Toxicities of Neonicotinoid Insecticides for Systemic Control of Brown Marmorated Stink Bug (Hemiptera: Pentatomidae) in Fruiting Vegetables[1]

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ABSTRACT The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive pest of various crops, including fruiting vegetables, throughout the mid-Atlantic U.S.A. Current control strategies for this pest rely almost exclusively on foliar applications of broad-spectrum insecticides, which disrupt IPM programs and cause secondary pest outbreaks. Systemic neonicotinoids applied to the root-zone via soil drench or chemigation may be a more IPM friendly tactic for insect control in vegetables. Laboratory bioassays that utilized a plant uptake method showed that the neonicotinoid insecticides clothianidin, dinotefuran, imidacloprid, and thiamethoxam were all toxic to *H. halys* nymphs, with estimated LC_{50} values of 0.077, 0.013, 0.068, and 0.018 ppm, respectively. Field efficacy experiments in Virginia showed that two soil applications of each of the aforementioned neonicotinoid insecticides significantly reduced stink bug damage to pepper and tomato. Field experiments conducted on tomatoes in North Carolina in 2012 and 2014 revealed a similar reduction in stink bug damage with a single-drip chemigation application of either dinotefuran or imidacloprid. In those trials, clothianidin was not efficacious and thiamethoxam was only effective in 2012. Our studies demonstrate the potential for soil applications of neonicotinoids to reduce stink bug damage to fruiting vegetables.

KEY WORDS *Halyomorpha halys,* soil applied, chemical control

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive pest native to eastern Asia (Lee et al. 2013). It was first discovered in the U.S.A. near Allentown, Pennsylvania, in the mid-1990s (Hoebeke & Carter 2003), and has since been detected in most of the continental U.S.A., parts of Canada, and Europe (Leskey et al. 2014). *Halyomorpha halys* is highly polyphagous and feeds on a wide variety of host plants, preferring to attack reproductive structures, such as developing and mature fruit and pods (Hoebeke & Carter 2003, Bergmann et al. 2013, Lee et al. 2013). In the mid-Atlantic U.S.A., this species has become a serious pest of fruiting vegetables, including pepper, *Capsicum* spp. (Solanales: Solanaceae); tomato, *Solanum*

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lycopersicum L. (Solanales: Solanaceae); okra, *Abelmoschus esculentus* (L.) Moench (Malvales: Malvaceae); and eggplant, *Solanum melongena* L. (Solanales: Solanaceae) (Kuhar et al. 2012a). Adults and nymphs invade fields during the summer months when fruiting bodies appear on plants. Feeding by *H. halys* causes white spongy areas on the skin and internal tissue damage to developed fruit, and it also may reduce fruit set and subsequent yield via abortion of flower buds and young fruiting bodies. Where this insect is established, such as southwest Virginia, damage to bell pepper has averaged between 20% to 40% in the absence of control measures (Kuhar et al. 2012b,c,d, 2013a,b,c).

Chemical control of stink bugs in agricultural crops has relied almost exclusively on the use of broad-spectrum insecticides, including pyrethroids, organophosphates, and carbamates (McPherson & McPherson 2000, Snodgrass et al. 2005, Temple et al. 2013). More recently, neonicotinoids have demonstrated a high level of activity against pentatomid pests, including various *Euschistus* species (Willrich et al. 2003, Cullen & Zalom 2007, Kamminga et al. 2009), *Chinavia* (=*Acrosternum*) *hilaris* (Say) (Willrich et al. 2003, Kamminga et al. 2009, 2012), *Murgantia histrionica* (Hahn) (Wallingford et al. 2012), and *H. halys* (Nielsen et al. 2008b, Kuhar et al. 2012c, Leskey et al. 2012a). In fruiting vegetable crop systems, several neonicotinoids, including imidacloprid, thiamethoxam, clothianidin, and dinotefuran, can be applied to root systems as a drench or injected directly into the drip irrigation system where they are translocated by the plant to green tissue to provide systemic control of above-ground feeding pests (Elbert et al. 2008, Ghidiu et al. 2012). Wallingford et al. (2012) achieved effective control of the harlequin bug, *M. histrionica*, using the aforementioned neonicotinoids applied to the root zone of collards, *Brassica oleracea* L. (Brassicales: Brassicaceae) plants. However, the harlequin bug is primarily a leaf-feeding stink bug. The efficacy of this strategy against *H. halys* is not known. Herein we investigated the relative toxicities of four neonicotinoids applied via a plant uptake bioassay, and we evaluated their field efficacy when applied to the soil for systemic control of *H. halys* on pepper and tomato.

