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Assaying the potential benefits of thiamethoxam and imidacloprid for phylloxera suppression and improvements to grapevine vigour

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ABSTRACT

Grape phylloxera (*Daktulosphaira vitifoliae*, Fitch) is an encroaching problem for the Australian wine industry with four new outbreaks recorded in the past 5 years. Whilst quarantine- and phylloxera-resistant rootstocks are the recommended long-term management strategies, there are at present no short-term options for recently infested vineyards. In this study, an assay for assessing the impact of the systemic insecticides thiamethoxam (Actara®; two applications at a concentration of 2000 ppm active ingredient) and imidacloprid (Confidor®; one and two applications at a concentration of 2000 ppm active ingredient) on suppression of phylloxera populations on grapevine roots was developed. Results from *in vitro* and *in planta* trials showed that both insecticides resulted in reduced phylloxera numbers and improved vine vigour. Imidacloprid was found to have a greater effect on phylloxera suppression and vine vigour improvement. Therefore imidacloprid has the potential to be successfully used for phylloxera suppression in the field, but will require further validation under field conditions before recommendations can be made. The assay developed provides a screening procedure for future chemical testing against phylloxera.

1. Introduction

Grape phylloxera, *Daktulosphaira vitifoliae*, has been a devastating pest of grapevines around the world and in Australia since 1877 (Adcock, 1902). Australia uses a combination of early detection, quarantine and management to restrict the movement of phylloxera from within a phylloxera-infested zone (PIZ) to outside a PIZ. Resistant rootstocks are the only recommended long-term strategy for phylloxera control and have been used since the early 1900s (Campbell, 2004). Resistant or tolerant rootstocks are bred from grapevines of American *Vitis* spp. where, unlike the susceptible *Vitis vinifera*, phylloxera feeding is restricted to the fibrous roots and leaves of the grapevine and does not affect vine health (Granett et al., 2001). While the exact mechanisms of resistance are poorly understood (Granett et al., 2001; Kellow, 2001), this fundamental difference between grapevine parentage has enabled the economic production of *V. vinifera* in phylloxera-infested regions. Nevertheless, Australia has seen a low adoption rate of rootstocks, with greater than 85% of vineyards planted on ungrafted *V. vinifera*, due to a combination of establishment costs, availability and additional management factors associated with grafted grapevines (Buchanan, 1990).

Phylloxera is found in a total of two per cent of Australian vineyards, restricted to five declared PIZs in Victoria and two PIZs in New South Wales. In order to prevent the spread of phylloxera to new regions, the success of the quarantine procedures is limited not only by detection and management options but also by the goodwill of people to uphold the National Phylloxera Management Protocols (NVHSC). This often difficult task, coupled with an increasing number of vineyard plantings each year, has not surprisingly seen breakdowns in quarantine as recently recorded in Victoria in the Upton (April 2000) and Buckland Valley (December 2003 and January 2004) and Yarra Valley (January 2006) regions. These outbreaks highlight the need for on-going research into alternative management options.

Alternative strategies previously explored for phylloxera control include methods such as the use of chemical insecticides (Cox et al., 1960; de Klerk, 1979; Williams, 1979; Rammer, 1980; Loubser et al., 1992; Weber et al., 1996), nutrient and irrigation management (Kopf et al., 2000), organic vineyard management (Lotter et al., 1999; Powell et al., 2003) and more general integrated phylloxera management (Granett et al., 2001).

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Chemical management remains the best potential candidate for short-term phylloxera control because, unlike other alternative management options, it can be rapidly implemented to potentially suppress phylloxera populations as well as to maximise vine vigour. Historically, however, chemical control methods have proven to be inefficient or ineffective. Chemical control measures, such as the use of carbon bisulphide, date back to the 1800s (Campbell, 2004) and trials conducted since this date in countries such as Australia, New Zealand, Europe and the USA have met with limited success (Coombe, 1963; Boubals 1966; de Klerk 1979; Rammer, 1980; King et al., 1983, Granett et al., 1986; Buchanan and Godden, 1989; Weber et al., 1996). While in some cases a reduction in phylloxera populations was achieved (Buchanan and Godden, 1989; Weber et al., 1996), conditions for plant recovery have not been attained or the environmental impacts were later deemed to be highly toxic to the environment and animals. The current constraints to available insecticide effectiveness (as reviewed in Granett et al., 2001) are due to (a) poor penetration of the given chemical through the soil to the vine roots; (b) phylloxera's potential for rapid population growth (a capacity that allows this insect to build up rapidly from a comparatively low population reservoir); and (c) slow repair of vine damage by phylloxera-infested vine roots, that ultimately may not be able to revert to a healthy status even after phylloxera numbers have decreased.

Recent improvements in the neonicotinoid chemical class of insecticides used against sap-sucking insects such as aphids have a mode of action that have a systemic action (i.e. the insecticide is taken up by roots or absorbed through the leaves into the plant). The benefits of using systemic insecticides over contact insecticides is that in most cases they provide continuous plant protection through most of the growing season without the need for repeat applications. In addition, systemic insecticides are not susceptible to ultraviolet light degradation or "wash off" during watering and minimised risk of overexposure to applicators.

