Lethal and Sublethal Effects of Organically-Approved Insecticides against *Bagrada hilaris* (Hemiptera: Pentatomidae)[1]

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Abstract *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae) is a stink bug species that preferentially attacks cruciferous crops. Because organic growers have limited insecticide tools for combating *B. hilaris*, the lethal and sublethal effects of organically approved insecticide products and their selected combinations against *B. hilaris* were examined in laboratory and field settings. In a topical spray assay, spinosad, either alone or in combination with pyrethrins, azadirachtin, and potassium salts, resulted in >95% mortality relative to the water-only treatment 48 h postapplication; whereas, stand-alone pyrethrins and azadirachtin failed to cause similar mortality rates. When adults were exposed to treated broccoli (*Brassica oleracea* var. *italica* Blenck) seedlings for 48 h after 0, 1, and 7 d of application, the number of feeding injury sites was significantly lower with the combined treatment of spinosad, pyrethrins, and azadirachtin than those on seedlings treated with water alone at 0 and 1 d postapplication. However, when adults were exposed to seedlings at 7 d postapplication, only the combined treatment with pyrethrins and azadirachtin reduced the number of feeding sites compared with the water control. When the locomotor activity of adults was evaluated after exposure to dried residues for 20 min and 2 h, adults were found to be more active when exposed to combined treatment with spinosad, pyrethrins, and azadirachtin than the individual treatments. In the field trials, although most of the insecticide products and their combinations failed to provide persistent *B. hilaris* control, the combined treatment of pyrethrins and potassium salts reduced the number of feeding sites compared with the nontreated control.

Key Words cruciferous crops, painted bug, spinosad, pyrethrins, azadirachtin, organic production

Bagrada hilaris (Burmeister) (Hemiptera: Pentatomidae) is an invasive insect pest that is native to South Africa, Asia, and Middle Eastern countries (Palumbo et al. 2016). After its first detection in North America in Los Angeles Co., CA, in 2008 (Palumbo and Natwick 2010), it has since been found in Arizona, New Mexico, Nevada, Texas, Oklahoma, and Hawaii (Bundy et al. 2012, Reed et al. 2013, Matsunaga 2014, Vitanza 2012), in addition to Mexico (Sanchez-Pena 2014). Since 2012, this pest has become established in Monterey Co., CA (Joseph 2014), and is a serious pest of cruciferous crops (Family: Brassicaceae) (Palumbo et al. 2016) which are grown on >34,390 ha in Monterey Co. and are valued at more than US$739 million (Monterey County Crop Report 2015). *Bagrada hilaris* feeds on

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germinating seeds and young seedlings of cruciferous crops, causing severe stunting and even plant mortality (Huang et al. 2014, Joseph et al. 2017b, Reed et al. 2013). The feeding injuries caused by *B. hilaris* on leaves appear as "starbursts" on leaf surfaces, which become chlorotic white blotches over time. This type of feeding injury on leafy cruciferous crops, such as bok choi (*Brassica rapa* L. var. *chinensis*), arugula (*Eruca sativa* L.), mizuna (*B. rapa* L. *nipposinica*), and kale (*B. oleracea* L. *acephala*), can affect their marketability. In broccoli (*B. oleracea* var. *italica* Plenck) and cauliflower (*B. oleracea* L. var. *botrytis*), *B. hilaris* adults feed on and damage the apical meristem of young seedlings, causing headless or blind head plants (Palumbo and Natwick 2010). The feeding of *B. hilaris* on plants also triggers growth of multiple secondary shoots, which produce unmarketable and undersized broccoli and cauliflower heads.

The management of *B. hilaris* primarily involves foliar spraying of synthetic pyrethroid, neonicotinoid, or carbamate insecticides on the seedling stages of cruciferous crops (Joseph and Godfrey 2016; Palumbo 2011a, 2011b, 2011c; Palumbo et al. 2013a, 2015). Growers with organically certified fields (referred to as "organic growers") cannot use synthetic insecticides and must rely on organically approved insecticides for pest management. Several organically approved insecticide products are available on the market, but information on their efficacy against *B. hilaris* is limited. Because the tools of organic growers are limited, determining the efficacy of organically approved insecticides or their combinations against *B. hilaris* is essential.

In previous studies, promising results against various stink bug pests have been obtained using several organically approved natural insecticides, including pyrethrins, azadirachtin, and spinosad (Durmusoglu et al. 2003, Kamminga et al. 2009, Mahdian et al. 2007, Morehead and Kuhar 2017, Trdan et al. 2006). Pyrethrins are naturally derived from dried and powdered flower heads of *Chrysanthemum* spp., particularly *C. cinerariaefolium* (Trev) Bocc (Casida 1980). Spinosad is the fermented product of *Saccharopolyspora spinosa* Mertz & Yao, primarily consisting of spinosyn A and D (Mertz and Yao 1990). Pyrethrins and spinosad target the nervous system of the insect. Azadirachtin is obtained from neem seed oils (*Azadirachta indica* A. Juss.), although its exact mode of action remains unknown (IRAC 2016). In a study involving other stink bugs, Kamminga et al. (2009) showed that certain rates of azadirachtin application had both repellent and antifeedant properties. Potassium salts of fatty acids, which are commonly referred to as insecticidal soaps, irritate the exoskeleton of insects (Asolkar et al. 2013). Similarly, kaolin clay is widely used due to its various horticultural benefits and for the purpose of arthropod pest management (Glenn and Puterka 2005) because it increases the contact between the arthropod and the insecticide material mixed with the clay. The use of mineral oil creates a physical barrier and interrupts arthropod respiration. Fermented solids of *Chromobacterium subtsugae* strain PRAA4-1^T Martin et al. and heat-killed *Burkholderia* spp. strain A396 cells have also been evaluated as naturally derived products that affect the insect nervous system. Although some of the stand-alone and combination treatments have been evaluated for adult *B. hilaris* control (Grasswitz 2013, Palumbo et al. 2013b), a thorough evaluation of organically approved insecticides is still lacking. Moreover, previous studies were conducted in other *Brassica* production regions, which have distinctly different environmental conditions as compared with central coast of

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California. Thus, the objective of the current study was to determine the lethal and sublethal effects of commonly available, organically approved insecticides and their selected combinations of these insecticides against *B. hilaris*.

