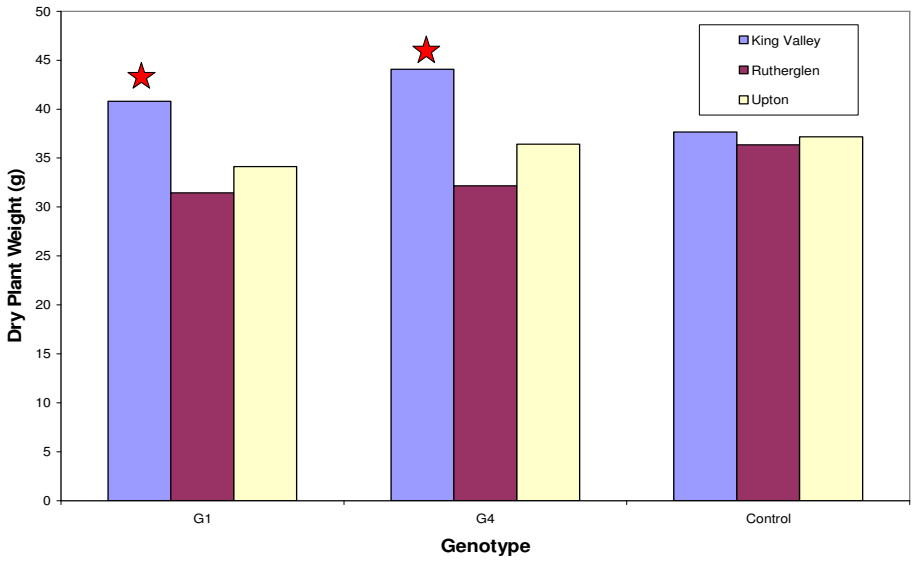


Fig. 7. Grapevine leaf and stem dry weight after 12 weeks infestation with either G1 or G4 phylloxera genotypes under three different soil types (King Valley, Rutherglen and Upton). The control treatment represents vines not infested with phylloxera. Stars indicate statistically significant differences (P> 0.05).

Soil chemistry

Examination of chemical analysis of the three soil treatments (Chapter 4; Powell *et al*., 2006) indicates that exchangeable exchangeable Aluminium capacity levels in the 'A' horizon of the Rutherglen soil were markedly lower compared to both the King Valley and Upton soils (Fig. 8). In addition K/Mg ratio at the Rutherglen site was markedly higher than the King Valley site (Fig. 9). Previous field-based studies have indicated a positive relationship with phylloxera population abundance and Aluminium exchange capacity of vineyard soils (Powell *et al*., 2003b) and a negative relation ship between phylloxera abundance and K/Mg ratio (Reisenzein *et al*., 2007). This could provide some explanation as to why phylloxera could establish on the potted vines in the King Valley soil type but not the Rutherglen soil type. It does not however explain why phylloxera could not establish on grapevines grown in the Upton soil type. Other factors including moisture holding capacity of the soil may be involved but further investigation is required before drawing any firm conclusions.