Systemic neonicotinoid-based insecticides kill insects via ingestion or contact and mimic the action of acetylcholine in the nerve synapse causing tremors, loss of coordination and eventual death (Gourment et al., 1994; Boiteau and Osborn, 1997). Recent improvements in the neonicotinoid chemical class of insecticides against sap-sucking insects, such as aphids, have seen a number of chemicals registered in the USA for phylloxera suppression (J. Granett, pers comm.). Field tests using both upwardly mobile and downwardly mobile systemic neonicotinoid insecticides by Omer and Granett (unpublished data) and Kocsis (unpublished data) significantly suppressed phylloxera populations in the vineyard (Granett et al., 2001). Futhermore, field tests in the USA using neonicotinoid-based insecticides have been shown not only to reduce phylloxera populations but also, more importantly, to provide the potential for grapevines to improve their root system while in the presence of the pest. Presently, the performance of these chemicals against Australian phylloxera genotypes under Australian conditions is unknown (Corrie et al., 2002).

This study was aimed at designing an *in planta* glasshouse-based assay to assess the effectiveness of chemicals in suppressing root-galling phylloxera populations and associated vine damage. The assay was tested with two systemic neonicotinoid insecticides previously not tested for phylloxera control in Australia.

2. Materials and methods

2.1. Glasshouse-based population trial

This experiment tested the development of phylloxera in a glasshouse assay to determine the timing of pesticide applications. Please note that all the glasshouse trials conducted (2.1–2.3) involved true biological replicates, such as fresh insecticide batches, grapevines and phylloxera populations. For this first glasshouse trial (2.1), 1-year-old grapevine (*V. vinifera* cv. Sauvignon Blanc clone FVH5V10) rootlings were obtained during April 2001 from Sunraysia Nurseries (Reg. No. N627) at Gol Gol, NSW, Australia. Rootlings were potted into autoclaved 20 cm plastic pots using a sterilised soil–perlite composite (80% potting mix, 20% perlite) and transferred to a controlled temperature glasshouse prior to phylloxera inoculation. The primary cane was pruned to 15 nodes with secondary, and subsequent canes removed so that each vine was standardised for the experiment. The trial was conducted over a 6 month (164 days) period in a temperature-controlled glasshouse, cycling between $24 \pm 2\,^{\circ}C$ (600–1800 h) and $20 \pm 2\,^{\circ}C$ (1800–600 h). This temperature range was optimal for both vine and phylloxera development during the course of the trial. Tinytag Ultra dataloggers were used to monitor temperature at 15 min intervals. A breakdown in the heating during days 139–140 resulted in mean temperature dropping between 6 and 10 °C before returning to previous levels. An automatic dripper watering system was established. Vines were watered once a day, until the pots just started to drain (equivalent to field capacity), with adjustments made as water use increased during the season. Growth lights were on for 12 h each day (0600–1800 h), with an additional spike from 000–100 h to offset dormancy due to changes in day length.

A randomised design was used to examine the effects of grapevine phylloxera infestation on *V. vinifera*. The trial comprised of two treatments (infested and uninfested grapevines), randomised over a 3×3 grid. The grid comprised of nine upturned wire crates, with three vines at random per crate. To prevent contamination of uninfested vines with phylloxera, pots were enclosed in individual 45 mm × 35 mm polyester/polyamide drawstring bags with an aperture size of 53 μm (Fig. 1). The root system of every vine was encased in this bag, which was secured to the vine trunk using a plastic cable tie. Tanglefoot™ was applied to the neck of the bag and around the cable tie as a preventative measure to prevent phylloxera escape.

A single G4 phylloxera genotype was selected for this study as it is one of the two most commonly found phylloxera genotypes present in Australia (Corrie et al., 2002). This genotype has been found to cause high-level damage to *V. vinifera* in the field. Insects were collected from a commercial vineyard in the King Valley region (within the north-east PIZ) and reared *in vitro* on excised root pieces using the method outlined by Granett et al. (1987). One hundred phylloxera eggs were placed on moistened filter paper (10 cm diameter) discs. The roots of the vines were exposed and the filter paper was placed in contact with the root system. The vines were then placed back into their respective pot and bagged (note that uninfested vines were bagged prior to the phylloxera infestation of the above-mentioned vines to ensure they remained phylloxera free). This point marked the beginning of the time course trial.

Two infested and two uninfested vines were destructively sampled at 40, 58, 79, 101, 122, 144 and 164 days post-infestation with phylloxera. At each sample date, infested vines were removed from their pots and the root system scored for the level of phylloxera damage (as per Boubals, 1966). Uninfested vines were also examined to ensure that no contamination had occurred. Root, shoot and leaf mass measurements were determined for each vine. Leaf area was also measured using a Paton™ electric planimeter. Three representative sub-samples (approximately 2 g wet weight) were taken from the root system of each vine and washed through a 60 μm mesh brass sieve with the collected filtrate containing phylloxera washed into a screw-top plastic container containing 70% ethanol. Phylloxera life-stages,

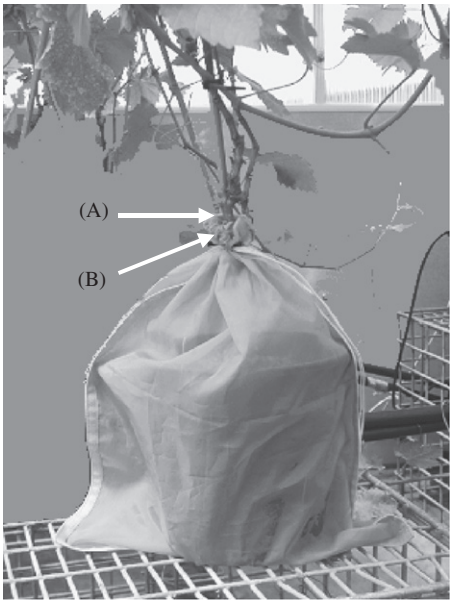

Fig. 1. Drawstring polyester/polyamide bags with 53 μm mesh size used to contain phylloxera genotypes in individual pots. (A) Site of Tanglefoot™ application at neck of bag and (B) plastic cable tie used to secure bag to vine trunk.

