Susceptibility of *Bagrada hilaris* (Hemiptera: Pentatomidae) to Insecticides in Laboratory and Greenhouse Bioassays

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ABSTRACT Field-collected nymphs and adults of *Bagrada hilaris* (Burmeister) (Hemiptera: Penatatomidae) from three locations were evaluated for susceptibility to insecticides representing 10 classes of insecticide chemistry. Although relative susceptibilities differed between leaf-spray and leaf-dip Petri dish bioassays, consistently low LC_{50} values were determined for chlorpyrifos, bifenthrin, and lambda-cyhalothrin. Fenpropathrin and methomyl had intermediate values. Susceptibility to dinotefuran varied depending on the bioassay, possibly owing to leaf substrates used in the two bioassays. In soil systemic bioassays, the LC_{50} value of dinotefuran was significantly greater than that of two other neonicotinoids, imidacloprid and thiamethoxam, and the anthranilic diamide, cyantraniliprole. Mortality and feeding damage of *B. hilaris* and plant growth on insecticide-treated plants in greenhouse trials were consistent with the laboratory bioassays; the best results were seen with bifenthrin, methomyl, and chlorpyrifos. Mortality to the neonicotinoids was not evident; however, feeding damage and plant growth responses on dinotefuran-treated plants damage were similar to the noninfested control. This highlights the apparent antifeedant properties of dinotefuran that may have prevented adults from injuring broccoli plants after exposure to foliar spray residues. Data presented serve as baseline susceptibilities that can be used to monitor for resistance development in field populations of *B. hilaris*.

KEY WORDS baseline data, insecticide, bioassay technique, invasive species

Bagrada hilaris (Burmeister) (Hemiptera: Penatatomidae), formerly known as *Bagrada cruciferarum* Kirkaldy and *Bagrada picta* (F.), is native to Africa, India, and Asia and is often referred to as the bagrada bug or painted bug (Howard 1906). An invasive stink bug species, *B. hilaris* was first discovered in North America in Los Angeles, CA, in 2008. Within a year, it was found attacking cole crops throughout the desert southwest of the United States (Palumbo and Natwick 2010). The small stink bug is now considered a serious economic pest of a variety of brassicaceous vegetable crops grown during fall and winter months in the agricultural valleys of Arizona and southern California (Reed et al. 2013). Surveys of growers from Yuma, AZ, have estimated that ~90% of fall-planted broccoli (*Brassica oleracea* L. var. italica) and cauliflower (*Br. oleracea* var. botrytis) acreage from 2010 to 2012 was infested with *B. hilaris*. These infestations resulted in average stand losses and plant injury as high as 10% (Palumbo 2014). Considering that the production of cole crops in Arizona and California was collectively valued at >US$1 billion in 2011–2012 (California Agricultural Statistics Review 2012, Arizona Agricultural Statistics 2011, U.S. Department of Agriculture–National Agricultural Statistics Service [USDA-NASS] 2012), the potential economic impact of *B. hilaris* outbreaks on the western vegetable industry could be substantial.

Direct-seeded and -transplanted brassicaceous crops are susceptible to feeding damage by *B. hilaris* adults, particularly during stand establishment (Huang et al. 2013, 2014a,b). Excessive feeding damage to apical meristems can result in their destruction, leading to either adventitious bud break (e.g., cabbage plants with multiple and unmarketable heads), or plants with reduced or nonreproductive heads (e.g., broccoli with no crowns; Palumbo and Natwick 2010). The potential for the pest to cause significant crop losses coupled with the lack of biological control alternatives for *B. hilaris* (Reed et al. 2013) has left little alternative but to use insecticides. Furthermore, because *B. hilaris* can rapidly damage seedling plants (Huang et al, 2014a), effective insecticide treatments applied in the field must act quickly. Currently, vegetable growers in Arizona and California rely heavily on frequent applications of pyrethroid insecticides to control *B. hilaris* adult infestations on seedling cole crops (Palumbo 2014). Because pyrethroids are used frequently for other pests in cole crops (Palumbo and Castle 2009), alternative insecticides are needed to protect desert cole crops from *B. hilaris* and conserve pyrethroid chemistry.

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A sustainable management program for *B. hilaris* requires a well-conceived insecticide use strategy that: 1) uses the most effective insecticides, 2) diversifies modes of action, and 3) promotes natural control by minimizing the use of broad-spectrum insecticides.

Because of the exotic nature of *B. hilaris*, little information is available on its control and management. Much of the published research detailing insecticidal control of *B. hilaris* is either outdated or not applicable to southwestern U.S. cropping systems. For example, many of the active ingredients previously evaluated for efficacy against *B. hilaris* are not currently available for use on cole crops in the United States (Hill 1975, Srivastava and Dixit 1977, Ahuja and Joshi 1995, Sachan and Purwar 2007). Pyrethroids have been shown to effectively control *B. hilaris* adults in a number of brassicaceous crops (Chauhan and Yadav 2007, Infantino et al. 2007, Obopile et al. 2008), and older organophosphate products such as chlorpyrifos, malathion, profenofos, and monocrotophos were also effective (Chauhan and Yadav 2007, Singh et al. 2009). Endosulfan was reported to be highly efficacious against *B. hilaris* adults on seedling mustard crops (Sachan and Purwar 2007, Ahuja et al. 2008), but the use of endosulfan is now prohibited on cole crops in the United States because of U.S. Environmental Protection Agency (EPA) regulations (EPA 2010). The neonicotinoids are known to provide residual systemic control of a number of piercing–sucking pests such as aphids and whiteflies on desert vegetable crops (Palumbo et al. 2001, Palumbo and Castle 2009), and have shown systemic activity against another pentatomid, the harlequin bug, *Murgantia histrionica* (Hahn), on collards and cabbage (Edelson and Mackey 2006, Kuhar and Doughty 2009). Singh et al. (2011) reported significant *B. hilaris* control and higher yields in mustard crops after foliar applications of imidacloprid and thiamethoxam.

The objectives of the present studies were to 1) establish baseline susceptibility levels through bioassays, and 2) to determine the relative toxicities of a number of insecticides on recently introduced populations of *B. hilaris*. The materials evaluated represent a range of active ingredients belonging to 10 classes of insecticides. Both conventional broad-spectrum and newer reduced-risk insecticides were selected based on preliminary field efficacy trials (Palumbo 2011a,b; Palumbo et al. 2013a). These insecticides also were evaluated to provide baseline data for certain reduced-risk insecticides (Palumbo 2012, Palumbo et al. 2013b) that could diversify a resistance management program while potentially reducing the impact on beneficial arthropods. Information gained from laboratory and greenhouse bioassays on relative toxicities of various insecticides will complement field trials to provide a broader foundation for establishing a sustainable chemical control program.

