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ORIGINAL PAPER

Susceptibility of germinating cruciferous seeds to *Bagrada hilaris* (Hemiptera: Pentatomidae) feeding injury

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Abstract *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae) is a serious pest that attacks both germinating and seedling stages of a variety of cruciferous crops grown in the Central Coast of California. *B. hilaris* feeding on germinating seeds can cause severe stunting and plant mortality, and little is known about the feeding preference of *B. hilaris* for germinating seeds of major cruciferous hosts and varieties of hosts. No-choice and choice experiments were conducted in which germinating seeds in soilless and soil settings were exposed to *B. hilaris* adults for 7 days. Susceptibility scores were developed using *B. hilaris* feeding injury sites, distorted leaves, and deformed and dead plants to determine the overall *B. hilaris* preference for germinating host seeds. Based on the scores, the order of preference was arugula (*Eruca sativa* L.) > turnip (*B. rapa* L. var. *rapa*) > mizuna (*B. rapa* L. *nipposinica*) > kale (*B. oleracea* L. *acephala*) > choi (*Brassica rapa* L. var. *chinensis*) > broccoli (*B.* oleracea var. italica Plenck) > cauliflower (*B. oleracea* L. var. *botrytis*) > lettuce (*Lactuca sativa* L.) > sweet alyssum (*Lobularia maritima* [L.] Desv.). The lowest feeding injury was recorded on germinating lettuce and sweet alyssum seeds. Furthermore, no-choice and choice experiments were conducted with four varieties each of arugula and mizuna, twelve varieties each of kale and choi, and nine varieties/types of leafy Asian greens. The arugula varieties 'Wild Rocket' and 'Spirit' were more damaged by *B. hilaris* than other varieties tested. Among mizuna varieties, 'Beira F1' was more attractive to *B. hilaris* than 'Scarlet' or 'Starbor F1.' The choi varieties 'Tokyo Bekana,' 'Feng Qing Choi F1,' 'Joi Choi F1,' and 'Win-Win Choi F1' were more attractive than 'Rosie F1.' The leafy Asian greens variety 'Carlton F1' was more attractive to *B. hilaris* than 'Yukina Savon,' 'Tatsoi OG,' 'Komatsuna Summerfest F1,' 'Red Rain F1,' and 'Shungiku.' Therefore, the results suggest that not all varieties were equally susceptible to *B. hilaris* feeding and possibly be utilized for further field evaluation as a trap crop or developing more resistant varieties to *B. hilaris*.

Keywords California's Central Coast · Brassicaceae · Asian leafy vegetables · Stink bug

Introduction

Bagrada hilaris (Burmeister) (Hemiptera: Pentatomidae) is an invasive stink bug species native to southern Africa, Middle East, and Asia (Reed et al. 2013). It was first detected in North America in Los Angeles Co., California in 2008 (Palumbo and Natwick 2010). Since then, it has been found in most of the counties in California and has become well established in the northern Central Coast of California since 2012 (Joseph 2014). In addition, *B. hilaris* has been detected or has established in Arizona, New Mexico, Nevada, Texas, Oklahoma, and Hawaii (Bundy et al. 2012; Vitanza 2012; Reed et al. 2013; Matsunaga 2014) as well as in Mexico (Sanchez-Pena 2014).

Bagrada hilaris preferentially feeds on cruciferous crops (Family: Brassicaceae), including broccoli (Brassica oleracea var. italica Plenck), cauliflower (*B. oleracea* L. var.

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botrytis), kale (*B. oleracea* L. *acephala*), choi (*B. rapa* L. var. *chinensis*), arugula (*Eruca sativa* Mill.), mizuna (*B. rapa* L. *nipposinica*), and turnip (*B. rapa* L. var. *rapa*) (Reed et al. 2013; Huang et al. 2014a). In California alone, cruciferous crops were valued at an estimated 1 billion USD in 2013 (U.S. Department Agriculture, NASS 2014). Cruciferous crops were grown on >4390 ha in 2013 and valued at 679 million USD, with leafy Asian salad crops in Monterey Co. valued at 64 million USD (Monterey County Crop Report 2014).

Many stink bug species cause economic damage by feeding on fruits or seeds on mature plants, with damage of germinating seeds and seedlings occurring infrequently (McPherson and McPherson 2000; Chocorosqui and Panizzi 2004). In contrast, *B. hilaris* is most problematic when feeding in freshly seeded or transplanted fields (Reed et al. 2013). *B. hilaris* kill seedlings, reduce plant stands, and severely stunt surviving plants when they feed in fields immediately following planting and during the early stages of plant growth (Reed et al. 2013; Huang et al. 2014b). The harlequin bug [*Murgantia histrionica* (Hahn)] is a specialist of plants in the family Brassicaceae and can also be a pest of cruciferous crops, but it tends to attack crops later in the growing cycle than *B. hilaris* (White and Brannon 1933). *Bagrada hilaris* feeds via a lacerate-and-flush method in which it repeatedly inserts its stylets between layers of epidermal cells (Reed et al. 2013). This feeding pattern physically injures the epidermal cellular tissue and the injury manifests as “starbursts” on leaf surfaces. With time, starburst damage changes into necrotic tissue. This feeding injury on leaves, even when primarily cosmetic, can render leafy cruciferous crops such as choi, arugula, mizuna, and kale unmarketable. In broccoli and cauliflower, economic injury also occurs when feeding by *B. hilaris* kills the apical meristem of young seedlings, causing plants to not produce a head (a “blind head”; Palumbo and Natwick 2010). On some plants, *B. hilaris* feeding also stimulates the growth of multiple secondary shoots, which produces unmarketable and undersized broccoli and cauliflower heads.

Germinating seeds provide a potential window of vulnerability for direct-seeded crops and are therefore a critical stage for evaluating damage by *B. hilaris*. The yield loss attributable to mortality or damage to germinating seeds has not been quantified for cruciferous crops. However, large numbers of adults and nymphs have been found in the soil cracks and crevices on production beds during germination or emergence of the seeds, and these high populations can inflict substantial damage (SVJ unpublished data). For instance, extensive feeding injury by *B. hilaris* was evident on newly germinated broccoli, arugula, and mizuna (SVJ unpublished data). These crops, as well as other leafy greens, are entirely direct seeded in the Salinas Valley in conventional production, while cauliflower is entirely transplanted. For instance, broccoli is typically planted “to stand” with ~15.2 cm spacing between seeds. Seedling mortality reduces the number of plants in the stand, and damage caused from feeding can cause plants to produce small and unmarketable heads. Plant mortality or stunting can cause nearby plants to produce larger, unmarketable heads, causing further yield loss. Typically, leafy greens like arugula and mizuna are densely planted and their tender foliage are harvested several times within six weeks after planting. *Bagrada hilaris* feeding injury symptoms on these tender foliage of these leafy crops reduce the leaf quality and can result in complete rejection of the harvested produce. Damage to germinating seeds and cotyledons just emerging from the soil is a significant threat for direct-seeded crops, and both heading and leafy cruciferous crops can suffer significant economic losses from *B. hilaris* feeding.