categorised as eggs, crawlers or 1st instars or post-crawlers (2nd, 3rd, 4th instars and apterous/alate adults), were determined using a dissecting microscope with a × 10 objective. To adjust phylloxera numbers for the amount of root tissue sampled, root pieces were oven dried and weighed, and phylloxera numbers expressed per gram of root tissue.

2.2. Effect of insecticides on eggs/crawlers

These trials were undertaken to assess the impact of different insecticide concentrations on phylloxera egg viability and subsequent crawler emergence. Egg viability (or crawler emergence) was used, rather than direct assessment of later life-stage development, due to the fact that phylloxera is extremely sensitive to changes in environment and is known to suffer high mortality rates when handled (K. Herbert, personal observation). It is also difficult to follow the fate of known numbers of insects on whole plants, or to test survival of insects in excised root bioassays because in some instances these can fail to adequately support phylloxera populations as well as healthy root tissue.

The thiamethoxam tested was Actara® (containing 250 g/kg of active ingredient, Syngenta Australia, North Ryde, NSW) and the imidacloprid tested was Confidor® (containing 0.25 g/kg active ingredient, Bayer CropScience, East Hawthorn, Victoria). Based on unpublished overseas laboratory results (K. Powell, pers comm.), rates tested for both chemicals were 250, 500, 750 and 2000 ppm active ingredient, as well as a water control. Five hundred G4 (confirmed by genotype studies by P. Umina, La Trobe, University, Victoria) phylloxera eggs (1–3 days old) were harvested from excised root bioassays and placed on moistened filter paper discs in groups of 20. Each replicate was transferred to a Millipore™ filtration apparatus (glass microanalysis system with stainless-steel support) fitted with 60 μm nylon filter and rinsed briefly with distilled water from a wash bottle. Insecticide solutions (or water control) were prepared independently for each replicate. Insecticide (or water) was drawn through the filter using a hand vacuum pump (Millipore™) immersing the eggs in solution. Eggs were immersed for a period of 3 min and the solution was then drained under vacuum. Treated eggs were again rinsed briefly in distilled water and transferred from the nylon filter to new moistened filter paper discs, placing eggs evenly spaced along the midline. Papers discs were placed inside 20 cm petri-dish containers and sealed with Parafilm™ and monitored twice daily over a 7-day period for egg viability. Crawler mortality was assessed visually using colour and observations of crawler mobility. Mobile crawlers or crawlers observed greater than 2 cm from the midline were scored as viable. Crawlers observed less than 2 cm from the midline, including those crawlers not fully emerged from their corresponding egg case, or eggs that appeared black in colour (having not hatched), were scored as non-viable.

2.3. Glasshouse-based insecticide screening

In this experiment insecticide effects were tested in the glasshouse screening assay. Sixty grapevine (*V. vinifera* cv. Sauvignon Blanc) rootlings were sourced and established and infested with G4 phylloxera as above. Results from the mean hatch rate trials determined a concentration of 2000 ppm as the optimal concentration for both insecticides. There were four treatments in total, with three replicates per treatment per sampling period: (1) single imidacloprid (Confidor®) applications at a concentration of 2000 ppm active ingredient; (2) double imidacloprid applications at a concentration of 2000 ppm active ingredient; (3) double applications of thiamethoxam (Actara®) at a concentration of 2000 ppm active ingredient; and (4) no insecticide application (control). The timing of the insecticide application was determined from the previous population dynamics study described earlier. These were at 80 days following initial infestation (first application) and 100 days following initial infestation (second application) at a rate of 500 ml per vine using a small watering can. Controls were watered with 500 ml distilled water. The destructive vine sampling intervals approximately followed the nominated intervals used in the previous glasshouse population study. That is, there was one initial vine sampling period after infestation at 79 days (post infestation) followed by the first application of insecticides at 80 days, destructive vine sampling at 98 days, the second application of insecticides at 100 days, followed by vine sampling at 122 and 143 and 164 days post-infestation, respectively. The duration of the insecticide trial was 42 days less than the duration of the population trial (164 days versus 204 days).

2.4. Analysis

All analyses were carried out with SPSS Version 11.5 on data initially tested for normality with Kolmogorov–Smirnov tests and transformed where appropriate. For the first glasshouse experiment, numbers of phylloxera life stages per gram were log (x+1) transformed for normality and then compared over time with one-way ANOVAs. Vine measurement data (untransformed) were assessed with two-way ANOVAs which included time of sampling and phylloxera infestation as fixed factors.

For the egg crawler data on root pieces, we analysed the number of eggs that had successfully hatched and the crawlers that had died by the last day of scoring. Both the proportion of eggs that had hatched and the proportion of crawlers that had died were angular transformed before analysis. One-way ANOVAs were used to assess whether the controls and the different concentrations of pesticides had a significant effect on egg hatch and crawler mortality.