Materials and Methods

Bioassays were conducted on *B. hilaris* collected from the Coachella Valley (Riverside County CA), Imperial Valley (Imperial County, CA), and Yuma Valley (Yuma County, AZ) in the fall and spring of 2010–2013. Hereafter, these populations will be referred to as Coachella, Imperial, and Yuma. Insects were collected from these three agricultural areas to cover the desert agricultural regions that have been most impacted by *B. hilaris*. Depending on the time of year and host availability, mixed life stages of *B. hilaris* were collected directly from the leaves and stems of broccoli and cauliflower, *Br. oleracea* L. var. acephala, London rocket (*Sisymbrium irio* L.), Sahara mustard (*Brassica tournefortii* Gouan), Indian mustard, *Brassica juncea* (L.) Czernajew, and short-pod mustard, *Hirschfeldia incana* (L.) Lagrèze-Fossat. Insects were transported to the greenhouse and placed on young broccoli, cabbage, or kale plants and held for 2–3 d before subjecting them to toxicity tests. Adults were evaluated for all insecticides, except in the case of spirotetramat and novaluron, where immature stages (third instar) were evaluated.

Preliminary laboratory bioassays were conducted on formulated insecticides (Table 1) to determine the appropriate concentration ranges for baseline toxicity studies. In each laboratory bioassay, serial dilutions of five to six concentrations of each insecticide were made on the same day of testing. Units for each insecticide in the laboratory bioassays were calculated as μg (AI) per ml. In greenhouse bioassays, the recommended field rates for each insecticide tested were used and calculated as kg (AI) per ha.

Leaf-Spray Bioassay. Susceptibility of the Coachella population to bifenthrin, fenpropathrin, chlorpyrifos, dinotefuran, spirotetramat, cyantraniliprole, pyrifluquinazone, and novaluron (Table 1) were assessed using an established leaf-spray technique (Prabhaker et al. 2006) with slight modification. Broccoli plants with three to four true leaves were selected for tests to provide uniformity of test plants. Leaves on each plant were sprayed until run-off with a specific concentration of each insecticide. Control plants were sprayed with water alone. At least five concentrations for each insecticide were applied to establish mortalities ranging from 5 to 95%. Spirotetramat was diluted in deionized water containing a nonionic wetter/spreader (Dyne-Amic, Helena Chemical Co., Memphis, TN) at a concentration of 0.1% (v/v). After the leaf surface dried for 1 h, five nymphs (novaluron and spirotetramat tests only) or adults were enclosed in clip cages (3.8 cm in diameter, 11.34-cm^2 surface area, 1.3 cm in height from the leaf) that were screened with nylon organdy on both sides for ventilation. For each concentration of insecticide or water control, five replicate clip cages, each containing five *B. hilaris*, were attached to the abaxial leaf surface and maintained at 27 ± 2°C and a photoperiod of 14:10 (L: D) h while tests were conducted. Tests were repeated two times for each insecticide. Numbers of dead *B. hilaris* (determined by the absence of any movement when prodded) were determined at 48 h from initial exposure.

Leaf-Dip Petri Dish Bioassay. Susceptibilities of Coachella, Imperial, and Yuma *B. hilaris* populations to contact activity of bifenthrin, dinotefuran, chlorpyrifos,

Table 1. Insecticides evaluated for *B. hilaris* susceptibility in laboratory and greenhouse bioassays

Class (IRAC[a] group)	Insecticide	Formulation	Company
Carbamate (1A)	Methomyl	Lannate SP	DuPont Crop Protection, Wilmington, DE
Organophosphate (1B)	Chlorpyrifos	Lorsban 50W	Gowan Co., Yuma, AZ
Pyrethroid (3)	Bifenthrin	Brigade 2F	FMC Corporation, Philadelphia, PA
	Fenpropathrin	Danitol 2.4EC	Valent USA Corp., Walnut Creek, CA
	Lambda-cyhalothrin	Warrior II 2.08CS	Syngenta Crop Protection, Greensboro, NC
Neonicotinoid (4A)	Dinotefuran	Venom 70WG	Valent USA Corp.
	Imidacloprid	Admire Pro 4.6F	Bayer CropScience, Research Triangle Park, NC
	Acetamiprid	Assail 30SG	United Phosphorus Inc., King of Prussia, PA
	Thiamethoxam	Platinum 50WG	Syngenta Crop Protection
	Clothianidin	Belay 2.13SC	Valent USA Corp.
Spinosyn (5)	Spinosad	Entrust 2SC	Dow AgroScience LLC
Selective Feeding Blocker (9)	Flonicamid	Beleaf 50WG	FMC Corporation
Inhibitors of Chitin synthesis (15)	Novaluron	Rimon 0.83EC	Makhteshim Agan, Raleigh, NC
Tetramic Acid derivative (23)	Spirotetramat	Movento 2F	Bayer CropScience
Anthranilic diamides (28)	Cyantraniliprole	Exirel 10SC	DuPont Crop Protection
Unclassified (Unk)	Pyrifluquinazon	NNI-0101 20SC	Nichino America, Inc, Wilmington, DE

[a] Insecticide Resistance Action Committee.

lambda-cyhalothrin, and methomyl (Table 1) were determined using a leaf-dip Petri dish technique adapted from an approved Insecticide Resistance Management Committee (IRAC) method (http://www.irac-online.org/content/uploads/Method_007_v3_june09 pdf). This bioassay was developed to measure contact activity of these insecticides. Cotton, *Gossypium hirsutum* L., was used for the leaf-dip bioassay because *B. hilaris* adults do not feed on cotton leaves (Reed et al. 2013) and intoxication from the insecticide occurred through contact on the treated leaf surface. Attempts of using a glass-vial bioassay similar to Nielson et al. 2008 resulted in unacceptable control mortality (>35%). Dosages were determined for each insecticide in preliminary tests, and five to six concentrations of each formulated insecticide were selected that established mortality ranging from 5 to 95%. Each solution contained a nonionic wetter/spreader (Dyne-Amic, Helena Chemical Co., Memphis TN) at a concentration of 0.1% (v/v).

