Soil application of neonicotinoid insecticides for control of insect pests in wine grape vineyards

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Abstract

BACKGROUND: Soil application of systemic neonicotinoid insecticides can provide opportunities for long-term control of insect pests in vineyards, with minimal risk of pesticide drift or worker exposure. This study compared the effectiveness of neonicotinoid insecticides applied via irrigation injection on key early-season and mid-season insect pests of vineyards in the eastern United States.

RESULTS: On vines trained to grow on drip irrigation, early-season application of imidacloprid, clothianidin, thiamethoxam and dinotefuran provided high levels of control against the potato leafhopper, *Empoasca fabae*. Protection of vines against Japanese beetle, *Popillia japonica*, and grape berry moth, *Paralobesia viteana*, was also observed after mid-season applications. Efficacy was poor in commercial vineyards when treatments were applied to the soil before irrigation or rain, indicating that vines must be grown with an irrigation system for efficient uptake of the insecticide.

CONCLUSIONS: In drip-irrigated vineyards, soil-applied neonicotinoids can be used to provide long residual control of either early-season or mid- to late-season foliage pests of vineyards. This approach can reduce the dependence on foliar-applied insecticides, with associated benefits for non-target exposure to workers and natural enemies.

Keywords: chemigation; systemic; integrated pest management; reduced risk

1 INTRODUCTION

Grapevines are at risk of infestation by insects that consume leaves, shoots, roots or fruit.\(^1\)\(^2\) In the eastern United States, this pest complex includes potato leafhopper (*Empoasca fabae* Harris), the nymphs and adults of which feed on leaves and shoots in late spring and early summer, Japanese beetles (*Popillia japonica* Newman), which defoliate vines during mid-late summer, and grape berry moth (*Paralobesia viteana* (Clemens)), which has 3–5 generations of larvae, depending on the region, which infest fruit from bloom to fruit maturity. When action thresholds are exceeded, these pests may be controlled through foliar application of insecticides, but the development of systemic insecticides, coupled with methods for their delivery to root systems, provides an alternative approach.\(^3\)\(^4\)

The advent of irrigation injection technology\(^5\)\(^6\) has provided a potential insecticide delivery system that, if effective, would have the additional benefits of reducing drift and non-target exposure, as well as avoiding the typical post-application degradation from wash-off by rain\(^7\) or by ultraviolet light.\(^8\)\(^9\)

The neonicotinoid class of insecticides contains members that have been shown to have high activity on homopteran pests of grapevines,\(^10\)\(^11\)\(^12\)\(^13\) in addition to beetle pests.\(^14\)\(^15\) Although imidacloprid has activity on these two groups, it is much less effective on lepidopteran pests. In contrast, the third-generation neonicotinoid dinotefuran has a broad activity spectrum, including Lepidoptera.\(^16\) This suggests that systemic delivery by root-targeted applications to vines may be able to provide control of a complex of grape insect pests.

Recent comparison of application routes of neonicotinoids to vines demonstrated longer residual control of the potato leafhopper by soil applications as opposed to foliar applications.\(^17\) Most of these studies were in potted vines, however, and the relevance to vineyard conditions remains unclear. In the present study, an irrigation injection system was employed to deliver different neonicotinoids to the roots of vines, and the degree of protection against key insect pests was measured. In vineyards without the equipment for irrigation injection, it may also be possible to use banded application under the vines to achieve application to the root zone. Therefore, tests were also conducted to establish whether banded applications could provide insect control in established vineyards.

2 METHODS

2.1 Potato leafhopper: irrigation injection applications

Irrigation injection of the soil formulations of three insecticides was tested in a vineyard at the Michigan State University Trevor Nichols Research Center (TNRC) in Fennville, Michigan. This vineyard was planted in 2005 with seven vines per row and 27 total rows. The
north 13 rows were planted with Vitis sp. cv. Chancellor vines, and the south 14 rows were planted with Vitis sp. cv. Aurora vines. All vines in this vineyard received regular drip irrigation from immediately after planting.