Materials and Methods

Insecticide plant uptake bioassay for LC_{50} determination. To start a colony of *Halyomorpha halys,* adults were collected from various locations throughout Virginia and Maryland in the fall of 2012 and 2013. These insects were held in artificial overwintering habitats consisting of 19-L buckets that were tightly packed with 12.7 mm polyethylene pipe insulation (Frost King®, Mahwah, NJ). Approximately 500 *H. halys* adults were placed into each overwintering container. Adults were maintained outdoors at Blacksburg, VA until early January for both years, at which time they were placed in a temperature chamber (Percival Scientific Inc., Perry, IA) and exposed to temperatures of 26° C ± 2, a 16:8 h L:D photoperiod, and a 50% relative humidity, which induced feeding and reproduction of the bugs after a few weeks (Nielsen et al. 2008a, Medal et al. 2012). Adults and nymphs *H. halys* were provided a water wick and maintained on a diet of snap beans, *Phaseolus vulgaris* L. (Fabales: Fabaceae); carrots, *Daucus carota* L. (Apiales: Apiaceae); and peanuts, *Arachis hypogaea* L. (Fabales: Fabaceae). Nymphs were starved for 24 h prior to use in bioassays.

'Ambrose' snap beans were planted in 1-L pots in a greenhouse located at Virginia Tech, Blacksburg, VA. *Phaseolus vulgaris* was chosen because of its ease of production in the greenhouse and because it is known that *H. halys* readily feed on the plant tissue when pods are not present. Commercial formulations of clothianidin (Belay®, Valent U.S.A. Corp., Walnut Creek, CA), dinotefuran (Venom® 70SG, Valent U.S.A. Corp., Walnut Creek, CA), imidacloprid (Admire® Pro™, Bayer CropScience U.S., Research Triangle Park, NC), and thiamethoxam (Platinum® 75SG, Syngenta Crop Protection Inc., Greensboro, NC) were obtained from manufacturers and were mixed with water to make concentrations of 0.0, 0.001, 0.01, 0.1 and 1.0 ppm of each active ingredient. Solutions were placed in 50 ml Falcon® (Becton Dickinson & Co., Franklin Lakes, NJ) sterile polystyrene conical tubes. At the three-leaf stage (17–21 d after germination), snap bean plants were excised at the base of the plant and the cut end submerged in each insecticide concentration for 24 h to allow the insecticide to be taken up by the plant and translocated throughout the tissue (Prabhaker et al. 2011). The plants were held upright in the centrifuge tubes using a disc of floral foam (FloraCraft®, Ludington, MI) cut to the inside diameter of the tube. For each experiment, 10 laboratory-reared 2^{nd}–4^{th} instars of *H. halys* were placed in 500 ml fine mesh bags that were fastened over the bean plants. Four bagged plants were established for each insecticide concentration (total = 40 bugs) and were evaluated after 72 h for mortality (dead + moribund bugs). The experiment was replicated on five separate dates for each insecticide. Abbott's formula was used to correct for control mortality (Abbott 1925). Mortality and morbidity data were combined for analysis. Dose-mortality data were entered into PoloPlus© Version 1.0 (LeOra Software Co., Petaluma, CA) to determine LC_{50} values (LeOra Software 2002).