Whole leaves (8–9 cm in diameter) were detached from cotton plants, dipped individually in serial dilutions of each insecticide for 15 s with gentle agitation, and placed on paper toweling, where they were allowed to dry for 1 h. Control leaves were dipped individually in water alone. The treated leaves were placed on wetted filter paper within glass 90-mm Petri dishes. Depending on the number of insects available at the time of the bioassay, 5 to 10 adult *B. hilaris* were placed in each Petri dish for exposure to the insecticide-treated leaf. A minimum of five concentrations per insecticide were evaluated with six replications of each concentration. Water-treated controls were maintained for each set of treatments. Mortality assessments were made after 48 h, using the same criterion as for the leaf-spray bioassay.

Soil Systemic Bioassay. Susceptibility of the Coachella population to three neonicotinoids (dinotefuran, imidacloprid, and thiamethoxam) and an anthranilic diamide (cyantraniloprole) was evaluated using a systemic uptake technique (Prabhaker et al. 2006). Potted broccoli plants with three to four true leaves in 4-inch pots were used. Five to six concentrations of each insecticide were prepared on the day of treatment and applied in 10-ml aliquots to the soil around the main stem of each plant. Control plants were treated similarly with aliquots of water. Uptake of each insecticide in each test plant was allowed for 24 h, after which *B. hilaris* were exposed to the treated leaves by enclosing them in clip cages (Prabhaker et al. 2006) attached to each leaf. Mortality counts were made after 48 h using the same criterion as the bioassays above.

Greenhouse Bioassay I. Whole plant bioassays evaluating bifenthrin, acetamiprid, flonicamid, cyantraniliprole, spinosad, spirotetramat, and pyrifluquinazone for knockdown efficacy were conducted in the greenhouse. Broccoli plants were direct-seeded into 12.5-cm diameter pots containing Miracle-Gro (Marysville, OH) potting soil and irrigated daily. Plants of uniform size at the two-leaf stage (20–22 d old) were selected for the bioassays. Adult insects used for the bioassays were collected from nontreated broccoli fields at the Yuma Agricultural Center, Yuma, AZ. Adults were starved for 24 h before being caged on plants used in the studies. The bioassay was conducted twice, with treatments replicated 8 times in the May 2011 test and 12 times in the November 2011 tests ($n = 20$). In each bioassay, two-leaf stage broccoli plants were taken outside the greenhouse and a foliar spray of the recommended labeled field rate for each insecticide was applied to each plant using a CO_2 operated back-pack sprayer delivering 205.3 liter/ha at 275.8 kPa similar to treatment of plants in small plots efficacy trials (Palumbo 2012). All sprays included an adjuvant, Dyne-Amic (Helena Chemical Co., Memphis, TN) at 0.25% vol/vol. After a 1-h drying period, insecticide-treated plants were placed inside of the greenhouse and enclosed individually within ventilated plastic containers to which four field-collected adult *B. hilaris* of unknown age were introduced. Average ambient temperatures in the greenhouse were held at 28.8 ± 5.5°C.

Adult mortality, described above, was assessed initially at 12 h after infestation, and then at 24-h intervals for a 120-h exposure period. After the exposure period, cages and any live adults were removed from each

plant. Feeding damage on leaf tissue and terminal growing points was measured by estimating the percentage of leaf surface on each plant that had feeding lesions (Huang et al. 2014a). The plants then were sprayed with bifenthrin (Brigade at 0.09 kg [AI]/ha) to eliminate the possibility of stray *B. hilaris* infesting the uncaged plants within the greenhouse. Periodic sprays were made at 5–7 d intervals to further ensure that insects did not damage plants. Plants were held in the greenhouse for 20 d to determine the impact of *B. hilaris* feeding on plant growth in each treatment. Plant growth responses were estimated by measuring total plant leaf area using a LI-3100 leaf-area meter (Li-Cor, Inc., Lincoln, NE), and dry weights for each plant were measured after drying all leaves, petioles, and stems for 48 h in a forced air oven (150°C).

Greenhouse Bioassay II. An additional greenhouse bioassay evaluated knockdown and residual efficacy of bifenthrin, methomyl, chlorpyrifos, dinotefuran, acetamiprid, and clothianidin (all registered on cole crops) on two-leaf stage cauliflower plants. Treated plants were infested with insects at 1 h, 3 d, and 5 d after insecticide application. Treatments were replicated six times for each infestation interval and the bioassay was conducted twice ($n = 12$). A nontreated, insect-infested control, and a noninsect infested control were included in each bioassay. The experimental design and all other experimental variables and measurements were the same as those used in greenhouse bioassay I.

Statistical Analyses. Results of the dose–mortality responses for each insecticide in the leaf-spray, leaf-dip Petri dish, and soil systemic bioassays were analyzed by probit regression analysis using the POLO program (Russell et al. 1977, LeOra 1987). Data are presented as LC_{50} values [μg (AI)/ml] with 95% confidence limits for each insecticide. Values were considered significantly different ($P < 0.05$) if CIs did not overlap. The slopes and chi-squares were computed and are presented to provide additional information regarding the degree of heterogeneity in the mortality response (slope) and as an indication of how well the mortality data fit the probit model (Robertson and Preisler 1992).

The greenhouse bioassays were arranged in a randomized complete block design. Because no interactions were found among trials, data were pooled and analyzed using analysis of variance (PROC GLIMMIX; SAS Institute 2009, Cary, NC). The response variables, adult mortality, and feeding damage on leaves were subjected to an arcsine transformation before analysis to meet the assumptions of analysis of variance (Zar 1999). Dry weights and leaf area were analyzed on nontransformed data. Insecticides were modeled as a fixed effect and replicates (greenhouse bioassay I: $n = 20$; greenhouse bioassay II: $n = 12$) were modeled as a random effect. Treatment means were separated using the Tukey (or Bonferroni) adjustment in LSMEANS (PROC LSMEANS/ ADJUST = TUKEY; SAS Institute 2009) at ($\alpha = 0.05$). Regardless of transformations, the original nontransformed data are presented in the text and tables.