To prevent damage by *B. hilaris*, growers typically rely on pyrethroid, neonicotinoid, or carbamate insecticides that are applied during the vulnerable seedling and young plant stages (Palumbo 2011a, b, c; Palumbo et al. 2015). Effective alternative methods to manage *B. hilaris* are still limited. Some growers have even transitioned from direct-seeding to transplanting for broccoli, which is more expensive, to reduce yield loss (Reed et al. 2013) or have avoided planting some crops during periods of greatest *B. hilaris* pressure (SVJ unpublished data). For many cruciferous crops, including baby leafy greens, direct-seeding remains a standard practice, and *B. hilaris* can therefore damage germinating seeds.

Because *B. hilaris* quickly find and colonize newly planted cruciferous crop fields and damage seeds as they germinate, there is an urgent need to reduce *B. hilaris* feeding on germinating seeds. A better understanding of plant damage by *B. hilaris* and the preference for different crops will facilitate more effective management of this pest and better predictions of when economic damage will occur. Previous work has determined how attractive and susceptible seedlings of different cruciferous crops are to *B. hilaris* using cotyledon-stage plants (Huang et al. 2014a). Adult *B. hilaris* were most attracted to radish (*Raphanus sativus* L.) seedlings, followed by those of red cabbage (*Brassica oleracea* L. var. *capitata*) and were less attracted to seedlings of arugula, Napa cabbage (*Brassica rapa* var. *pekinensis* [Lour.], and green mustard (*Brassica juncea* var. *rugosa* [Roxb.]) (Huang et al. 2014a). For red and green cabbage, the combined characteristics of high attractiveness, but lower susceptibility to damage compared to radish, suggested these crops may be good candidates as a trap crop (Huang et al. 2014a).

In contrast, susceptibility of germinating seeds to *B. hilaris* feeding is not well understood, nor is the preference of *B. hilaris* for these seeds. In addition, varieties of individual may vary in their susceptibility and attractiveness.

This information is necessary to predict damage to recently seeded crops. Additionally, differences in preference among germinating seeds could be used for developing sustainable management tactics. Trap cropping is a tactic used to attract or divert invading pests using a preferred plant host or cultivar (Hokkanen 1991; Shelton and Badenes-Perez 2006; Wallingford et al. 2013). This tactic could be used to divert or concentrate colonizing *B. hilaris* protecting the germinating main crop by planting seeds of more attractive host plants. Laboratory screening experiments can serve as a first step to selecting trap crop candidates by determining the attractiveness of various potential hosts to the targeted pest, and can be complemented by subsequent field tests (Shelton et al. 1991; Shelton and Nault 2004; Cook et al. 2006). It will be necessary to evaluate various stages of a potential trap crop (germinating, cotyledon, seedling). Developing non-chemical tactics to reduce *B. hilaris* feeding (e.g., trap cropping) is necessary for organically managed farms and could increase the sustainability of conventional farms.

Thus, the major objectives of this study were to (1) assess the relative feeding preference of *B. hilaris* for different germinating cruciferous crop types and susceptibility of the crop types to feeding injury and to (2) evaluate the preference of *B. hilaris* for different germinating leafy cruciferous crop varieties and susceptibility of the varieties to *B. hilaris* injury.

Materials and methods

General methods and insects

We conducted no-choice and choice assays for both types of crops and crop varieties for certain crops (Table 1). In the crop type experiments, we tested eight crops in no-choice and choice tests: broccoli ('Heritage'), cauliflower ('Herman'), turnip ('Tokyo'), choi ('Mei Qing Choi'), kale ('Red winter'), arugula ('Astro'), mizuna ('unknown'), and lettuce (*Lactuca sativa* L., 'Rouge d'hiver'), as well as the insectary plant sweet alyssum (*Lobularia maritima* [L.] Desv., Snow Seeds Co., Salinas, California). Lettuce, which typically does not incur damage by *B. hilaris*, was included as a non-host. No-choice and choice assays were conducted both with and without soil substrate for the seeds. Lettuce and kale were not included in the choice test with soil, while sweet alyssum was only included in this test. Because *B. hilaris* encounter soil when they interact with planted seeds, experiments were conducted with and without a soil substrate. The soil type used was "Clear lake clay" and was collected from a field in Salinas, California. For both assay types, only adult bugs, rather than nymphs, were used because adults are more mobile and are the life stage that typically colonizes newly planted beds.

Crop variety experiments were conducted with multiple varieties of five crop types: arugula, mizuna, kale, choi, and leafy Asian greens. We used four varieties each of arugula and mizuna, twelve varieties each of kale and choi, and nine varieties/types of leafy Asian greens. The varieties used were arugula: 'Sprint,' 'Astro,' 'Wild Rocket,' and 'Roquette Cultive'; mizuna: 'Mustard,' 'Mizuna OG,' 'Kyoto,' and 'Dark Purple'; kale: 'Scarlet,' 'Beira F1,' 'Starbor F1,' 'Olympic Red OG,' 'Premier OG,' 'Toscano,' 'Red Russian OG,' 'Siberian,' 'Black Magic,' 'Lacinato,' 'Redbor F1,' and 'Rogue'; choi: 'Tokyo Bekana,' 'Rosie F1,' 'Gunsho F1,' 'Red Choi F1,' 'Black Summer F1,' 'Feng Qing Choi F1,' 'Joi Choi F1,' 'Win-Win Choi F1,' 'Red PAC F1,' 'White Flash F1,' 'Shiro F1,' and 'Mei Qing Choi'; assorted leafy Asian greens: 'Yukina Savon' (*B. rapa*), 'Hon Tsai Tai' (*B. rapa*), 'Koji F1' (*B. rapa*), 'Tatsoi OG' (*B. rapa* var. *narinosa*), 'Vitamin Green' (*B. rapa* var. *narinosa*), 'Komatsuna Summerfest F1' (*B. rapa* var. *perviridis*), 'Carlton F1' (*B. rapa* var. *perviridis*), 'Red Rain F1' (*B. juncea*), and 'Shungiku' (*Glebionis coronaria* (L.) Cass. ex Spach) (Johnny's Seeds, Winslow, Maine [USA], Snow Seeds Co., Salinas, California, USA).

All assays were conducted in plastic tool organizer boxes, each of which contained a number of individual open-top plastic compartments (Table 1). For each assay, an individual compartment served as a replicate, and *B. hilaris* adults were given access to seeds within the compartment. *Bagrada hilaris* adults were restricted to the individual compartments by securing the tops with no-see-um mesh (Bioquip, Rancho Dominguez, California, USA) held in place with a rubber band or Velcro, or a plastic lid with twelve 2-mm-diameter holes to ensure airflow. Treatments were randomly assigned to compartments of different crop types and varieties were randomly arranged within compartments. Compartments varied in size for different assays, as did the number of compartments per organizer box (Table 1). Compartments were larger for the choice tests compared to the no-choice tests to accommodate multiple types of seeds and more *B. hilaris* individuals (one in no-choice vs. five in choice). For the crop type assays, both no-choice and choice tests were performed with and without soil, whereas the crop variety assays were only conducted using a potting mix substrate (Sun Gro, Sunshine Aggregate Plus Mix 4, Agawam, Massachusetts, USA) for the seeds to rest on. Groups of replicates were completed in successive trials and number of trials and replicates are listed in Table 1. All assays were evaluated after seven days of exposure time to *B. hilaris* to allow sufficient time for seeds to germinate and for feeding damage to become visible. After three days of exposure time, any dead *B. hilaris* were replaced with healthy adults. This mortality was likely due primarily to old age because adults used in the experiment were of varying ages. All experiments were conducted in a controlled

