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Efficacy of Selected Insecticides for Management of the Multicolored Asian Lady Beetle on Wine Grapes Near Harvest

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Abstract

As one component of an Integrated Pest Management program for Midwestern wine grapes, we examined the efficacy of several insecticides on adults of the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). For field trials, percentages of clusters with at least one *H. axyridis* were recorded for each plot before treatment and on the day of harvest. We also examined the efficacy of insecticides under laboratory conditions via two routes of exposure, topical application and insecticide residues. In the 2004 field trials, the percentage of clusters infested with *H. axyridis* adults at harvest was statistically lower in plots treated with bifenthrin applied 7 days before harvest (DBH), carbaryl 10 DBH, and in plots covered with floating row cover compared to the untreated plots. In 2005, the percentage of clusters infested with *H. axyridis* adults at harvest in plots treated with zeta-cypermethrin 7 DBH, bifenthrin 22 and 7 DBH, and imidacloprid 1 DBH was statistically lower than beetle infestation in the untreated plots. In the laboratory, bifenthrin, carbaryl, and thiamethoxam were lethal to *H. axyridis* adults 7 days after treatment. Based on the efficacy results, labeled insecticides, pre-harvest intervals, and the late-season influx of *H. axyridis* infestations, chemical control is currently limited to carbaryl, malathion, and/or imidacloprid.

Introduction

The multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), has recently become an economically significant contaminant pest in the wine making process. *H. axyridis* adults tend to aggregate on clusters with injured berries (Figs. 1 and 2) just prior to harvest, and, eventually, they may be incorporated with the grapes during wine processing (5,10). Once crushed, *H. axyridis* release their foul smelling hemolymph that, depending on the level of infestation, can taint the flavor and aroma of the resulting wine (10). A component of *H. axyridis* hemolymph, 2-isopropyl-3-methoxypyrazine (IPMP), was recently identified as one of the key compounds responsible for affecting wine flavor (9). The sensory threshold for the white grape variety 'Riesling' was approximately 1.76 *H. axyridis* adults per kg of grapes (8), or 0.4 per cluster assuming an average weight of 0.23 kg per cluster. Such a low threshold demonstrates the potential damage that this pest could cause to the wine industry. In addition, wine made from red grape varieties could be even more sensitive to taint and therefore may have an even lower threshold (8). Tainted wine and the unacceptable taste associated with it could lead to economic losses for the wine industry in US states and Canadian provinces in the Great Lakes region.

Fig. 1. Aggregation of *Harmonia axyridis* adults on clusters of 'Leon Millot,' Hastings, MN, 2005.

Fig. 2. *Harmonia axyridis* adult feeding on injured berry, Hastings, MN, 2005.

Although remediation of tainted wine by adding oak chips, activated charcoal, and deodorized oak has decreased *H. axyridis*-related taint or IPMP concentration, it has not completely removed the taint from contaminated wine (7). Remediation of tainted wine has two other shortcomings. First, it may not be appropriate for all wine grape varieties. Second, legislative restrictions on the use of remediation products may limit the application of this option (7). Therefore, the use of control measures, such as insecticides, to manage *H. axyridis* before it can become a wine contaminant is essential for reducing the economic impact of this pest on the wine industry.

Although several laboratoy studies have shown the efficacy of some insecticides for *H. axyridis* in wine grapes (e.g., 15,16), we are aware of only one field study (17). This field study was limited to the assessment of three insecticides, including imidacloprid, azadirachtin, and acetamiprid (17). Other compounds, such as camphor and menthol, have been shown to repel *H. axyridis* adults on the outside of buildings, but the repellent effects of these materials only lasted 48 h (11). Therefore, there is a need to assess the field efficacy of additional insecticides for *H. axyridis* control in wine grapes. In addition, laboratory toxicity studies addressing the route of exposure and residual activity can also be useful in making final decisions for insecticide choice for *H. axyridis* management in wine grapes. The objectives of this paper were: (i) to investigate the efficacy of currently labeled insecticides against *H. axyridis*; and (ii) to evaluate the efficacy of selected insecticides for possible registration or reduction of their pre-harvest interval (PHI) for wine grapes. For example, thiamethoxam and zeta-cypermethrin are not currently registered for use in grapes, and bifenthrin was applied at a shorter PHI than indicated on the label.