Table 2. Susceptibility of the Coachella population of *B. hilaris* adults and nymphs to insecticides evaluated in the leaf-spray and soil systemic bioassays, 2011

Insecticide	*n*	LC_{50} (μg [AI]/ml) (95% Fiducial limits)	Slope ± SE	χ^2
Leaf-spray bioassay: adults				
Bifenthrin	300	27.05 (17.08–44.60)	2.10 ± 0.23	8.62
Dinotefuran	215	24.32 (13.60–41.28)	1.30 ± 0.20	4.30
Chlorpyrifos	200	15.09 (8.53–24.96)	1.50 ± 0.22	4.25
Fenpropathrin	250	51.10 (29.51–85.25)	1.50 ± 0.24	6.25
Cyantraniliprole	252	313.01 (151.20–683.30)	0.80 ± 0.16	6.54
Pyrifluquinazone	200	259.05 (167.00–551.00)	1.30 ± 0.29	8.36
Leaf-spray bioassay: nymphs				
Spirotetramat	250	386.05 (278.00–571.00)	0.49 ± 0.24	7.45
Novaluron	205	53.86 (35.75–77.45)	1.70 ± 0.31	6.43
Soil systemic bioassay : adults				
Dinotefuran	261	31.88 (21.39–46.88)	1.10 + 0.13	9.54
Imidacloprid	382	85.17 (66.57–104.74)	3.80 + 0.78	6.39
Thiamethoxam	380	77.69 (60.87–94.19)	4.05 + 0.83	8.28
Cyantraniliprole	200	557.00 (292.23–739.05)	1.20 + 0.39	5.25

Results

Leaf-Spray Bioassay. In this bioassay, the nontreated control mortality never exceeded 8%. The chi-square goodness-of fit statistics showed the data in all the laboratory bioassays adequately conformed to the probit model. There were significant differences in the LC_{50} between the tested insecticides, with the lowest concentrations in chlorpyrifos, dinotefuran, and bifenthrin (Table 2). Bifenthrin and fenpropathrin (the two pyrethroids evaluated) had intermediate LC_{50} values and they were not significantly different from each other. The newest insecticides cyantraniliprole (anthranilic diamide) and pyrifluquinazone (unclassified chemistry) had significantly higher LC_{50} s than the other insecticides.

The LC_{50} between spirotetramat and novaluron, both insecticides with modes of action disruptive to immature development, was significantly different (Table 2). The LC_{50} was seven times greater when *B. hilaris* immatures were exposed to spirotetramat than when exposed to novaluron. The slopes of spirotetramat were low at 0.49 ± 0.24 as compared with 1.7 ± 0.31 for novaluron. These lower slope values represent a heterogeneous response, which usually is considered to be a function of genetic heterogeneity in the test population. However, in the case of spirotetramat that interferes with lipid metabolism and arrests development, it may be because of the amount of time necessary to realize the impact of the insecticide on mortality. In this case, an evaluation period > 48 h might have resolved mortality effects more conclusively, resulting in higher slope values.

Leaf-Dip Petri Dish Bioassay. In this experiment, the nontreated control mortality never exceeded 4%. Of the five insecticides evaluated, the pyrethroids (bifenthrin and lambda-cyhalothrin) and chlorpyrifos had the lowest LC_{50} values (Table 3). LC_{50} values for these three insecticides were not significantly different across the populations and years evaluated in the study. This was not the case for methomyl, in which the LC_{50} values varied significantly and was more than three

Table 3. Susceptibility of Yuma, Imperial, and Coachella populations of *B. hilaris* adults to insecticides evaluated in the leaf-dip Petri dish bioassay, 2010–2013

Insecticide	Population	Year	*n*	LC_{50} (μg [AI]/ml) (95% Fiducial limits)	Slope ± SEM	χ^2
Bifenthrin	Yuma	2010	192	0.25 (0.14–0.39)	1.16 ± 0.16	11.71
Bifenthrin	Yuma	2011	240	0.74 (0.38–1.44)	1.24 ± 0.13	50.99
Bifenthrin	Yuma	2012	300	0.56 (0.29–1.03)	1.15 ± 0.13	41.12
Bifenthrin	Imperial	2010	240	0.61 (0.28–1.01)	1.26 ± 0.22	21.55
Bifenthrin	Imperial	2012	240	0.80 (0.35–1.42)	1.05 ± 0.16	15.52
Bifenthrin	Coachella	2013	240	0.45 (0.24–0.79)	0.85 ± 0.11	20.40
Dinotefuran	Yuma	2010	192	8.93 (5.28–14.45)	1.09 ± 0.13	17.38
Dinotefuran	Yuma	2011	240	6.09 (3.11–10.76)	0.91 ± 0.11	26.24
Dinotefuran	Yuma	2012	300	16.33 (8.24–31.53)	1.05 ± 0.11	42.45
Dinotefuran	Imperial	2010	192	14.46 (8.16–24.77)	0.92 ± 0.11	18.44
Dinotefuran	Imperial	2012	240	10.04 (5.08–18.54)	0.94 ± 0.12	26.01
Dinotefuran	Coachella	2013	240	10.98 (6.04–18.95)	0.90 ± 0.11	10.34
Chlorpyrifos	Yuma	2011	240	1.91 (1.56–2.35)	3.50 ± 0.38	9.24
Chlorpyrifos	Yuma	2012	300	2.67 (2.05–3.49)	2.13 ± 0.20	38.72
Chlorpyrifos	Coachella	2013	240	2.06 (1.22–3.19)	0.91 ± 0.13	17.90
Lambda-cyhalothrin	Yuma	2010	240	2.09 (1.33–3.29)	1.21 ± 0.13	14.19
Lambda-cyhalothrin	Yuma	2011	192	2.63 (1.16–5.31)	0.88 ± 0.09	10.14
Lambda-cyhalothrin	Yuma	2012	300	0.88 (0.50–1.44)	1.06 ± 0.13	22.84
Methomyl	Yuma	2010	240	11.45 (7.87–16.87)	1.74 ± 0.22	21.60
Methomyl	Yuma	2012	300	3.04 (1.67–5.58)	1.19 ± 0.12	40.44
Methomyl	Coachella	2013	240	8.77 (3.22–21.31)	0.53 ± 0.08	9.31

times higher in 2010 than in 2012. The Coachella population had an LC_{50} value that was intermediate between the two Yuma populations. Overall, dinotefuran had the highest LC_{50} values and there were no significant differences in the susceptibility of the five populations.

Soil Systemic Bioassay. In this experiment, the nontreated control mortality never exceeded 8%. Dinotefuran had a significantly lower LC_{50} than the other insecticides (Table 2). The other two neonicotinoids, imidacloprid and thiamethoxam, had statistically higher LC_{50} values than dinotefuran and they were statistically similar to each other. Cyantraniloprole had the highest LC_{50} of all insecticides in the soil systemic bioassay, with values ranging from 6.5 (imidacloprid) to 17.5 (dinotefuran) times higher than the neonicotinoids.