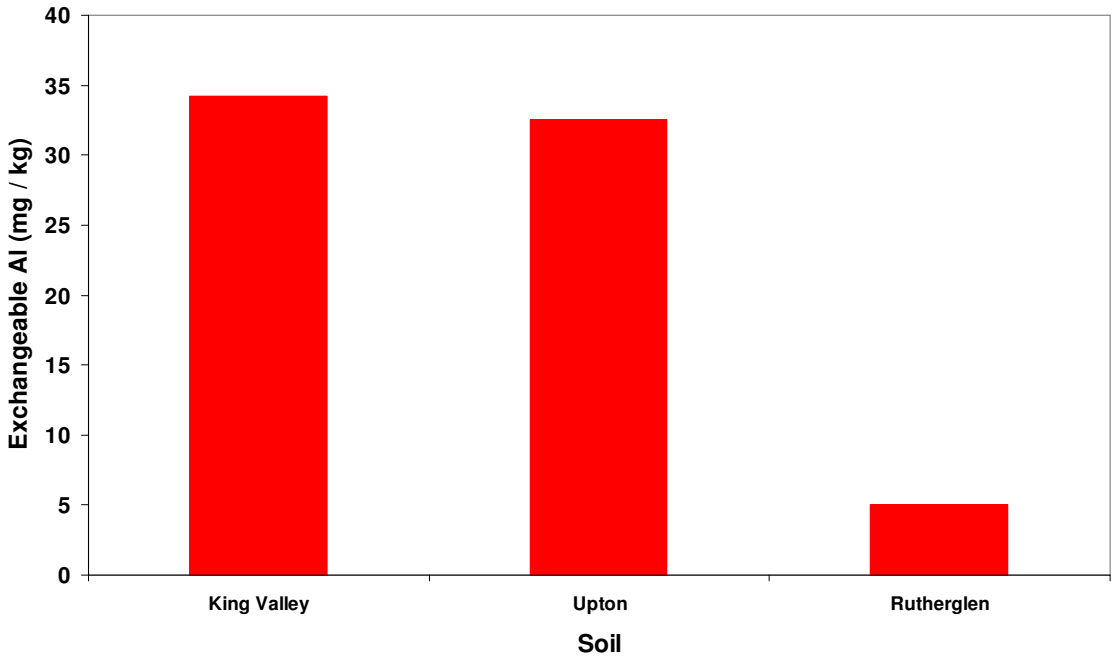


Fig. 8. **Mean Aluminium exchange capacity of three soil types collected from phylloxera-infested vineyards and used in the glasshouse trial.**

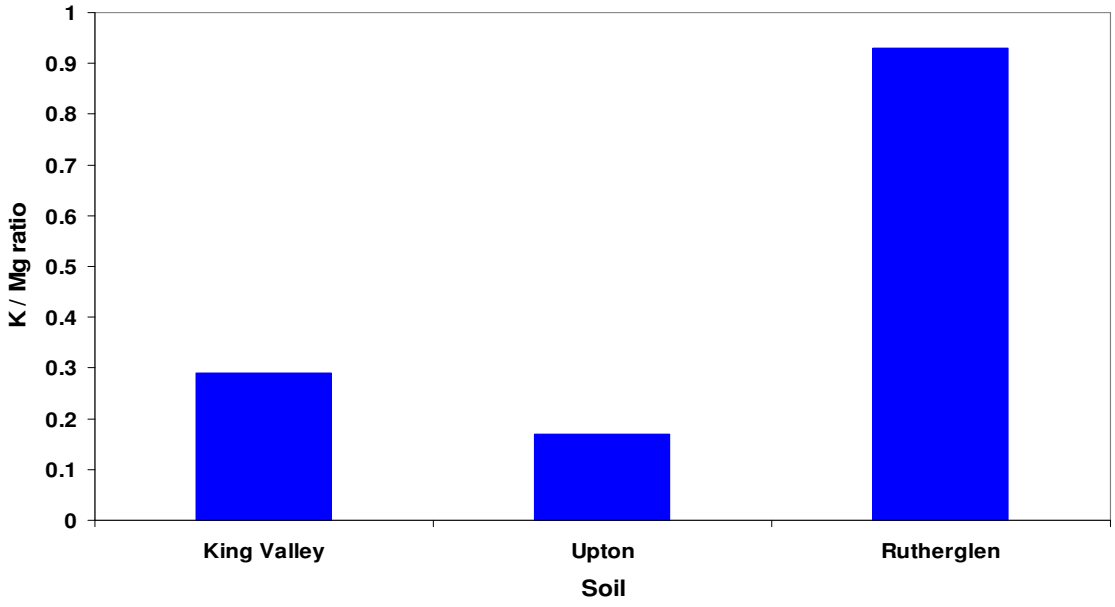


Fig. 9. **Mean potassium:magnesium ratio of three soil types collected from phylloxera-infested vineyards and used in the glasshouse trial.**

DISCUSSION AND RECOMMENDATIONS

Most studies on phylloxera soil interactions have been conducted under field conditions and focus either on soil samples taken to assess the distribution of phylloxera (Nougaret *et al*, 1928) or root or trap surveys (Powell *et al*., 2000; 2003a). There are very few studies under controlled conditions to assess the impact of soil type on phylloxera population development. The effects of soil type on phylloxera establishment under glasshouse conditions has recently been examined by Chitowski *et al*. (2005). In their study six vineyard soils and two artificially prepared soils were examined when infested with two different phylloxera populations over a 12-week period. Their study did not involve DNA typing the phylloxera populations so they may have been working with mixed genetic populations. It is very important to determine the genetic lineage of the phylloxera as it is known that population development is influenced by which genetic strain is being used. The method used for infestation and post-trial sampling also differed as they used 60 eggs per potted vine (i.e. three times the rate of our treatment inoculum), did not use root enclosures or quarantine mesh bags to prevent cross contamination between pots and sampled over a three week period rather than a single time point.