Insecticide applications to the vineyard were made using a randomized complete block design with four replications (two in cv. Chancellor and two in cv. Aurora). The following insecticide treatments were each applied to seven-vine rows via irrigation injection on 6 June 2006, timed for when potato leafhoppers first appeared on yellow sticky sentinel traps placed in the vineyard: clothianidin 160 g kg⁻¹ WG (Belay WG; Valent USA, Walnut Creek, CA; 1400 g ha⁻¹, 224 g AI ha⁻¹); imidacloprid 240 g L⁻¹ F (Admire 2F; Bayer CropScience, Research Triangle Park, NC; 1.17 L ha⁻¹, 281 g AI ha⁻¹); thiamethoxam 240 g L⁻¹ SC (Platinum 2SC; Syngenta Crop Protection, Inc., Greensboro, NC; 1.17 L ha⁻¹, 281 g AI ha⁻¹); and a control that received no injections. Chemicals were injected into the existing irrigation drip line by connecting a 2 gal spray canister (R&D Sprayers) containing the insecticide to the drip line via quick couplers. Canisters were pressurized with CO₂ to 28 T m⁻² (40 psi), and the irrigation system was maintained at a constant 14 T m⁻² (20 psi). The insecticide mixtures (1 gal), as measured by weighing the spray canister, were applied to each row over the course of 0.5 h. Rows were irrigated for a total of 4 h, including 0.5 h before application, 0.5 h during application and 3 h after application, to ensure that the chemical was watered in effectively. Untreated control plots received the same irrigation regime without any chemicals being injected into the drip lines.

Potato leafhopper assessments were conducted from the time chemicals were applied until leafhopper populations naturally declined at the end of July. Assessments took place every 2–5 days by examining 30 randomly selected leaves (six leaves on each of the middle five vines) in each seven-vine row, and counting the total number of potato leafhopper adults and nymphs on those 30 leaves. Injury to vines caused by potato leafhopper was measured on 6 July 2006 by assessing the amount of potato-leafhopper-induced leaf yellowing on 20 randomly selected leaves per plot (four leaves on each of the middle five vines). Leaves were considered to be injured when yellowing was present around the margins of the leaf.

### 2.2 Potato leafhopper: banded soil applications

A second experiment was set up to test the effectiveness of banding insecticides under vines in vineyards receiving different irrigation programs. Two insecticides were tested at three commercial wine grape vineyards in 2007. Two vineyards (site 1 and site 2, cv. Pinot Noir) were located near Traverse City, Michigan, and one vineyard (site 3, cv. Chardonnay) was located near Fennville, Michigan. Site 1 was trained on a drip irrigation system that was used only during periods of hot dry weather, while site 2 and site 3 had no irrigation systems in place. Four replicates of five-vine plots were laid out in a randomized complete block design near the edge of each vineyard with the following treatments: imidacloprid 552 g L⁻¹ SC (Admire Pro; Bayer CropScience, Research Triangle Park, NC; 0.51 L ha⁻¹, 282 g AI ha⁻¹); imidacloprid 552 g L⁻¹ SC (Admire Pro; 1.02 L ha⁻¹, 564 g AI ha⁻¹); dinetrafuran 700 g kg⁻¹ WG (Venom 70SG; Valent USA, Walnut Creek, CA; 245 g ha⁻¹, 350 g AI ha⁻¹); dinetrafuran 700 g kg⁻¹ WG (Venom 70SG; 294 g ha⁻¹, 420 g AI ha⁻¹); and a untreated control. Chemicals were applied to the soil in a 47 cm band directly underneath the trellis wire for the length of the plot. All applications were applied using a CO₂-powered backpack sprayer fitted with a single head boom and a TeeJet 8003 VS nozzle operating at 35.1 T m⁻² (50 psi) and delivering spray solution equivalent to 655.8 L h⁻¹. Applications took place on 1 June 2007 at sites 1 and 2, and on 5 June 2007 at site 3. Treatments at site 1 received 3.8 cm of water immediately post-treatment using a drip irrigation system, whereas treatments at site 2 were watered in with 0.77 cm of rain later in the day of the treatment. Treatments at site 3 were irrigated immediately after application using a hose to apply 3.8 cm of water where chemicals were applied.