For glasshouse trial on pesticide effects, counts of the egg phylloxera stages were normally distributed without transformation and analysed by ANOVA with time of sampling and the pesticide treatments (control, thiamethoxam, 1 × imidacloprid,

2 × imidacloprid) as fixed factors. For the crawler and post-crawler numbers, data were log (x+1) transformed before analysis. The design was not completely balanced because the 2 × imidacloprid treatment was not sampled on the first day. Vine morphology was also assessed by two-way ANOVAs on untransformed data.

3. Results

3.1. Glasshouse-based population trial

A summary of mean life-stage numbers, taken from subsamples of individual vine roots, is shown in Fig. 2. The numbers of eggs and crawlers increased in the first seven sampling periods, apart from the 143-day sampling date. A breakdown in the heating system 2 days prior to this sample date probably accounted for the low numbers at this time. Numbers peaked for egg and crawler life stages at day 164 and then decreased for the two remaining sample dates. One-way ANOVAs indicated significant effects of infestation time on both egg numbers and crawler numbers ($F_{(8,9)} = 6.79$, $P = 0.005$, $F_{(8,9)} = 7.58$, $P = 0.003$, respectively) and the combined post-crawler life-stage numbers also changed significantly over time ($F_{(8,9)} = 5.54$, $P = 0.01$).

There were significant effects of phylloxera infestation on all measures of vine morphology (Table 1). For leaf area there was a significant overall effect of time and phylloxera infestation and a marginally significant interaction between these two factors. For whole root weight and stem weight, significant time and treatment effects were also recorded but there was no interaction between these two factors. Phylloxera infestation significantly decreased leaf area, root and stem weight (Fig. 3). The effects of phylloxera infestation were evident on leaf area and root weight assessments sooner than effects on stem weight; control and treated vines differed significantly in one-way ANOVAs for leaf area and root weight assessments 79 days after infestation, compared to 101 days after infestation for mean stem width.

These results were used to identify optimum application timings for tested pesticides. Insecticide treatments would need to be applied before 164 days after infestation, prior to the rapid increase in phylloxera numbers. Therefore insecticide applications (as indicated with arrows in Fig. 2) were subsequently applied at

Table 1
Results of two-way ANOVAs testing the effects of phylloxera infestation and time of sampling on vine morphology variables in the first glasshouse assay

Vine measurement	Effect	df	MS	F	P
Leaf area (cm^2)	Time	8	1302.065	26.970	<0.001
	Infestation	1	2422.772	50.184	<0.001
	Interaction	8	127.048	2.632	0.042
	Error	18	48.278		
Stem weight (g)	Time	8	54.096	6.164	<0.001
	Infestation	1	112.148	12.779	0.002
	Interaction	8	19.516	2.224	0.076
	Error	18	8.776		
Root weight (g)	Time	8	1781.489	49.210	<0.001
	Infestation	1	691.865	19.112	<0.001
	Interaction	8	32.560	0.899	0.537
	Error	18	36.201		

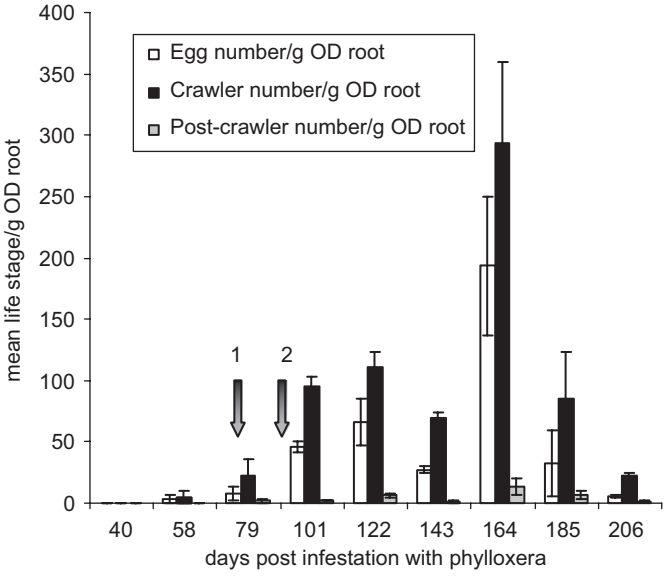

Fig. 2. Mean numbers of phylloxera life stages per oven dried (OD) gram of root sampled. Post-crawler life stages refer to 2nd–4th instars and adults that were pooled. Arrows indicate estimated optimum timing of insecticides at around days 80 and 100 post-infestation.