Materials and Methods

Insects. A colony of *B. hilaris* was maintained at the University of California Cooperative Extension (UCCE) Monterey Co. laboratory in Salinas, CA. The rearing procedure and conditions are described in Joseph et al. (2017c).

Topical spray assay. This assay was conducted in 2016. The insecticides used in the assay and their rates of application are listed in Table 1. Additionally, the following selected combinations were used in this assay: pyrethrins + azadirachtin, pyrethrins + spinosad, pyrethrins + azadirachtin + spinosad, pyrethrins + spinosad + insecticidal soap, and pyrethrins + spinosad + insecticidal soap + kaolin clay. The same rates indicated in Table 1 were used for the combined insecticide treatments. The pyrethroid insecticide zeta-cypermethrin and water served as positive and negative controls, respectively. The water volume of 373.9 L/ha was selected for the assays.

Ten *B. hilaris* adults were randomly selected from the laboratory colony and released on a paper towel placed in a 53 × 40-cm tray for insecticide application. The insecticide solutions were topically applied to adults on the paper towel (approximately 5.896 $\mu l/cm^2$) using a hand-operated pressure sprayer. The adults were immediately collected into a translucent polypropylene cup (6-cm diameter and 7.1-cm depth) using a paintbrush. A piece of broccoli crown (avg. 2.5 g) was added to the cup as a food and water source. The top inner edge of the cup was coated with a 2-cm band of fluoropolymer resin (PTFE-30) or fluon (Insect-a-Slip; BioQuip, Rancho Dominguez, CA) to prevent the escape of *B. hilaris*. The lids of the cups were modified with no-see-um fabric mesh glued over an opening (4-cm diameter; BioQuip) that was cut for air circulation. These cups were then placed in an environmentally controlled chamber at 26°C, under approximately 20% relative humidity and a 24:0 h (light:dark) photoperiod. This assay was arranged in a completely randomized design with five replications, where each cup served as a replicate. This assay was conducted twice for a total of 10 replications. The adults were evaluated for moribund posture and mortality at 4, 24, and 48 h posttreatment. They were classified as moribund when their bodies were positioned upside down with only the legs and antennae moving. When none of their body parts moved, the adult stink bugs were marked as dead.

Residual spray assay. In 2016, 'Heritage' broccoli seeds were planted in seedling trays, and the seedlings were grown in a commercial greenhouse (Growers Transplant Inc., Salinas, CA). After 5 weeks, the seedling trays were transferred to the UCCE greenhouse and were temporarily held there for 2 d. Cylindrical cages (22.5-cm height, 9-cm diameter) were built using clear 0.381-mm Dura-Lar film (Grafix, Maple Heights, OH) with no-see-um mesh fabric (catalog number 7250NSW, Rancho Dominguez, CA) glued on the top for air circulation as described in Joseph et al. (2016).

On 25 August 2016, broccoli seedlings were transplanted to 101.6-cm-wide beds with 17.8-cm spacing in a vegetable field in Gonzales, CA. The insecticides used in

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Table 1. Insecticides tested against *Bagrada hilaris*.

Active Ingredient (a.i.)	Product (% a.i.)	Manufacturer	Tested Rate[a] (g a.i./ha)
Thiamethoxam	Actara® (25%)	Syngenta Agrochemical Company, Basel, Switzerland	70.0†
Azadirachtin	Aza-Direct® (1.2%)	Gowan Company LLC, Yuma, AZ	27.6*†‡§
Spinosad	Entrust® (80%)	Dow AgroScience LLC, Indianapolis, IN	140.0*†‡§
Bacterial extract[b]	Grandevo® (30%)	Marrone Bio Innovations Inc., Davis, CA	1,008.3*‡
Mineral oil	Horticultural & dormant spray oil (98%)	Bonide Products Inc., Oriskany, NY	5,493.3*
Potassium salts of fatty acids[c]	M-Pede® SL (49.0%)	Gowan Company LLC, Yuma, AZ	2,553.9 (low)*, 3,405.5 (high)§
Zeta-cypermethrin	Mustang® (17.1%)	FMC Corporation, Philadelphia, PA	55.9*†‡
Pyrethrins	PyGanic® 5.0 (5.0%)	MGK, Minneapolis, MN	62.1*†‡§
Kaolin clay	Surround® WP (95.0%)	Novasource, Phoenix, AZ	10,643.5 (low)§, 21,287.1 (high)*§
Bacterial extract[d]	Venerate® XC (94.4%)	Marrone Bio Innovations Inc., Davis, CA	17,663.9*
Dinotefuran	Venom® (70%)	Valent USA, Walnut Creek, CA	196.1†‡

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Table 1. Continued.