Field Trials

Field experiments were conducted in 2004 and 2005 at a commercial vineyard in east-central Minnesota, near Hastings. The insecticide trials were set up in an established vineyard of 'Leon Millot'. In 2004, insecticide treatments included carbaryl (Sevin XLR Plus, Bayer CropScience, Kansas City, MO) at 56 oz/acre or 1.71 kg a.i./ha; bifenthrin (Capture 2EC, FMC Corporation, Philadelphia, PA) at 2.56 oz/acre or 0.045 kg a.i./ha; malathion (Malathion 5, Agriliance, St. Paul, MN) at 48 oz/acre or 1.90 kg a.i./ha, pyrethrin (Pyganic EC 1.4, MGK Company, Minneapolis, MN) at 32 oz/acre or 0.031 kg a.i./ha; and an untreated check. All insecticides were applied within 16 days of anticipated harvest and within the respective pre-harvest intervals. Carbaryl was applied 16 and 10 days before harvest (DBH) (September 1 and 7); bifenthrin at 7 DBH (September 10); malathion at 10 and 7 DBH (September 7 and 10); pyrethrin at 1 DBH (September 16). The floating row cover (FRC) was set on trellises on the same day that the first insecticide treatment was applied (16 DBH, September 1), and it was not removed until the harvest day. FRC covered the entire foliage and clusters of the vines, and it was tied with staples at 0.30 m from the ground.

In 2005, treatments included: carbaryl (Sevin XLR Plus) at 64.0 oz/acre or 1.97 kg a.i./ha; bifenthrin (Capture 2EC) at 6.4 oz/acre or 0.112 kg a.i./ha; imidacloprid (Provado Solupak 75% WP, Bayer CropScience) at 1.0 oz/acre or 0.053 kg a.i./ha, thiamethoxam (Actara WG, Syngenta Crop Protection Inc., Greensboro, NC) at 3.5 oz/acre or 0.061 kg a.i./ha; zeta-cypermethrin (Mustang Max, FMC Corporation) at 4.0 oz/acre or 0.027 kg a.i./ha; and an untreated check. All insecticides were applied within 22 days of anticipated harvest, and within the respective pre-harvest intervals. Carbaryl, thiamethoxam, and zeta-cypermethrin were applied 7 days before harvest (DBH) (August 24); bifenthrin at 22 and 7 DBH (August 9 and 24, respectively); and imidacloprid at 1 DBH (August 30).

For both years, treatments were arranged in a randomized complete block design with four replications and each plot consisted of one trellis and spanned the length of four vines. A single untreated trellis separated plots. Standard production practices were followed for disease and weed control and pruning (19). Insecticides were applied using a CO_2-pressurized backpack sprayer with a 0.91-m (3-ft) boom with 2 nozzles (XR-Teejet 8002 flat fan with no screen). The sprayer was calibrated to deliver 467.74 liters/ha (50 gal/acre) at 242.32 kPa (35 psi). Plots were sampled for *H. axyridis* adults and injured berries on 31 August (pre-treatment sample), 17 September 2004 (at harvest sample), and 23 (pre-treatment sample) and 31 August 2005 (at harvest sample). *H. axyridis* adults were identified using a diagnostic guide (13). On each sample date in 2004 and 2005, 10 and 15 randomly-selected clusters from each plot were sampled, respectively, using visual whole-cluster inspection; percentages of clusters with at least one *H. axyridis* or one injured grape berry were recorded for each plot. Percentages were averaged within treatments for each sample date, giving a mean percentage per plot for each treatment on each sample date. Mean percentages were then transformed by arcsine square root (15). Transformed percentages of infested clusters were analyzed by date using analysis of covariance (Proc GLM, SAS Institute Inc., Cary, NC). The analysis of covariance model included main effects for treatment and the covariate (i.e., transformed percentages of clusters with at least one injured grape berry). Mean square errors (MSE) of the analysis of covariance for all sample dates were smaller than the MSE of analysis of covariance increasing the precision of the analysis. If the mean effect for treatment for each sample date was significant ($P < 0.05$), then differences among treatments were tested using least square means for each pairwise combination of treatments.

