Toxicity of Insecticides to *Halyomorpha halys* (Hemiptera: Pentatomidae) Using Glass-Vial Bioassays

ANNE L. NIELSEN,[1] PETER W. SHEARER, AND GEORGE C. HAMILTON

Department of Entomology, Rutgers University, 93 Lipman Drive, New Brunswick, NJ 08901

J. Econ. Entomol. 101(4): 1439–1442 (2008)

ABSTRACT A scintillation glass-vial bioassay was used to test technical grade insecticides against the non-native stink bug *Halyomorpha halys* (Stål). *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is emerging as an important pest in the Mid-Atlantic States, especially in tree fruits and as a homeowner nuisance during the winter. Pyrethroid insecticides, especially bifenthrin, caused mortality against *H. halys* at low doses, with LC_{50} values of 0.03–0.49 (μg [AI]/cm^2) (mg body mass^{-1}). Three nicotinoids were tested against adults with LC_{50} values ranging between 0.05 and 2.64 (μg [AI]/cm^2) (mg body mass^{-1}). Phosmet had LC_{50} values that were up to 3.6-fold higher than other classes of insecticides tested. Fifth instars of *H. halys* were evaluated against selected chemicals, and they were generally susceptible at lower rates than the adults. Due to significant differences in weight, males and females were individually weighed, tested, and analyzed separately. Sex-related differences in susceptibility were found in the responses to thiomethoxam with males being less susceptible despite having a smaller body mass.

KEY WORDS stink bug, LC_{50}, sex-related response, insecticide

Stink bugs are becoming increasingly abundant pests of a variety of crops, including soybean, cotton, and tree fruit (Leskey and Hogmire 2005, Snodgrass et al. 2005). In the eastern United States, the stink bug complex in tree fruit consists primarily of *Euschistus servus* (Say), *Euschistus tristigmus* (Say), and *Acrosternum hilare* (Say) (Hemiptera: Pentatomidae). Their feeding damage varies depending on the crop phenology when attacked. In peaches, early season feeding by stink bugs produces deformed or "catfaced" fruit (Mundinger and Chapman 1932, Rings 1957), whereas feeding late in the development of fruit causes discolored necrotic tissue and may lead to dimpling and discoloration on or near the fruit surface. Similar late season damage occurs in apples (*Malus* spp.) and pears (*Pyrus* spp.), although it is easily mistaken for physiological disorders (Brown 2003). In soybean, feeding by stink bugs during the R–IV and R–V stages of pod formation may result in deformed seeds and reduced oil yield. Regardless of the crop, stink bug feeding can introduce pathogens at the site of stylet insertion (McPherson and McPherson 2000).

Stink bugs have historically been managed using organophosphorus insecticides, however, changes in insecticide chemistries and U.S. Environmental Protection Agency decisions that limit or prohibit the use of this class of insecticides has led researchers to investigate other management options. A recently introduced species, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) (Hoebeck and Carter 2003), is the newest member of the stink bug pest complex in the Mid-Atlantic states, causing economic losses in apples and pears in New Jersey and Pennsylvania (A.L.N., unpublished data). Populations also have become established in Oregon (2005), California (2006), Ohio (2007), and Mississippi (2007).

H. halys is also a considerable homeowner nuisance, increasing the scope of interest in its insecticide susceptibility. As photoperiod and temperatures decline in September and October, adult *H. halys* move to overwintering sites. Unlike many stink bug species, the preferred overwintering location of *H. halys* is in artificial structures, such as houses (Watanabe et al. 1994, Hamilton et al. 2008). Large numbers of stink bugs congregating on the exterior or interior of houses have caused concern in residential areas and raised demands for finding management solutions for this pest. The chemicals used to control *H. halys* in agriculture also may be effective for control in and around homes, emphasizing the need to investigate reduced-risk insecticides. Overwintering populations in Japan were reduced with pyrethroid insecticide applications of permethrin, phenothrin, and cyphenothrin (Watanabe et al. 1994). Previous success with pyrethroids against *H. halys* and changes in insecticide uses in the United States led us to test primarily this class of insecticide by using a scintillation glass-vial bioassay. In addition, we evaluated one organophosphorus and three neonicotinoid insecticides. The glass-vial bioassay is a quick tool to determine insecticide suscepti-

[1] Corresponding author, e-mail: annielse@rci.rutgers.edu.

bility in the laboratory and has been successfully used to evaluate susceptibility of various pentatomid species to pyrethroid and organophosphorus insecticides (Willrich et al. 2003, Snodgrass et al. 2005). It is necessary to establish baseline mortality data for *H. halys* so that its susceptibility to insecticides can be monitored as it expands its geographic range. This is the first study on insecticide susceptibility for the U.S. population of *H. halys,* and one of the few laboratory investigations evaluating stink bugs against technical grade neonicotinoid insecticides.