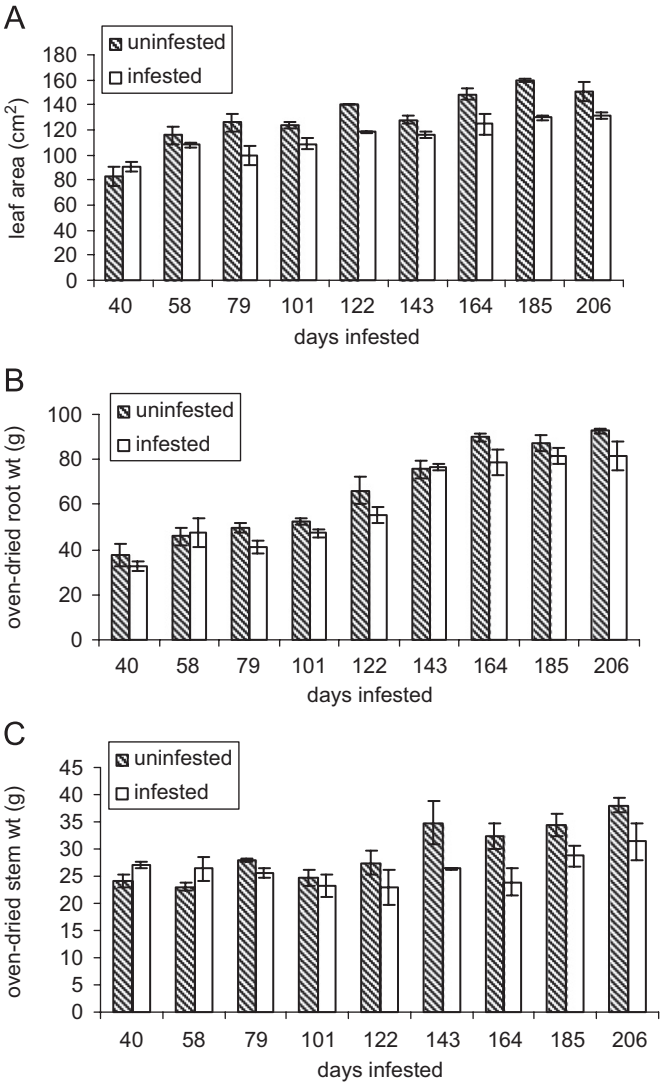

Fig. 3. Differences in: (A) mean leaf area (cm^2); (B) mean root weight; and (C) mean stem weight (g) of phylloxera-infested grapevines recorded at nine sampling periods.

day 80 and (in the case of two applications) also at day 100 after phylloxera infestation.

3.2. Effect of insecticides on eggs/crawlers

Pesticide concentrations did not significantly influence egg hatch rates in the thiamethoxam treatment ($F_{(4,25)} = 2.05$, $P = 0.118$) but did influence hatch rates in the imidacloprid comparison ($F_{(4,25)} = 14.49$, $P < 0.001$). Hatch rates decreased to 70% in the 500 ppm treatment but high in the controls and the 2000 ppm treatment (Fig. 4). The reason for this decrease at intermediate concentrations is unclear.

Crawler mortality was influenced by pesticide concentrations for both the thiamethoxam ($F_{(4,25)} = 53.25$, $P < 0.001$) and imidacloprid ($F_{(4,25)} = 104.92$, $P < 0.001$) treatments. For both chemicals, crawler mortality increased with concentration of the pesticides (Fig. 4), with a significant difference between 750 and 2000 ppm concentrations ($F_{(2,11)} = 10.49$, $P = 0.001$). A significant difference between single and double imidacloprid applications was also found ($F_{(5,25)} = 105.25$, $P < 0.001$). At 0 ppm, crawler mortalities of 16% (thiamethoxam) and 13% (imidacloprid) were observed, indicating that some crawlers died even when water was applied. It is possible that the combined egg transfer and treatment procedure in the filtration apparatus may have affected egg viability; however, phylloxera is known to exhibit high crawler mortalities (Granett et al., 1997). Because both chemicals produced relatively higher mortalities at the highest concentration, 2000 ppm was selected for the glasshouse *in planta* trial. This concentration was within the manufacturer's recommended concentrations; higher levels were not tested to minimise toxicity and residual levels.

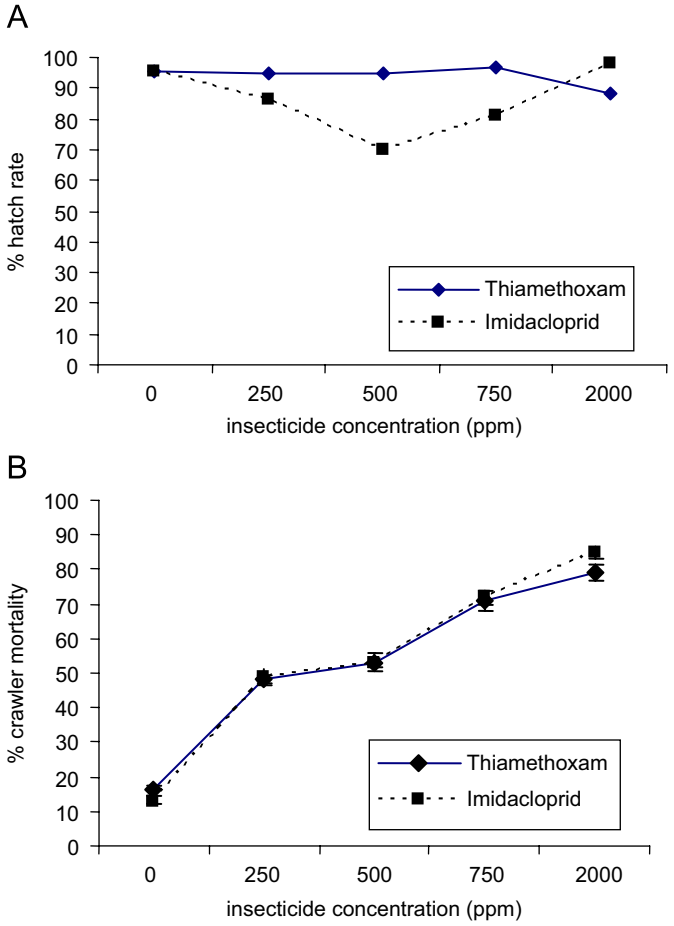

Fig. 4. Mean hatch rate and percentage crawler mortalities following thiamethoxam and imidacloprid application after a 7-day period. For the control treatment (0 ppm), eggs were treated with distilled water. (A) Hatch rate and (B) crawler mortality.