Active Ingredient (a.i.)	Product (% a.i.)	Manufacturer	Tested Rate[a] (g a.i./ha)
Lambda-cyhalothrin	Warrior II® (22.8%)	Syngenta Agrochemical Company, Basel, Switzerland	34.9†

[a] The concentrations of insecticides were determined based on a water volume of 373.9 L/ha. The tested rates are recommended field rates.
[b] *Chromobacterium subtsugae* strain PRAA4-1^{T}.
[c] Also referred to as insecticidal soap or soap.
[d] Heat-killed *Burkholderia* spp. strain A396 cells.
* Direct spray (topical) assay.
† Indirect spray (residues) assay.
‡ Behavioral (horizontal movement) assay.
§ Field trials.

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the assay and their rates of application are listed in Table 1. The insecticide combinations employed in this assay were azadirachtin + pyrethrins and azadirachtin + pyrethrins + spinosad. The same rates indicated in Table 1 were used for the combined insecticide treatments. The water volume of 373.9 L/ha was selected for the assays. The pyrethroid insecticides zeta-cypermethrin and lambda-cyhalothrin and the neonicotinoid insecticides dinotefuran and thiamethoxam were included in the assay as positive control treatments. A water treatment served as the negative control in the assay. On 13 September 2016, the insecticides were applied directly on the broccoli transplants in the field using a spray bottle at (1 ml of insecticide solution per seedling). Adults collected from the rearing colony were individually placed in the cylindrical cages with broccoli transplants (one adult per cage) for 48 h. The adult stink bugs were not differentiated by sex. The bottom edge (2.5-cm diameter) of the cylindrical cage was inserted into the soil to secure and seal the cage. The adults were caged with the seedlings at 0 (immediately), 1, and 7 d postapplication, on 13, 14, and 20 September, respectively. The treatments were arranged in a completely randomized block design with 10 replications. Treatments were assigned for all three periods of time (0, 1, and 7 d posttreatment) within each block. After 48 h of exposure of the adult stink bugs in each residual time period, the seedlings were destructively sampled and transported to the laboratory. In the laboratory, the number of *B. hilaris* feeding injury sites on both the cotyledons and true leaves of each seedling was documented. The feeding injury sites observed on broccoli leaves are described in Joseph et al. (2017b).

Behavioral (horizontal movement) assay. The horizontal walking behavior of adult *B. hilaris* when exposed to insecticide residues was examined in the UCCE laboratory. Insecticides were sprayed on both inner surfaces of polystyrene petri dishes (100 × 15 mm; Fisher Scientific, Hampton, NH) at ~5.896 μl/cm^2. The insecticides used in this assay and their rates of application are listed in Table 1. The insecticide combinations used in this assay were azadirachtin + spinosad, spinosad + pyrethrins, and azadirachtin + spinosad + pyrethrins. The same rates indicated in Table 1 were employed in the combined insecticide treatments. Zeta-cypermethrin and dinotefuran served as the positive controls, whereas water was the negative control. After application, the insecticide residues on the petri dishes were dried for 20 min in an environmentally controlled chamber at 26°C. Adult *B. hilaris* were placed in the insecticide-treated petri dishes (one adult per dish) for 20 min, 2 h, or 4 h. The petri dishes were only partially sealed to provide ventilation. After exposure of the adults to the dried insecticide residues for the specified time interval (20 min, 2 h, or 4 h), they were individually transferred into arenas consisting of polystyrene petri dishes (100 × 15 mm). These dishes were placed on the base of a document visualizer (Visualizer re-350; Canon USA, Inc., Melville, NY), which was backlit by fluorescent lights. Video recordings of the arenas were captured with an Ethernet camera (acA1300-60gm; Basler, Inc., Exton, PA). After the adult stink bugs were introduced to the petri dishes (one adult per dish), 20-min videos were captured. The walking parameters of the adult stink bugs, including the total distance covered (cm), average speed (cm s^{-1}), and percentage of time in a mobile state, were calculated from the videos using Noldus EthoVision XT software (Version 11.5; Noldus Information Technologies, Wageningen, Netherlands). For each insecticide exposure interval (20 min, 2 h, or 4 h), 40 adults were individually examined per insecticide or insecticide combination. Four mini-digital thermo-

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Table 2. Number of *B. hilaris* feeding sites on true leaves of mizuna plants in a field trial in Hollister, CA.

	Mean (± SE) Number of *B. hilaris* Feeding Sites[b]			
Treatment[a]	13 October	17 October	21 October	30 October
Azadirachtin	11.3 ± 2.3a	25.8 ± 3.8a	32.8 ± 4.1a	113.0 ± 20.1a
Pyrethrins	10.3 ± 1.6a	12.5 ± 4.1a	20.8 ± 2.1a	143.3 ± 16.6a
Pyrethrins + kaolin clay (high)	8.5 ± 1.8a	22.8 ± 3.9a	43.3 ± 3.8a	74.8 ± 15.4a
Azadirachtin + kaolin clay (high)	9.3 ± 1.4a	16.3 ± 5.2a	40.3 ± 7.8a	82.5 ± 19.7a
Kaolin clay (high)	10.5 ± 1.8a	23.3 ± 4.9a	43.3 ± 10.6a	85.3 ± 15.3a
Kaolin clay (low)	11.8 ± 1.9a	36.3 ± 10.7a	48.7 ± 14.5a	153.5 ± 32.7a
Nontreated	13.0 ± 1.2a	28.8 ± 3.4a	35.0 ± 3.7a	141.8 ± 16.2a
F (df1, df2)	0.9 (6, 18)	1.4 (6, 18)	1.9 (6, 18)	2.3 (6, 18)
P	0.493	0.266	0.146	0.093

[a] Surfact 50 (organically approved surfactant) was added to all treatments at 0.25% v/v. Mizuna seeds were planted on 1 October 2014. Insecticides were applied on 7, 13, 16, 20, and 23 October 2014. The water volume was 373.9 L/ha.

