Research Article

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Comparison of foliar and soil formulations of neonicotinoid insecticides for control of potato leafhopper, *Empoasca fabae* (Homoptera: Cicadellidae), in wine grapes

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Abstract

BACKGROUND: The potential of systemic neonicotinoid insecticides to control potato leafhopper, *Empoasca fabae* (Harris), a damaging pest of wine grapes in the eastern United States, was investigated. Soil or foliar applications were made to potted or field-grown vines, and the response of leafhoppers was determined in clip cages over the following month on young or mature leaves.

RESULTS: Foliar application of imidacloprid caused immediate and long-lasting reductions in *E. fabae* survival on both leaf ages, whereas the activity of soil-applied imidacloprid was delayed. Clothianidin, imidacloprid and thiamethoxam all provided long-lasting reduction in leafhopper survival on young and mature foliage when applied through either delivery route. However, the percentage of moribund nymphs was significantly greater on foliar-treated vines and increased over time in mature and immature leaves compared with soil-treated vines. Residue analysis of foliar-applied imidacloprid showed an 89% decline in mature leaves from day 1 to day 27, and a 98% decline in immature leaves over the same time period. Comparison of soil-applied clothianidin, imidacloprid and thiamethoxam in field-grown vines showed significant reduction in *E. fabae* only on mature leaves of vines treated with thiamethoxam.

CONCLUSIONS: Neonicotinoids can control *E. fabae* in small vines, even in rapidly expanding foliage where this pest causes greatest injury. Soil application provides superior long-term vine protection because declining residues on foliar-treated vines lead to suboptimal activity within 2–3 weeks. Vineyard managers of susceptible cultivars may take advantage of this approach to *E. fabae* management by using foliar applications of the three neonicotinoids tested here, or by using soil-applied thiamethoxam.

Keywords: systemic; vineyard; grape; IPM; reduced risk; neonicotinoid; growth dilution; residue profile

1 INTRODUCTION

The potato leafhopper, *Empoasca fabae* (Harris), is a cosmopolitan pest that migrates northwards each spring from subtropical overwintering habitats carried by spring weather systems to reach temperate regions of North America.[1] Precipitation within synoptic weather patterns deposits *E. fabae* onto vegetation where they establish and reproduce. *Emposaca fabae* is highly polyphagous, and the adult leafhoppers feed on host plants, causing economic injury to field crops, vegetables and fruit crops. Adult and immature leafhoppers exhibit preference for the younger tissues, thereby concentrating their feeding distally on shoots. In vineyards, this immigration typically coincides with the period of shoot expansion, and their feeding can cause stunted growth, particularly on young vines.

Phloem feeding by *E. fabae* causes a hypersensitive response in many plant species,[2,3] mediated by rupturing of plant cells and enhanced by the saliva injected during feeding.[2] In grapevines (*Vitis* spp.) this response varies from highly apparent leaf yellowing and cupping in some sensitive European *Vitis vinifera* L. cultivars[4] to a complete absence of symptoms in North American *V. labrusca* L. and related species. Hybrid cultivars tend to be intermediate in their sensitivity. In the highly sensitive cultivars or in vines with high levels of infestation, reduction in shoot growth can be severe, negatively affecting vine establishment and training.

Applications of relatively short-lived contact insecticides, such as carbaryl, are used to protect sensitive vines from injury by *E. fabae*. However, repeated leafhopper reinvasions during the spring, coupled with this insect's propensity for feeding on

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rapidly growing shoots, result in the need for reapplication. The neonicotinoid class of insecticides has physical properties making them highly suitable for use against insects feeding on foliage or plant sap.[5] Translaminar movement after foliar application and systemic movement from roots to foliage after soil application both provide a means of reaching the feeding sites of insects that are otherwise challenging to treat with sufficient insecticide residues for control of *E. fabae*.

Systemic insecticides have many benefits for use against sucking insect pests, and these have led to their widespread adoption in agriculture.[5] Absorption into foliage can protect insecticide molecules from rain and UV degradation and provides increased levels of human safety compared with broad-spectrum contact insecticides that are applied to the foliage. In grapevine canopies where hand labor is a typical part of horticultural management, avoiding the use of broad-spectrum contact insecticides that have only surface residues will have obvious benefits to worker safety. In California, for example, imidacloprid has become a valuable tool for control of the glassy-winged sharpshooter and vine mealybug, two destructive invasive insect pests of vineyards.[6,7]

Recent registration of multiple neonicotinoid insecticides for use in vineyards may provide an opportunity for improved control of *E. fabae*, although it is not clear whether foliar-applied or soil-applied formulations will provide superior protection of the young foliage which is the preferred feeding site of *E. fabae*. The three insecticides, clothianidin, imidacloprid and thiamethoxam, have formulations that can be applied to foliage or soil, enabling a comparison of relative efficacy among the different insecticides and between the two application methods. Neonicotinoids absorbed into leaves and stems after foliar application or that move to foliage from a soil application can act on pest insects through non-lethal modes of action after leaf contact, and members of this class have been shown to provide crop protection against insect pests through sublethal effects that include repellency and antifeedant activity.[8–11]

The objectives of this study were to determine the effectiveness of soil and foliar applications of three neonicotinoid insecticides for control of *E. fabae* on young and mature grape foliage. Experiments were designed to measure insecticide activity over time and to determine the extent to which these treatments caused mortality or moribund symptoms under potted vine conditions and in a vineyard utilizing an irrigation injection system. To understand the impact of residue degradation and plant growth dilution on residual insecticide activity, leaves that were mature and immature at the time of application were compared, and residues and leaf expansion over time were measured.