3.3. Glasshouse-based insecticide screening

The mean numbers of each life stage observed (Fig. 5) were lower than in the initial experiment to determine the timing of pesticides (Fig. 2). The reason for this difference is not clear, but might reflect minor changes in root development as a consequence of different vine planting material sourced from the nursery in different years. Nevertheless, the chemical treatments had clear-cut effects on phylloxera. There was no significant effect of the insecticide treatments on the egg life stage (Table 2), but insecticide treatments influenced the number of crawler and post-crawler stages ($P < 0.001$), and there were significant effects of sampling time for both these stages but no interactions between time and treatment (Table 2).

The insecticide treatments markedly reduced the abundance of phylloxera on the roots (Fig. 5). Imidacloprid treatments were particularly effective, although there was no detectable effect of two imidacloprid applications compared to a single application. Pesticides altered the relative number of crawlers and post-crawler life stages when compared to the controls. With no pesticide application, the proportions of egg and crawler life stages were fairly uniform across the sampling periods, but for the imidacloprid treatment the proportion of eggs almost doubled, while crawler numbers were reduced more than 50% after 122 days following infestation (data not shown). Overall the imidacloprid treatments reduced the number of crawlers and later stages by more than 90% at 143 days after infestation.

While vine morphology tended to vary across the treatments, there were overall significant effects of time and treatment on leaf area and root mass but no interaction between these factors (Table 2). Pesticide treatments and in particular the double imidacloprid treatment led to a larger leaf area and a larger root mass (Fig. 6). The amounts of fibrous root growth for the imidacloprid treatments were much more abundant compared to vine roots that received no insecticide application. Stem weight was not influenced by insecticide treatment (Table 2) although there was a tendency for the insecticide treatments to have a higher stem weight particularly for the double imidacloprid treatment.

4. Discussion

This study represents one of the few published attempts to investigate chemical effects on phylloxera in under controlled laboratory conditions. The egg hatching bioassay was similar to the one outlined by Granett et al. (1997). However, in their assay, crawler mortality and life-stage counts were assessed via an excised root bioassay method. Results from the egg bioassay and glasshouse trials indicate that Confidor® but not Actara® significantly affected egg hatch at 500 ppm. It has been well documented that eggs are often the life stage most resistant to insecticides (Sutter et al., 1990; Gubran et al., 1992 and Devine et al., 1999). In the current study, hatched crawlers were killed after contact with the egg case. For effective phylloxera population control it is imperative that the insecticide dramatically reduces at least directly one or more life stages.

Using a pot-based glasshouse trial was an effective means of evaluating both insecticide efficacy as well as assessing vine health. Similar assays of this nature have been used successfully for other pest problems (Barratt et al., 1995; Ninkovic et al., 2001;