Greenhouse Bioassay I. Bifenthrin was the most efficacious insecticide in this bioassay where 100% adult knockdown mortality was observed at 12 h after placement of adults on the treated plants (Fig. 1). None of the other insecticides used in this study caused this level of *B. hilaris* mortality. The next most effective insecticides were acetamiprid, which provided adult mortality greater than the nontreated control beginning 72 h after infestation ($F = 152.9$; df = 7,133; $P = <0.0001$) and spinosad at 96 h post-infestation ($F = 53.9$; df = 7,133; $P = < 0.0001$). The rapid adult mortality provided by bifenthrin resulted in negligible feeding damage, and significantly greater plant growth responses were observed for bifenthrin-treated plants compared with the other spray treatments and the nontreated control plants (Table 4). Among the other insecticide treatments only acetamiprid-, spirotetramat-, and pyrifluquinazone-treated plants had significantly less feeding damage and significantly greater dry weight and leaf area than nontreated control plants.

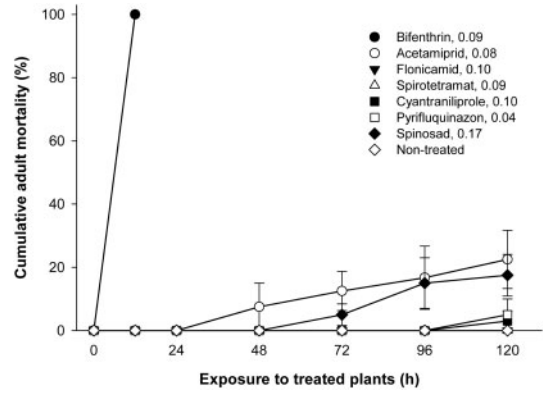

Fig. 1. Mean cumulative percent mortality (± SEM) of *B. hilaris* adults after exposure on plants sprayed with insecticides (kg[AI]/ha) in greenhouse bioassay I.

Greenhouse Bioassay II. Plants treated with methomyl and bifenthrin and infested with *B. hilaris* adults 1 h after spray treatments caused 100% mortality at or before 24 h of exposure, which was significantly different from the other insecticides and the nontreated control ($F = 116.8$; df = 6,66; $P = < 0.0001$; Fig. 2A). Mortality due to chlorpyrifos treatment was significantly different from the other treatments after 24 h of exposure ($F = 116.8$; df = 6,66; $P = < 0.0001$) and all insects in this treatment were dead after 48 h. Plants sprayed with the neonicotinoids (dinotefuran, acetamiprid, and clothianidin) had significantly lower mortality than bifenthrin, methomyl, and chlorpyrifos at 48 h ($F = 138.3$; df = 6,66; $P = < 0.0001$). Dinotefuran and clothianidin provided 72.9 and 66.7% mortality, respectively, after 120 h exposure, and mortality on acetamiprid-treated plants was significantly lower at

Table 4. Plant damage and growth responses associated with *B. hilaris* adults on broccoli plants treated with insecticides in greenhouse bioassay I

Treatment, rate (kg[AI]/ha)	Mean feeding signs[a] (% ± SEM)	Mean plant responses[b] (± SEM)	
		Dry weight (g)	Leaf area (cm^2)
Bifenthrin, 0.09	0.6 ± 0.2d	1.3 ± 0.0a	277.6 ± 7.5a
Acetamiprid, 0.08	22.9 ± 1.4c	0.7 ± 0.1b	153.0 ± 15.7b
Flonicamid, 0.10	62.0 ± 3.9ab	0.4 ± 0.1c	91.9 ± 11.3de
Cyantraniliprole, 0.10	59.5 ± 3.4ab	0.4 ± 0.0c	72.1 ± 7.1e
Spinosad, 0.17	62.5 ± 2.6ab	0.5 ± 0.0c	111.3 ± 7.1cd
Spirotetramat, 0.09	56.0 ± 3.1b	0.4 ± 0.0c	98.5 ± 9.5de
Pyrifluquinazon, 0.04	30.0 ± 2.4c	0.6 ± 0.0b	126.8 ± 8.2c
Nontreated	64.8 ± 2.2a	0.4 ± 0.0c	83.4 ± 6.9e
$F_{7,133}$	108.12	61.68	52.51
$P > F$	<0.0001	<0.0001	<0.0001

Means followed by the same letter are not significantly different ($P < 0.05$).

[a] Percentage of leaf tissue with visible feeding damage was estimated at 120 h after infestation.

[b] Total plant dry weights and leaf areas were estimated 20 d after the adults were removed.

37.5% ($F = 73.78$; df = 6,66; $P < 0.0001$). The rapid adult mortality provided by bifenthrin, methomyl, and chlorpyrifos resulted in significantly less leaf feeding, and growth responses were similar to the noninfested plants (Table 5). Although dinotefuran did not provide adult mortality comparable with bifenthrin, the percentage of feeding signs was not different from the bifenthrin-treated and noninfested control plants. Both clothianidin and acetamiprid had significantly more feeding damage and less plant growth than the noninfested control based on dry weight and leaf area measurements.

On plants infested 3 d after insecticide sprays, none of the insecticides provided 100% mortality of *B. hilaris* adults at 72 h after initial exposure (Fig. 2B). The most efficacious products were chlorpyrifos, which provided 100% mortality after 96 h of exposure, and bifenthrin, which caused 100% mortality 120 h after infestation. Methomyl provided marginal residual mortality (66.7%) and did not differ from dinotefuran (75.6%) at 120 h ($F = 38.3$; df = 6, 66; $P = < 0.0001$). Among the neonciotinoids, clothianidin provided significantly less residual efficacy than dinotefuran, and adult mortality on acetamiprid-treated plants did not differ from the nontreated control throughout the 120 h bioassay. Only bifenthrin and dinotefuran-treated plants had negligible feeding damage on leaves, and plant growth responses did not differ from noninfested control plants (Table 6). The amount of feeding measured on leaves in the acetamiprid- and methomyl-treated plants was significantly greater than all other spray treatments, but was lower than on the nontreated control plants. Average growth responses for the spray treatments were significantly greater than the nontreated control, with the exception of dry weight for the acetamiprid-treated plants (Table 6).