Each of the sites was assessed for potato leafhopper just prior to the treatment applications and then at 3, 11 and 24 days after treatment (DAT). The total number of potato leafhopper adults and nymphs were counted on 30 randomly selected leaves per plot (ten leaves on the middle three vines). Potato-leafhopper-induced leaf yellowing was measured at 25 DAT by assessing the percentage of leaves examined for potato leafhopper adults and nymphs that exhibited potato-leafhopper-induced leaf yellowing symptoms.

#### 2.3 Mid-season application for Japanese beetle control

The irrigation injection experiments conducted for potato leafhopper in 2006 were repeated in 2007 to test for control of Japanese beetle. The methods for this experiment were the same as in the 2006 potato leafhopper irrigation injection experiment, except that insecticide applications targeting potato leafhopper took place on 6 June 2007, and potato leafhopper populations were extremely low in this vineyard in 2007, so those data are not presented.

A reaplication was made on 6 July 2007 at the start of Japanese beetle activity with the following treatments: 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; 1.02 L ha⁻¹, 563 g AI ha⁻¹); and a control that received no injections. These treatments were watered in using 2.5 cm of water immediately post-application.

Japanese beetles on vines were assessed by counting the total number of adults on each row every 2–6 days, starting at adult emergence in mid-June and continuing through to the end of August. The health of individual beetles was assessed as either healthy or moribund (behaviors including leg twitching, rear legs dragging behind body and sluggish movements). Japanese beetle leaf feeding injury was assessed on 10 August 2007 (35 days after the second treatment) by estimating the total percentage of leaf area removed (5% increments) on 50 leaves per plot (ten leaves on each of five vines). Because this insect exhibits top-down feeding preferences on foliage,18 leaves were randomly selected from the five highest shoots on each vine. In addition to field sampling, laboratory bioassays were conducted to measure feeding on four uninjured leaves per treatment plot. Laboratory bioassays were conducted once a week, beginning on 2 July 2007 (4 days prior to the second irrigation injection treatments) and ending on 14 September 2007 (70 days after the second irrigation injection treatments). Individual leaves were collected and placed directly into a water pick (Aquapic® brand, No. 49-47, 10 cm length) that was inserted into a hole cut into the bottom of a 32 oz deli cup. One female Japanese beetle, obtained the same day using green bucket-style traps baited with sex pheromone and floral lure (Trécé Inc., Adair, OK), was then added to each cup and allowed to feed on the leaf for 96 h before being removed. Beetle health was assessed just prior to removal from the cup using the following rankings: 1 = dead, 2 = moribund, 3 = no visible signs of chemical poisoning. Leaves were taped to white printer paper and scanned.
in as jpeg images using a Canon Color imageRUNNER C3380i copy machine. Leaf area removed (cm²) was measured using computer software (Scion Image for Windows, Alpha Release 4.0.3.2, Scion Corporation, Frederick, MD).19

2.4 Grape berry moth infestation

Infestation of grape clusters by grape berry moth was assessed in the irrigation injection experiments timed for control of potato leafhopper and Japanese beetle. Grape berry moth was assessed on 28 July 2006 during the potato leafhopper irrigation injection experiments and on 24 August 2007 in the Japanese beetle irradiation injection experiments. Assessments in 2006 were made by counting the number of infested clusters out of all clusters (13 clusters per plot on average) on the middle five vines, while assessments in 2007 consisted of counting the number of infested clusters out of 25 clusters per plot (five clusters on each of the middle five vines).