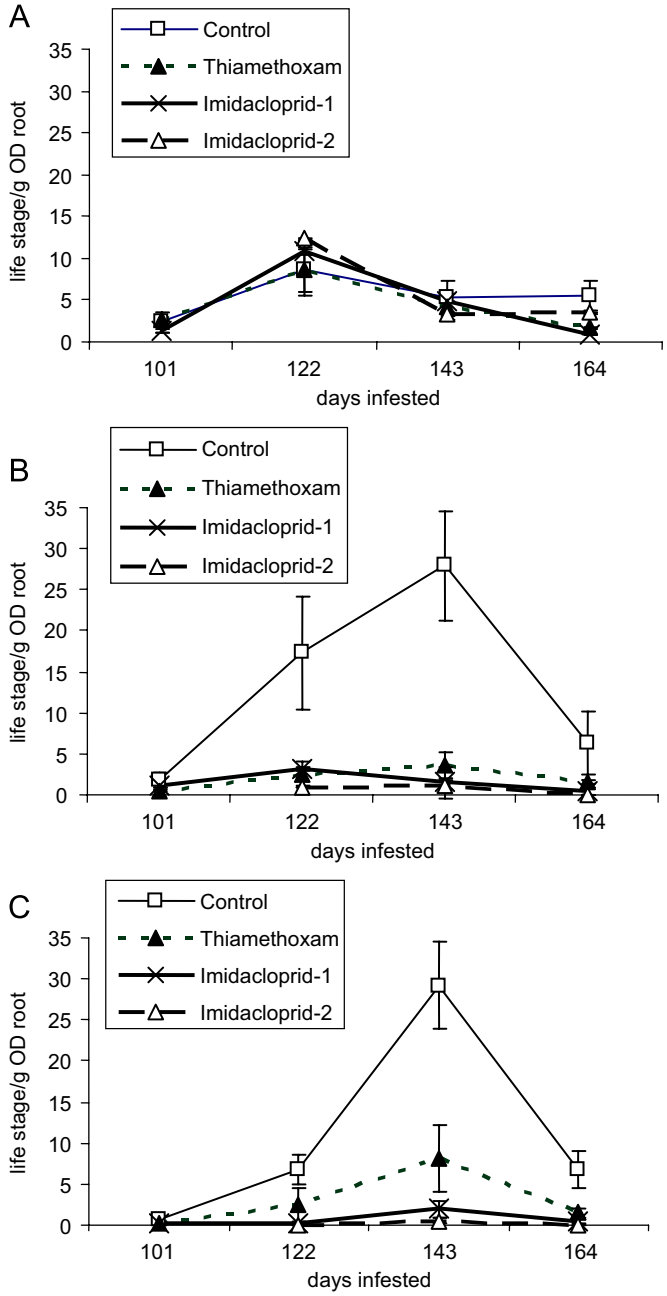

Fig. 5. Mean counts of different life stages per gram OD root for thiamethoxam and imidacloprid glasshouse *in planta* trial (Control = water treatment, thiamethoxam = 2000 ppm application, imidacloprid-1 = single application at 2000 ppm, imidacloprid-2 = double application at 2000 ppm). (A) Eggs, (B) crawler, (C) post-crawler (= 2nd, 3rd, 4th instars and adults).

Lígia et al., 2004). In this study changes in leaf square area between infested and non-infested treatments and also between insecticide-treated vines were the best physiological monitor of phylloxera infestation. Leaf parameters, such as premature yellowing and stunted growth, are known to be one of the first (above-ground) symptoms of phylloxera infestation (Granett et al., 2001). Significant changes in leaf area were seen 79 days after commencement of the potted trial and may represent the best indicator of vine health.

Omer et al. (2002) found a relationship between the plant growth stage on the overall performance and proportion of phylloxera life stages. They reported higher levels of crawlers and eggs in the vegetative and mid-ripening vine stages

Table 2
Two-way ANOVAs comparing the effects of pesticide treatment and time since phylloxera establishment on numbers of life stages per gram of OD root and on vine morphology

	Effect	df	MS	F	*P*
Egg stage	Time	3	263.80	9.94	<0.001
	Treatment	3	65.39	2.46	0.082
	Interaction	8	13.19	0.50	0.849
	Error	30	26.54		
Crawler stage (log transformed)	Time	3	3.033	7.72	<0.001
	Treatment	3	7.236	18.41	<0.001
	Interaction	8	0.540	1.37	0.247
	Error	30	0.393		
Post-crawler stage (log transformed)	Time	3	4.573	15.07	<0.001
	Treatment	3	8.633	28.45	<0.001
	Interaction	8	0.498	1.64	0.155
	Error	30	0.303		
Leaf area (cm^2)	Time	3	274.05	5.52	<0.001
	Treatment	3	472.55	9.51	<0.001
	Interaction	8	36.33	0.73	0.66
	Error	32	49.68		
Stem weight (g)	Time	3	184.41	29.92	<0.001
	Treatment	3	15.81	2.57	0.07
	Interaction	8	4.91	0.80	0.61
	Error	32	6.16		
Root weight (g)	Time	3	1141.62	6.05	<0.001
	Treatment	3	1272.32	6.74	<0.001
	Interaction	8	155.98	0.83	0.59
	Error	32	49.68		

(approximately 60 days post-bud burst) compared to post-harvest stages (greater than 125 days). However, it is difficult to compare the findings of Omer et al. (2002) with those in the present study because vines remained in a vegetative growth stage for the duration of the glasshouse trial. Future pot trials over a longer time course, including harvest periods (and with a greater range of phylloxera infestation), could be undertaken to further test the utility of this measure. Field studies are required to assess the ability of the given insecticides to penetrate different soil types and spread to root systems several metres deep in the soil. Field studies are required to assess the ability of the given insecticide to penetrate different soil types and spread to root systems several metres deep in the soil. For upwardly mobile systemic insecticides in particular, it is important to determine how effectively these chemicals penetrate to root systems deep in the different soil types and readily absorbed by the plant root systems.

Phylloxera has a high potential for rapid population growth from comparatively low population reservoirs. Generation time may be less than a month, giving three to ten generations per year (Granett et al., 2001). Hence, high mortality is critical to negate this reproductive potential. Two applications of imidacloprid had the highest mortality: 85% of total crawlers in the bioassay trial were killed and there was also a significant reduction in crawler and post-crawler life stages. Since 100% effectiveness was not achieved, multiple applications of insecticide will be required to achieve continued suppression and to avoid phylloxera increasing again from resistant egg and other life stages.

A reduction in crawler number was observed following two applications of imidacloprid. This has implications for effective quarantine of recently infested vineyards. First instar phylloxera, or crawlers, represent the most active dispersive stage of the insect and have been found in relatively high numbers, both below and above ground, during the vine-growing season (Powell