Materials and Methods

Insect Source. Adult and immature *H. halys* were collected from July to October in 2005 and 2006 from the Rodale Working Tree Farm in Allentown, PA. The site is an organic arboretum containing native and exotic ornamental trees and shrubs that are maintained by the Rodale Corporation, and it has had a large population of *H. halys* because at least 2000. Ornamental trees and shrubs were sampled using a beating sheet (71 cm^2, BioQuip Products Inc., Rancho Dominguez, CA) to dislodge specimens. Collected insects were held in BugDorm2 rearing cages (60 by 60 by 60 cm; Megaview Science Education Services Co., Ltd., Taichung, Taiwan) and provided green beans and peanuts for a minimum of 24 h before treatment.

Bioassay. A scintillation glass-vial bioassay was chosen to evaluate residual insecticidal activity on *H. halys.* This method allows for rapid dosing of large numbers of insects while using small amounts of product (Snodgrass 1996, Willrich et al. 2003, Snodgrass et al. 2005). Three classes of insecticides were evaluated: organophosphorus (phosmet [Gowan Company, LLC, Yuma, AZ]); pyrethroid (β-cyfluthrin, cyfluthrin [Bayer CropScience, Research Triangle Park, NC], fenpropathrin [Valent U.S.A. Corporation, Walnut Creek, CA], bifenthrin [FMC Corporation, Philadelphia, PA], and λ-cyhalothrin [Syngenta, Greensboro, NC]); and neonicotinoid (acetamiprid [Cerexagi, Inc., King of Prussia, PA], dinotefuran [Valent U.S.A. Corporation], and thiomethoxam [Syngenta]). For each insecticide, a known quantity of technical grade active ingredient was dissolved in acetone (Fisher, Atlanta, GA) and serially diluted to desired concentrations. A minimum of five graduated doses for each chemical were assayed on at least two different dates with 10 treated vials per dose per insect group (male, female, or fifth instar). A minimum of 90 male, female, and fifth instars of *H. halys* were tested per chemical, with >4,000 individuals tested. Each individual was weighed before testing to correct insecticide dose for mean body mass. The limited availability of fifth instars prevented them from being tested with every insecticide used against the adults.

The glass-vial bioassay was prepared by pipetting 0.5 ml of insecticide and acetone solution into each 20-ml scintillation vial (Wheaton Science Products, Millville, NJ). Vials treated with acetone alone served as controls. To ensure an even insecticide residue, vials were rolled on a modified hotdog roller with the heat unit disconnected (Helman Group Ltd., Oxnard, CA) in a fume hood until the acetone evaporated. Individual insects were weighed (APX-203, Denver Instruments, Denver, CO) and then randomly placed into a treated scintillation vial, which was then plugged with cotton. The vials were laid on their side under ambient laboratory conditions ($\approx$25°C) and supplemented with fluorescent lighting that provided a photoperiod of 16:8 (L:D) h. Mortality and morbidity was checked at 24 and 48 h. Morbidity was defined as the inability of the insect to cling to the side of the vial or the insect being unable to right itself when inverted. The pyrethroid mode-of-action causes an initial knockdown from which it may recover, during which the insect seems moribund. If an individual scored as "moribund" at 24 h recovered at the 48-h observation, it was reclassified as "alive." This revised 24-h data set was used for Probit analysis. Mortality and morbidity data were combined for analysis.

Data Analysis. Mean body mass of males, females, and fifth instars for each insecticide concentration and replicate were calculated using the PROC MEANS statement and compared using PROC GLM, with Tukey's mean separation (SAS version 9.1.3, SAS Institute 2002–2003) for each chemical evaluated. Insecticide concentration was then corrected for mean body mass [(μg [AI]/cm^2)(mg body $mass^{-1}$)] and the insect response at 24 h was analyzed using Probit analysis (PoloPlus version 1.0, LeOra Software, El Cerrito, CA) for males, females, and fifth instars. Higher doses were excluded from analysis if 100% mortality had already been reached. For each chemical, the male/female/immature LC_{50} ratio was tested for significance according to Robertson and Preisler (1992) to determine differences at $P \geq 0.05$. This was achieved by calculating the 95% fiduciary limits, if the limit of the tolerance ratio at the LC_{50} includes 1.0, then there is no significant difference between the sexes or stages (Robertson and Preisler 1992).