[b] Data were log-transformed ($\ln[x + 1]$) before analysis. Means within a column with the same letters are not significantly different based on LSD test ($P > 0.05$).

hygrometers (Avianweb, Mims, FL) placed at the four corners of the visualizer surface were used to record air temperatures. The assays were initiated when the room temperature averaged 23.8°C, and all of the assays were conducted at temperatures between 23.8 and 29°C.

Field trials. Mizuna 'mizuna' and arugula 'Astro' seeds were broadcast applied in 203.2-cm-wide beds on 1 and 11 October 2014, respectively. These beds were sprinkler irrigated immediately after sowing. An experimental plot of 7.6 m in length in a bed (203.2 cm wide). The insecticides used in these trials and their rates of application are listed and highlighted in Table 1, and the insecticide treatments (including insecticide combinations) included in the trials are listed in Tables 2 and 3. The insecticides were applied five times on the mizuna plants, on 7, 13, 16, 20, and 23 October, and three times on the arugula plants on 17, 21, and 24 October. All of the insecticides were applied using a manual pneumatic sprayer (25.754 μl/cm^2). The insecticide applications and plant sampling were discontinued 2–3 d before the first cutting (harvest). The treatments were arranged in a randomized complete block design with four replications. The water volume for the applications was 373.9 L/ha. An organically approved spreader-acidifier/buffer (Surfact 50; Northwest Agricultural Products, Inc., Pasco, WA) was added at 0.25% (v/v) to all of the treatments. Twenty seedlings were randomly sampled from each plot on 13, 17, 21, and 30 October in the mizuna trial, while plant samples in the arugula trial were

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Table 3. Mean (± SE) number of *B. hilaris* feeding sites on true leaves of arugula plants in a field trial in Hollister, CA.

Treatment[a]	Mean (± SE) Number of *B. hilaris* Feeding Injury Sites[b] 20 October	24 October	27 October
Kaolin clay (high) + spinosad	6.3 ± 2.7bc	9.8 ± 3.5a	16.3 ± 8.4a
Kaolin clay (high) + pyrethrins	15.0 ± 2.7a	10.0 ± 1.6a	31.6 ± 3.5a
Kaolin clay (high) + azadirachtin	15.0 ± 2.7a	9.5 ± 2.7a	20.3 ± 5.2a
Soap (high)[c] + spinosad	9.5 ± 1.4ab	7.5 ± 2.9a	26.0 ± 3.5a
Soap (high) + pyrethrins	4.3 ± 2.2c	7.2 ± 3.0a	18.6 ± 5.4a
Soap (high) + azadirachtin	12.8 ± 2.6ab	4.3 ± 1.6a	13.0 ± 3.6a
Nontreated	10.8 ± 1.3ab	11.5 ± 3.1a	18.0 ± 2.1a
F (df1, df2)	4.3 (6, 18)	0.8 (6, 18)	1.3 (6, 18)
P	0.008	0.583	0.325

[a] Surfact 50 (organically approved surfactant) was added to all treatments at 0.25% v/v. Arugula seeds were planted on 11 October 2014. Insecticides were applied on 17, 21, and 24 October 2014. The water volume was 373.9 L/ha.
[b] Data were log-transformed (ln[x + 1]) before analysis. Means within a column with the same letters are not significantly different based on LSD test ($P > 0.05$).
[c] Potassium salts of fatty acids are also referred to as insecticidal soap or soap.

collected on 20, 24, and 27 October. The plants were evaluated for the number of fresh *B. hilaris* feeding injury sites on true leaves (noncotyledon leaves). The cotyledons were not included in this evaluation because *B. hilaris* can feed on germinating seeds (Joseph et al. 2017a). The symptoms of *B. hilaris* feeding injury on broccoli leaves appear as a "starbursts"; however, because the shape of the starbursts varies, and they eventually coalesce, only individual, freshly discolored (chlorotic) feeding injury sites were quantified.

Statistical analyses. All of the data from the different assays and trials were analyzed using the SAS statistical software package (SAS Institute 2012). The number of moribund and dead adults in the topical spray assay (at 4, 24, and 48 h posttreatment); the number of feeding sites in the residual assay; the distance traveled and speed of movement in the behavioral assay; and the number of feeding sites on true leaves in the field trials were natural log(x+1) transformed. The data on the proportion of adult mobility were arcsine square root transformed. All of the transformed data were subjected to an analysis of variance using a general linear model (PROC GLM). The different individual insecticides and their combinations were the treatments. The means were separated using LSD test for comparisons of the treatments. All statistical comparisons were considered significant at $\alpha = 0.05$.

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Results

Topical spray assay. At 4 h postapplication, the number of dead adults was significantly greater in the pyrethrins + azadirachtin + spinosad, pyrethrins + spinosad, pyrethrins + spinosad + soap, pyrethrins + spinosad + soap + kaolin clay, and zeta-cypermethrin treatments than in the water treatment ($F = 9.7$; df = 14, 136; $P < 0.001$; Fig. 1A). Additionally, the number of moribund adults was significantly greater in the treatments involving pyrethrins, spinosad, pyrethrins + azadirachtin, pyrethrins + spinosad, pyrethrins + azadirachtin + spinosad, pyrethrins + spinosad + soap, pyrethrins + spinosad + soap + kaolin clay, and zeta-cypermethrin than in the azadirachtin, soap, kaolin clay, *Chromobacterium* sp., *Burkholderia* spp., horticulture oil, and water treatments ($F = 69.2$; df = 14, 136; $P < 0.001$; Fig. 1A).