Table 1 Treatments and experimental design for choice and no-choice tests with *B. hilaris* in no-choice and choice tests

Crop type or variety test	Choice or no-choice	Treatments	Substrate	Box mfr.	No. compartments	Compartment size (cm)	Trials	Replicates per treatment[d]	No. *B. hilaris* per compartment
Crop type	No -choice	Arugula, broccoli, choi, cauliflower, kale, lettuce, mizuna, and turnip	Paper towel	NA[a]	16	5.5×4×4.2	4	16	1
		Arugula, broccoli, choi, cauliflower, kale, lettuce, mizuna, and turnip	Soil	Stanley[b]	48	5.0×3.5×3.8	3	12	1
	Choice	Arugula, broccoli, choi, cauliflower, mizuna, turnip, alyssum	Paper towel	Tactix[c]	18	7.0×10.0×6.0	2	36	5
		Arugula, broccoli, choi, cauliflower, kale, lettuce, mizuna, and turnip	Soil	Tactix[c]	9	12.2×8.3×9.7	4	36	5
Variety	No-choice	Varieties of arugula and mizuna	Potting mix	NA[a]	16	5.5×4×4.2	2	8	1
		Varieties of kale and choi and types of leafy Asian greens	Potting mix	Stanley[b]	48	5.0×3.5×3.8	2	12	1
	Choice	Varieties of arugula and mizuna	Potting mix	Tactix[c]	18	7.0×10.0×6.0	2	28	5
		Varieties of kale and choi and types leafy Asian greens	Potting mix	Tactix[c]	9	12.2×8.3×9.7	2	18	5

[a]Manufacturer unknown, but purchased from Walmart, Salinas, CA

[b]Stanley Tools, New Britain, CT

[c]Meridian International Co., Shanghai, China

[d]All the replicates were combined by trial type for analysis purpose

environmental chamber (21 ± 1 °C, 45% relative humidity, and L:D 16:8 photoperiod). Seeds used in the experiments were not treated with any pesticides.

The experiments were conducted in University of California Cooperative Extension-Monterey County Laboratory, Salinas, California. *Bagrada hilaris* adults were collected from broccoli fields in Salinas and Chualar, California and maintained in an environment chamber at 21 ± 1 °C; 45% relative humidity; and L:D 16: 8 photoperiod in cages. Two- to five-week-old potted-broccoli plants were provided as food source for the *B. hilaris* and the plants were changed every week.

Crop type studies

No-choice assays

In the no-choice assay, single *B. hilaris* were given access to only one type of seed. For the assay without soil, three seeds of a host plant were placed inside the compartment in a seed holder. The seed holder was constructed from a plastic vial lid (1.5 cm diam.) that had been inverted and glued to the center of a 5.3 × 3.8 cm piece of cardboard and lined with 2 × 2 cm of moistened paper towel and followed by introduction of one *B. hilaris* adult into each compartment to start the assay. The paper towel within the inverted vial tops was moistened three times during the 7 days period with 0.5 mL of distilled water starting from the set-up.

For the no-choice assays with soil, the seed holder consisted of a modified single well of a plastic seed sprouting tray (1.5 × 1.5 × 1.5 cm; Hummert Greenhouse Supplies, Topeka, Kansas, USA), which was glued directly in the center of the compartment. The well was modified by removing the top of the well to produce a total height of 1.5 cm. Each well was filled with oven-dried soil to 3 mm from the top, and three seeds of each crop type were placed on the soil surface. One *B. hilaris* adult was introduced into each compartment to start the assay and water was added.

Choice assays

Bagrada hilaris were given a choice among different crop types in the choice assays. In the choice assay without soil, the seed holder in the compartment comprised eight inverted plastic vial lids (1.5 cm diameter) glued to a piece of cardboard in two rows with 1.5 cm between rows and 1 cm between lids within rows. Each vial lid was lined with folded 2 × 2 cm of paper towel moistened with distilled water. Three seeds of each crop type were placed in each inverted vial top. Five *B. hilaris* adults were introduced into the compartment at the start of the assay. The paper towel within the inverted vial tops was moistened three times during the 7 days period with 0.5 mL of distilled water.

The experiment with soil used a group of 24 wells of a plastic seed sprouting tray (12 × 8.5 × 3.7 cm, 2 × 2 cm per well) as the seed holders. The group of wells completely filled the bottom of the compartment. Six of the 24 wells were used for the assay and the rest of the wells were closed using paper tape. Open wells were spaced 2 cm apart. Each well was filled with oven-dried soil up to 3 mm from the top, and three seeds of each crop type were placed on the soil surface. Five *B. hilaris* adults were introduced to each compartment at the start of the assay.

Crop variety test

No-choice assays

In the no-choice assays, *B. hilaris* were given access to only one crop variety at a time. For the assay with arugula and mizuna, the seed holder consisted of a single well of a seed tray (1.5 × 1.5 × 1.5 cm) glued directly to the center of a piece of cardboard (5.3 × 3.8 cm). The well was filled with potting mix to 3 mm from the top, and three seeds of each variety were added to the surface of the potting mix. One *B. hilaris* adult was introduced into each compartment at the start of the trial.

The no-choice assays for kale, choi, and leafy Asian greens also used a well of a seed tray (1.5 × 1.5 × 1.5 cm) as the seed holder, although it was glued directly in the center of the compartment. Each well was filled with 0.37 g of potting mix. Three seeds of each species/variety were placed on the surface of the potting mix. One *B. hilaris* adult was introduced into the compartment to start the trial.

Choice assays

Choice assays for arugula and mizuna used seed tray wells (1.5 × 1.5 × 1.5 cm) glued to a piece of cardboard (7 × 10 cm). There were four wells per replication arranged 2 × 2 and each well was filled with potting mix. Three seeds of each host species were placed in each well on the potting mix. At the start of the assay, five *B. hilaris* adults were introduced into the compartment.

For choice assays with kale, choi, and leafy Asian greens, the seed holder consisted of multiple wells from a seed sprouting tray cut and glued to the piece of cardboard (12.2 × 8.3 cm). The number of wells per compartment depended on the number of crop varieties for a given crop type, which varied (Table 1). The wells were arranged in two rows in each compartment. Three seeds of each variety were placed on the surface of the potting mix in the different wells. Five *B. hilaris* adults were introduced into a cell to start the assay.