Results and Discussion

Many insects exhibit sexual dimorphism with one sex, usually the female, being larger. The implications this has for pest management are rarely taken into consideration (Shearer and Usmani 2001). *Halyomorpha halys* females are significantly heavier than their male counterparts. Mean ± SE weight for males (0.09 ± 0.001 g; n = 1614) and females (0.12 ± 0.001 g; n = 1631) was significantly different ($F_{1,\ 3244}$ = 615.54; $P < 0.0001$). To exclude any effects due to differences in body mass on susceptibility to insecticides, the insecticide concentration per unit area of treated vials was corrected for mean body mass (μg [AI]/cm^2)(mg body $mass^{-1}$) in our study for each sex, chemical, replicate, and dose. Generally, males had higher LC_{50} values than females for most chemicals tested (Table 1). This difference was only significant for thiomethoxam ($P \leq 0.05$) and may indicate sex-related differences in response to this chemical.

Table 1. Toxicity of selected insecticides to *H. halys* from residual 24-h exposure

Insecticide	Sex or stage	*n*	Slope ± SE	LC_{50} [a,b]	95% FL	χ^2 [c]	df
Phosmet	Male	119	4.08 ± 0.98	12.24a	9.28–15.12	3.83	4
	Female	110	4.85 ± 1.45	10.23a	6.96–12.95	0.75	4
Dinotefuran	Male	110	1.62 ± 0.31	0.14a	0.02–0.50	3.80	3
	Female	100	1.60 ± 0.50	0.08a	0.02–0.16	1.73	2
	Fifth instar	210	1.22 ± 0.17	0.15a	0.04–0.49	7.76	4
Acetamiprid	Male	160	1.13 ± 0.22	1.62a	0.17–3.95	9.25	6
	Female	140	1.20 ± 0.21	2.64a	0.80–5.52	8.21	6
	Fifth instar	109	1.73 ± 0.56	0.09b	0.01–0.20	1.50	3
Thiomethoxam	Male	185	1.02 ± 0.16	0.13a	0.02–0.55	7.23	4
	Female	185	1.43 ± 0.22	0.05b	0.03–0.08	4.00	4
	Fifth instar	124	1.22 ± 0.27	0.02c	0.01–0.03	2.73	3
β-cyfluthrin	Male	100	2.27 ± 0.50	0.49a	0.11–0.95	8.67	6
	Female	90	4.64 ± 1.36	0.41a	0.26–0.54	3.26	5
	Fifth instar	120	1.77 ± 0.36	0.03b	0.02–0.05	0.53	4
Cyfluthrin	Male	130	1.38 ± 0.44	0.18a	0.02–0.41	0.45	4
	Female	129	1.81 ± 0.44	0.06a	0.02–0.11	1.83	4
Fenpropathrin	Male	103	1.04 ± 0.21	0.12bc	0.06–0.28	2.75	4
	Female	114	1.06 ± 0.20	0.06c	0.03–0.12	2.30	4
	Fifth instar	160	1.03 ± 0.19	0.20ab	0.03–1.02	7.79	4
Bifenthrin	Male	110	1.81 ± 0.45	0.03a	0.01–0.05	1.72	3
	Female	110	1.81 ± 0.42	0.03a	0.02–0.06	1.28	3
λ-Cyhalothrin	Male	119	1.26 ± 0.29	0.12a	0.04–0.25	1.59	2
	Female	114	1.40 ± 0.26	0.06a	0.03–0.11	2.22	3
	Fifth instar	188	1.41 ± 0.28	0.12a	0.05–0.22	0.51	4

[a] LC_{50} values are in (micrograms [AI]/cm^2) (insect $weight^{-1}$).
[b] For each chemical, LC_{50} values within a column followed by the same letters are not significantly different (Roberston and Priesler (1992); $P > 0.05$).
[c] All χ^2 values fit the model at $P > 0.05$.

H. halys adults had lower LC_{50} values to the pyrethroid insecticides than to the other chemicals (Table 1). Knockdown and recovery were observed for all pyrethroids tested. Overall, the LC_{50} value for the organophosphorus insecticide, phosmet, was higher than for all neonicotinoids and pyrethroids evaluated, with phosmet having LC_{50} values up to 3.6-fold higher than for bifenthrin. The LC_{50} values estimated for *H. halys* by using pyrethroid insecticides ranged from 0.03 to 0.49 (μg [AI]/cm^2)(mg body $mass^{-1}$) for adults, supporting reports that *H. halys* is more susceptible to pyrethroid than to organophosphorus insecticides (Watanabe et al. 1994). Of the five pyrethroid insecticides tested, bifenthrin was most toxic to *H. halys* with an LC_{50} value of 0.03 (μg [AI]/cm^2) (mg body $mass^{-1}$) for both males and females. Native species of pentatomids demonstrate similar responses to bifenthrin, λ-cyhalothrin, and cyfluthrin (Willrich et al. 2003, Snodgrass et al. 2005). Those studies ranked λ-cyhalothrin as having the lowest LC_{50} values, whereas in our study, λ-cyhalothrin was ranked as the second most toxic pyrethroid. Differences in toxicity between the chemicals may be slight, however, and laboratory evaluations are needed to compare susceptibility of *H. halys* with native species.