2.5 Data analysis

All statistical analyses were performed using Systat 13 (Systat Software, Inc., Chicago, IL). For potato leafhopper irrigation injection experiments, the total number of potato leafhopper adults and nymphs for each sampling date as well as the season totals were log (x + 1) transformed to meet the assumptions of normality and were subsequently analyzed using analysis of variance (ANOVA). Post hoc comparisons were made using a Fisher’s protected least significance (PLS) test. Leaf yellowing data in the irrigation injection experiments for potato leafhopper control did not fit the assumptions of normality and were analyzed using a Kruskal–Wallis test followed by a Conover–Inman test for post hoc comparisons.20 Data on the total number of Japanese beetles per row were log (x + 1) transformed before analysis using ANOVA followed by Fisher’s PLS for post hoc comparisons. Moribund beetle data did not fit the assumptions of normality and were analyzed using a Kruskal–Wallis test followed by a Conover–Inman test for post hoc comparisons. Japanese beetle proportion feeding damage estimate data were arc sine transformed before analysis using ANOVA followed by Fisher’s PLS for post hoc comparisons. In the leaf feeding bioassays, the defoliation data were analyzed using ANOVA followed by Fisher’s PLSD test for post hoc comparisons. In the banding experiments against potato leafhopper, the number of adult and nymph leafhoppers did not fit the assumptions of normality and were analyzed using a Kruskal–Wallis test followed by a Conover–Inman test for post hoc comparisons. The proportion of clusters infested with moth larvae were arc sine transformed and analyzed using ANOVA followed by Fisher’s PLS for post hoc comparisons. A significance value of 0.05 was used for all tests, and untransformed means and percentages are presented ± SEM for all experiments.

3 RESULTS

3.1 Potato leafhopper: irrigation injection applications

A comparison of clothianidin, imidacloprid and thiamethoxam applied to soil via irrigation injection showed significant differences in abundance of potato leafhopper adults from 16 to 27 DAT and nymphs on vines from 7 to 28 DAT (Fig. 1). The biggest treatment effect was to the populations of leafhopper nymphs, which dropped to near zero by the sixth day after treatment on both clothianidin- and thiamethoxam-treated vines, while nymphs on imidacloprid-treated vines took an additional week to drop off. When the seasonal totals of nymphs were analyzed, all three insecticide treatments had significantly fewer nymphs per plot than the untreated controls (F = 48.11, P < 0.001; untreated: 235.0 ± 46.0, clothianidin: 4.3 ± 1.3, imidacloprid: 41.0 ± 8.7).

Potato leafhopper adults were relatively scarce on all treatments until 15 DAT, when adult abundance increased on untreated vines. Adults in the three insecticide treatments increased gradually until 29 DAT, when they reached levels similar to untreated vines. Seasonal totals of adult potato leafhoppers per plot were not significantly different from each other (F = 2.91, P = 0.078; untreated: 39.0 ± 16.4, clothianidin: 12.8 ± 3.1, imidacloprid: 27.8 ± 4.8, thiamethoxam: 17.8 ± 4.3). However, there were significant differences at 10 DAT (clothianidin significantly lower than imidacloprid; F = 4.60, P = 0.023), 20 DAT (imidacloprid, clothianidin and thiamethoxam significantly lower than controls; F = 5.15, P = 0.016), 27 DAT (clothianidin and thiamethoxam significantly lower than imidacloprid and controls; F = 10.76, P = 0.001) and 34 DAT (clothianidin significantly lower than imidacloprid and thiamethoxam; F = 3.73, P = 0.042).

Figure 1. Average number of adult (A) and nymph (B) potato leafhoppers found on 30 leaves per plot. Vines received one of three insecticide treatments applied via irrigation injection on 6 June 2006. Asterisks indicate significant differences among some treatments using an alpha value of 0.05.
3.2 Potato leafhopper: banding applications
The total number of potato leafhopper adults and nymphs on vines was not significantly different among treatments on any of the assessment dates (Table 1). Leaf yellowing levels also did not differ significantly among banding treatments at any of the three sites ($F < 0.54, P > 0.71$).

3.3 Mid-season application for Japanese beetle control
There were fewer Japanese beetles on treated vines at 1, 14 and 24 DAT. At 10 days after the second irrigation injection there were significantly fewer beetles on imidacloprid-treated vines than on thiamethoxam-treated and untreated vines (Fig. 2A) ($F = 3.98, P = 0.035$). At 14 and 24 DAT there were significantly fewer beetles on vines in the imidacloprid treatment than in each of the other three treatments (Fig. 2A) (14 DAT: $F = 7.29, P = 0.005$; 24 DAT: $F = 4.77, P = 0.021$). There were no significant differences among treatments on any of the other sampling dates (Fig. 2A) ($F = 0.18, P > 0.91$).