Evaluation

Seeds or newly germinated seedlings (maximum of two leaves) or were evaluated after 7 days of exposure to *B. hilaris*. We determined the number of germinated seeds, seedlings with *B. hilaris* feeding injury on leaves, discrete feeding injury sites on leaves, distorted leaves with discrete feeding sites coalesced, and completely deformed or dead plants. Seeds and seedlings were evaluated with a dissecting microscope at 10 × magnification. Seeds were determined to be germinated if the radicle had emerged from the seed coat. Intact seeds and seeds with a cracked seed coat but no visible radicle were grouped as un-germinated seeds. Because there were several variations of "starburst" injury on leaves, all discrete discolored spots caused by *B. hilaris* feeding on intact leaves were quantified as injury sites (Fig. 1a, b). Distorted or misshapen leaves with completely discolored dead tissue were quantified as distorted leaves (Fig. 1c, d). Severe *B. hilaris* feeding can deform the seedling to the point where no leaves are present. Those completely destroyed seedlings were classified as deformed or dead (Fig. 1e, f). Individual feeding injury sites were indistinguishable for both distorted leaves and deformed seedlings.

Bagrada hilaris feeding on various crops and varieties was evaluated using three measures of feeding damage: number of feeding sites on leaves, distorted leaves, and dead or deformed seedlings. There could be subtle differences in expression of *B. hilaris* feeding on these hosts as measured by these different types of feeding damage. To accommodate these differences and provide a measure of overall susceptibility levels to *B. hilaris* feeding, susceptibility scores were generated where the number of feeding injury sites per germinated seeds was converted to a scale (0–9) denoted as 0 (0); 1 (0.1–1.0); 2 (1.1–2.0); 3 (2.1–3.0); 4 (3.1–4.0); 5 (4.1–5.0); 6 (5.1–6.0); 7 (6.1–7.0); 8 (7.1–8.0); and 9 (≥8.1). The number of distorted leaves and dead or deformed seedlings per germinated seeds were converted to a scale (0–9) denoted as 0, (0); 1 (0.01–0.25); 2 (0.26–0.50); 3 (0.51–0.75); 4 (0.76–1.00); 5 (1.01–1.25); 6 (1.26–1.50); 7 (1.51–1.75); 8 (1.76–2.00); and 9 (≥2.01).

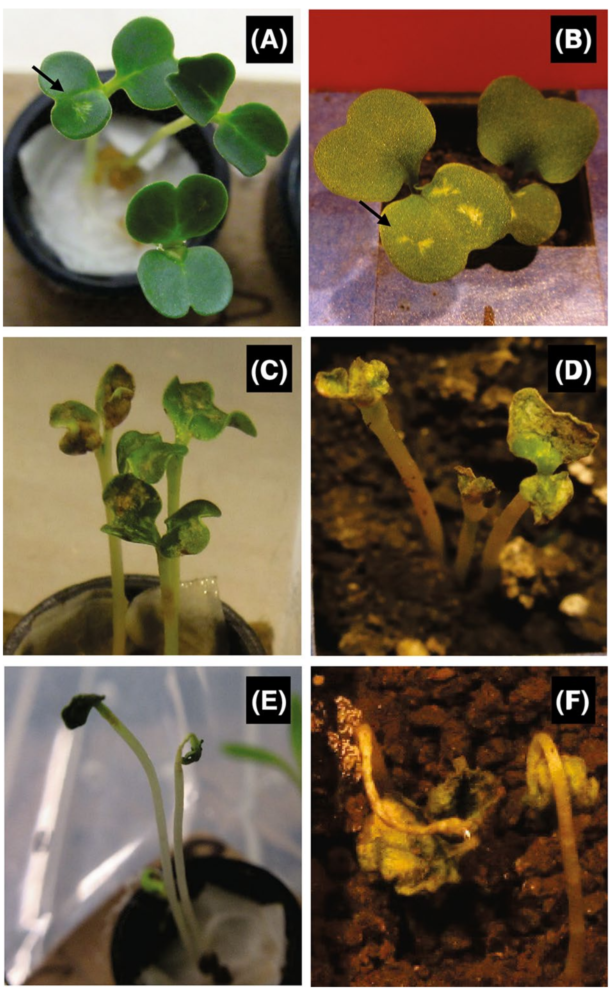

Fig. 1 *Bagrada hilaris* feeding injury types: **a, b** starburst or discolored spots as pointed out using black arrows, **c, d** distorted leaves, and **e, f** completely destroyed leaves or dead seedlings

$$\mathrm{SC} = \frac{\mathrm{FS}\,(\mathrm{Scale}) + \mathrm{DL}\,(\mathrm{Scale}) + \mathrm{DS}\,(\mathrm{Scale})}{\mathrm{Sum\,of\,maximum\,scale\,value}} \times 100. \quad (1)$$

The abbreviations are as follows: SC, susceptibility score; FS, number of feeding sites; DL, number of distorted leaves; DS, number of deformed plus dead seedlings. To generate susceptibility scores, the sum of individual scores for the two or three parameters was divided by the sum of the maximum values for each scale (27 for no-choice assays and 18 for choice assays) and percentage was calculated for each replication (1). Susceptibility scores were calculated for assays with or without soil in both the no-choice and choice tests.

Statistical analyses

SAS was used for all statistical analyses (SAS Institute 2012). Analyses were conducted for each assay type (choice/no-choice) for each trial type (crop type/crop varieties), as well as for each substrate type (soil/soilless) for crop type experiments and crop type (e.g., arugula) for crop variety experiments (see Table 1). Number of seeds germinated per well among crop types and crop varieties were analyzed with analysis of variance (ANOVA) using a general linear model (PROC GLM), and means were separated using Tukey's HSD test. All statistical comparisons were considered significant at $\alpha = 0.05$. The number of *B. hilaris* feeding injury sites, distorted leaves, and dead/deformed seedlings were standardized for the number of seeds that germinated by dividing them by the number of germinated seeds per well. For some damage measures, data were not available for certain replicates because high levels of some damage measures (e.g., no seeds germinated) precluded data collection for other damage measures (e.g., distorted leaves), and these data were excluded from the analyses. Each damage measure was analyzed with ANOVA using the PROC GLM procedure. Susceptibility scores were analyzed with ANOVA after arcsine square root transformation using the PROC GLM procedure. Means were separated using Tukey's HSD method. Averages of scores by crop type or variety were ranked to determine the overall susceptibility of each host to *B. hilaris* feeding. Because kale and sweet alyssum were not included in the choice soil assay, the assay was excluded from these averages.

Results

Crop type studies

No-choice assays

In soilless assays, cauliflower seeds germinated at the lowest rate (<50%) after exposure to *B. hilaris* compared to all other crop types, with the exception of choi, which was intermediate to cauliflower and the other crops (Table 2). The number of *B. hilaris* feeding sites was significantly greater for arugula than for kale, cauliflower, and lettuce, although number of feeding sites was similar between arugula, broccoli, mizuna, and choi ($F=6.8$; df = 7, 130; $P<0.001$; Fig. 2a). The number of leaves deformed by *B. hilaris* feeding in arugula was significantly greater than in broccoli, cauliflower, kale, lettuce and turnip; however, the number of distorted leaves was not significantly different between arugula, mizuna and choi (Fig. 2b) ($F=2.5$; df = 7, 130; $P=0.017$). The number of dead or deformed seedlings was highest for arugula and turnip compared to all other hosts except mizuna, for which the difference was not significant (Fig. 2c) ($F=3.2$; df = 7, 130; $P=0.003$). There was no significant difference in number of deformed or dead seedlings between cauliflower and lettuce.

