Effects of the Insect Growth Regulators Novaluron and Diflubenzuron on the Brown Marmorated Stink Bug

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Abstract

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is an invasive pest from China that causes economic damage to field crops, vegetables, and tree fruit. Due to its destructive potential, applications of broad-spectrum insecticides have escalated. Researchers are trying to identify options for controlling BMSB that have less of a negative impact on non-target species. Chitin biosynthesis inhibitors are more selective than the commonly used pyrethroids and organophosphates. They are active on the larval stage of the insect and are reported as having sublethal effects such as reducing adult fecundity. In our studies, bioassays were completed with chitin biosynthesis inhibitors novaluron and diflubenzuron to evaluate the effectiveness of these insecticides on adult mortality, nymphal growth, adult fecundity, and egg hatch. Our data indicate that treatments of novaluron at 362.2 g ai/ha or diflubenzuron at 280.2 g ai/ha effectively controlled BMSB nymphs. However, the insecticides were not effective at reducing egg hatch, adult fecundity, or adult life span. If novaluron or diflubenzuron are used in agriculture for BMSB control, then the nymphal stage should be targeted.

Introduction

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), (Fig. 1) is an exotic insect pest that likely arrived in the US via shipping cargo from China (8). It was first detected near Allentown, PA, in the mid-1990s (9) and is currently spreading rapidly across the continental US (8). In the mid-Atlantic region of the US, this stink bug has become a major pest of tree fruit (15,16) and vegetables (12), and the number of applications of broad-spectrum insecticides such as pyrethroids, carbamates, and organophosphates have increased on these crops in an effort to control this invasive pest (14). However, multiple applications of broad-spectrum insecticides can have deleterious environmental risks, reduce natural enemy populations, and cause secondary pest outbreaks (7,13,18,19). Researchers are searching for a less disruptive strategy to control BMSB and stink bug pests in general (11). Insect growth regulators (IGRs) are considered to be more selective than many traditionally used insecticides because they typically target the

Fig. 1. Brown marmorated stink bug adult on grape.

immature stages of insects with modes of action (MOA) that interfere with molting and growth (7). Diflubenzuron and novaluron are types of IGRs that belong to the benzoylphenyl urea (benzoylurea) class of chemicals (IRAC Group 15). These insecticides disrupt chitin biosynthesis of the larval stages in susceptible insects (10,20). Applications of novaluron or diflubenzuron may result in mortality by disrupting the molting process through inhibiting the formation of chitin. Additionally, reduced egg hatch has been documented from eggs treated with benzyl ureas as well as a sublethal ovicidal effect from females fed treated food (1,5).

The objective of this study was to determine the lethal and sublethal effects of novaluron and diflubenzuron against BMSB adults, nymphs, and eggs.

Bioassays on BMSB Nymphs

Brown marmorated stink bug lab colony. All insects used in the bioassays were from a colony maintained in a laboratory in the Department of Entomology at Virginia Tech, Blacksburg, VA. The colony was initiated from adult insects that were collected from houses near Roanoke and Bedford, VA, in January 2011. Adults and nymphs were maintained in a growth chamber at 60 to 80% RH, $27 \pm 2^\circ\text{C}$, with a photoperiod of 16:8 (L:D) and were provided with carrot (*Daucus carota* L.), green beans (*Phaseolus vulgaris* L.), raw shelled peanuts (*Arachis hypogaea* L.), and a water wick.

Insecticide solutions. All experiments conducted had three treatments consisting of two insecticide treatments and a water control. The insecticide concentrations used for the laboratory bioassays were based on the highest labeled rates of each compound using a 935.3-liter/ha (100-gal/acre) water output. The insecticide treatments were novaluron (Rimon 0.83EC at 363.2 g ai/ha, Chemtura AgroSolutions, Lawrenceville, GA) and diflubenzuron (Dimilin 2L at 280.2 g ai/ha, Chemtura AgroSolutions). A surfactant was not used in the bioassays.