2 METHODS

2.1 General methods

Laboratory experiments took place at Michigan State University's Trevor Nichols Research Complex (TNRC) in Fennville, MI, during 2004 and 2005. One-year-old cv. Chardonnay vines were planted in sandy soil in plastic pots (28 cm height, 28 cm diameter) and grown until they had four leaves on at least one shoot, at which point they were brought to a laboratory and maintained at $21.9 \pm 0.5\,^{\circ}C$ for the duration of the experiments. Application of soil-applied insecticides to the pots was done via a soil drench in water (473 mL), while foliar insecticides were applied with a 700 mL handheld spray mister (Sprayco, Farmington Hills, MI) until all leaves were wet (approximately 60 mL water per vine). Field experiments took place at TNRC in 2007 in a three-year-old cv. Chancellor and cv. Aurore vineyard with a drip irrigation system. All experiments were conducted using *E. fabae* nymphs collected from untreated alfalfa fields in southwest Michigan during June and July. Nymphs were collected using sweep nets and were aspirated into vials containing sprigs of alfalfa. Vials were transported in a cooler until use in experiments later the same day.

Clip cages were utilized to contain *E. fabae* nymphs on grape leaves. Each clip cage consisted of a piece of PVC tubing (1.9 cm diameter) cut to 0.64 cm in length. On one side, a piece of Darice® mesh (Darice, Inc., Strongville, OH) was attached with hot glue. On the opposite side of the PVC tube, a small strip of Frost King® camper mounting tape (7.9 cm long and 3.2 mm wide; Thermwell Products Co., Mahwah, NJ) was placed to prevent nymphs from escaping from the cage. A large hair clip (Salon Care®; Brentwood Beauty Labs International Inc., Hillside, IL) was used to clip the cage to the grape leaves. A circular piece of plastic (4.1 cm diameter) was glued to the top prong of the hair clip in order to minimize leaf damage when the clip was attached to the leaf. During the experiments, the PVC tube cage was placed on the underside of the grape leaf and kept in place with the hair clip. Extra clips were added as needed (up to three per cage) to ensure an even seal on the leaf. Nymphs were either placed briefly in the freezer (2004) or exposed briefly to CO_2 gas (2005 and 2007) to facilitate transfer to the clip cages. Five nymphs were transferred to each cage using fine paint brushes, and cages were affixed to grape leaves before nymphs had a chance fully to revive. Assessment of nymph survival involved classifying insects as alive, moribund or dead. Alive nymphs were those that were moving normally and appeared healthy, while dead nymphs were those that were completely immobile. Moribund nymphs were still alive but showing signs of toxicity such as twitching legs or slow uneven movements.

2.2 Experiment 1: foliar-applied versus soil-applied imidacloprid

Six replicates of the following three treatments were set up on 12 July 2004: soil-applied imidacloprid 240 g L^{-1} SC (Admire 2F; Bayer CropScience, Research Triangle Park, NC), 3.5 L ha^{-1}, 840 g AI ha^{-1}; foliar-applied imidacloprid 750 g kg^{-1} WP (Provado 75WP; Bayer CropScience, Research Triangle Park, NC), 70 g ha^{-1}, 52.5 g AI ha^{-1}; an untreated control.

Clip cages with nymphs were applied to vines at 1, 7, 14, 21 and 27 days after application of insecticides. Two clip cages were attached to one shoot on each of the vines. One cage was attached to a leaf that was fully expanded at the time of the insecticide application ('mature') and the other cage was attached to the smallest leaf on the shoot that had not yet expanded when the insecticide was applied ('immature'). The only exceptions for the immature leaves were those used during the first two (1 and 7 day) time periods, where partially expanded leaves were used because new leaves had not unfolded since the insecticides were applied. Leaves on separate shoots were used for each time period until no more shoots were available on the vine, at which point an old shoot was reused. No shoot was used more than 3 times during the length of the experiments, and individual leaves were never reused. Cages were left on the leaves for 24 h, after which they were taken off and nymph survival was assessed.

A parallel series of mature and immature leaf samples were taken from the imidacloprid foliar-treated grape vines for residue analysis at each of the five post-application timings. A single sample of six leaves was collected and combined for analysis. Leaves were frozen and transported to the Michigan State University

Pesticide Analytical Laboratory in East Lansing, Michigan. Samples were placed in a −20 °C freezer until extraction. A pesticide extraction procedure was used to separate dislodgeable residues on the surface of the leaves from the subsurface residues in order to provide a spatial profile of residues over time. To determine the amount of residue on the leaf surfaces, samples were placed in 150 mL of HPLC-grade acetonitrile (EMD Chemicals, Inc., Gibbstown, NJ) and sonicated for 10–15 s. The acetonitrile was decanted through 5 g of reagent-grade anhydrous sodium sulfate (EMD) to remove water. The sample was dried via rotary evaporation and brought up in acetonitrile for HPLC analysis.

