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TOXICITY AND FIELD EFFICACY OF FOUR NEONICOTINOIDS ON HARLEQUIN BUG (HEMIPTERA: PENTATOMIDAE)

Anna K. Wallingford[1], Thomas P. Kuhar[1] and Peter B. Schultz[2]
[1]Virginia Tech, Department of Entomology, 216 Price Hall, Blacksburg, VA 24061-0319

[2]Virginia Tech Hampton Roads Agricultural Research and Extension Center, 1444 Diamond Springs Rd., Virginia Beach, VA 23455

*Corresponding author; E-mail: tkuhar@vt.edu

Abstract

Harlequin bug, *Murgantia histrionica* (Hahn), is a pest of cole crops in the USA. Laboratory toxicity assays revealed that the neonicotinoid insecticides imidacloprid, thiamethoxam, dinotefuran, and clothianidin are toxic to harlequin bug nymphs; LC_{50} = 0.57, 0.52, 0.39, and 0.39 ppm, respectively. Field experiments were conducted to evaluate the efficacy of these insecticides over time when applied as a one-time soil drench. Each of the 4 neonicotinoids provided significant control of harlequin bug for at least 14 d after application.

Key Words: *Murgantia histrionica*, imidacloprid, thiamethoxam, dinotefuran, clothianidin

Resumen

El chinche Arlequín, *Murgantia histrionica* (Hahn), es una plaga de cultivos de col en los Estados Unidos. Los ensayos de toxicidad de laboratorio revelaron que los insecticidas neonicotinoides imidacloprid, tiametoxam, dinotefurano y clotianidina son tóxicos a las ninfas del chinche Arlequín; CL_{50} = 0.57, 0.52, 0.39 y 0.39 g ia / L, respectivamente. Se realizaron experimentos de campo para evaluar la eficacia de estos insecticidas con el tiempo de aplicación cuando se usa una sola vez como un regado al suelo. Cada uno de los 4 neonicotinoides proveyeron un control significativo contra el chinche Arlequín por lo menos 14 días después de la aplicación.

Palabras Clave: Murgantia histrionica, imidacloprid, tiametoxam, dinotefurano, clotianidina

Harlequin bug, *Murgantia histrionica* (Hahn) (Hemiptera: Pentatomidae), is a specialist herbivore of cruciferous vegetables (Brassicaceae) and is an important pest of cole crops (Brassicale: Brassicaceae) in the USA (McPherson & McPherson 2000; Wallingford et al. 2011). The piercing-sucking feeding of adults and nymphs create white blotches on leaves, making vegetables sold as greens unmarketable, and under heavy pest pressure, can kill plants or entire fields of cabbage or broccoli (Paddock 1915; Ludwig & Kok 2001). Although most broad-spectrum insecticides such as organophosphates, carbamates, and pyrethroids provide effective control (Rogers & Howel 1972; Wang 1978; Edelson & Mackey 2005a,b; McLeod 2005; Walgenbach & Schoof 2005; Kuhar & Doughty 2009), these insecticides are also detrimental to important natural enemies in the crucifer crop agroecosystem (Xu et al. 2001, 2004; Hill & Foster 2003; Cordero et al. 2007).

Neonicotinoid insecticides offer a less-disruptive alternative for controlling hemipteran insects; they are water soluble and can be taken up by plants through the roots and translocated through the xylem vessels to plant tissues, exposing herbivores to the toxin only when they feed (Sur & Stork 2003; Tomizawa & Casida 2005). Neonicotinoids target the nicotinic acetylcholine receptors in insects, which over-stimulate neurons leading to paralysis and the ultimate failure of the central nervous system (Thomson 2000).

The neonicotinoid insecticides acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam have been found to be effective in controlling harlequin bug when used as a foliar spray (Edelson 2004; Edelson & Mackey 2005c, 2005d, 2006; Walgenbach & Scoof 2011). However, soil application of neonicotinoids could allow for greater residual efficacy against the target pest while reducing non-target effects by not leaving surface residues on foliage as occurs with foliar application. The objectives of this study were to compare and contrast the relative toxicity of 4 neonicotinoid insecticides on the harlequin

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bug and to assess the residual efficacy of these compounds when applied as a drench to the soil surface in the field.