After 24 h of application, treatment with pyrethrins + spinosad, pyrethrins + azadirachtin + spinosad, pyrethrins + spinosad + soap, pyrethrins + spinosad + soap + kaolin clay, and zeta-cypermethrin resulted in significantly greater numbers of dead adults than the water or other treatments ($F = 22.7$; df = 14, 136; $P < 0.001$; Fig. 1B). The number of moribund adults was significantly greater in the spinosad, pyrethrins + azadirachtin, and zeta-cypermethrin treatments than in the other treatments ($F = 17.8$; df = 14, 136; $P < 0.001$; Fig. 1B).

After 48 h of application, the number of dead adults was significantly greater under treatment with spinosad, pyrethrins + spinosad, pyrethrins + azadirachtin + spinosad, pyrethrins + spinosad + soap, pyrethrins + spinosad + soap + kaolin clay, and zeta-cypermethrin than in the other treatments, which included the stand-alone pyrethrins and azadirachtin treatments ($F = 18.7$; df = 14, 111; $P < 0.001$; Fig. 1C). Significantly more moribund adults were observed in the zeta-cypermethrin and spinosad treatments than in the other treatments ($F = 13.6$; df = 14, 111; $P < 0.001$; Fig. 1C).

Residual spray assay. When adult stink bugs were individually caged with broccoli seedlings immediately after insecticide application (0 d), the number of feeding injury sites was significantly lower in treatments with pyrethrins, spinosad, pyrethrins + azadirachtin, pyrethrins + azadirachtin + spinosad, zeta-cypermethrin, lambda-cyhalothrin, dinotefuran, and thiamethoxam than in the water treatment after 48 h ($F = 10.9$; df = 9, 76; $P < 0.001$; Fig. 2A). One day after application, the number of feeding injury sites was significantly lower in the treatments with pyrethrins, pyrethrins + azadirachtin, pyrethrins + azadirachtin + spinosad, zeta-cypermethrin, lambda-cyhalothrin, dinotefuran, and thiamethoxam than in those involving water, azadirachtin, and spinosad after 48 h ($F = 5.9$; df = 9, 74; $P < 0.001$; Fig. 2B). Seven days after insecticide application, the number of feeding injury sites was significantly lower in the azadirachtin + pyrethrins, zeta-cypermethrin, lambda-cyhalothrin, dinotefuran, and thiamethoxam treatments than under the water and other treatments after 48 h ($F = 5.7$; df = 9, 73; $P < 0.001$; Fig. 2C).

Behavioral (horizontal movement) assay. After 20 min of exposure to dried residues of insecticides, the adult stink bugs in the azadirachtin + pyrethrins + spinosad and pyrethrins + spinosad treatment groups traveled significantly longer distances than those in the azadirachtin, pyrethrins, spinosad, azadirachtin + spinosad, dinotefuran, and water treatment groups (Table 4). Their speed of

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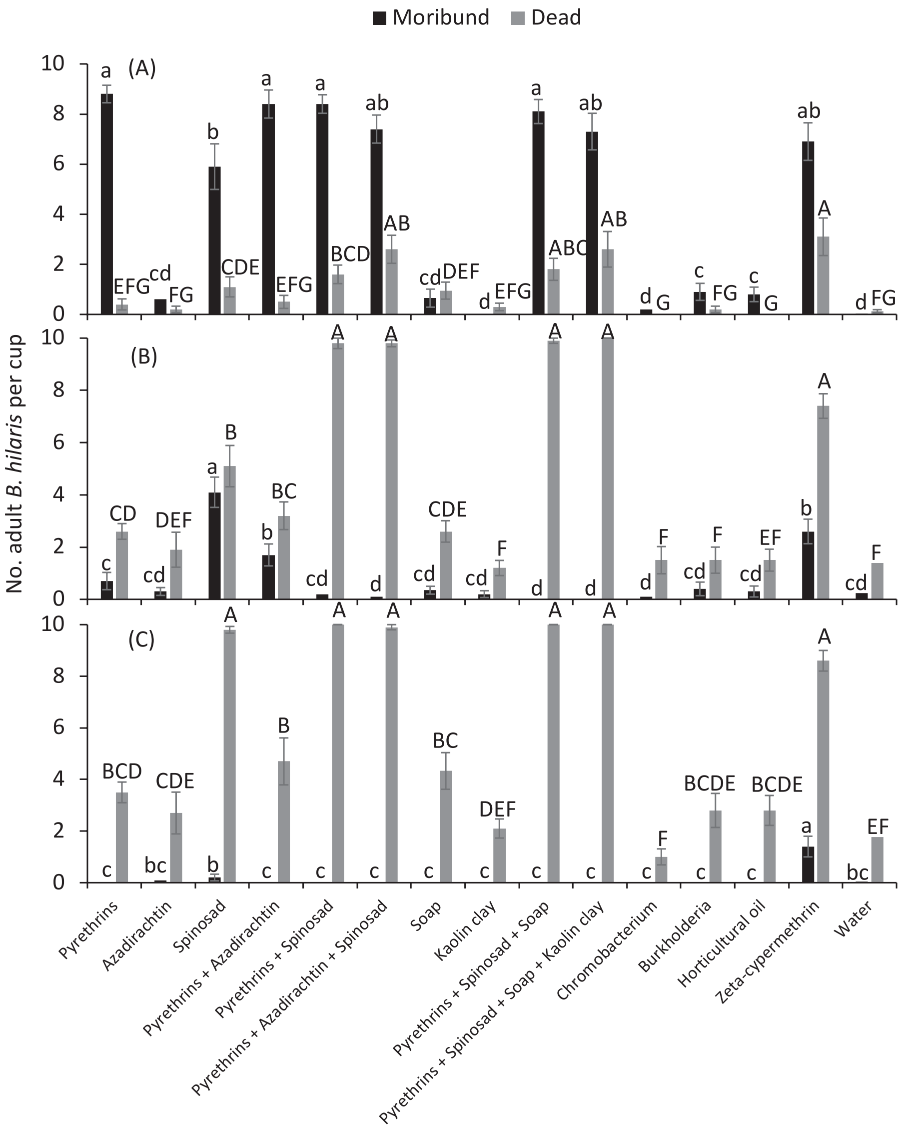