Laboratory Tests

Harmonia axyridis adults were obtained from a laboratory colony established from adults collected during October 2004 at the University of Minnesota Outreach, Research, and Education (UMORE) Park, Rosemount, MN. Following collection, beetles were held in 1.96-liter plastic dishes with ~200 beetles per dish, and maintained at 10 ± 1°C and a photoperiod of 16:8 (L:D) h. Prior to experimentation, the dishes containing beetles were warmed to 25 ± 1°C with a photoperiod of 16:8 (L:D) h (these rearing conditions were used throughout the rest of the studies), and the beetles were allowed to mate for 14 days. Beetles were provided an *ad libitum* supply of live soybean aphids, *Aphis glycines* Matsumara, a diet made from freeze-dried honey bee, *Apis mellifera* L., drone pupae (6), and water in 0.5-ml plastic microcentrifuge tubes plugged with cotton. After the mating period, adult females were maintained individually in plastic Petri dishes (60 mm × 15 mm) lined with 55-mm filter paper disks. The Petri dishes containing females were checked daily for oviposition. If eggs were found, the females were removed and transferred to new Petri dishes (60 mm × 15 mm) provisioned with food and water. After egg hatch and dispersal of larvae from egg clusters (i.e., ~1 day after hatching), single larvae were placed into separate plastic Petri dishes (60 mm × 15 mm), and reared to adults on a diet of freeze-dried *A. mellifera* drone pupae.

The experiment was conducted as a randomized complete block design for adults (7 ± 2 days after emergence) of *H. axyridis*. The experiment consisted of eight treatments, three replications over time, with seven individuals per replication, and two routes of exposure (topical application and insecticide residues). Treatments included carbaryl (Sevin XLR Plus) at 48 oz/acre or 1.47 kg a.i./ha; bifenthrin (Capture 2 EC) at 4.8 oz/acre or 0.084 kg a.i./ha; imidacloprid (Provado Solupak) at 0.9 oz/acre or 0.047 kg a.i./ha; azadirachtin (Ecozin EC, AMVAC Chemical Corporation, Los Angeles, CA) at 9.0 oz/acre or 0.019 kg a.i./ha; thiamethoxam (Actara WG) at 4.0 oz/acre or 0.07 Kg a.i./ha; and an untreated check (i.e., water). Treatments were applied using a motorized spray chamber (Department of Agronomy, University of Minnesota) with a single XR-Teejet 8002 flat fan nozzle (e.g., 3,4). The sprayer was calibrated to deliver the equivalent of 467.70 liters/ha (50 gal/acre) at 242.32 kPa (35 psi).

Routes of exposure included topical application and insecticide residue. For the topical application exposure, seven individuals were placed into plastic Petri dish bottoms (150 mm × 15 mm) covered with a fine metal mesh to retain the insects in the dish, and then placed into the spray chamber. After treatment, the Petri dish bottoms were removed from the spray chamber and allowed to dry for 1 h before individuals were transferred to clean plastic Petri dishes (60 mm × 15 mm). For insecticide residue exposure, insecticides and water were applied inside the tops and bottoms of seven plastic Petri dishes (60 mm × 15 mm) using the spray chamber. After treatment, the tops and bottoms of Petri dishes were removed from the spray chamber and allowed to dry for 1 h, 1, 3, and 7 days before one adult was transferred to each treated Petri dish.

Food and water were not provided for any treatment in the first 24 h post-exposure. Petri dishes with adults were held at 25 ± 1°C and a photoperiod of 16:8 (L:D) h. After 24 h, mortality was recorded, and live individuals were transferred to clean plastic Petri dishes (60 mm × 15 mm) lined with 55-mm filter paper disks. In these new Petri dishes, an *ad libitum* supply of freeze-dried *A. mellifera* drone pupae and water were provided as described above. Mortality was recorded again 2 and 7 days after exposure, with mortality defined as the immobility of insects after stimulation with a fine camel-hair brush.

Mean percentage survival of adults for each replication was transformed by arcsine square root. Transformed percentages were analyzed across time (i.e., 1, 2, and 7 days after exposure) using repeated measures analysis of variance with a first order autoregressive covariance structure (Proc mixed) (3,4,12). The analysis of variance model included main effects for time, treatment, and the interaction of the main effects. If the main effect for treatment (across time) was significant ($P < 0.05$), then differences among treatments were tested using Bonferroni adjusted contrasts of the least squares means for each pairwise combination of treatments.