One factor which could have influenced our results is potential drying and/or soil compaction due to the water regime used, which was the same for all treatments even though the water holding capacity of the three soils would have been different. A large proportion of our treated vines were found to be not supporting a phylloxera population at the end of the 12-week period. Was this due to soil suppressive properties, the impact of compaction or soil moisture or a relatively low initial phylloxera inoculation level? Several authors suggest that under dry conditions phylloxera is more abundant (Helm *et al*., 1991); whereas in the pots we examined from the King Valley the soils appeared relatively moist and had higher phylloxera population levels. Further analysis of the impact of soil moisture on phylloxera establishment is therefore required. Our inoculum level of twenty phylloxera eggs per pot was considered adequate as in previous studies where a mixture of potting mixture and Perlite™ were used this level of inoculum was sufficient for good phylloxera establishment. The risk of transfer levels above this are considered likely.

Clay loam and clay-based soil have relatively small pore sizes and higher moisture potential and therefore poorer aeration (limited oxygen supply) than sandy soils. Because pore spaces are small in clay soils movement of small pests could also be restrictive. Whether this had an influence on phylloxera establishment in this trial is unclear. The Upton A horizon soil does have relatively high sand content which could have restricted establishment. Interestingly these features have been highlighted as the reason why some entomopathogenic nematodes have higher survival and activity in sandy soils compared to clay soils (Kung *et al*., 1990).

Soil textural and chemical characteristics clearly do play some role in phylloxera infestation or risk of establishment. However other factors could also influence the insect-host plant interactions and physiology of both pest and host including water and nutrient availability, rootstock variety, vine health and vine management (in particular irrigation and fertigation) and climate.

CHAPTER SIX

Phylloxera Education and Awareness Activities

Objective: To facilitate knowledge transfer on research activities through presentations at the Annual National Phylloxera Management Workshops and related extension and awareness activities

SUMMARY

A key component of any research project is to ensure that research outputs are disseminated to a diverse range of stakeholders in a timely, accessible and coordinated manner. Throughout the project period, October 2005-September 2007, communication activities related to this project encompassed a range of activities including written through industry publications and conference proceedings and orally through international and national conferences and annual phylloxera workshops. As a result of phylloxera outbreaks in the Yarra Valley and Murchison in 2006 additional extension and awareness activities were conducted.

INTRODUCTION

Communication activities were conducted throughout the course of the project both orally through presentations at a National and International level and in a variety of written articles.

1. Annual Phylloxera Workshops and Grower Forums

Annual Phylloxera Identification and Management Workshops (Korosi *et al*., 2005) aim to inform viticulture industry personnel of ways to manage phylloxera and highlight protocols to reduce the risk of phylloxera movement to uninfested vineyards and regions. Annually the workshop format predominantly followed a one–day format, with the exception of a two-day bus tour format for South Australian

participants which was organised in conjunction with the Phylloxera and Grape Industry Board of South Australia. Two major components of the workshop format were "**Early detection and surveillance"** and **"Risk Management"** which relate directly to the outputs of this project**.** Stakeholders were provided information on progress and outputs of the project though delivery of oral presentations by project staff at six phylloxera workshops conducted in Nagambie, Murchison, Rutherglen, Milawa, King Valley and Yarra Valley. The workshops were publicised annually through industry publications, local and interstate newspapers, mail-outs to regional organisations, ABC Radio, and through the Department of Primary Industries-Victoria facilitated Grapecheque groups. Workshops included oral presentations on Phylloxera surveillance and detection and influence of soil factors on population abundance, establishment and dispersal, and facilitating practical training in phylloxera detection for both growers and survey team personnel. In addition phylloxera research updates were provided at 4 grower forums at the request of the growers in the Yarra Valley and Mornington Peninsula following the outbreak of phylloxera in the Yarra Valley in December 2006.

Workshop Timetable

- **February 2006** a two-day Phylloxera Workshop, held at DPI Rutherglen and the King Valley in August 2007, in conjunction with the Phylloxera and Grape Industry Board of South Australia (total of 30 participants).

- **January 2007** a two-day Phylloxera Identification and Management workshop at Yarra Valley, Glenrowan and DPI Rutherglen (total of 26 participants mainly from South Australian and Victorian wine regions).