To determine the subsurface residues, the remaining solid leaf samples (5 ± 3 g) were ground in 200 mL of HPLC-grade dichloromethane (Burdick & Jackson, Muskegon, MI). The extracts were then vacuum filtered, and the filtrate was passed through 5 g of anhydrous sodium sulfate. The samples were dried via rotary evaporation and brought up in acetonitrile. Any remaining particulates were removed by passing the sample through a 0.45 µm Acrodisc® 13 mm syringe filter (Pall, East Hills, NY).

Samples were analyzed for imidacloprid residue (parent compound) with a Waters 2690 separator module HPLC equipped with a Waters 2487 dual-wavelength absorbance detector set at 270 nm, and a C18 reversed-phase column (150 × 4.6 mm bore, 5 µm particle size; Restek, Bellefonte, PA) (Bayer 1998). The mobile phase was water/acetonitrile (80 : 20) at 55 °C. The HPLC level of quantification was 0.42 µg g^{-1} (ppm) of active ingredient, and the level of detection was 0.13 µg g^{-1} (ppm).

Before analysis, the dimensions of six mature and immature leaves were measured to document leaf expansion over the five post-application time periods. These dimensions were used to calculate the change in relative leaf surface area so as to determine the contribution of plant growth to the change in plant residues over time. To determine the amount of active ingredient on the leaf surface per cm^2, the total micrograms per composite sample was divided by 6 for the replicates and then divided by the average surface area.

2.3 Experiment 2: comparison of neonicotinoids

Foliar and soil formulations of three neonicotinoid compounds were compared in 2005 using similar methods to experiment 1, and using labeled rates of the tested insecticides, with the rates similar across treatments. Each of the following treatments was applied to six replicate potted grapevines on 19 July 2005: clothianidin, foliar: 500 g kg^{-1} WG (Clutch 50WG; Valent USA, Walnut Creek, CA), 105 g ha^{-1}, 52.5 g AI ha^{-1}; soil: 160 g kg^{-1} WG (Belay 16WG; Valent USA, Walnut Creek, CA), 1400 g ha^{-1}, 224 g AI ha^{-1}; imidacloprid, foliar: 750 g kg^{-1} WP (Provado 75WP), 70 g ha^{-1}, 52.5 g AI ha^{-1}; soil: 240 g L^{-1} SC (Admire 2F), 1.17 L ha^{-1}, 281 g AI ha^{-1}; thiamethoxam, foliar: 250 g kg^{-1} WG (Actara 25WG; Syngenta Crop Protection, Inc., Greensboro, NC), 210 g ha^{-1}, 52.5 g AI ha^{-1}; soil: 240 g L^{-1} SC (Platinum 2SC; Syngenta Crop Protection, Inc., Greensboro, NC), 1.17 L ha^{-1}, 281 g AI ha^{-1}; a control that received water only. Clip cage methods and insect assessments were the same as those applied in experiment 1.

2.4 Experiment 3: field efficacy of soil-applied neonicotinoids

Soil formulations of the neonicotinoid compounds tested in experiment 2 were tested in the field in 2007 utilizing an irrigation injection system attached to the drip irrigation system. The irrigation injection system consisted of a 3 gal (11.35 L) beverage container (R & D Sprayers, Opelousas, LA) pressurized to 40 psi with a canister of compressed CO_2. Chemicals were injected into the drip irrigation line at 23 psi via a hose connected to a quick coupler (Amflo Products, Ontario, CA) located at the beginning of each row. Vines were irrigated for a total of 1 h before, 0.5 h during and 2.5 h after chemical injections to ensure that the insecticide solution reached the root zone. Four replicate seven-vine rows (two on Chancellor grapes and two on Aurore grapes) of the following treatments were applied on 6 June 2007: clothianidin 500 g kg^{-1} WG (Clutch 50WG), 448 g ha^{-1}, 224 g AI ha^{-1}; thiamethoxam 240 g L^{-1} SC (Platinum 2SC), 1.17 L ha^{-1}, 281 g AI ha^{-1}; imidacloprid 552 g L^{-1} SC (Admire Pro; Bayer CropScience, Research Triangle Park, NC), 1.02 L ha^{-1}, 563 g AI ha^{-1}. The latter compound's rate was double that in experiment 2, to test the highest label rate of this insecticide for its performance. Chemicals were injected into the drip irrigation system in 3.78 L of water. Clip cage experiments were similar to experiments 1 and 2, but cages were placed on one shoot on the middle vine of each seven-vine row. Each shoot had a clip cage attached to an immature leaf and mature leaf. Cages were attached to the vines at 1600 hours on 2 July 2007 (26 days after treatment) and taken off at 0900 hours the next morning, at which point nymph survival was assessed. Cages were deployed overnight in order to minimize heat stress to *E. fabae* during the middle of the day. Temperatures in the vineyard overnight ranged from 16.7 to 22 °C.