The initial assessment of moribund beetles in early July indicated significant differences among treatments, even though the initial potato-leafhopper-targeted applications had been applied at least 1 month before (Fig. 2B), but the abundance of these insects was very low at this time. Later assessments showed significantly more moribund beetles on imidacloprid-treated vines than with the other treatments at all assessment periods except 10, 14 and 19 DAT (0, 3, 24, 28, 35, 39, 46, 56 DAT: $H > 10.9, P < 0.01$). This trend became even more pronounced, beginning with the 24 DAT assessment, and continued to the end of assessments at 56 DAT (Fig. 2B). At 35 DAT, all insecticide treatments were significantly different from each other except for the clothianidin and thiamethoxam treatments, and at 46 DAT and 56 DAT all treatments were significantly different from each other except for the clothianidin and untreated treatments (Fig. 2B).

Feeding damage estimates, as measured by the estimated percentage leaf area fed on and removed from vines at 35 DAT, showed that leaves on imidacloprid-treated vines were significantly less damaged than with any of the other treatments ($F = 21.32, P < 0.0001$) (imidacloprid: $4.43 \pm 0.5$, thiamethoxam: $27.67 \pm 7.9$, clothianidin: $48.30 \pm 2.4$, untreated: $50.97 \pm 7.2$). Thiamethoxam-treated leaves were also significantly less damaged than clothianidin-treated and untreated leaves. Clothianidin treatments did not differ significantly from the untreated controls.

Laboratory bioassays using leaves from vines in these experiments confirmed this pattern of the lowest level of leaf feeding damage occurring in the imidacloprid treatments, thiamethoxam with the second lowest amount of damage and clothianidin showing no significant differences from the untreated controls (Fig. 3) (0, 14, 21, 28, 35, 42, 49, 63 DAT: $F > 6.0, P < 0.01$; 56 DAT: $F > 4.1, P < 0.03$). In the laboratory bioassays, imidacloprid-treated leaves continued to show significant reductions in Japanese beetle feeding through the 63 DAT bioassays.

3.4 Grape berry moth infestation
There were no significant differences in the number of clusters infested with grape berry moth during the early-season irrigation injection experiments (Table 2). However, in the mid-season irrigation injection experiments timed for control of Japanese beetle, infestation of grape berry moth was reduced from 11%
Untreated suggests that imidacloprid may be binding to the soil organic matter, thereby providing lower titers of active ingredient in the leaves and slower control of the adult leafhoppers compared with the other treatments. It is also possible that the observed activity on adult potato leafhopper reflects lower sensitivity of this insect species to imidacloprid than to other neonicotinoids. Imidacloprid has been used in vineyards for control of other sucking pests, including sharpshooters and mealybugs, indicating that it is an effective insecticide for vine protection against other homoptera. In spite of the slower activity in imidacloprid treatments, it should be noted that, by 2 weeks after treatment, there were similarly high levels of control of potato leafhopper in all three insecticide treatments. In spite of the control of potato leafhopper observed in the young irrigated vines, there was no activity of neonicotinoid treatments applied to mature vineyards. Whether application was banded under the vine in a site with or without drip irrigation, no significant reduction in leafhopper densities or in injury symptoms was observed. The vineyards used for this experiment were all more mature than those used in the irrigation injection study, and only one of them had a drip irrigation system, but it was used only during infrequent periods of high water stress. The contrasting results between the two vineyard situations suggests that, in regions of grape production with high summer rainfall, unless vines are irrigated regularly, the roots will remain widely distributed and uptake of insecticide banded sprays will be too low to achieve sufficient control of canopy-feeding insect pests. Studies on grapevine root distribution under different irrigation regimes emphasize the link between irrigation distribution and root distribution.