Field efficacy experiments. Field efficacy experiments were conducted on bell pepper in 2012 and 2013 in Virginia, and on tomato in 2013 in Virginia and in 2012 and 2014 in North Carolina. In early June 2012 and 2013, transplants of 'Aristotle' bell peppers were planted on raised beds covered with black polyethylene mulch at Virginia Tech's Kentland Farm near Blacksburg, VA. 'Baby Cake' tomatoes also were tested in 2013 at the Virginia site. Pepper and tomato plants were spaced 0.3 and 0.5 m, respectively, within rows. Plots were one row by six meters long. Each experiment was set up in a randomized complete block design replicated four times, and each of the four neonicotinoids was applied at the highest labeled soil application rate. Treatments included an untreated control, imidacloprid (0.426 kg ai/ha), clothianidin (0.224 kg ai/ha), dinotefuran (0.291 kg ai/ha), and thiamethoxam (0.190 kg ai/ha). In addition, we included a soil application of clothianidin (0.224 kg ai/ha) with a weekly foliar application of the pyrethroid fenpropathrin (0.347 kg ai/ha) (Danitol®, Valent U.S.A. Corp., Walnut Creek, CA). The first soil applications were made approximately 21 d after planting by placing 40 ml of the insecticide solution directly at the base of each plant within its respective plot using a single nozzle pump action Solo® sprayer (Newport News, VA). A second application of all treatments was made 30 d after the first application. All plots were irrigated approximately once per week using 10-mil Aqua-Traxx® drip tape (Toro®, Bloomington, MN) with 30.5 cm spacing and were given approximately the same volume of water. Foliar applications of fenpropathrin were made on 6, 14, 21, and 29 August in 2012, and on 30 July, 6, 14, and 26 August in 2013 with a 3-nozzle boom equipped with D3 spray tips and 45 cores and powered by a CO_2 backpack sprayer at 275.8 kPa delivering 355 L/ha. On three harvest dates in August in both years of the study, twenty randomly-selected mature fruit were hand-picked from each plot and inspected for stink bug feeding damage. This damage is indicated by a whitish or yellow spongy area on the fruit (Kuhar et al. 2012a).

Tomato trials were conducted at North Carolina State University's Mountain Horticultural Crops Research Station near Mills River, NC. Five-week-old 'Biltmore' and 'Florida 47' tomato transplants were set on 31 and 15 May in 2012 and 2014, respectively. Plants were set in raised beds covered with black plastic mulch and Chapin Drip Tape™ (Jain Irrigation Inc., Watertown, NY) buried about 6 cm below the soil surface. Drip tape emitters were spaced 30 cm apart and they delivered 372 L/h/100 m at a flow of 68.9 kPa. Plots consisted of two 7.6-m long rows on 1.5 m centers, with a non-treated row separating treatment rows. Plants were spaced 0.4 m within rows, and treatments were replicated four times and arranged in a randomized complete block. Treatments consisted of an untreated control and the following insecticides applied to the soil via drip tape: imidacloprid at 0.423 kg/ha, clothianidin at 0.224 kg/ha, dinotefuran at 0.294 kg/ha, and thiamethoxam at 0.193 kg/ha. Insecticides were applied on 2 and 8 July in 2012 and 2014, respectively, with a CO_2 injector into one-inch ploy tube connected to individual treatment drip lines. In 2012, chlorantraniliprole (Coragen® Insect Control, DuPont® Crop Protection, Wilmington, DE) was applied at 0.058 kg/ha via drip tape to all treatments except the control on 20 June and to the imidacloprid and dinotefuran treatments on 25 July. In 2014, the same rate of chlorantraniliprole was applied via drip tape to all treatments, including the control, on 2 June and 4 August. Chlorantraniliprole was applied to control lepidopteran pests; however, this insecticide does not exhibit activity against *H. halys* (JFW unpublished). The entire plot was on the same drip irrigation schedule, which consisted of irrigating two times per week during which 1 to 3 cm of water was applied per week. Mature fruit were harvested from all plots on 8 and 22 August and 6 and 20 September in 2012, and on 31 July, 14, 21 and 28 August in 2014. The time intervals between treatment applications and harvests in 2012 and 2014 were 37 to 80 d and 23 to 51 d, respectively.

When necessary, the proportion of damaged peppers and tomatoes from each treatment was transformed using an arcsine-square root transformation to normalize the variances (Sokal & Rohlf 1995), and then analyzed using ANOVA, JMP version 10.0 (SAS Institute, Cary, NC). Means were separated using Fisher's Protected LSD at the $P < 0.05$ level of significance. Data are presented as original means.

Results

Insecticide plant uptake bioassay for LC_{50} determination. All four of the neonicotinoid insecticides were highly toxic to *H. halys*, killing nearly 100% of the nymphs at a concentration of 1.0 ppm. Systemic LC_{50} values were lowest for dinotefuran (0.013 ppm) and thiamethoxam (0.018), but, based on non-overlapping 95% fiducial limits, only the LC_{50} value for dinotefuran was statistically lower than that of clothianidin (0.068 ppm) or imidacloprid (0.068 ppm) (Table 1).