2.5 Analysis

All statistical analyses were performed using Statview® v.5.0.1 (Abacus Concepts Inc., Berkeley, CA). For experiments 1 and 2, the proportion of leafhoppers surviving or the proportion moribund in each treatment were corrected for ties where appropriate, then analyzed using a Kruskal–Wallis test followed by a Mann–Whitney *U*-test for *post hoc* comparisons among treatments. A significance value of 0.05 was used for Kruskal–Wallis tests, while Mann–Whitney means separation significance values were adjusted using the 1995 Benjamini and Hochberg false discovery rate procedure[12,13] and were regarded as significant if $P_i < (i)(\alpha)/m$, where $\alpha = 0.05$, m is the number of tests performed and i is the test number. Mann–Whitney *U*-tests were used for foliar and soil treatment comparisons in experiment 2. Clip cages where more than two nymphs escaped during the 24 h time period were not included in any analyses. For experiment 3, the arcsine-transformed proportion of alive leafhoppers was analyzed using analysis of variance, followed by Fisher's protected least significance test for *post hoc* comparisons. Untransformed percentages are presented ± SEM for all experiments.

3 RESULTS

3.1 Experiment 1

3.1.1 Foliar-applied versus soil-applied imidacloprid

Significant differences in nymph survival were seen among insecticide treatments and the untreated control. At the 1 day assessment on mature leaves, the soil imidacloprid treatment and control were not significantly different, and both had significantly higher nymph survival than the foliar imidacloprid treatment (Fig. 1A). For the rest of the time periods, the percentage of leafhopper nymphs surviving was significantly lower on both the soil and foliar imidacloprid-treated vines than on the controls (Fig. 1A). Survival of leafhopper nymphs on immature leaves also

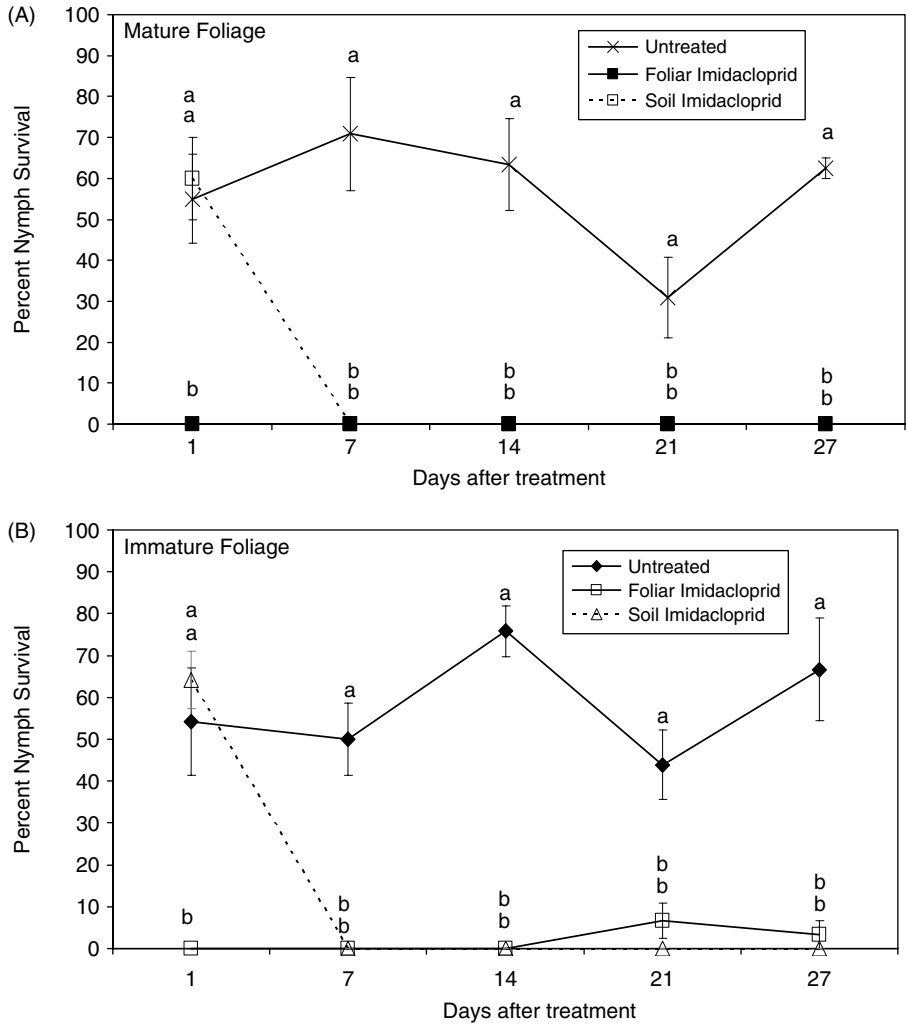

Figure 1. Average percentage (± SEM) of *Empoasca fabae* nymphs surviving (not including nymphs showing moribund effects) in clip cages on potted grape vines on either (A) mature or (B) immature grape leaves. Leaves were treated with foliar-applied (Provado 75WP) or soil-applied (Admire 2F) imidacloprid or were untreated. Nymphs were exposed to leaves for 24 h at each time interval after insecticide application. Values within a time period with the same letter are not significantly different.

showed significant differences among the treatments (Fig. 1B). Results were similar to those for mature leaves with both imidacloprid treatments, showing significantly lower survival for all days except 1 day after treatment (DAT), where the soil imidacloprid treatment was not significantly different from the control.

The percentage of nymphs on mature leaves that exhibited moribund symptoms was low (less than 5% for any treatment or time period), with no significant differences among treatments for any of the time periods. On immature leaves, the percentage of moribund nymphs increased steadily in the foliar treatment over the course of the experiment, from 9.4% at 7 days up to 26.7% at 21 days, although this increase was not significant.