In the assays with soil, the number of seeds that germinated for cauliflower and mizuna were significantly lower than for lettuce (Table 2). The number of *B. hilaris* feeding sites for seeds that did germinate was significantly greater in kale than in broccoli and lettuce (Fig. 2d) ($F=3.3$; df = 7, 73; $P=0.004$). There were no differences in number of feeding sites between arugula, cauliflower, kale, mizuna, choi, and turnip. A similar number of leaves were deformed by *B. hilaris* feeding across hosts, although no leaves of lettuce were fed upon (Fig. 2e) ($F=1.8$; df = 7, 73; $P=0.089$). The number of dead or deformed seedlings was significantly greater in mizuna than in broccoli, cauliflower, lettuce, and choi (Fig. 2f) ($F=2.4$; df = 7, 73; $P=0.029$), and similar numbers of seedlings did not successfully germinate for arugula, kale, mizuna, and turnip.

Choice assays

In soilless assays, the number of seeds that had germinated after 7 days was greater in arugula than choi and cauliflower (Table 2). Among germinated seeds of arugula, broccoli, kale, mizuna, turnip, and lettuce, there were no significant differences. After 7 days, number of feeding injury sites was significantly greater in turnip and arugula than in broccoli, cauliflower, kale, mizuna, and lettuce ($F=26.7$; df = 7, 236; $P<0.001$), but was not significantly different from choi (Fig. 3a). Number of leaves distorted by *B. hilaris* feeding was significantly greater in mizuna than in kale and lettuce ($F=5.2$; df = 7, 236; $P<0.001$) and it was not significantly differently among arugula, broccoli, choi, cauliflower, and turnip (Fig. 3b).

In the assays with soil, the number of germinated choi seeds after 7 days was significantly lower than germinated broccoli and sweet alyssum seeds (Table 2). Significantly more feeding sites were found in turnip than in choi, cauliflower, and sweet alyssum but number of feeding sites in turnip was not different from arugula, broccoli, and mizuna ($F=11.2$; df = 6, 140; $P<0.001$) (Fig. 3c). The number of distorted leaves was significantly less in sweet alyssum than in all other hosts ($F=3.2$; df = 6, 140; $P=0.005$) (Fig. 3d).

Susceptibility scores

In the no-choice assay without soil, the susceptibility scores were significantly lower in kale, lettuce, and cauliflower than in other hosts (Table 3). However, in the no-choice assays with soil, the susceptibility score was only lower in lettuce than other hosts. In choice assays without soil, susceptibility scores were significantly greater in turnip than in kale, cauliflower, and lettuce, whereas there was no difference in susceptibility scores in choice assays with soil.

Table 2 Mean (±SE) number of seeds germinated out of three after 7 days in the test arena

Host plant	No-choice		Choice	
	Soilless	Soil	Soilless	Soil
Arugula	2.35 ± 0.18a	2.16 ± 0.27abc	2.88 ± 0.05a	2.32 ± 0.12ab
Broccoli	2.70 ± 0.13a	2.83 ± 0.11ab	2.83 ± 0.06ab	2.74 ± 0.11a
Choi	2.20 ± 0.22ab	2.50 ± 0.19ab	2.47 ± 0.11bc	2.00 ± 0.19b
Cauliflower	1.45 ± 0.22b	1.25 ± 0.25c	2.33 ± 0.13c	2.44 ± 0.15ab
Kale	2.45 ± 0.17a	2.50 ± 0.15ab	2.74 ± 0.09ab	–
Mizuna	2.35 ± 0.18a	1.91 ± 0.28bc	2.56 ± 0.12abc	2.57 ± 0.14ab
Turnip	2.70 ± 0.13a	2.25 ± 0.30ab	2.60 ± 0.12abc	2.60 ± 0.12ab
Lettuce	2.65 ± 0.18a	2.91 ± 0.08a	2.77 ± 0.07 ab	–
S. alyssum	–	–	–	2.86 ± 0.26a
F (df1, df2)	5.7 (7, 137)	6.3 (7, 77)	4.2 (7, 236)	3.2 (6, 140)
P	<0.001	<0.001	<0.001	0.004

Means followed by the same letters within a column are not significantly different (Tukey's HSD Test, $\alpha=0.05$)

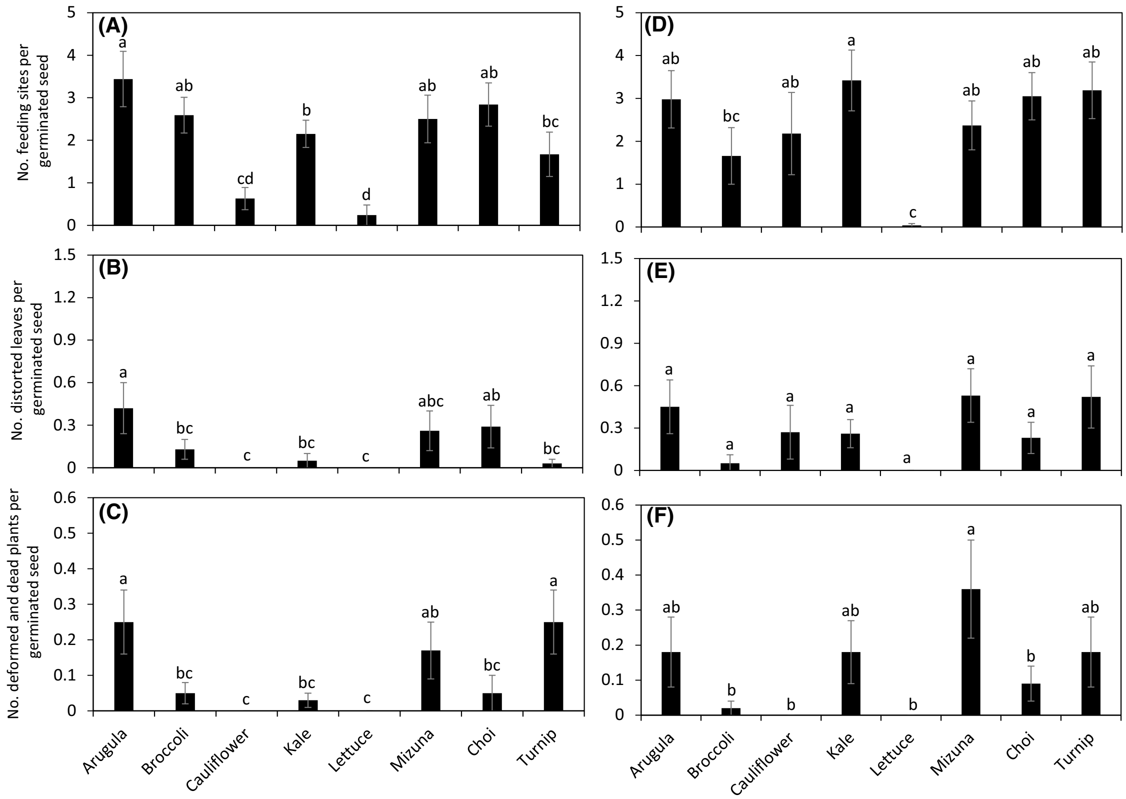

Fig. 2 *Bagrada hilaris* feeding injury (mean ± SE) on germinating seeds in the no-choice assays using of different crop types without soil (**a**–**c**), and with soil (**d**–**f**). Assays were evaluated after 7 days of *B. hilaris* exposure. Bars with the same letter within a figure are not significantly different ($P > 0.05$)