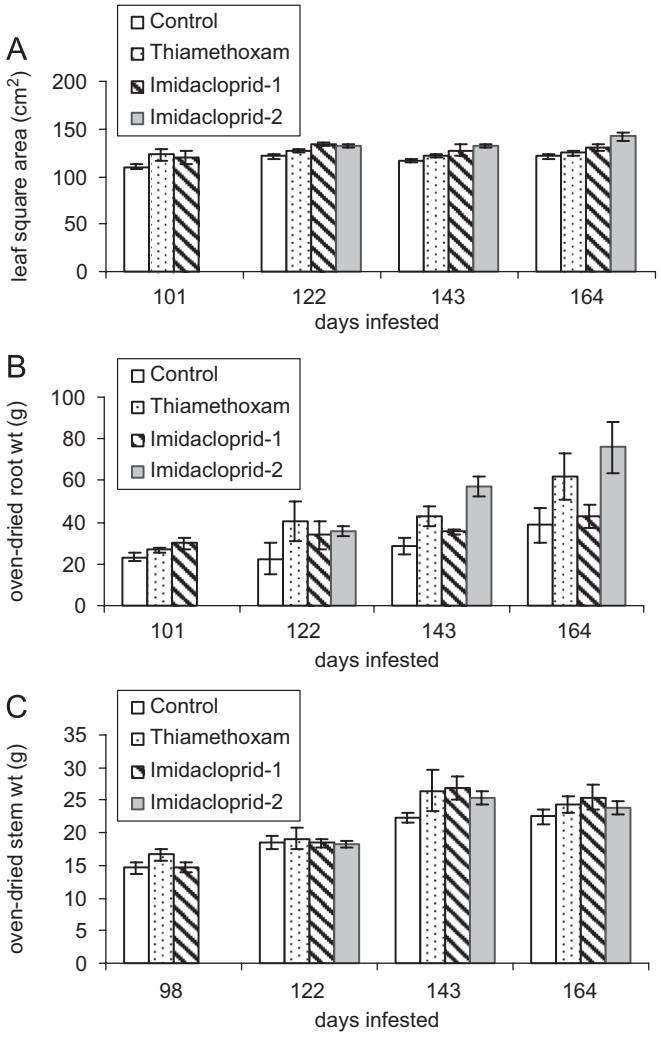

Fig. 6. Vine morphology comparisons of: (A) mean leaf square area (cm^2); (B) mean root wt (g); and (C) mean stem wt (g) for the insecticide treatments. Control = no insecticide application; thiamethoxam = 2000 ppm application; imidacloprid-1 = 2000 ppm single application and imidacloprid-2 = 2000 ppm double application.

et al., 2000). A reduction in numbers of this life stage would reduce the substantial risk of transfer from infested to uninfested vines, strengthening quarantine measures within an existing vineyard and also neighbouring vineyards. It is unlikely that growers would consider chemical control for short-term phylloxera control before significant vine damage was observed. Therefore, the effectiveness of systemic chemical delivery to the vine roots would be significantly affected as compared to healthy roots, emphasising the importance of both insecticide timing and application number.

In California, where imidacloprid is registered for phylloxera suppression, two applications between budbreak and the pea-berry stage are recommended to reduce phylloxera populations and allow for an increase in root growth (UC Pest Management Guidelines, 2003). Apart from its use to suppress phylloxera on grapevines, this chemical is also registered for use in the US on cotton, potato, fruiting vegetables, Brassica, leafy vegetables, canola, grapes, cucurbits, tuberous vegetables, citrus and pecan (UC Pest Management Guidelines, 2003). Imidacloprid is highly soluble in water, has moderate binding affinity to organic materials in soils and a relatively long half life in soils (Zalom et al., 2005). Thiamethoxam is registered in California for phylloxera suppression on grapes. This product is also registered in the US for use in melons, fruiting vegetables, cotton and tuberous vegetable (UC Pest Management Guidelines, 2003). Thiamethoxam is less water soluble, has a low binding affinity to organic materials in soil and has a greater persistence in soils (Zalom et al., 2005). Soil type and irrigation practices will therefore be important considerations for growers in order to optimise neonicotinoid efficacy, while preventing unwanted chemical effects.

While imidacloprid has potential to control phylloxera and is also an effective strategy for aphid control in a number of crops, this chemical has a mixed reputation regarding its safety to natural enemies of pests. It has low toxicity to spiders, some predatory beetles (Carabids, Staphylinids) (Kunkel et al., 1999; James and Vogele, 2001), predatory bugs (Anthocorids, Lygaeids, Pentatomids, Reduviids) (Elzen, 2001; James and Vogele, 2001) and soil inhabiting decomposers. However studies by other researchers, particularly when applied directly to the plant canopy, showed that imidacloprid to be toxic to these organisms as well as to parasitoids (e.g. Stark et al., 1995; Sclar et al., 1998; James and Vogele, 2001; Hewa-Kapuge et al., 2003; Kreutzweiser et al., 2008). Thiamethoxam also has mixed effects on beneficials; while it appears to have low toxicity to parasitoids, it can have harmful effects on beneficial predators such as on mirids (Bostanian et al., 2005; Kilpatrick et al., 2005). Both chemicals would therefore need to be applied cautiously within an IPM framework.

In summary, the results of this study suggest that the use of imidacloprid and thiamethoxam causes a reduction of crawler and egg life stages leading to some significant improvements in vine vigour. Field trials of both these chemicals, conducted within a smaller concentration range to that tested in this study (e.g. 1000–2000 ppm a.i and 1000–1500 ppm a.i), need to be undertaken to assess the overall economic benefit of using this insecticide as a cost-effective interim management strategy and so that exact recommendations can be developed.

Several questions concerning the use of the insecticides for short-term phylloxera control remain, surrounding the efficiency of transport into the plant root system, residual presence in xylem and any effects that chemicals may have on grape quality and safety for consumption. Future chemical field trials should also incorporate integrated pest management programs to further investigate the interactions between neonicotinoid compounds and pest/beneficial organisms. Ultimately, the adoption of insecticides as an interim management strategy for phylloxera control would require a continued suppression of phylloxera populations to ensure long-term economic effects on vine vigour and the maintenance of productivity of the vines.

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