Fig. 1. Mean (± SE) number of dead and moribund *B. hilaris* adults at (A) 4 h, (B) 24 h, and (C) 48 h after topical spraying of insecticides. Potassium salts of fatty acids are referred to as soap. Bars with the same fill color with letters of the same case are not significantly different (LSD test, $\alpha = 0.05$).

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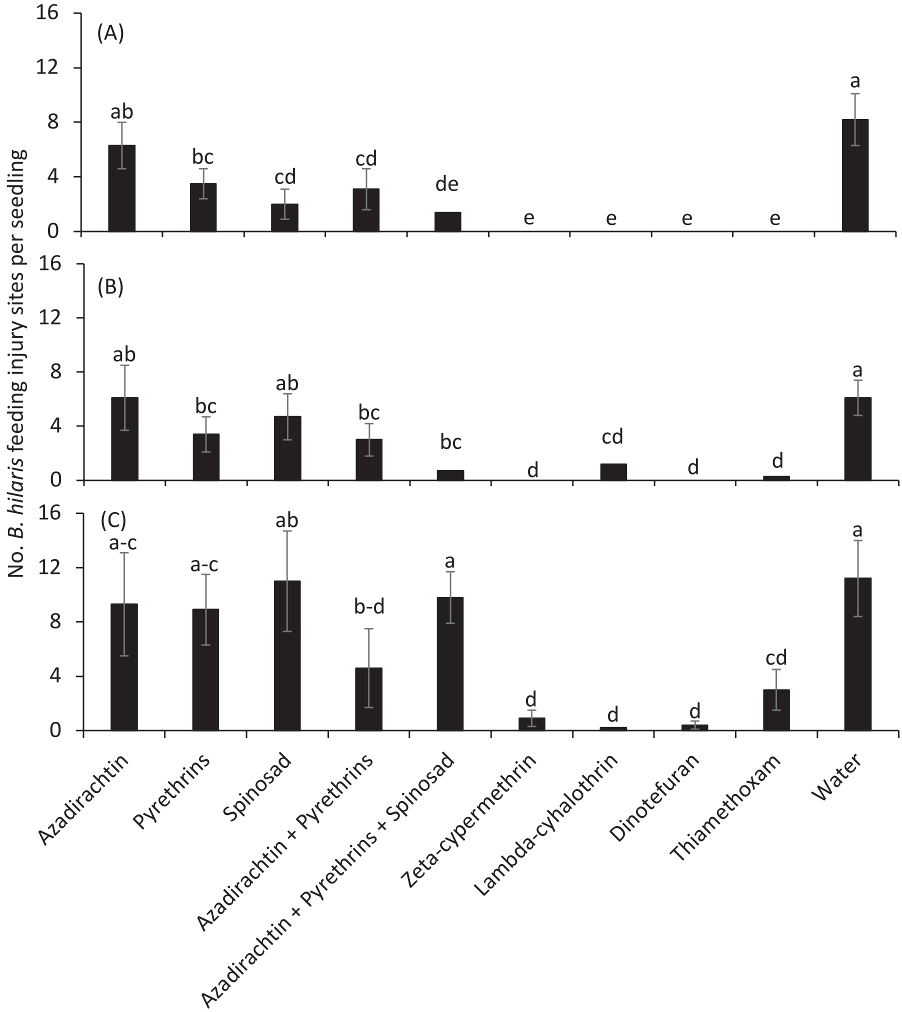

Fig. 2. Mean (± SE) number of *B. hilaris* feeding sites on broccoli foliage when adults were exposed for 48 h after (A) 0 h, (B) 24 h, and (C) 7 d of insecticide spraying on seedlings. Bars with the same fill color with the same letters are not significantly different (LSD test, $\alpha = 0.05$).

movement and percentage of time in motion were significantly greater in the azadirachtin + pyrethrins + spinosad treatment than in any other treatment.

After 2 h of exposure to the dried residues of insecticides, the adult stinkbugs in spinosad, pyrethrins + spinosad, and water treatment groups traveled significantly longer distances than those in the pyrethrins, azadirachtin + spinosad, zeta-cypermethrin, and dinotefuran treatment groups (Table 4). Stink bugs exposed to spinosad or pyrethrins + spinosad exhibited a significantly greater speed of travel and percentage of time in motion than those treated with azadirachtin, pyrethrins,

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Table 4. Mean (± SE) distance traveled, speed, and the percentage of time in a mobile state over 20 min when *B. hilaris* adults were exposed to insecticides alone and in combinations in a horizontal arena for 20 min, 2 h, or 4 h.