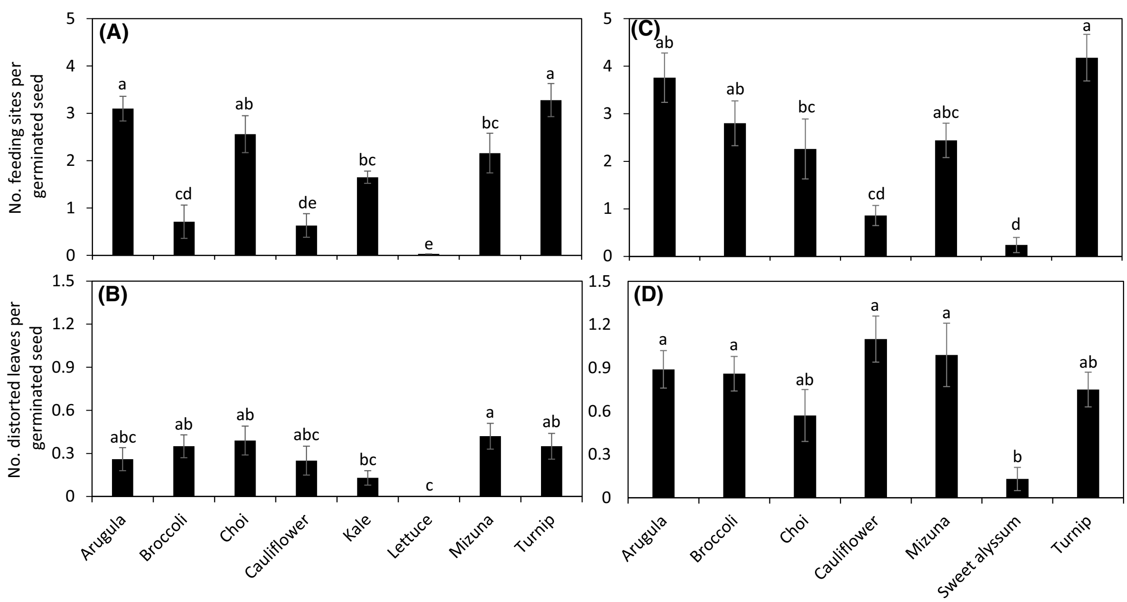

Fig. 3 *Bagrada hilaris* feeding injury (mean ± SE) on various germinating host seeds in the choice assay in the absence (**a, b**) and presence of soil (**c, d**) when evaluated after 7 days of exposure. No plants were severely deformed or dead and therefore these data are not included in the figure. Within a figure, *bars marked* with the *same letter* are not significantly different ($P > 0.05$)

The average of mean susceptibility scores by host indicated that the order of preference/susceptibility to *B. hilaris* feeding from highest to lowest was as follows: arugula > turnip > mizuna > kale > choi > broccoli > cauliflower > lettuce (Table 3).

Assays with crop varieties

On arugula, the number of feeding sites, distorted leaves, and dead or deformed seedlings was significantly lower in 'Wild Rocket' than other varieties in the no-choice assay (Table 4). In the choice assay, a significantly lower number of seeds germinated in 'Roquette Cultive' variety than 'Wild Rocket' and 'Sprint.' The number of feeding sites was significantly greater on 'Spirit' than on 'Wild Rocket' and 'Roquette Cultive,' although there was no difference between 'Spirit' and 'Astro' and or 'Sprint.' The number of distorted leaves was significantly greater on 'Wild Rocket' than on 'Spirit' and 'Roquette Cultive.' The dead and deformed seedlings were significantly greater on 'Wild Rocket' than on 'Astro' and 'Roquette Cultive.'

On mizuna, there were no significant differences among varieties in both no-choice and choice assays (Table 4). On kale in the no-choice assay, the varieties did not differ for number of germinated seeds, feeding injury sites, distorted leaves, and dead or deformed seedlings. In the choice assay, number of seeds germinated among varieties was not significantly different. The number of feeding injury was significantly greater on 'Beira F1' than 'Scarlet' and 'Starbor F1.' Similarly, number of distorted leaves was significantly greater on 'Beira F1' than on 'Starbor F1.' There was no difference among varieties for dead and deformed seedlings.

In no-choice assays with choi, numbers of germinated seeds, feeding injury sites, and distorted leaves were similar among varieties, although dead and deformed seedlings were significantly greater on 'Rosie F1' than 'Mei Qing Choi' (Table 4). In the choice assay, number of seeds germinated was not different among varieties. The number of feeding injury sites was significant greater on 'Tokyo Bekana,' 'Feng Qing Choi F1,' 'Joi Choi F1,' 'Win-Win Choi F1' than on 'Rosie F1,' while the number of distorted leaves was significantly more on 'Win-Win Choi F1' than on 'Rosie F1.' The choi variety 'Joi Choi F1' had significantly greater number of dead and deformed seedlings compared to 'Rosie F1.'

For the no-choice assay with leafy Asian greens, number of germinated seeds, feeding injury sites, distorted leaves, and dead or deformed seedlings did not differ significantly among varieties (Table 4). In choice assay, number of germinated seeds and dead or deformed seedlings was not significantly differently among varieties but number of feeding injury sites and distorted leaves varied with varieties. The feeding injury sites were significantly greater on 'Carlton F1' than on 'Yukina Savon,' 'Tatsoi OG,' 'Komatsuna Summerfest F1,' 'Red Rain F1,' and 'Shungiku.' The leaves distorted after *B. hilaris* feeding were significantly greater on 'Hon Tsai Tai' and 'Carlton F1' than on 'Shungiku.'

Discussion

We assessed the relative susceptibility of germinating seeds of cruciferous species and varieties to *B. hilaris* feeding injury under controlled environmental conditions. First, the ability of *B. hilaris* to feed on germinating cruciferous host

Table 3 Relative susceptibility of germinating host plant seeds to *B. hilaris* feeding injury based on susceptibility scores (%)

Plant host	No-choice		Choice		Ave. susceptibility score[a]	Rank[b]
	Soilless	Soil	Soilless	Soil		
Arugula	23.8 ± 3.8a	21.9 ± 5.5a	25.4 ± 3.1ab	43.9 ± 4.8a	23.70	1
Turnip	11.2 ± 1.8ab	23.9 ± 6.9a	28.1 ± 2.5a	43.9 ± 4.8a	21.06	2
Mizuna	15.9 ± 2.9ab	23.6 ± 6.1a	23.6 ± 2.6abc	36.2 ± 3.9a	21.03	3
Kale	9.9 ± 1.6bc	20.9 ± 3.9a	14.6 ± 1.7 cd	–	15.13	4
Choi	16.7 ± 2.8ab	17.9 ± 3.7a	10.6 ± 2.6ab	27.4 ± 5.9a	15.06	5
Broccoli	13.8 ± 2.9ab	8.3 ± 3.1ab	16.5 ± 2.2bc	36.9 ± 4.6a	12.87	6
Cauliflower	2.8 ± 1.0 cd	12.1 ± 6.2ab	10.6 ± 2.6d	32.9 ± 3.9a	8.50	7
Lettuce	1.0 ± 1.0d	0.3 ± 0.3b	0.3 ± 0.2e	–	0.53	8
Sweet alyssum	–	–		5.2 ± 2.9b	–	–
F (df1, df2)	8.2 (7, 130)	4.9 (7, 73)	21.9 (7, 236)	7.5 (6, 140)		
P	<0.001	<0.001	<0.001	<0.001		