The present results from the Japanese beetle trials emphasize how pest management goals (in this case, reduced leaf feeding) may be achieved without causing complete mortality of the pest. The neonicotinoid insecticides tested in the irrigation injection vines caused little effect on beetle abundance on the vines, although imidacloprid-treated vines had consistently lower abundance. When beetle behavior was closely observed, however, those on imidacloprid-treated vines demonstrated a much higher level of moribund symptoms, which included uncoordinated movement and slow walking on the leaves. Leaves from treated vines that were brought into the laboratory for exposure to untreated beetles provided a standardized method for comparing effects of previous treatments. In this study, such an approach indicated that, in spite of observations in the vineyard suggesting no reduction in beetle abundance, leaves from vines treated with imidacloprid, and to a lesser extent thiamefoxam, were much less likely to be fed upon. Such effects could translate into vineyard-level protection against Japanese beetles in drip-irrigated vineyards, but further large-scale trials would be needed to determine the level of insect control achieved.

The control on grape berry moth in this study suggests that neonicotinoids delivered mid-season by root-targeted injection can result in sufficient residues in the fruit to control this pest. This result was unexpected, as root-targeted insecticide applications would depend on the xylem stream for translocation to primary sink tissues associated with transpiration, and grape berries receive a relatively low proportion of the xylem flow after fruit ripening begins.

The ability to control multiple insect pests with one insecticide application is of great interest to vineyard managers who are concerned about the environmental impact, cost and efficiency of their pest control programs. In eastern US grape production regions, emergence of Japanese beetle adults typically begins a month after the period of rapid shoot growth, when potato

![Figure 3. Average grape leaf area (cm²) removed by Japanese beetles in laboratory feeding bioassays. Beetles were given grape leaves treated with one of three insecticide treatments applied via irrigation injection on 6 June 2007 and 6 July 2007. Bioassays began 4 days prior to the treatment and ended 70 days later. Asterisks indicate significant differences among some treatments using an alpha value of 0.05.](chart)

<table>
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<th>Treatment</th>
<th>Early-season irrigation injection</th>
<th>Mid-season irrigation injection</th>
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<td>Untreated</td>
<td>34.3 ± 4.4 a</td>
<td>11.0 ± 1.9 a</td>
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<td>Clothianidin</td>
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<td>Imidacloprid</td>
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<td>1.0 ± 1.0 b</td>
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<td>Thiamefoxam</td>
<td>25.3 ± 13.1 a</td>
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<td>$F$</td>
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<td>$P$</td>
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<td>0.002</td>
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in the control treatment to 1% in the imidacloprid treatment (Table 2). The two other insecticides were intermediate, but not significantly different from the untreated control.

4 DISCUSSION

This study demonstrates the potential for long-term insect control in eastern US vineyards with soil-applied neonicotinoid applications. High levels of insect control were found using an injection system to deliver insecticides to the roots of vines grown using irrigation. Control was observed during the early season, when potato leafhopper was active, with both clothianidin and thiamefoxam providing rapid initial control followed by a month of protection against nymphs and approximately 3 weeks of protection against adult leafhoppers. While imidacloprid provided similar protection against adult leafhoppers, the effect did not start as rapidly as for the other treatments. This may reflect a slower movement of imidacloprid from the roots to the leaves and shoots of the vine or lower absolute toxicity, or it may be an effect of the rates registered for use in vineyards. All three of these insecticides were shown to be similarly effective at controlling potato leafhopper in recent studies using soil application to potted vines, but only thiamefoxam was effective in field trials. This suggests that imidacloprid may be binding to the soil organic
leafhopper control may be needed. Additionally, pest pressure from grape berry moth increases in July, and, if that generation is not controlled, the third generation before harvest can cause significant cluster infestation and berry loss.\textsuperscript{25} This mid-season period of insect activity may be amenable to control using irrigation injection systems such as that demonstrated in this study. Combined protection against coleopteran and lepidopteran insect pests through irrigation treatment would reduce the need to drive machinery through the vineyard for insecticide application while also reducing pesticide exposure to workers during periods of manual shoot positioning and leaf pulling, especially in premium winegrapes. Although irrigation injection during periods of manual shoot positioning and leaf pulling, application while also reducing pesticide exposure to workers need to drive machinery through the vineyard for insecticide treatment would reduce the study. Combined protection against coleopteran and lepidopteran irrigation injection systems such as that demonstrated in this period of insect activity may be amenable to control using neonicotinoid insecticides (Hymoptera: Mymaridae). BioControl 43:130–135 (2007).