Further evaluation of insecticide residual activity showed a lower mortality rate curve for all chemicals when insects were placed on plants 5 d after spray treatments were applied (Fig. 2C). None of the treatments caused 100% mortality at any time during the 120 h bioassay. However, chlorpyrifos, bifenthrin, and dinotefuran provided > 75% mortality after 72-h exposure on the treated plants and reached levels exceeding 90% mortality after 120 h of exposure. Neither clothianidin nor acetamiprid provided mortality comparable with the other insecticides, although adult mortality was greater than on the nontreated control after 120 h of exposure on treated plants ($F = 54.1$; df = 6,66; $P < 0.0001$). Among all treatments, only the dinotefuran- and bifenthrin-treated plants had feeding damage similar to the noninfested control (Table 7), but all treatments had significantly less feeding damage than the nontreated control. Growth responses were greatest for dinotefuran, whereas acetamiprid and clothianidin had the least growth response among the sprayed insecticide treatments.

Discussion

Data presented in the present study provides baseline susceptibilities, relative toxicities, and plant protection capabilities of insecticides that are being used and potentially may be used in the future to control *B. hilaris*. We approached this work under controlled conditions in two ways. First, several types of laboratory bioassays were used to establish baseline susceptibilities, which are critical for evaluating resistance in future populations of the bagrada bug. Bioassay monitoring of *B. hilaris* populations in India showed that LC_{50} values of organophosphate and organochlorine insecticides increased significantly over a 25-yr span (Swaran Dhingra 1998). The baseline data generated from our present study will be increasingly important in the future given the reliance on pyrethroids and organophosphates for control of other key insect pests on desert vegetable crops (Palumbo and Castle 2009). Second, we used greenhouse bioassays that closely mimicked field exposures, allowing us to determine the relative knockdown and residual toxicities of insecticides. In addition, information on the damage and growth response of plants treated with different insecticides was gathered.

In the laboratory bioassays, there were differences in the relative LC_{50} values between methodologies and consistently higher LC_{50}s were generated in the leaf-spray bioassay than in the leaf-dip Petri dish bioassay. A number of factors may have contributed to these relative differences. These include insecticide coverage in the two studies, different *B. hilaris* populations that were tested, utilization of different substrates (broccoli in leaf-spray and cotton in leaf-dip Petri dish), and arena type (ventilated clip cage in leaf-spray and nonventilated petri dish in leaf-dip Petri dish). Despite these differences in bioassays, there were consistencies in the relative LC_{50} values of the tested materials. Chlorpyrifos and bifenthrin had the lowest LC_{50}s in both bioassays. These results mimic field trials and support the use of bifenthrin that has been the primary pyrethroid used by growers and pest control advisors for reducing infestations of *B. hilaris* and protecting

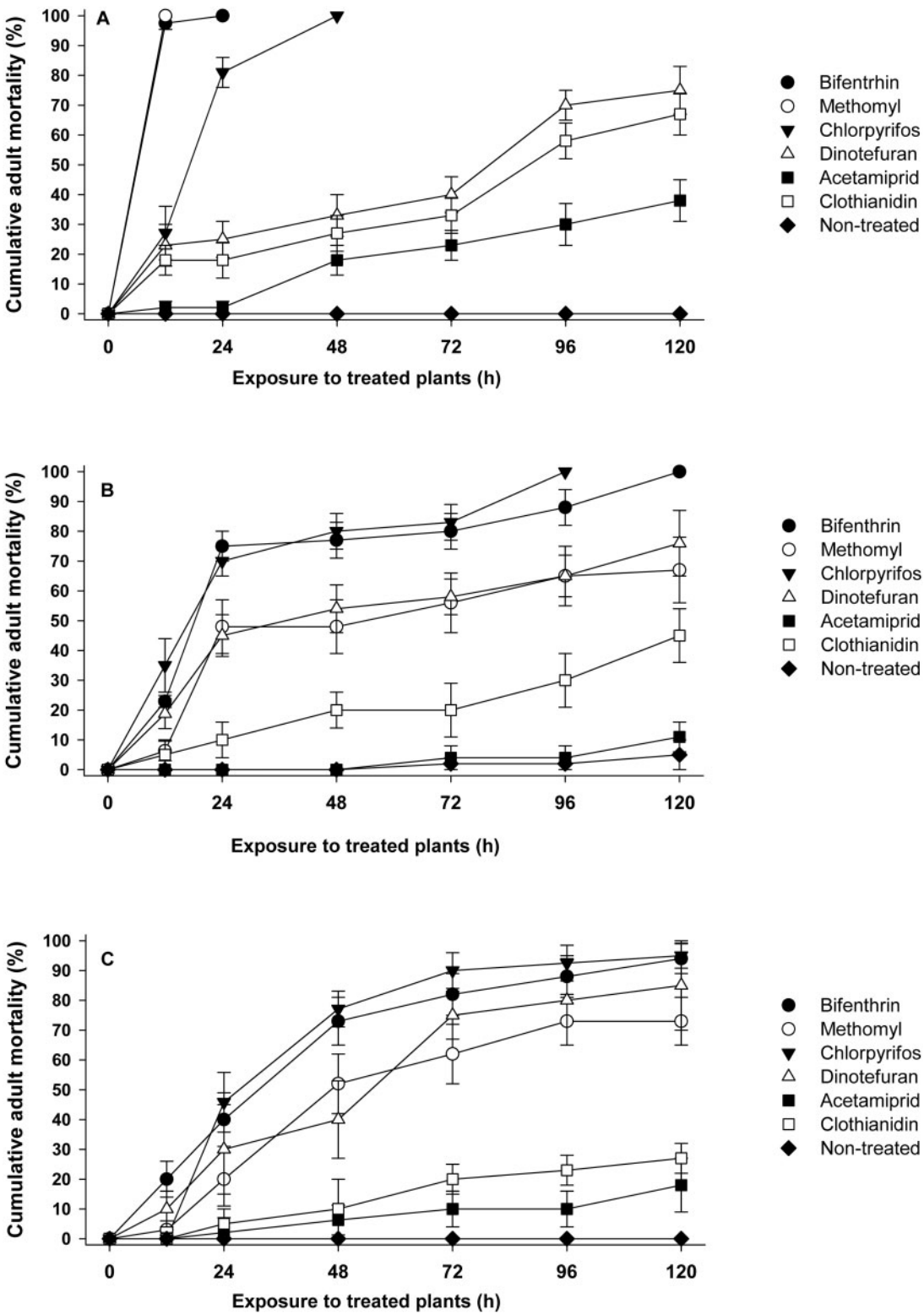

Fig. 2. Mean cumulative percent mortality (±SEM) of *B. hilaris* adults after exposure on plants sprayed with insecticides in greenhouse bioassay II. Plants infested with insects (A) 1 h after foliar sprays applied; (B) 3 d after foliar sprays applied, and; (C) 5 d after foliar sprays applied.