- **January 2007** a one-day workshop on Phylloxera Identification and Management, held at Murchison, included vineyard training in phylloxera surveying for a total of 21 participants from Victoria.

- **August 2007** a two-day Phylloxera Workshop, held at DPI Rutherglen and the Yarra Valley in, in conjunction with Phylloxera and Grape Industry Board of South Australia (total of 25 participants) for Grape Liaison Officers.

Survey team training

- **January 2006** a one-day Phylloxera Workshop and Ground Survey training exercise for Queensland Phylloxera Survey team leaders held in Milawa and the King Valley (5 participants).

- **November 2006** Training in phylloxera surveying techniques for Victorian Ground Survey teams held at Murchison (20 participants).

Grower Forums

- **December 2006 – January 2007** presentations and open discussion forum given by project leader at phylloxera updates for the Yarra Valley Growers groups at Swinburne TAFE (150 participants), Healesville (70 participants), Healesville (25 participants) and Mornington Peninsula (55 participants).

2. National and International conference presentations

Presentations related to specific outputs of this project were made at the following conferences and meetings:

i. National Industry Conferences

- **July-August 2007.** 13th Australian Wine Industry Technical Conference, Adelaide, South Australia.

Poster Presentations (project team members in **bold**):

Bruce R.J., Mackie A.M., Lamb D.W., Runting J., **Powell K.S.** (2007) *Development of a targeted surveillance approach for detection of grapevine phylloxera Daktulosphaira vitifoliae.*

Powell K.S., Korosi G.A., **Mackie A.M.** (2007) *Monitoring grape phylloxera outbreaks using simple non-destructive trapping systems.*

Oral presentation:

Powell, K.S. (2007) *Grapevine phylloxera – early detection using non-destructive techniques.*

ii. National Scientific Conferences

- Australian Entomological Society 38th AGM and Scientific Conference, 24/9/07 to 26/9/07 at Beechworth.

Oral presentation (project team members in **bold**):

Mackie A.M., Powell K.S. (2007) *Grape phylloxera development in different soils under controlled conditions*

- *National Phylloxera Technical Reference Group meetings*

Regular project updates were made to the NPTRG during 2006 and 2007 meetings.

iii. International Scientific Conferences

- [1]Plant Protection Office, Wageningen, The Netherlands, 17th September 2007.

- [2]Fourth International Phylloxera Symposium in Keselthy, Hungary 20-21st September 2007.

Oral presentations:

[1]**Powell, K.S.** (2007). *Grapevine phylloxera - Detection and protection from the world's worst grapevine insect pest*

[2]**Powell K.S**., Korosi G.A., Trethowan C.J., **Mackie, A.M.** (2007). *Monitoring grape phylloxera populations using simple non-destructive trapping systems.*

Poster Presentation:

[2]**Bruce R.J., Mackie A.M., Lamb D.W., Powell K.S.** (2007) *Development of a targeted surveillance approach for detection of grapevine phylloxera Daktulosphaira vitifoliae.*

3. Media Activities

Phylloxera awareness and research activities have also been highlighted with members of the research team being interviewed for local radio channels (ABC radio – 2007) and regional newspapers including the Border Mail, Corowa Free Press, Weekly Times and Wangaratta Chronicle.

4. Publications

Selected industry and scientific publications produced during the course of the project include the following:

i. Industry Articles

Powell K.S., Herbert K.S., Hoffmann A.A. (2007) *Grapevine Phylloxera – Opportunities for monitoring and early detection using non-destructive techniques. Australian Viticulture* 11(6): 50-53.

ii. Scientific journal articles

Bruce R.J., Mackie A.M., Lamb D.W., Powell K.S. (2007) *Development of a targeted surveillance approach for detection of grapevine phylloxera Daktulosphaira vitifoliae*. Submitted to Acta *Horticulturae* - November 2007.

Powell K.S., Korosi G.A., Trethowan C.J., **Mackie A.M**. (2007). Monitoring grape phylloxera populations using simple non-destructive trapping systems. Submitted to *Acta Horticulturae* - November 2007.

5. Team Awards and Recognition

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

October 2006 - Team Finalists in Daniel McAlpine Outstanding Achievement Award - DPI Science Awards.

October 2007 - Winners of the Daniel McAlpine Outstanding Achievement Award - DPI Science Awards – for holistic approach to phylloxera management. Award ceremony at Presentation at Melbourne Museum.

APPENDIX 1

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