Efficacy of Insecticides to *H. axyridis* under Field Conditions

In the 2004 field trials, the mean percentage of clusters infested with *H. axyridis* adults at harvest was significantly lower ($P < 0.05$) in plots treated with bifenthrin applied 7 days before harvest (DBH), carbaryl 10 DBH, and in plots covered with the floating row cover (FRC), compared with untreated plots (Table 1). Applications of carbaryl 16 DBH, malathion 10 and 7 DBH, and pyrethrin 1 DBH did not provide statistically significant reductions of beetle-infested clusters compared to the untreated plots (Table 1).

In 2005, the mean percentage of clusters infested with *H. axyridis* adults at harvest were significantly reduced in plots treated with zeta-cypermethrin 7 DBH, bifenthrin 22 and 7 DBH, and imidacloprid 1 DBH, compared with untreated plots (Table 2). However, infestations of clusters with *H. axyridis* adults at harvest were not significantly reduced by thiamethoxam 7 DBH or carbaryl 7 DBH relative to the untreated check (Table 2).

Table 1. Mean percentage (± SEM) of clusters infested with at least one *H. axyridis* adult in wine grapes ('Leon Millot') treated with insecticides, Hastings, MN, 2004.

Treatment[x]	**Rate** (kg a.i./ha)	**Mean percentage (±SEM) of infested clusters**	
		Pre-treatment sample (31 August)	Harvest sample (17 September)
Bifenthrin 7 DBH[y]	0.045	0.0 ± 0.0	0.0 ± 0.0a
Carbaryl 10 DBH	1.54	0.0 ± 0.0	22.5 ± 11.0ab
FRC[z] 16 DBH		2.5 ± 2.5	20.0 ± 14.0abc
Carbaryl 16 DBH	1.54	5.0 ± 5.0	32.5 ± 8.5bcd
Malathion 7 DBH	1.90	2.5 ± 2.5	22.5 ± 13.0bcd
Pyrethrin 1 DBH	0.031	0.0 ± 0.0	40.0 ± 11.0cd
Malathion 10 DBH	1.90	0.0 ± 0.0	37.5 ± 13.0d
Untreated check	—	5.0 ± 2.9	52.5 ± 7.5d
P		0.4908	0.0057
F		0.95	4.15
df		7,20	7,20

Means, within a sample date, followed by same letters do not differ significantly ($P < 0.05$) least squares means. Mean percentages were transformed by arcsine square root; non-transformed mean percentages are presented.

[x] Plots were treated on 1 (Carbaryl 16 DBH), 7 (Carbaryl 10 DBH and Malathion 10 DBH), 10 (Bifenthrin 7 DBH and Malathion 7 DBH), and 16 (Pyrethrin 1 DBH) September 2004.

[y] DBH = days before harvest.

[z] FRC = floating row cover, set on trellises 1 September 2004.

Table 2. Mean percentage (± SEM) of clusters infested with at least one *H. axyridis* adult in wine grapes ('Leon Millot') treated with insecticides in 2005, Hastings, MN.

Treatment[x]	**Rate** (kg a.i./ha)	**Mean percentage (±SEM) of infested clusters**	
		Pre-treatment sample (23 August)	Harvest sample (31 August)
Zeta-cypermethrin 7 DBH[y]	0.027	6.7 ± 6.7	0.0 ± 0.0a
Bifenthrin 22 DBH	0.112	0.0 ± 0.0	0.0 ± 0.0ab
Imidacloprid 1 DBH	0.053	3.3 ± 1.9	5.0 ± 3.0ab
Bifenthrin 7 DBH	0.112	0.0 ± 0.0	6.7 ± 6.7ab
Thiamethoxam 7 DBH	0.061	1.7 ± 1.7	11.7 ± 9.6abc
Carbaryl 7 DBH	1.97	5.0 ± 1.7	22.0 ± 9.0bc
Untreated check	—	5.0 ± 5.0	23.0 ± 4.0c
P		0.5717	0.0296
F		0.82	3.13
df		6,17	6,17

Means, within a sample date, followed by same letters do not differ significantly ($P < 0.05$) least squares means. Mean percentages were transformed by arcsine square root; non-transformed mean percentages are presented.

[x] Plots were treated on 9 (Bifenthrin 22 DBH), 23 (Carbaryl 7 DBH, Bifenthrin 7 DBH, Thiamethoxam 7 DBH, and Zeta-cypermethrin 7 DBH), and 29 (Imidacloprid 1 DBH) August 2005.