3.1.2 Imidacloprid residue profiles

Imidacloprid residues detected in mature and immature leaves were highest in the time periods first following the foliar application, and gradually declined over the duration of the experiment (Table 1). One day after treatment, the proportion of imidacloprid residues on the surface of both mature and immature leaves was approximately 5 times greater than subsurface residues, reflecting the time lag of translaminar penetration. The proportion of imidacloprid residues measured as subsurface peaked between 7 days and 14 days post-application for both immature and mature leaves. Even though the surface area of immature leaves was initially only about one-fourth that of mature leaves, the residue levels were higher.

3.2 Experiment 2: comparison of neonicotinoids

Significant differences were found among treatments in the proportion of *E. fabae* surviving and exhibiting moribund symptoms. The percentage of nymphs surviving on mature leaves was significantly lower in all chemical treatments than in the controls for all time periods (Fig. 2). This was true for both the foliar formulations (Fig. 2A) and the soil applications (Fig. 2B). Leafhopper survival in cages on mature leaves remained at or near zero in all foliar and soil applications for the duration of the experiment. Survival of nymphs on immature leaves was similar to that on mature leaves, with controls showing a significantly higher percentage of live nymphs than any of the chemical treatments (Fig. 2). As in the mature leaves, this was true for both foliar (Fig. 2C) and soil formulations (Fig. 2D).

Table 1. Residue profile of foliar-applied imidacloprid on grape leaves over 1 month post-application. Residue levels and the area of sampled leaves were measured at 1, 7, 14, 21 and 27 days post-application[a]

	Immature leaves					Mature leaves[b]				
	Residue (μg g^{-1})					Residue (μg g^{-1})				
Days post-application	Surface	Subsurface	Total	Leaf area (cm^2)	Mean AI (± SEM) (μg cm^{-2})	Surface	Subsurface	Total	Leaf area (cm^2)	Mean AI (± SEM) (μg cm^{-2})
1	159.21	35.77	194.98	31.5	6.18 (±4.98)	109.5	18.04	127.53	112.9	1.13 (±0.27)
7	46.68	47.93	94.62	34.0	2.78 (±2.62)	147.74	31.52	179.26	134.5	1.33 (±0.67)
14	23.36	13.3	36.66	25.6	1.43 (±1.3)	71.24	25.66	96.9	99.7	0.97 (±0.28)
21	5.14	2.21	7.35	55.8	0.13 (±0.02)	33.18	12.09	45.27	119.2	0.38 (±0.09)
27	4.04	0.0	4.04	29.2	0.14 (±0.06)	0.3*	13.92*	14.22	109.9	0.13 (±0.12)

[a] HPLC detection levels; LOQ = 0.42 μg g^{-1}, LOD = 0.13 μg g^{-1}.
[b] * 27 day mature leaf residue values estimated using regression.