Table 1. Observed LC_{50} levels of four neonicotinoid insecticides on *Halyomorpha halys* nymphs 72 h after exposure on treated snap bean plants in an insecticide plant uptake bioassay.

Active ingredient	LC_{50}	n	χ^2	95% FL	Slope ± SE
Clothianidin	0.077	461	127.91	0.033–0.282	1.301 ± 0.111
Dinotefuran	0.013	400	74.61	0.004–0.031	2.160 ± 0.347
Imidacloprid	0.068	393	46.12	0.032–0.198	1.489 ± 0.141
Thiamethoxam	0.018	539	147.76	0.009–0.041	1.634 ± 0.166

Field efficacy experiments. For all insecticides applied at the Virginia location to pepper in 2012 ($F = 6.72$, df = 5, 18, $P < 0.05$) and 2013 ($F = 3.09$, df = 5, 18, $P < 0.05$) and to tomato in 2013 ($F = 5.02$, df = 5, 18, $P < 0.05$), there were significant treatment effects on *H. halys* feeding damage to fruit at harvest over the course of the season, resulting in less damage than the untreated control (Figures 1 and 2). On average, stink bug damage to fruit was reduced approximately 50% by the soil applications of neonicotinoids. In all experiments, the addition of four weekly foliar applications of the pyrethroid fenpropathrin to a systemic application of clothianidin did not result in significant additional reduction of stink bug damage except in peppers in 2012, where the combination of clothianidin and fenpropathrin had significantly less stink bug damage compared with imidacloprid.

In the tomato trials in North Carolina in 2012 ($F = 5.28$, df = 4, 12, $P = 0.011$) and 2014 ($F = 3.29$, df = 4, 12, $P = 0.048$), the lowest levels of fruit damage were in the dinotefuran and imidacloprid treatments (Figure 3). In 2012, under relatively low stink bug pressure, clothianidin was the only treatment that did not significantly reduce damage below the control. In 2014, when stink bug populations were higher, only dinotefuran significantly reduced damage below the control, although damage levels in the imidacloprid treatment did not differ from that of dinotefuran. The most effective drip line treatments in the North Carolina studies reduced damage by approximately 50% below the control, similar to that observed with drench applications in the Virginia peppers and tomatoes.

Discussion

Chemical control of *H. halys* has been a topic of increasing interest ever since this invasive stink bug was recognized as a serious agricultural threat in the U.S.A. (Nielsen & Hamilton 2009, Leskey et al. 2012b). Several recent studies have assessed the efficacy of insecticides on *H. halys* using glass vial bioassays (Nielsen et al. 2008b), treated glass surface assays (Leskey 2012a), direct-contact topical applications (G. Krawczyk, unpublished data), bean dip bioassays (Kuhar et al. 2012f), and field efficacy trials (Kuhar et al. 2012b,c,d,e, Bergh 2013a,b,c, Hull et al. 2013a,b, Kuhar et al. 2013a,b,c). To our knowledge, this paper is the first published research on the systemic activity of neonicotinoid insecticides on *H. halys*. The plant uptake bioassay method, adapted from Prabhaker et al. (2011), was an effective and efficient method for discriminating insecticide concentrations to determine LC_{50} levels for four neonicotinoids on *H. halys*. The effectiveness of this bioassay technique confirms *H. halys* will feed on leaves and