[y] DBH = days before harvest.

Efficacy of Insecticides to *H. axyridis* under Laboratory Conditions

Under laboratory conditions, bifenthrin, carbaryl, and thiamethoxam resulted in significant mortality of *H. axyridis* adults 7 days after treatment via both routes of exposure (Table 3). Imidacloprid was more lethal to *H. axyridis* adults 7 days after treatment, via both routes of exposure, compared to the untreated control (i.e., water), but less lethal than bifenthrin, carbaryl, and thiamethoxam (Table 3). Azadirachtin, known to be non-toxic to several lady beetle species (e.g., 13), did not cause any mortality to *H. axyridis* adults 7 days after treatment via both routes of exposure (Table 3).

Table 3. Mean percentage (± SEM) survival of *H. axyridis* adults 7 days after treatment in the laboratory.

Treatment	Rate (kg a.i. /ha)	Topical application	Insecticide residues			
			1 h	1 day	3 days	7 days
Bifenthrin	0.048	0.0 ±0.0a	0.0 ±0.0a	0.0 ±0.0a	0.0 ±0.0a	0.0 ±0.0a
Carbaryl	1.47	0.0 ±0.0a	0.0 ±0.0a	0.0 ±0.0a	0.0 ±0.0a	0.0 ±0.0a
Thiamethoxam	0.07	0.0 ±0.0a	9.0 ±4.0a	5.0 ±4.0a	5.0 ±5.0a	19.0 ±12.0a
Imidacloprid	0.047	66.0 ±2.0b	66.0 ±11.0b	71.0 ±14.0b	66.0 ±20.0b	86.0 ±0.0b
Azadirachtin	0.019	90.0 ±4.0c	66.0 ±20.0bc	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0c
Untreated check	--	100.0 ±0.0c	85.0 ±14.0c	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0c
P		<.0001	<.0001	<.0001	<.0001	<.0001
F		44.41	25.07	76.38	64.04	79.59
df		5,36	5,36	5,36	5,36	5,36

Mean percentage were analyzed across time (i.e., 1, 2, and 7 days after exposure) using repeated measures analysis of variance and Bonferroni adjusted contrasts; mean percentages followed by the same letter do not differ significantly ($P < 0.05$). The mean percentages were transformed by arcsine square root; non-transformed mean percentages are presented.

Summary

Based on field and laboratory studies, carbaryl, bifenthrin, zeta-cypermethrin, thiamethoxam, and imidacloprid showed either a toxic and/or repellent effect on *H. axyridis*. However, of these insecticides, only carbaryl and imidacloprid are currently labeled for use on wine grapes within 7 days of harvest, the period in which *H. axyridis* typically reaches high densities (1). Even though bifenthrin has demonstrated excellent efficacy against *H. axyridis* infestations under field conditions and high toxicity in the laboratory, the 30-day PHI currently precludes its use for managing *H. axyridis*, as populations do not start to build up until 2 wk before harvest. Moreover, at 30 DBH the extent of potential infestations of *H. axyridis* at harvest cannot be predicted, and spraying bifenthrin at this time will likely result in unnecessary costs and environmental impacts. Although the FRC provided some suppression of *H. axyridis* in 2004, the method proved to be too labor-intensive for practical use and was not evaluated in 2005.

Our results for carbaryl and imidacloprid are in agreement with previous studies in Ohio (e.g., 15,16,17). Based on these results possible chemical control options for *H. axyridis* could include carbaryl and imidacloprid, and these products could serve as the primary insecticides when developing an IPM program for *H. axyridis*. However, as new insecticides receive registrations for new uses, or possibly lose current registrations, the availability of insecticides may change. One example is the addition of cyfluthrin (Baythroid 2, Bayer CropScience, Kansas City, MO), a pyrethroid insecticide that recently gained approval for use on wine grapes with a 3-day PHI. This emphasizes the need for on-going research on the efficacy of currently registered insecticides and insecticides with the potential to be registered in the near future. For example, future research should evaluate the efficacy of other insecticides not labeled for grapes, but have shown high toxicity to *H. axyridis* such as lambda-cyhalothrin and chlorpyrifos (3). Insecticides, however, are only needed when beetles are present, as indicated by direct sampling of clusters in each variety of a vineyard (1,2).

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