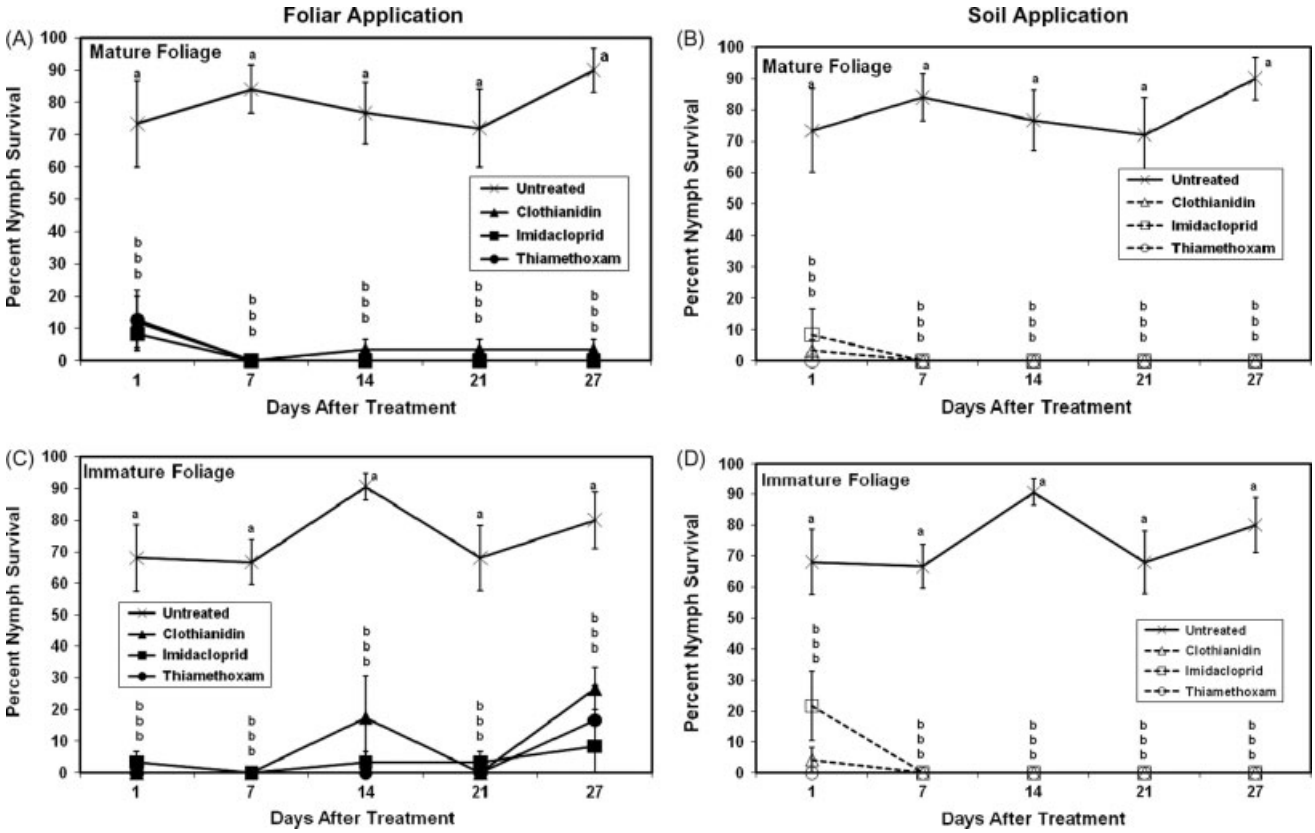

Figure 2. Average percentage (± SEM) of *Empoasca fabae* nymphs surviving (not including nymphs showing moribund effects) in clip cages on potted grape vines on mature or immature grape leaves treated with either foliar or soil formulations of clothianidin, imidacloprid or thiamethoxam or only receiving water. Nymphs were exposed to leaves for 24 h at each time interval after insecticide application. Values within a time period with the same letter are not significantly different.

Post hoc comparisons of survival of *E. fabae* between application methods found that on mature foliage there was no significant difference between foliar-treated and soil-treated vines over the course of this experiment (Fig. 2) ($U > 116.0$, $P > 0.10$). On immature foliage, significant differences in survival between application methods were evident at 1 DAT ($U = 112.0$, $P = 0.049$) when foliar applications were more effective at controlling *E. fabae*, and at 27 DAT ($U = 90.0$, $P < 0.003$) when soil applications were more effective at controlling *E. fabae* (Fig. 2). There was no difference between application methods at 7, 14 or 21 DAT ($U > 126.0$, $P > 0.07$). Effects on the number of *E. fabae* in a moribund state were much more evident on foliar-treated vines than on those treated through the soil. On foliar-treated vines there was no difference in these symptoms between application methods at 1 or 7 DAT ($U > 140.0$, $P > 0.48$), but significant differences were evident at 14 DAT ($U = 88.0$, $P < 0.003$), 21 DAT ($U = 102.0$, $P = 0.01$) and 27 DAT ($U = 64.0$, $P < 0.001$), with a higher proportion of moribund nymphs on mature foliage. There was a somewhat different pattern on immature foliage, with a higher proportion of moribund nymphs on foliar-treated than on soil-treated vines on all dates except 1 DAT (1 DAT: $U = 127.5$, $P = 0.08$; 7 DAT: $U = 106.5$, $P < 0.03$; 14 DAT: $U = 104.5$,