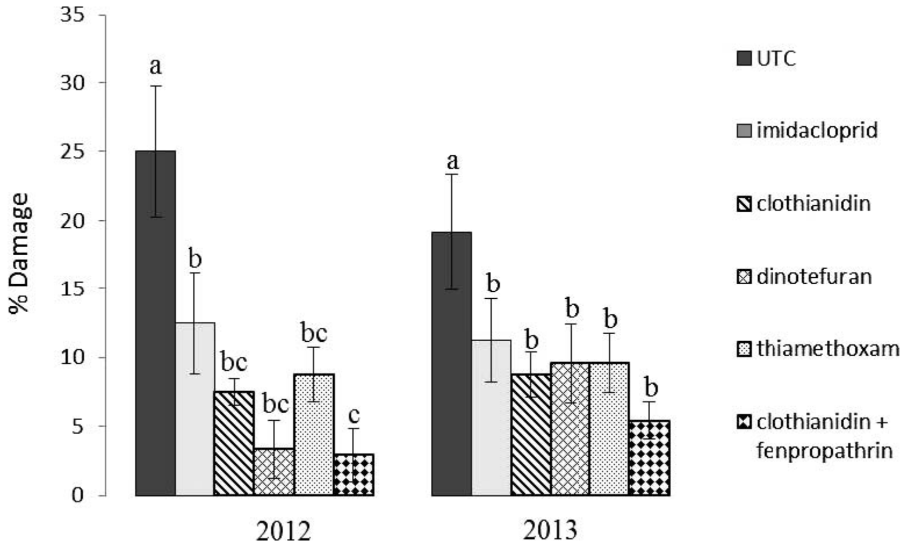

Fig. 1. Mean (±SE) cumulative percentage stink bug damage to pepper fruit from soil-drench insecticide field-efficacy experiments conducted near Blacksburg, Virginia. All neonicotinoids were applied twice as a soil drench 30 d apart. Fenpropathrin was applied as four weekly foliar sprays. Bars within years with a letter in common are not significantly different according to Fisher's Protected LSD ($P > 0.05$).

stem tissue of snap bean and that this bioassay method is a sufficient way to measure mortality in future studies to achieve similar objectives.

While all neonicotinoids were toxic to *H. halys* nymphs in our bioassays, imidacloprid and clothianidin had slightly higher (4–6 times) LC_{50} levels than those of dinotefuran and thiamethoxam. It is noted, however, that we do not know if the different neonicotinoids were taken up at the same rate by bean plants, so these values should not be used to compare relative toxicity of the products to *H. halys*. Nielsen et al. (2008a) previously reported toxicity levels for dinotefuran and thiamethoxam on *H. halys*, but, because a treated glass vial assay was used, the LC_{50} values are based on μg [AI]/cm^2, rather than ppm concentration of ingested solution.

In the field, soil applications of the aforementioned neonicotinoids at their highest recommended labeled rates were efficacious, significantly reducing stink bug damage to peppers and tomatoes in Virginia by about 50%. However, it should be noted that we evaluated two soil applications of these products made approximately 30 d apart in order to compare the relative toxicities throughout multiple harvests. This application strategy would violate the label restrictions by the U.S.A. Environmental Protection Agency (EPA) for some of the products. For instance, clothianidin cannot be applied to the soil on fruiting vegetables more than once per season, and the postharvest interval for imidacloprid, dinotefuran, and clothianidin is 21 d, and thiamethoxam is 30 d to harvest. Those intervals would probably eliminate the possibility of a second application of these insecticides in order to not interfere with proper harvest dates. However, in the

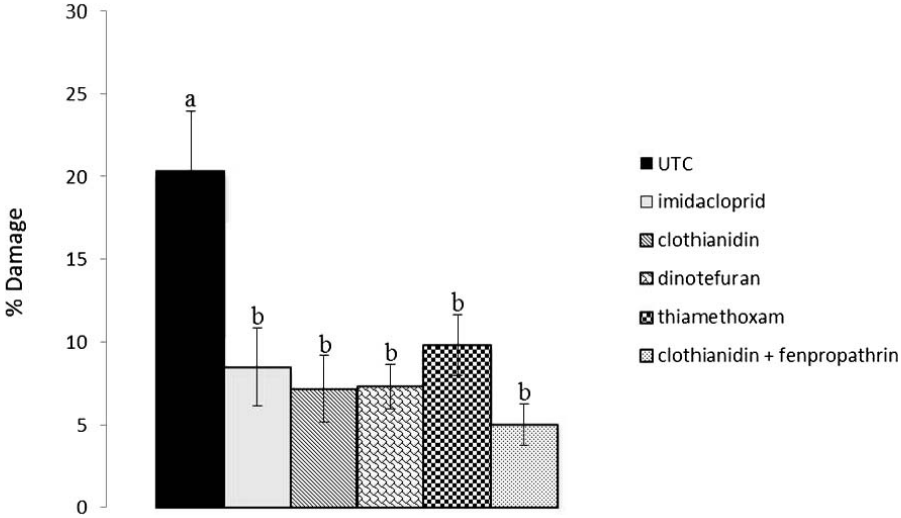

Fig. 2. Mean (±SE) cumulative percentage stink bug damage to tomato fruit from soil-drench insecticide field-efficacy experiments conducted near Blacksburg, Virginia, in 2013. All neonicotinoids were applied twice as a soil drench 30 d apart. Fenpropathrin was applied as four weekly foliar sprays. Bars within years with a letter in common are not significantly different according to Fisher's Protected LSD ($P > 0.05$).