Our study is one of the first laboratory evaluations of technical grade neonicotinoids against pentatomid species and demonstrates that this class may be a suitable chemical option for stink bug control in the field. Acetamiprid, dinotefuran, and thiomethoxam all had lower LC_{50} values than phosmet, with adult LC_{50} values ranging between 0.05 and 2.64 (μg [AI]/cm^2) (mg body $mass^{-1}$). Consistent with field trials of formulated products against native pentatomid species, *H. halys* was not as susceptible to low doses of acetamiprid compared with the other neonicotinoids tested (Willrich et al. 2002, Herbert et al. 2006, Tillman 2006).

Fifth instars of *H. halys* were tested against six insecticides; dinotefuran, acetamiprid, thiomethoxam, β-cyfluthrin, fenpropathrin, and λ-cyhalothrin. Generally, immature *H. halys* were significantly more susceptible to the insecticides tested compared with adult response ($P \leq 0.05$). This is consistent with results for *A. hilare*, *Nezara viridula* (L.), and *E. servus* nymphs observed previously (Willrich et al. 2003). Because toxicity differences that could have been due to variations in body mass were excluded (when the insecticide dose was corrected for mass), any significant differences in susceptibility may be due to absorption rates resulting from the softer bodied nymphs or decreased levels of detoxification enzymes. Dinotefuran and λ-cyhalothrin, however, were equally toxic to the adults and fifth instars. For reasons that are unclear, fenpropathrin was significantly more toxic to female *H. halys* than to the nymphs. Chi-square comparisons revealed that the responses did not deviate from the expected results for either adult or immature *H. halys* ($P \leq 0.05$).

No significant differences were found between LC_{50} ratios for males and females and the y-intercept and slopes were equal for all chemicals evaluated except for thiomethoxam. The results for thiomethoxam yielded different slopes and intercept values for males, females, and nymphs, as well as having significantly different LC_{50} ratios. Susceptibility to thiomethoxam was greater for *H. halys* females than males when doses were corrected for body mass, indicating that body mass is not a factor in the response to this chemical. Because sex-related differences were not apparent for uncorrected concentrations (data not shown), we hypothesize that increased tolerance in males may be due to differences in detoxifying enzymes or target-site insensitivity. Additional research is needed to test these hypotheses.

H. halys has substantially enlarged both its range and the local population density since its introduction (Hamilton et al. 2008). In certain crops, *H. halys* has established itself as the predominant stink bug species, and we are finding high levels of stink bug damage in commercial apple and pear orchards (A.L.N., unpublished data). As *H. halys* ' range continues to expand, we predict that management of this species will need to be incorporated into control programs for other stink bug pests. Field testing of Danitol (fenpropathrin), Baythroid (β-cyfluthrin), Assail (acetamiprid), Imidan (phosmet), and Warrior (λ-cyhalothrin) against catfacing insects, including stink bugs, in New Jersey peaches showed that only Danitol significantly reduced late-season catfacing damage (Nielsen et al. 2007). However, the use of pyrethroids in an orchard system can disrupt integrated pest management (IPM) programs by causing a resurgence of phytophagous mites and scale insects post application (Croft 1990). Because insecticides that do not disrupt IPM programs are especially desirable, some neonicotinoid insecticides may provide *H. halys* control with minimal impacts to biological control agents. Further field testing and research are required to evaluate field efficacy of these chemicals for *H. halys* management.

Acknowledgments

We thank Gowan Company, LLC, Valent U.S.A. Corporation, Cerexagi, Inc., Syngenta, and Bayer Crop Science for donating material used in this study. This work was supported in part by funds from the Pennsylvania Department of Agriculture (ME444449) and Valent U.S.A. Corporation. We also thank M. Duva, D. Gupta, and A. Rucker for technical assistance. This is New Jersey Agricultural Experiment Station Publication D-08-08294-02-08.

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Received 17 March 2008; accepted 5 May 2008.