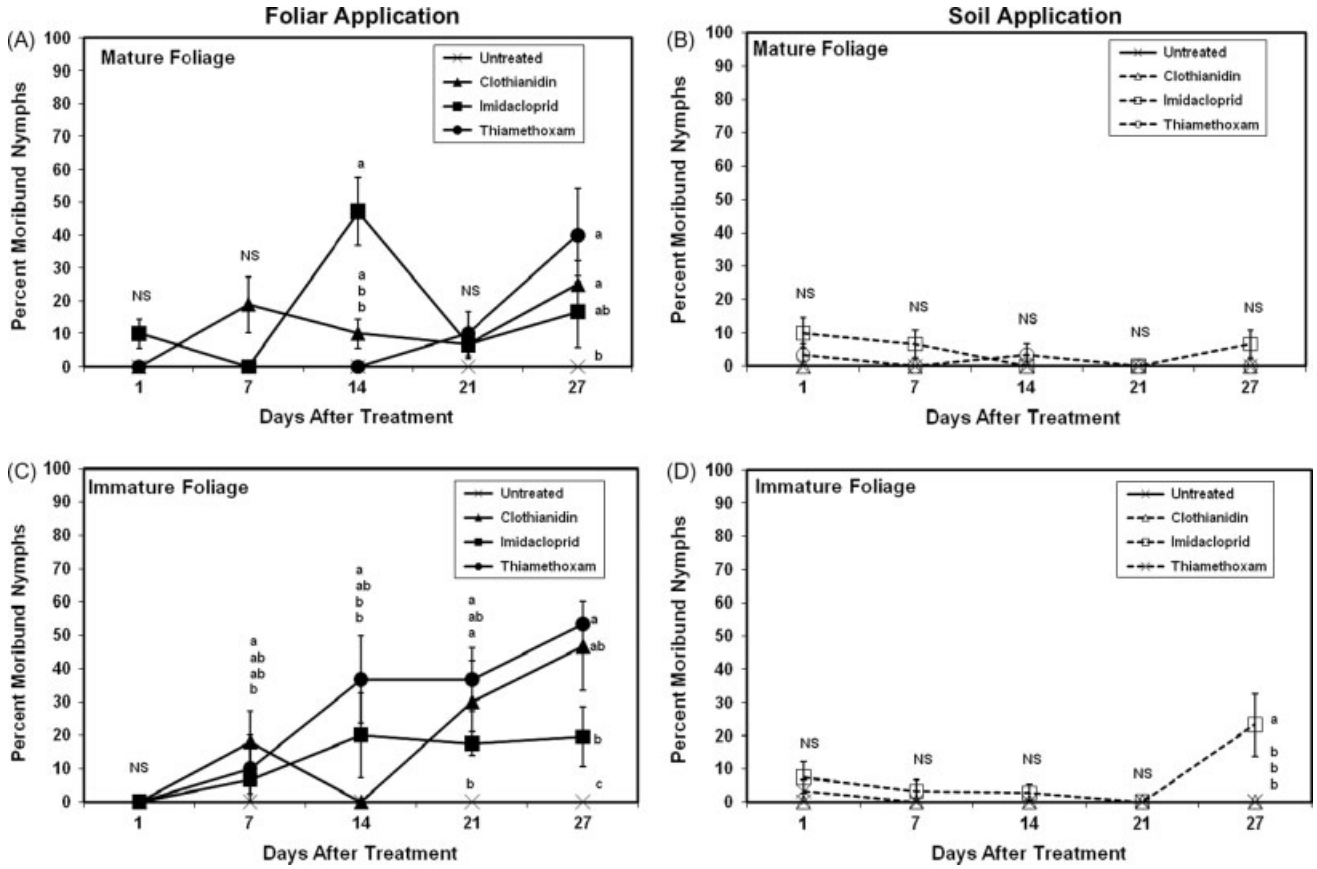

Figure 3. Average percentage (± SEM) of *Empoasca fabae* nymphs in clip cages on potted grape vines that were moribund on mature and immature grape leaves treated with either foliar or soil formulations of clothianidin, imidacloprid or thiamethoxam, or only receiving water. Nymphs were exposed to leaves for 24 h at each time interval after insecticide application. Values within a time period with the same letter are not significantly different.

$P = 0.01$; 21 DAT: $U = 36.0$, $P < 0.001$; 27 DAT: $U = 48.0$, $P < 0.001$) (Fig. 3).

The percentage of nymphs exhibiting moribund symptoms varied depending on the chemical formulation, leaf type and time period. The proportion of nymphs exhibiting these symptoms increased over time on mature leaves receiving foliar applications (Fig. 3A), but this remained low for the soil formulations (Fig. 3B). Nymphs on immature leaves showed the same patterns as on the mature leaves, with higher percentages of moribund nymphs in the foliar formulations over the course of the experiment (Fig. 3C) and less than 10% for the soil formulations, except for 27 DAT where the soil formulation of imidacloprid had a significantly higher percentage of moribund nymphs than the other three treatments (Fig. 3D).

3.3 Experiment 3: field efficacy of soil-applied neonicotinoids

Field-based clip cage experiments revealed significant differences among treatments in the level of *E. fabae* survival. The percentage of nymphs surviving on mature foliage in the thiamethoxam treatment was significantly lower than in any of the other treatments (Table 2). Clothianidin and imidacloprid also had a lower percentage of nymphs surviving than the untreated controls, although this difference was only significant for clothianidin, in spite of the high rate of imidacloprid used in this experiment. On immature foliage, thiamethoxam had a lower percentage of nymphs surviving than the other three treatments, although this difference was not significant (Table 2). None of the treatments had any moribund nymphs, except for thiamethoxam, where an average of 6.3 ± 6.3% of nymphs were moribund.

Table 2. Average percentage survival (± SEM), not including nymphs showing moribund effects, of *Empoasca fabae* nymphs in clip cages on grape vines in the field on mature and immature grape leaves. Soil formulations of clothianidin, imidacloprid and thiamethoxam were injected into drip irrigation lines via an irrigation injection system. Nymphs were exposed to leaves for 17 h overnight at 26 days after the insecticide application. Values with the same letter are not significantly different at $\alpha = 0.05$

	Nymph survival (%)	
Treatment	Mature foliage	Immature foliage
Untreated control	90.0 (±5.8) a	68.8 (±12.6) a
Clothianidin	53.8 (±8.5) b	75.0 (±9.6) a
Imidacloprid	63.8 (±9.0) ab	72.5 (±11.1) a
Thiamethoxam	20.0 (±14.1) c	33.8 (±6.9) a
$F_{3,12}$	8.49	2.27
P	0.0027	0.13

4 DISCUSSION

This study demonstrates the potential of neonicotinoid insecticides to provide long-lasting control of *E. fabae* on grapevines.

Under laboratory conditions, foliar- and soil-applied imidacloprid provided high levels of control on both mature and immature leaves for almost a month. This indicates that potato leafhoppers are highly sensitive to imidacloprid, and that this compound has sufficient penetrative and mobility characteristics to reach these insects even when feeding at the shoot tips. The results also suggest, however, that growth dilution of residues related to actively growing plant tissues could have a significant effect on overall performance of foliar-applied imidacloprid if application rates are lower or insects become less sensitive through resistance mechanisms.