tomato experiments conducted in North Carolina, a single application was made through the drip irrigation line more than 21 d before harvest. In those experiments, application of either dinotefuran or imidacloprid significantly reduced stink bug fruit damage; whereas, clothianidin was not efficacious, and thiamethoxam was only effective in 2012 and not 2013. Additional late season control of stink bugs could be provided by foliar insecticide applications. In our study, the percentage of stink bug damage to fruit in plots treated with a soil application of clothianidin followed by four weekly foliar applications of fenpropathrin was significantly less in one of the three experiments. However, foliar applications of pyrethroids also kill natural enemies and other beneficial insects (Croft & Whalon 1982), which can lead to outbreaks of secondary pests, such as spider mites and aphids (Leskey et al. 2012b). In peppers in the mid-Atlantic U.S.A., for instance, multiple applications of pyrethroids often result in serious pest outbreaks of green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Chapman et al. 2009, Ghidiu & Kuhar 2012, Kuhar et al. 2012b). Thus, because systemic neonicotinoids are highly efficacious at controlling aphids and many other sucking insect pests (Elbert et al. 2008), their use as one of the insecticide applications for control of *H. halys* during the season should prevent the problems with aphid outbreaks in crops like pepper.

The use of neonicotinoid insecticides have been under scrutiny in recent years as being detrimental to honeybees, *Apis mellifera* L., and other pollinators (Laycock et al. 2012, Stoner & Eitzer 2012, Fairbrother et al. 2014). While the active ingredients of all of the soil-applied neonicotinoids that we tested are highly toxic to bees when applied topically or orally, the assumed risk to

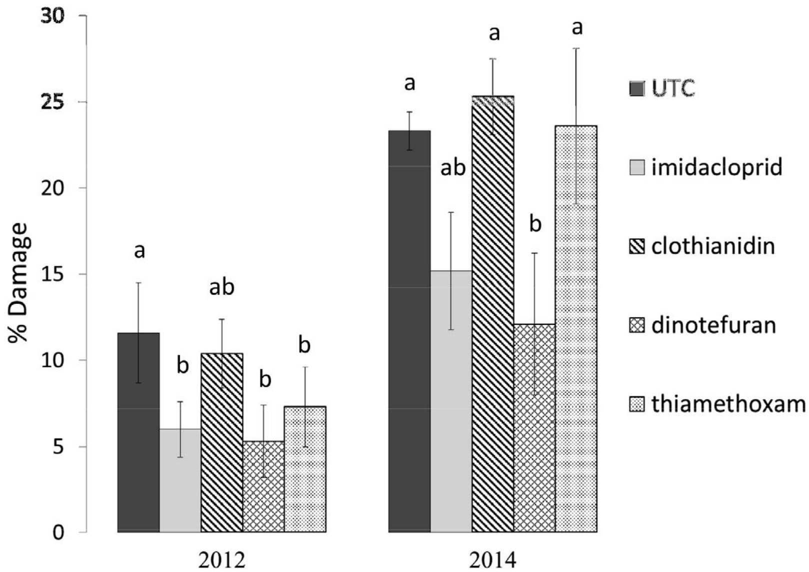

Fig. 3. Mean (±SE) cumulative percentage stink bug damage to tomato fruit resulting from drip-line application of soil insecticides conducted near Mills River, North Carolina. Bars within years with a letter in common are not significantly different according to Fisher's Protected LSD ($P > 0.05$).

pollinators will likely be reduced when these products are applied to the root systems via chemigation or soil drench. Dively & Kamel (2012) found that the levels of neonicotinoid insecticides in pollen and nectar of cucurbit crops varied with the timing of soil applications, but, under worst case scenarios, levels were not acutely lethal to honeybees and were often below the no-observable-effect level. We believe that this strategy should pose less threat to pollinators than any of the alternative chemical control approaches, which invariably include multiple foliar sprays of insecticides, such as pyrethroids, carbamates, organophosphates. It is our conclusion that soil application of the neonicotinoid insecticides imidacloprid, dinotefuran, clothianidin, or thiamethoxam is an effective method to manage *H. halys* in fruiting vegetables efficiently, while attempting to minimize impacts on non-target species.

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