[a]Generated by averaging the percentage susceptibility scores in each tests excluding soil test in choice assay

[b]Generated based on the average susceptibility score. Highest susceptibility score corresponds to higher levels of damage. Values followed by the same letter within the column are not significantly different (Tukey's HSD Test, $\alpha = 0.05$)

Table 4 *B. hilaris* feeding injury on germinating seeds of leafy cruciferous crop varieties

Host varieties	No-choice				Choice			
	Germinated seeds	Feeding sites	Distorted leaves	Dead seedlings	Seeds germinated	Feeding sites	Distorted leaves	Dead seedlings
Arugula[a]								
'Sprint'	3.0 ± 0.0a	5.7 ± 1.4a	1.2 ± 0.3a	0.6 ± 0.2a	2.8 ± 0.1a	7.4 ± 0.7a	0.8 ± 0.1b	1.6 ± 0.9ab
'Astro'	2.6 ± 0.2a	5.7 ± 1.4a	1.3 ± 0.3a	0.6 ± 0.2a	2.6 ± 0.2ab	5.8 ± 0.6ab	0.6 ± 0.1bc	0.3 ± 0.1b
'Wild Rocket'	2.5 ± 0.3a	0.1 ± 0.1b	0.1 ± 0.1b	0.0 ± 0.0b	2.9 ± 0.1a	4.4 ± 0.5b	1.6 ± 0.2a	4.7 ± 1.5a
'Roquette Cultive'	2.6 ± 0.2a	5.4 ± 1.1a	1.7 ± 0.1a	0.9 ± 0.1a	2.1 ± 0.2b	0.3 ± 0.1c	0.1 ± 0.1c	0.2 ± 0.2b
$F_{df1,df2}$; P	$F_{3, 21} = 1.5$; $P = 0.252$	$F_{3, 18} = 7.9$; $P = 0.001$	$F_{3, 21} = 10.8$; $P < 0.183$	$F_{3, 21} = 10.8$; $P < 0.001$	$F_{3, 71} = 4.6$; $P = 0.006$	$F_{3, 67} = 21.6$; $P < 0.001$	$F_{3, 67} = 13.4$; $P < 0.001$	$F_{3, 69} = 4.8$; $P = 0.005$
Mizuna[b]								
'Mustard'	1.9 ± 0.3	3.8 ± 1.4a	0.3 ± 0.2a	0.5 ± 0.2a	2.3 ± 0.1a	6.5 ± 1.0a	0.8 ± 0.1a	0.6 ± 0.1a
'Mizuna OG'	1.3 ± 0.3	6.0 ± 1.3a	1.0 ± 0.4a	0.7 ± 0.2a	2.0 ± 0.2a	7.2 ± 0.8a	0.8 ± 0.1a	0.6 ± 0.1a
'Kyoto'	2.0 ± 0.3	4.1 ± 1.1a	0.5 ± 0.2a	0.6 ± 0.1a	2.3 ± 0.2a	6.4 ± 0.8a	0.9 ± 0.2a	0.6 ± 0.1a
'Dark Purple'	2.3 ± 0.2	3.6 ± 0.6a	0.5 ± 0.1a	0.3 ± 0.1a	2.4 ± 0.1a	7.6 ± 0.9a	0.9 ± 0.1a	0.5 ± 0.1a
$F_{df1,df2}$; P	$F_{3, 33} = 2.1$; $P = 0.113$	$F_{3, 13} = 1.2$; $P = 0.325$	$F_{3, 12} = 1.9$; $P = 0.183$	$F_{3, 26} = 1.1$; $P = 0.387$	$F_{3, 80} = 1.6$; $P = 0.203$	$F_{3, 42} = 0.8$; $P = 0.515$	$F_{3, 47} = 0.1$; $P = 0.938$	$F_{3, 77} = 0.3$; $P = 0.809$
Kale[c]								
'Scarlet'	2.1 ± 0.3a	6.3 ± 1.1a	0.8 ± 0.3a	0.5 ± 0.2a	2.1 ± 0.3a	1.2 ± 0.4c	0.2 ± 0.1bc	0.2 ± 0.1a
'Beira F1'	2.3 ± 0.4a	6.2 ± 0.7a	0.8 ± 0.3a	0.5 ± 0.1a	2.8 ± 0.1a	6.8 ± 1.1a	0.8 ± 0.1a	0.4 ± 0.1a
'Starbor F1'	2.8 ± 0.2a	3.2 ± 1.2a	0.2 ± 0.1a	0.2 ± 0.1a	2.5 ± 0.2a	1.6 ± 0.4c	0.1 ± 0.1c	0.1 ± 0.0a
'Olympic Red OG'	2.6 ± 0.4a	2.5 ± 0.9a	0.2 ± 0.1a	0.2 ± 0.1a	2.3 ± 0.2a	3.4 ± 0.6bc	0.5 ± 0.2abc	0.2 ± 0.1a
'Premier OG'	1.8 ± 0.3a	3.8 ± 1.2a	0.2 ± 0.1a	0.3 ± 0.2a	2.4 ± 0.2a	3.1 ± 0.5bc	0.4 ± 0.1abc	0.2 ± 0.1a
'Toscano'	1.8 ± 0.5a	3.8 ± 1.5a	0.8 ± 0.3a	0.6 ± 0.2a	2.6 ± 0.2a	3.2 ± 0.5bc	0.4 ± 0.1abc	0.2 ± 0.0a
'Red Russian OG'	1.9 ± 0.5a	2.8 ± 0.9a	0.8 ± 0.4a	0.4 ± 0.2a	2.3 ± 0.2a	3.3 ± 0.4bc	0.6 ± 0.1abc	0.3 ± 0.1a
'Siberian'	2.1 ± 0.3a	6.1 ± 0.7a	0.8 ± 0.2a	0.5 ± 0.2a	2.6 ± 0.1a	4.8 ± 0.9ab	0.5 ± 0.1abc	0.3 ± 0.1a
'Black Magic'	2.4 ± 0.4a	4.8 ± 1.4a	0.6 ± 0.3a	0.3 ± 0.1a	2.4 ± 0.2a	2.9 ± 0.6bc	0.3 ± 0.1abc	0.3 ± 0.1a
'Lacinato'	2.3 ± 0.4a	2.6 ± 1.5a	0.5 ± 0.2a	0.5 ± 0.2a	2.6 ± 0.1a	3.5 ± 0.5bc	0.7 ± 0.1abc	0.3 ± 0.1a
'Redbor F1'	1.8 ± 0.4a	3.8 ± 1.9a	0.6 ± 0.2a	0.4 ± 0.2a	2.4 ± 0.2a	3.3 ± 0.7bc	0.3 ± 0.1abc	0.2 ± 0.1a
'Rogue'	2.4 ± 0.3a	6.1 ± 1.5a	0.6 ± 0.2a	0.3 ± 0.1a	2.5 ± 0.1a	3.6 ± 0.6bc	0.4 ± 0.1abc	0.2 ± 0.1a
$F_{df1,df2}$; P	$F_{11, 77} = 0.9$; $P = 0.474$	$F_{11, 53} = 1.6$; $P = 0.132$	$F_{11, 57} = 1.1$; $P = 0.356$	$F_{11, 68} = 0.7$; $P = 0.710$	$F_{11, 187} = 0.9$; $P = 0.555$	$F_{11, 172} = 5.1$; $P < 0.001$	$F_{11, 176} = 2.9$; $P = 0.002$	$F_{11, 182} = 1.7$; $P = 0.076$
Choi[d]								
'Tokyo Bekana'	2.8 ± 0.2a	2.3 ± 1.1a	0.5 ± 0.3a	0.3 ± 0.1ab	2.3 ± 0.2a	7.3 ± 0.8a	1.0 ± 0.3ab	0.2 ± 0.1ab
'Rosie F1'	2.0 ± 0.4a	2.0 ± 1.0a	1.6 ± 0.3a	0.8 ± 0.1a	2.7 ± 0.1a	2.5 ± 0.7b	0.3 ± 0.1b	0.1 ± 0.1b
'Gunsho F1'	1.8 ± 0.4a	3.0 ± 1.3a	0.5 ± 0.3a	0.2 ± 0.2ab	2.4 ± 0.2a	5.3 ± 0.8ab	0.8 ± 0.2ab	0.3 ± 0.1ab
'Red Choi F1'	2.2 ± 0.4a	4.8 ± 1.6a	0.9 ± 0.2a	0.5 ± 0.1ab	2.4 ± 0.2a	5.1 ± 1.1ab	0.5 ± 0.1ab	0.2 ± 0.1b
'Black Summer F1'	2.8 ± 0.2a	3.6 ± 1.2a	0.9 ± 0.3a	0.5 ± 0.2ab	2.3 ± 0.2a	5.3 ± 1.2ab	0.4 ± 0.1ab	0.2 ± 0.1b
'Feng Qing Choi F1'	2.6 ± 0.3a	3.7 ± 0.9a	0.6 ± 0.3a	0.4 ± 0.2ab	2.7 ± 0.1a	6.9 ± 1.2a	0.6 ± 0.1ab	0.3 ± 0.1ab
'Joi Choi F1'	2.6 ± 0.3a	5.6 ± 1.7a	0.9 ± 0.3a	0.9 ± 0.3ab	2.8 ± 0.1a	7.3 ± 1.2a	0.8 ± 0.2ab	0.5 ± 0.1a