Materials and Methods

Leaf Disk Bioassays to Estimate LC_{50} Values

Dose-mortality was estimated for 4 insecticides; the formulations and registered field rates for vegetables are listed in Table 1. Insecticide solutions were prepared as a serial dilution in distilled water at concentration of 0, 0.001, 0.01, 0.1 and 1 ppm. Leaf disks (8.5 cm diameter) were cut from the wrapper leaves of store bought cabbage heads, surface sterilized in 10% bleach water and triple rinsed prior to insecticide treatment. Disks were dipped for 10 s in each insecticide solution and allowed to dry for 2 h. Dry leaf disks were placed into individual 9-cm diam Petri dishes along with 5 harlequin bug 3rd-4th instars (n = 4). Participant insects were field-collected from untreated collard plots grown at Virginia Tech's Agriculture Research and Extension Center at Painter, Virginia. Mortality was determined after 48 and 72 h of exposure to treated disks at room temperature. Nymphs were considered dead when no movement was observed when prodded. The experiment was repeated 3 times for each insecticide.

Excised Collard Leaf Toxicity Bioassays to Determine Residual Efficacy in the Field

Collards (*Brassica oleracea* cv 'Vates'; Brassicale: Brassicaeae) were planted in May, 2010 and again in Jul, 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center at Painter, Virginia. Collards were direct seeded at 3 m spacing between rows and 0.11 m within each row, and managed on bare ground with minimal inputs other than weed management, which were applied according to conventional management practices (Wilson et al. 2010). Temperatures ranged from 3-34 °C and 12-38 °C through May and Jul experiments, respectively. Insecticide drenches were applied to 6 m, single-row plots in a randomized block design once plants had reached at least 1 true leaf (n = 4; Table 1). Leaves were removed from plots 7, 14, 21 and 28 d after treatment and 5 harlequin bug 3rd-4th instars were isolated to these leaves in Petri dishes (9 cm diam). Insects were observed for mortality or signs of intoxication after 48 h of exposure. Nymphs were considered dead when no movement was observed when prodded, and considered moribund when unable to right themselves.

Statistical Analysis

Analysis of variance was conducted using JMP (SAS Institute, Cary, North Carolina) to test significant difference between percent mortality of treatments in leaf disk bioassays (1 ppm concentration), and both excised-leaf bioassays, and means separation was determined using Tukey's HSD. Control mortality was using Abbott's formula, and then dose-mortality was estimated for each insecticide using probit analysis (EPA Probit Software 2010).

Results

Leaf Disk Bioassays to Estimate LC_{50} Values

There was no difference in mortality among insecticides in leaf disk bioassays (α = 0.05). Assays resulted in 60-70% mortality at 1 ppm concentrations for all insecticides (Table 2). Moribund nymphs exposed to clothianidin took up to 72 h before they were reliably determined dead, while 48 h was sufficient for the other 3 insecticides. The LC_{50} for each insecticide was less than 1 ppm; below the equivalent of the registered field rate for all 4 products, with the exception of the product containing thiamethoxam, which contains a lower concentration of active ingredient compared to the other products assayed (Table 2).

Excised Collard Leaf Toxicity Bioassays to Determine Residual Efficacy in the Field

All insecticides provided significant mortality relative to the control in bioassays conducted 7 and 14 d after treatment (Table 3) in the May experiment (F = 20.27; df = 6, 21; P < 0.0001, F = 17.68; df = 6, 21; P <0.0001, respectively) and in the Jul experiment (F = 4.89; df = 6, 21; P = 0.0028, F = 12.18; df = 6, 21; P < 0.0001, respectively). Imidacloprid was an exception, as this

Table 1. Insecticides evaluated and their application rates in field experiments. Rates used were the high end of the registered label rates.

MANUFACTURER	PRODUCT	ACTIVE INGREDIENT	SOIL RATE
Bayer (Research Triangle Park, NC)	Admire PRO	Imidacloprid	10.5 fl oz/A
Syngenta (Greensboro, NC)	Platinum 75SG	Thiamethoxam	11.0 fl oz/A
Valent (Libertyville, IL)	Venom 70SG	Dinotefuran	6 oz/A
	Belay	Clothianidin	12 fl oz/A

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Table 2. Percent mortalities, LC_{50} (ppm) values, and the field rate equivalent to each neonicotinoid LC_{50} value for harlequin bug nymphs exposed to cabbage leaves dipped in serial dilutions of 4 different neonicotinoid insecticides (N = 3).