When imidacloprid was compared with the two other neonicotinoids, thiamethoxam and clothianidin, these also provided long-lasting control in potted vine studies, although there was some early indication of the foliar residues starting to decline, allowing low levels of survival at 27 DAT. Although not significant, survival was higher in clothianidin than in thiamethoxam, with the lowest levels in the imidacloprid-treated vines. Surviving leafhoppers also exhibited poisoning symptoms as the foliar residues declined over 27 days. This was most evident in imidacloprid at 14 DAT and clothianidin and thiamethoxam at 27 DAT in the mature leaves, and at 27 DAT in immature leaves. The general pattern was for increasing levels of non-lethal activity over the course of the experiment in foliar-treated vines. In contrast, with the soil-treated vines, the high levels of mortality resulted in no moribund leafhoppers during the course of this experiment. Previous studies of soil-applied neonicotinoids have shown similar high levels of control of various plant-feeding pests.[14–17]

Moribund symptoms were observed in leafhoppers on leaves treated using both delivery methods, and it is expected that insects affected in this way would not have been able to recover, thereby protecting vines. These symptoms were more common on foliar-treated vines than on those receiving soil applications. This difference may be due to decline of neonicotinoid residues after foliar application, leading to concentrations that were insufficient for rapid control of *E. fabae*. Indeed, an 89% decline was detected in imidacloprid residues in mature leaves from day 1 to day 27, and in immature leaves a 98% decline over the same time period. Vines receiving a single soil treatment as in this experiment are expected to have a reservoir of active ingredient at the roots that is gradually depleted, and this likely caused the lethal levels of insecticide in the leaves over the duration of the study which declined with time. The greater amount of leafhoppers with moribund symptoms observed on foliar-treated leaves may also be related to the distinct penetration profile and residue degradation pattern characteristic of foliar treatment, as opposed to the profile resulting from systemic delivery of insecticide through soil treatment. Whereas soil treatments rely primarily on leafhopper feeding to deliver the insecticide, foliar treatments provide a combination of contact and ingestion routes of exposure. Further research to observe *E. fabae* feeding behavior on vines treated in both ways could help elucidate the mechanisms of these effects.[18]

The residue analysis results for foliar-treated grape leaves suggest that the physical characteristics of immature grape leaves may have greater absorption potential for imidacloprid than mature leaves. Additionally, the surface area expansion for immature leaves was much greater than that for mature leaves, nearly doubling over the duration of the experiment. As the size of mature grape leaves remained relatively uniform, the decline in imidacloprid residue concentrations over the 28 day period can be attributed primarily to environmental degradation. For immature leaves, however, the decline of residues reflects a combination of environmental degradation and plant growth dilution that may help explain the challenge of controlling *E. fabae* on rapidly expanding vine shoots. The combination of bioassay and residue analysis approaches also provided a surprising insight into the high toxicity of the tested insecticides, in that leaves with a 90% reduction in residue still had less than 10% survival of nymphs.

Soil application methods help mitigate the challenge of maintaining sufficient residue in vine canopies because they can minimize photolytic degradation and provide long-lasting delivery of systemic insecticides via the root system. The rate of application has a direct influence on the concentration of imidacloprid that reaches grapevine canopies from soil applications,[19] and a 562 g ha^{-1} rate was required to provide season-long concentrations above the threshold for killing glassy-winged sharpshooters in California vineyards.[6] In the present study, even this high rate of drip-applied imidacloprid did not provide significant reduction in *E. fabae* on these grapevines. Delayed uptake of imidacloprid has been reported in mature vines,[19] and this may provide immigrating *E. fabae* adults with sufficient time to cause the hypersensitive response that stunts vines. Therefore, regular scouting of susceptible vineyards during spring, and particularly after rainstorms, can identify the immigration of adults and allow growers to protect their vines from initial feeding. Coupling this with soil application of a neonicotinoid will then protect the vines from further *E. fabae* injury and will save multiple tractor trips through the vineyard. Field testing of this strategy is needed, to determine whether this can effectively reduce *E. fabae* injury to vines.

Once neonicotinoid insecticides are applied to crop canopies or soil under field conditions, they undergo various forms of environmental degradation[20–22] that reduce their residual activity. In the present field trial to compare soil-applied neonicotinoids, the high activity observed in the potted vines was largely lost for imidacloprid and clothianidin. In contrast, thiamethoxam treatment provided greater control of *E. fabae* at 26 days after treatment than any of the other treatments. Nauen *et al.*[23] demonstrated that thiamethoxam is converted to clothianidin in pest insects and in plants, so this result is unlikely to be caused by differences in inherent toxicity, although there was a slightly higher rate of active ingredient in the thiamethoxam compared with the clothianidin treatment. Given the need for insecticide to move from vine roots to the canopy, the efficacy of thiamethoxam is expected largely to be a function of its 8–10-fold higher water solubility.[24,25] Environmental stability parameters may also contribute to the intertreatment variation in field performance.

Increasing integration of neonicotinoids into grape IPM programs is expected for control of key pests, while also providing improved environmental and worker safety. Much of this will be through use of foliar formulations now registered for control of vine pests such as beetles, leafhoppers and aerial phylloxera. As new neonicotinoid products with a greater spectrum of activity become available, they are expected to provide even greater utility to farmers seeking broader-spectrum pest control. For those neonicotinoids that can be applied to the soil, dependence on this chemical class will be further facilitated as growers learn more about the benefits of delivery of systemic insecticides to the vine through injection into drip irrigation systems.[26]

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