Table 5. Plant damage and growth responses associated with *B. hilaris* adults on cabbage plants treated with insecticides in greenhouse bioassay II

Treatment, rate (kg[AI]/ha)	Mean feeding signs[a] (% ± SEM)	Mean plant responses[b] (± SEM)	
		Dry weight (g)	Leaf area (cm^2)
Bifenthrin, 0.09	0.0d	0.61 ± 0.02a	166.7 ± 6.6ab
Methomyl, 1.01	0.0d	0.56 ± 0.03ab	142.7 ± 6.4b
Chlorpyrifos, 0.75	3.8 ± 2.4d	0.64 ± 0.03a	160.1 ± 9.9ab
Dinotefuran, 0.17	0.3 ± 0.1d	0.60 ± 0.03a	148.2 ± 10.1b
Acetamiprid, 0.08	39.2 ± 8.3b	0.35 ± 0.05cd	79.1 ± 7.9c
Clothianidin, 0.07	12.6 ± 3.5c	0.48 ± 0.04bc	100.0 ± 7.3c
Noninfested	0.0d	0.64 ± 0.03a	177.5 ± 5.6a
Nontreated	89.6 ± 2.6a	0.22 ± 0.05d	42.9 ± 5.8d
$F_{7,77}$	97.64	16.4	36.17
$P > F$	<0.0001	<0.0001	<0.0001

Adults were exposed to plants 1 h after foliar sprays were applied.

Means followed by the same letter are not significantly different ($P < 0.05$).

[a] Percentage of leaf tissue with visible feeding damage was estimated at 120 h after infestation.

[b] Total plant dry weights and leaf areas were estimated 20 d after the adults were removed.

Table 6. Plant damage and growth responses associated with *B. hilaris* adults on cabbage plants treated with insecticides in greenhouse bioassay II

Treatment, rate (kg[AI]/ha)	Mean feeding signs[a] (% ± SEM)	Mean plant responses[b] (± SEM)	
		Dry weight (g)	Leaf area (cm^2)
Bifenthrin, 0.09	0.0e	0.59 ± 0.03a	151.0 ± 7.7a
Methomyl, 1.01	39.6 ± 9.3bc	0.41 ± 0.05bc	105.8 ± 12.9b
Chlorpyrifos, 0.75	6.1 ± 2.1cd	0.46 ± 0.02ab	122.9 ± 6.3b
Dinotefuran, 0.17	0.4 ± 0.1de	0.58 ± 0.02a	147.2 ± 6.5a
Acetamiprid, 0.08	55.4 ± 5.4b	0.27 ± 0.02cd	68.4 ± 8.6c
Clothianidin, 0.07	13.6 ± 3.1cd	0.41 ± 0.01bc	102.1 ± 4.4b
Noninfested	0.0e	0.60 ± 0.03a	165.4 ± 4.5a
Nontreated	79.2 ± 7.6a	0.18 ± 0.04d	35.2 ± 10.0d
$F_{7,77}$	42.96	22.22	29.86
$P > F$	<0.0001	<0.0001	<0.0001

Adults were exposed to plants 3 d after foliar sprays were applied.

Means followed by the same letter are not significantly different ($P < 0.05$).

[a] Percentage of leaf tissue with visible feeding damage was estimated at 120 h after infestation.

[b] Total plant dry weights and leaf areas were estimated 20 d after the adults were removed.

Table 7. Plant damage and growth responses associated with *B. hilaris* adults on cabbage plants treated with insecticides in greenhouse bioassay II

Treatment, rate (kg[AI]/ha)	Mean feeding signs[a] (% ± SEM)	Mean plant responses[b] (± SEM)	
		Dry weight (g)	Leaf area (cm^2)
Bifenthrin, 0.09	5.4 ± 1.6de	0.59 ± 0.02b	146.1 ± 5.6ab
Methomyl, 1.01	29.6 ± 4.6bc	0.51 ± 0.02cd	131.5 ± 6.3b
Chlorpyrifos, 0.75	15.9 ± 2.3cd	0.53 ± 0.02bc	132.8 ± 5.1b
Dinotefuran, 0.17	0.6 ± 0.2e	0.70 ± 0.02a	168.4 ± 4.8a
Acetamiprid, 0.08	47.1 ± 4.1b	0.37 ± 0.03d	79.9 ± 7.1c
Clothianidin, 0.07	30.0 ± 3.9c	0.43 ± 0.03cd	92.3 ± 5.3c
Noninfested	0.0e	0.69 ± 0.02a	171.1 ± 4.1a
Nontreated	72.5 ± 6.5a	0.21 ± 0.03e	43.6 ± 6.1d
$F_{7,77}$	60.97	49.64	64.19
$P > F$	<0.0001	<0.0001	<0.0001

Adults were exposed to plants 5 d after foliar sprays were applied.

Means followed by the same letter are not significantly different ($P < 0.05$).

[a] Percentage of leaf tissue with visible feeding damage was estimated at 120 h after infestation.

[b] Total plant dry weights and leaf areas were estimated 20 d after the adults were removed.