Green bean dip bioassays. Green bean pods were dipped in the insecticide solution for 5 sec and allowed to dry for approximately $\frac{1}{2}$ h under a fume hood. One green bean was then placed with 5 BMSB nymphs (3rd instars) per 9-cm Petri dish (Fig. 2). Four Petri dishes were set up per treatment for a total of 20 insects per experiment. In the first bioassay, the green beans were dipped on 3 November 2011 and mortality (dead + moribund) was assessed after 72 h. Insects were considered moribund if they were unable to right themselves when flipped onto their backs. On 3 February 2012, a second bioassay was initiated using methods similar to those described above, except the treatments were also assessed at 120 and 168 h after treatment. Freshly dipped beans were added at 120 h after treatment.

Fig. 2. Brown marmorated stink bug nymphs in a green bean dip bioassay.

Proportion data was arcsine square root transformed prior to analysis as a one-way ANOVA using JMP (version 9.0) software (SAS Institute Inc., Cary, NC). Tukey's mean separation test was used to separate the means at the $P \leq 0.05$ level of significance.

In the first bioassay, the insecticide-treated green beans resulted in significantly higher mortality of nymphs than the control at 72 h after treatment (Table 1). In the second bioassay, green beans treated with novaluron resulted in significantly higher mortality than the control at 72 h, and at 120 and 168 h, both insecticide treatments had higher mortality than the control (Table 1; Fig. 3). Also, the novaluron treatment resulted in significantly higher mortality than the diflubenzuron treatment. Mortality was observed in 100% of the nymphs that attempted to molt in treated groups during the duration of both bioassays. These data confirm that novaluron and diflubenzuron are lethal to BMSB nymphs and could be used as part of the management strategy for BMSB.

Table 1. Mean percent mortality $\pm$ SEM of brown marmorated stink bug nymphs after exposure to insecticide treated green beans for two bioassays.

Treatment	Rate (g ai /ha)	Mean % dead and moribund $\pm$ SEM			
		Bioassay 1	Bioassay 2		
		72 h	72 h	120 h	168 h
Control	–	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 c
Novaluron	362.2	65.0 $\pm$ 17.1 a	40.0 $\pm$ 8.1 a	75.0 $\pm$ 9.6 a	90.0 $\pm$ 5.8 a
Diflubenzuron	280.2	60.0 $\pm$ 11.5 a	5.0 $\pm$ 5.0 b	35.0 $\pm$ 9.6 b	65.0 $\pm$ 5.0 b

Means within a column followed by the same letter are not significantly different according to Tukey's HSD ($P < 0.05$).

Fig. 3. Dead brown marmorated stink bug nymph after unsuccessful molting from feeding on a diflubenzuron-treated green bean.

Egg Mass Immersion Bioassays

Freshly-laid egg masses (< 24 h old) were collected in groups of two or three from the BMSB laboratory colony on 3 dates (5, 7, and 11 April 2011). Two to three egg masses (from 50 to 85 eggs) were evaluated for each treatment on each date. Each egg mass was randomly assigned a treatment (water, novaluron, or diflubenzuron) and was treated using a hand pump spray bottle until runoff. Egg masses were checked every 24 h for hatching. The number of emerging nymphs from each egg mass was recorded. Proportion egg hatch data were arcsine square root-transformed prior to analysis and analyzed as a completely randomized design using JMP (version 9.0) software (SAS Institute Inc.) with date as the blocking factor (Table 2). The analysis determined that there was no significant treatment effect on egg hatch. These results suggest that there is little to no penetration of the insecticides across the egg chorion, or novaluron and diflubenzuron have no effect on embryonic development of BMSB.

Table 2. Mean percent egg hatch $\pm$ SEM of the brown marmorated stink bug after insecticide solution application to egg masses.

Treatment	Rate (g ai/ha)	Total # eggs	Mean % egg hatch $\pm$ SEM
Control	–	193	70.7 $\pm$ 13.4
Novaluron	362.2	192	75.4 $\pm$ 5.8
Diflubenzuron	280.2	196	72.1 $\pm$ 5.8

Bioassays on Adult Mortality, Natality, and Transovarial Effects

Three bioassays were conducted using BMSB adults that were collected from dwellings in Bedford, VA, or sweet corn and soybeans late in the season (3rd bioassay only). The first bioassay was conducted over 18 days beginning 11 April, the second bioassay from 11 May until 22 June (42 days), and the third bioassay began on 5 September and was discontinued after 60 days.