	20 min		
Insecticide	Distance (cm)	Speed cm^{-1}	Mobility (%)
Azadirachtin	99.1 ± 20.5cde	0.13 ± 0.02bcd	17.7 ± 2.3bcd
Pyrethrins	80.1 ± 12.9cde	0.17 ± 0.03bc	20.9 ± 2.5cd
Spinosad	90.6 ± 15.6de	0.11 ± 0.02cd	15.6 ± 1.9bcd
Azadirachtin + spinosad	92.4 ± 15.3de	0.14 ± 0.02bcd	20.5 ± 2.4bcd
Azadirachtin + spinosad + pyrethrins	203.6 ± 31.0a	0.27 ± 0.03a	30.0 ± 2.3a
Spinosad + pyrethrins	133.0 ± 23.9ab	0.17 ± 0.02b	21.3 ± 2.5b
Chromobacterium	106.2 ± 20.4bcd	0.12 ± 0.02bcd	15.8 ± 1.8bcd
Zeta-cypermethrin	99.5 ± 9.9bc	0.13 ± 0.01bcd	19.2 ± 1.9bcd
Dinotefuran	60.2 ± 11.7e	0.11 ± 0.02cd	16.2 ± 2.4d
Water	75.1 ± 15.3e	0.09 ± 0.01d	14.8 ± 2.0d
F (df1, df2)	5.9 (9, 349)	5.6 (9, 349)	4.1 (9, 349)
P	<0.001	<0.001	<0.001

azadirachtin + pyrethrins, azadirachtin + pyrethrins + spinosad, zeta-cypermethrin, or dinotefuran. When the adult stink bugs were exposed to dried insecticide residues for 4 h, the distance that they traveled, their speed, and percentage of time in motion were not significantly different among the treatments (Table 4).

Field trials. In the mizuna trial, the feeding injury sites on the true leaves were not significantly different among the treatments on any of the sampling days postapplication (Table 2). In the arugula trial, the insecticidal soap (high) + pyrethrins treatment resulted in significantly lower numbers of feeding injury sites than the kaolin + pyrethrins or the azadirachtin and insecticidal soap + azadirachtin or spinosad treatments at 3 d postapplication. No significant differences in the observed feeding injury sites were detected among the treatments on the following two sample dates (Table 3).

Discussion

A thorough understanding of lethal and sublethal effects of organically approved insecticides against *B. hilaris* is important for organic growers who use no or limited synthetic insecticides for pest management. In the current study, direct and indirect exposure to organically approved insecticides achieved some control of adult *B.*

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Table 4. Extended.

2 h		
Distance (cm)	**Speed cm^{-1}**	**Mobility (%)**
60.5 ± 10.4bcd	0.08 ± 0.02bc	12.7 ± 2.1cd
57.8 ± 11.2cd	0.08 ± 0.01bc	13.9 ± 2.0c
169.5 ± 39.3a	0.19 ± 0.04a	22.6 ± 3.7a
36.6 ± 4.1d	0.04 ± 0.00c	6.1 ± 0.8de
70.5 ± 9.3abc	0.07 ± 0.01bc	12.0 ± 1.3cd
121.7 ± 24.2ab	0.17 ± 0.03a	22.3 ± 3.7ab
—	—	—
46.3 ± 7.4d	0.06 ± 0.01c	10.9 ± 1.7cde
52.7 ± 14.8e	0.05 ± 0.02c	7.8 ± 2.0e
131.3 ± 29.6a	0.13 ± 0.03ab	15.0 ± 2.8bc
7.1 (8, 312)	5.9 (8, 312)	5.4 (8, 312)
<0.001	<0.001	<0.001

hilaris compared with the water control. For some of the treatments, the efficacy was comparable to that of the pyrethroid zeta-cypermethrin. The topical application of spinosad in combination with pyrethrins and azdirachtin was more lethal to the adults than stand-alone treatment with pyrethrins or azdirachtin. Spinosad caused >95**%** adult mortality at 2 d, which was greater than the mortality observed at 4 h or 1 d postapplication (Fig. 1). The topical application of pyrethrins was not as lethal as the topical application of spinosad because most of the initially moribund adults treated with spinosad recovered at 2 d postapplication. In the combined treatment with spinosad and pyrethrins, the initially moribund adults did not recover at 2 d postapplication. In previous studies examining the use of organically approved insecticides against *B. hilaris*, the efficacy of various combinations of spinosad, pyrethrins, and azadirachtin was not thoroughly evaluated, as it was in the current study. These studies showed that the use of pyrethrins or azadirachtin as a stand-alone treatment and the combination of insecticidal soap and spinosad achieved acceptable adult control compared with untreated controls (Grasswitz 2013, Palumbo et al. 2013b). However, in the current topical assay, stand-alone treatments with pyrethrins or azadirachtin was not found to be lethal to adult *B. hilaris* compared with untreated controls.

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Table 4. Extended.