RATE (ppm)	IMIDACLOPRID (48 h)	THIAMETHOXAM (48 h)	DINOTEFURAN (48 h)	CLOTHIANIDIN (72 h)
0.001	5.2%	5.3%	2.1%	5.3%
0.01	4.0%	8.0%	2.1%	7.4%
0.1	14.6%	31.1%	16.0%	32.9%
1	67.7%	73.4%	65.8%	61.9%
LC_{50} (lower - upper)	0.57 (0.32 - 1.26)	0.52 (0.28 - 1.06)	0.39 (0.20 - 0.92)	0.39 (0.16 - 1.50)
Equivalent Field Rate	62%	126%	61%	81%

Mortality adjusted using Abbott's Formula. LC_{50} and lower and upper 95% confidence intervals calculated according to probit analysis (EPA Software 2010).

insecticide provided only 7 d of residual efficacy in the May experiment, and mortality due to imidacloprid treatments was not different from the control 7 d after treatment in the Jul experiment, although mortality was higher than the control 14 d after treatment (Table 3). No insecticide resulted in levels of mortality significantly higher than the control at 21 or 28 d after treatment in either experiment.

Discussion

The neonicotinoids imidacloprid, clothianidin, dinotefuran, and thiamethoxam were all toxic to harlequin bug nymphs with LC_{50} levels below 1 ppm. Soil drench-treated plants were found to result in residual mortality of nymphs for roughly 2 wk, compared to approximately 10 d of control by foliar treatments (Walgenbach & Scoof 2011). Imidacloprid-treated collard plants provided significant mortality for 7 and 14 d in May and Jul experiments, respectively. This was shorter than 29 d of protection in the field reported by Kuhar & Doughty (2009). However, in their experiment, as well as any other that uses natural pest populations, residual efficacy is very difficult to assess because the timing and duration of pest infestations is variable.

Critical to the use of neonicotinoids as a systemic insecticide is delivery to the root zone, accomplished via seed treatment, drench application, chemigation, or in transplant water. Residual efficacy over time will be influenced by how quickly the insecticide can be taken up into the plant and the life of the insecticide in the soil, whether it will be leached away or bind to the soil, and how quickly it degrades in the environment. Imidacloprid, thiamethoxam, dinotefuran, and clothianidin are all water soluble, but imidacloprid is less water soluble than the rest, slower to be taken up by plants, and the most likely to bind to soil (Byrne et al. 2007, 2010; Ali & Caldwell 2010).

Imidacloprid had a shorter period of efficacy than the other insecticides in the May experiment and was also slower to provide control in the Jul experiment. A slow uptake of imidacloprid can be expected due to its higher affinity to soil. All insecticides demonstrated shorter than anticipated residual efficacy, and it is possible that the volume of water used in these drench treatments was not adequate to deliver the full rate of the insecticide to the root-zone, or insecticide percolated to areas beyond the root structure.

In conclusion, neonicotinoids provide effective control of harlequin bug nymphs and, while lethal

Table 3. Percent mortalities (dead + moribund) of harlequin bug nymphs exposed to excised collard leaves at 7, 12, 21, and 28 d after treatment (DAT) by soil drench of each of 4 neonicotinoid insecticides at their highest labeled rates.

	MAY EXPT. (% MORTALITY)				JUL EXPT. (% MORTALITY)			
INSECTICIDE	7 DAT	14	21	28	7 DAT	14	21	28
untreated	5 b	3 c	18	8	3 c	10 b	0	5
imidacloprid	80 a	45 b	20	15	25 bc	95 a	25	0
thiamethoxam	100 a	100 a	48	40	63 ab	95 a	25	10
dinotefuran	98 a	100 a	75	63	83 a	90 a	10	5
chlothianidin	93 a	88 a	28	18	68 ab	90 a	20	25

Data within a column followed by the same letter are not significantly different according to ANOVA followed by Tukey HSD test of means separation (n = 4, α = 0.05); there was no significant treatment effect on mortality at 21 and 28 d after treatment.

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concentrations were not different among the insecticides assayed, the residual efficacy by drench application was variable. A method of application that puts the active ingredient directly in the root zone may be preferred (e.g. seed treatment or transplant water).

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