For each of the three bioassays, 15 BMSB adult males and females were placed into a 4-liter plastic container labeled with the respective treatment. Adults were fed either untreated or insecticide-treated green beans and carrots. Each container also received a saturated cotton dental wick as a moisture source that was either treated with the respective insecticide solution or tap water for the control treatment. Any dead adults or egg masses were recorded and removed daily from the containers; egg masses were placed into labeled Petri dishes. Adult mortality was not recorded for the second bioassay. The dishes containing the eggs were kept in the growth chamber at 16:8 (L:D) photoperiod. Egg masses were checked daily for egg hatch.

In bioassay 1, more than 50% of the adults died in containers with water and diflubenzuron treated food, and more than 90% died in the container with novaluron treated food by day 18. In bioassay 3, 40% of the adults died in the control and diflubenzuron-treated containers, and 60% in the novaluron container by day 60. Based on the similar mortality of the three treatments across the three bioassays, it appears that these insecticides have no effect on adult survivorship. Rather, the high mortality observed in the control containers for all bioassays may demonstrate the variable life span of BMSB adults in the laboratory.

Across the three bioassays, percent hatch of eggs ranged from 82.5 to 100% from novaluron-fed adults, 85.7 to 100% from diflubenzuron-fed adults, and 70.6 to 100% for water-fed adults (Table 3). The data indicate that there are no treatment effects on the number of eggs deposited or the percentage of those eggs hatching.

Table 3. Egg mortality and transovarial effects on brown marmorated stink bugs after prolonged exposure of adults to insecticide treated food and water.

Treatment	Rate (g ai/ha)	Bioassay 1 (18 days)		Bioassay 2 (42 days)		Bioassay 3 (60 days)	
		# egg masses	% egg hatch	# egg masses	% egg hatch	# egg masses	% egg hatch
Control	–	6	100	4	70.6	4	98.1
Novaluron	362.2	10	100	3	82.5	5	95.8
Diflubenzuron	280.2	7	100	2	100	4	85.7

Considerations for Using Novaluron Or Diflubenzuron To Manage BMSB

Insect growth regulators are valuable tools for integrated pest management. However, targeting the correct life stage of the pest is vital for the effective use of novaluron and diflubenzuron. Erler et al. (6) reported a reduction in the emergence of the mushroom sciarid fly, *Lycoriella ingenua* (Dufour), when the larval populations were targeted with a soil drench using either diflubenzuron

or novaluron. However, other reports have indicated that benzyl urea treatments are not effective at reducing some larval pest populations (17). This may occur when insect larvae bore into plant tissue; thus, avoiding contact with the insecticide. Based on the present study, novaluron and diflubenzuron are effective against BMSB nymphs by disrupting the molting process and resulting in mortality. Proper applications of these insecticides should target the early instars.

Benzyl ureas have been shown to be toxic to insect eggs. Alyokhin and Choban (1) reported that treatment of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), eggs that were < 24 h old with novaluron resulted in reduced egg hatch; however, eggs > 96 h old were no longer susceptible. Additionally, diflubenzuron was also more effective against freshly-laid eggs than older eggs of the codling moth, *Cydia pomonella* L. (3). Our results indicated that these insecticides had no effect on BMSB eggs, even those that were freshly laid.

The reproductive potential of adult females has been negatively influenced when fed benzyl urea-laced solutions (1,4). Researchers have reported that chitin biosynthesis inhibitors can have transovarial effects on gravid females and may reduce egg hatch in the Colorado potato beetle (1,2). In our bioassays, neither diflubenzuron nor novaluron showed any transovarial effects on BMSB; with adult females depositing viable eggs. Additionally, adult BMSBs that were fed the insecticide solutions did not exhibit increased mortality compared to the untreated adults. Therefore, diflubenzuron and novaluron do not appear to have a lethal or sublethal effect on BMSB adults. Our results indicate that novaluron and diflubenzuron applications should target the immature stages of BMSB.

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