4 h		
Distance (cm)	**Speed cm^{-1}**	**Mobility (%)**
46.2 ± 5.7a	0.04 ± 0.01a	3.5 ± 0.7a
32.0 ± 4.1a	0.05 ± 0.01a	7.0 ± 1.9a
62.9 ± 14.4a	0.06 ± 0.01a	6.5 ± 1.6a
49.1 ± 7.0a	0.04 ± 0.01a	5.3 ± 0.9a
47.8 ± 6.8a	0.06 ± 0.01a	7.7 ± 1.3a
51.4 ± 8.2a	0.04 ± 0.01a	3.9 ± 1.0a
—	—	—
51.1 ± 8.3a	0.05 ± 0.01a	5.8 ± 1.0a
34.4 ± 4.8a	0.04 ± 0.00a	4.3 ± 0.8a
54.9 ± 12.2a	0.04 ± 0.01a	6.1 ± 1.5a
1.3 (8, 303)	0.7 (8, 303)	1.7 (8, 303)
0.272	0.686	0.101

The results of the current study on *B. hilaris* are not completely consistent with those of previous laboratory studies involving other stink bugs. Pyrethrins have been reported to be effective against the green stink bug, *Chinavia hilaris* (Say) (Kamminga et al. 2009), and the brown marmorated stink bug, *Halyomorpha halys* Stål (Lee et al. 2014, Morehead and Kuhar 2017), but were not found to be effective against the brown stink bug, *Euschistus servus* (Say) (Kamminga et al. 2009). Azadirachtin was not observed to be effective against *H. halys* (Lee et al. 2014), but treatment with pyrethrins + azadirachtin was effective (Morehead and Kuhar 2017). In laboratory studies, spinosad was shown to be effective against *C. hilaris* and *E. servus* (Kamminga et al. 2009) and *H. halys* (Lee et al. 2014) but was less effective against the predatory stink bug, *Picromerus bidens* (L.) (Mahdian et al. 2007).

In the caged field experiments, pyrethrins in combinations involving spinosad or azadirachtin reduced the severity of *B. hilaris* feeding damage on seedlings compared with untreated seedlings. However, the superior residual effects of these organically approved insecticides diminished at 7 d postapplication, as the levels of feeding damage for most treatments, except the pyrethrins + azadirachtin-treated seedlings, were similar to those of untreated seedlings. In the positive controls, the synthetic insecticides (pyrethroids and neonicotinoids) were effective at suppressing *B. hilaris* feeding injury on the seedlings, even at 7 d postapplication (Fig. 2).

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Based on these results, even the effective organically approved materials could only achieve shorter-term control of *B. hilaris* adults, and organic growers should, therefore, consider multiple applications of these effective organically approved insecticides at closer intervals.

When the efficacy of organically approved materials was evaluated in a cage-less setting following multiple applications (3–5 applications) immediately after seedling emergence, pyrethrins, spinosad, and azadirachtin, either alone or combined with soap or kaolin clay, did not reduce *B. hilaris* feeding damage to the leaves. The combination of pyrethrins with soap reduced *B. hilaris* feeding injury on the true leaves of arugula in the short term, but this effect did not persist because nymphs and adults of *B. hilaris* continuously entered the field. In contrast, a previous field study on *B. hilaris* showed that fresh feeding injury was reduced after spraying with spinosad + potassium salts compared with untreated controls (Palumbo et al. 2013a). However, field trials against *H. halys* demonstrated that none of the organically approved insecticides tested were effective after multiple applications (Morehead and Kuhar 2017). In the field, organically approved materials are vulnerable to photodegradation, and following application, residues can be easily washed off with water (Schmutterer 1990, Zehnder et al. 2007). Salad crops, such as mizuna and arugula, have a relatively short production cycle, as they are typically harvested beginning approximately 21 d after seed planting. These young seedlings are frequently sprinkler irrigated through the short growing period. Therefore, the insecticide residues on their foliage were washed off, leaving tender leaves unprotected from *B. hilaris*, which immediately entered the beds after seed planting and caused feeding damage.

Other organically approved insecticides were also evaluated in the various assays and field trials performed in this study, both as stand-alone treatments and in combination with pyrethrins, spinosad, and azadirachtin. Potassium salts and kaolin clay caused low mortality among adult *B. hilaris* and failed to reduce feeding damage on the foliage as stand-alone treatments. In contrast, the mortality of *H. halys* was found to be high with treatment of potassium salts in a submersion assay in the laboratory (Morehead and Kuhar 2017). No lethal or residual effects against *B. hilaris* were observed in our study with the other tested organically approved materials of mineral oil and extracts of *C. subtsugae* and *Burkholderia* spp. tested in our study. Previous studies have shown that *C. subtsugae* is effective against the southern green stink bug, *Nezara viridula* (L.) (Martin et al. 2007), and *Burkholderia* spp. exhibits activity against sucking insects (Asolkar et al. 2013).

The results of the present study showed that the adults exposed to dried residues of pyrethrins and spinosad, either alone or in combination, traveled farther at a faster pace than following exposure to the other organically approved insecticides. This finding is consistent with results reported for *H. halys*, whose movement was found to increase with exposure to pyrethrins (Lee et al. 2014). In the current study, none of the *B. hilaris* adults were moribund or died after exposure to the stand-alone or combined treatments with approved organic insecticides for 20 min or 2 or 4 h. However, moribund adults were observed with the dinotefuran treatment, which likely affected their movement compared with the water treatment. None of the treatments had an effect on the movement of adult *B. hilaris* after 4 h of exposure, which could be attributed to the breakdown of insecticide material.

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In summary, the current study showed that organically approved insecticides, particularly spinosad and pyrethrins applied in combination with other insecticidal materials, such as potassium salts, can be lethal to *B. hilaris* adults and may reduce the feeding damage caused by these insects and alter their normal locomotor activity. However, these effects exhibited low persistence, particularly under field conditions, which suggests that certified organic growers should consider multiple applications of effective insecticides at shorter intervals to achieve reasonable *B. hilaris* control. Future studies will continue to evaluate potential organically acceptable products as they become available for research. Additionally, the potential use of some of these organically approved insecticides integrated with other IPM tactics, such as intercropping, trap or barrier cropping, and the management of weed hosts adjacent to crop fields, will be examined in future research.

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