Brassica plantings (Palumbo 2014). In addition, lambda-cyhalothrin had low LC_{50} values in the leaf-dip Petri dish bioassay, while fenpropathrin and methomyl showed intermediate LC_{50} values. Interestingly, the LC_{50} for dinotefuran was among the lowest in the leaf-spray bioassay, but it was the highest in the leaf-dip Petri dish bioassay. This likely is related to the substrate used in the two bioassays. In the leaf spray bioassay, broccoli leaves, a preferred feeding host of *B. hilaris* were used, and in the leaf-dip Petri dish bioassay, cotton leaves, a nonpreferred feeding host, was used. Insects in the leaf-dip assay would not be induced to feed on the plant, thus they may not have been exposed to lethal dosages of the dinotefuran, which may need to be ingested to cause maximum mortality. This hypothesis is supported by the results of the soil systemic bioassay, in which dinotefuran had a significantly lowest LC_{50} than the other insecticides. This result with dinotefuran demonstrates the importance of matching the appropriate bioassay technique to the insecticide mode of action. For the xylem-mobile neonicotinoid and anthranilic diamide insecticides, a systemic uptake bioassay was used to simulate the type of exposure that *B. hilaris* would experience from feeding on a brassicaceous plant treated with a soil-applied systemic insecticide. As mentioned, dinotefuran had the lowest LC_{50} values among the neonicotinoids, and cyantraniliprole had dramatically higher LC_{50} values. Leskey et al. (2012) indicated that cyantraniliprole was minimally efficacious against brown marmorated stink bug, *Halyomorpha halys* (Stål), adults in a glass Petri dish assay. Similar response to cyantraniliprole was demonstrated in the present study in the leaf-spray bioassay and when applied as a foliar spray on plants in the greenhouse.

Among insecticides that exert an inhibitory effect on developing insects, the benzoylurea-type insecticide novaluron was relatively effective against *B. hilaris* immatures, whereas spirotetramat had little mortality impact on nymphs in the leaf-spray bioassay, or against adults on treated plants in the greenhouse. Kamminga et al. (2012) showed that novaluron was effective against brown marmorated stink bug nymphs in bean-dip bioassays. The lack of toxicity with spirotetramat in our bioassays is consistent with its slow activity against immature stages and lack of adult mortality shown against other sucking hemipterans (Nauen at al. 2008, Leskey et al. 2012). Pyrifluquinazone, which is highly efficacious against *Bemisia* sp. whiteflies on vegetable crops (Castle et al. 2009), was not effective against *B. hilaris* adults. Similarly, flonicamid and spinosad provided little to no adult mortality in the greenhouse bioassay. These observations are consistent with results from recent small plot trials where field applications of these insecticides were not effective at controlling

B. hilaris adults or preventing feeding damage (Palumbo et al. 2013b).

The toxicity studies conducted in the greenhouse provided important data not only on the speed of knockdown for each insecticide tested but also on the persistence of the mortality effect and plant protection over increasing intervals of exposure to treated foliage. Both of these concepts are critical to a full appraisal of the potential of insecticides to prevent damage by *B. hilaris*. Considering the nature of attack by *B. hilaris* adults and the extensive damage that occurs to inadequately protected young *Brassica* crops, insecticides that provide rapid knockdown activity and continue to protect plants during seedling growth will be most valuable in defending against *B. hilaris*. The importance of protecting seedlings is clearly evident from the low nominal action threshold currently being recommended for *B. hilaris* control on broccoli, where plants should be treated when populations exceed one adult per two row meters of seedling plants (https://extension.arizona.edu/sites/extension.arizona.edu/files/resources/091714%20Bagrada%20Bug%20Management%20Tips_VegIPMUpdate_2014b.pdf) accessed January 2015.

In the greenhouse trials, the pyrethroid bifenthrin had superior knockdown and residual performance as 100% mortality was demonstrated within 24 h when *B. hilaris* adults were exposed to plants 1 h posttreatment. Mortality was slower with exposures at 3 and 5 d posttreatment, but still exceeded a mean of 90% after 5 d of exposure, indicating residual activity of bifenthrin. Methomyl also provided 100% mortality within 24 h at 1 h posttreatment exposure, and chlorpyrifos provided 100% mortality within 48 h at 1 h posttreatment exposure. Methomyl had less residual activity with 3 and 5 d posttreatment exposure compared with bifenthrin and chlorpyrifos. In contrast, adult mortality to dinotefuran at 24 h at all intervals posttreatment was limited, but increased with longer exposures of 72 and 120 h. Interestingly, neither methomyl nor chlorpyrifos reduced feeding damage or prevented reductions in plant growth compared with bifenthrin, dinotefuran, or the noninfested control when plants were exposed to *B. hilaris* at 3 or 5 d after application of insecticides.

Most of the newer insecticides with novel modes of action and translaminar routes of activity (i.e., neonicotinoids, flonicamid, cyantraniliprole, spinosad, spirotetramat, and pyrifluquinazone) appear to be poor candidates for *B. hilaris* control, as shown in the present studies and preliminary field evaluations (Palumbo 2012, Palumbo et al. 2013b). Among the neonicotinoids, dinotefuran was more effective than either acetamiprid or clothianidin, which did not provide comparable adult mortality or plant protection in the greenhouse bioassays. Other studies with neonicotinoids showed that *H. halys* was not as susceptible to acetamiprid compared with dinotefuran (Nielson et al. 2008, Lesky et al. 2012). Dinotefuran demonstrated contact activity against *B. hilaris* in the leaf-dip laboratory bioassay, and although adult mortality on dinotefuran-treated plants was significantly slower than for bifenthrin in the greenhouse bioassay, both materials provided comparable residual plant protection for up to 5 d. While dinotefuran did not show particularly strong knockdown or residual efficacy, it did protect the plants from bagrada bug feeding, resulting in plant responses that were similar to the noninfested controls. This suggests that dinotefuran has an antifeedant property that would be effective in a *B. hilaris* management program. Leskey et al. (2014) similarly reported that antifeeding effects of dinotefuran may have played a role in preventing fruit injury from *H. halys* in apples where spray applications of the insecticide did not result in high adult mortality.

This research illustrates the importance of considering the effects of *B. hilaris* feeding on young leaf tissue rather than relying solely on insect mortality when evaluating insecticide activity. The insecticides that produced the highest mortality within 24 h resulted in the best plant protective capabilities. The organophosphates and pyrethroids appear to be quite effective; however, measured plant responses indicate that neonicotinoids such as dinotefuran offer an alternative for managing *B. hilaris* that may fit into the integrated pest management programs for *Brassica* crops. Coordination of rapid knockdown insecticides, such as methomyl, with slower acting insecticides with residual activity, such as dinotefuran, could represent an insecticide use strategy for preventing damage during the vulnerable emergence stage of crop phenology. This approach would provide needed relief from pyrethroids, which are the most widely used insecticides on produce crops grown in the desert southwest (Palumbo and Castle 2009). Finally, the information gained from these laboratory and greenhouse bioassays on relative toxicities of various insecticides complements field tests (Palumbo 2011a, 2012, 2013a,b) and provides a broader foundation for establishing a sustainable pest management program for *B. hilaris* on *Brassica* crops.

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