Table 4 (continued)

Host varieties	No-choice				Choice			
	Germinated seeds	Feeding sites	Distorted leaves	Dead seedlings	Seeds germinated	Feeding sites	Distorted leaves	Dead seedlings
'Win–Win Choi F1'	2.8 ± 0.2a	3.5 ± 1.4a	0.4 ± 0.2a	0.4 ± 0.2ab	2.5 ± 0.2a	8.5 ± 1.0a	1.2 ± 0.3a	0.4 ± 0.1ab
'Red PAC F1'	2.3 ± 0.3a	3.1 ± 0.9a	0.5 ± 0.3a	0.5 ± 0.3ab	2.3 ± 0.2a	4.3 ± 0.8ab	0.4 ± 0.2ab	0.2 ± 0.1ab
'White Flash F1'	2.4 ± 0.2a	3.2 ± 0.9a	0.4 ± 0.2a	0.3 ± 0.1ab	2.4 ± 0.2a	4.9 ± 1.0ab	0.6 ± 0.2ab	0.3 ± 0.1ab
'Shiro F1'	2.8 ± 0.2a	5.8 ± 1.6a	1.2 ± 0.3a	0.6 ± 0.1ab	2.4 ± 0.2a	6.3 ± 1.3ab	0.5 ± 0.2ab	0.3 ± 0.1ab
'Mei Qing Choi'	2.6 ± 0.2a	2.8 ± 1.3a	0.1 ± 0.1a	0.0 ± 0.0b	2.7 ± 0.2a	6.9 ± 1.2ab	0.7 ± 0.1ab	0.3 ± 0.1ab
$F_{df1,df2}$; P	$F_{11, 77} = 1.7$; $P = 0.094$	$F_{11, 56} = 0.7$; $P = 0.771$	$F_{11, 64} = 1.6$; $P = 0.109$	$F_{11, 74} = 2.1$; $P = 0.034$	$F_{11, 187} = 0.9$; $P = 0.539$	$F_{11, 168} = 3.1$; $P = 0.001$	$F_{11, 168} = 2.1$; $P = 0.022$	$F_{11, 180} = 2.7$; $P = 0.003$
Leafy Asian greens[e]								
'Yukina Savon'[f]	2.3 ± 0.4a	2.4 ± 0.7a	0.1 ± 0.1a	0.1 ± 0.1a	2.2 ± 0.2a	2.1 ± 0.7bc	0.5 ± 0.1ab	0.2 ± 0.1a
'Hon Tsai Tai'[f]	2.3 ± 0.3a	1.9 ± 0.9a	0.3 ± 0.3a	0.1 ± 0.1a	2.6 ± 0.2a	3.5 ± 1.1ab	0.9 ± 0.2a	0.4 ± 0.1a
'Koji F1'[f]	1.9 ± 0.4a	5.2 ± 2.6a	0.6 ± 0.4a	0.2 ± 0.2a	2.6 ± 0.2a	3.6 ± 0.6ab	0.6 ± 0.2ab	0.2 ± 0.1a
'Tatsoi OG'[g]	1.2 ± 0.4a	1.5 ± 1.5a	0.4 ± 0.4a	0.2 ± 0.2a	1.8 ± 0.2a	1.8 ± 0.6bc	0.6 ± 0.2ab	0.3 ± 0.1a
'Vitamin Green'[g]	2.0 ± 0.4a	3.8 ± 1.8a	0.9 ± 0.4a	0.4 ± 0.2a	2.6 ± 0.2a	1.7 ± 0.5bc	0.3 ± 0.1ab	0.1 ± 0.1a
'Komatsuna Summerfest F1'[h]	1.5 ± 0.4a	1.8 ± 1.1a	0.6 ± 0.3a	0.4 ± 0.2a	2.6 ± 0.2a	1.7 ± 0.6bc	0.6 ± 0.2ab	0.2 ± 0.1a
'Carlton F1'[h]	1.1 ± 0.3a	4.2 ± 1.8a	1.0 ± 0.4a	0.7 ± 0.2a	2.4 ± 0.2a	5.7 ± 1.2a	0.9 ± 0.2a	0.5 ± 0.1a
'Red Rain F1'[i]	1.9 ± 0.2a	5.3 ± 1.3a	1.0 ± 0.4a	0.4 ± 0.2a	2.3 ± 0.2a	1.6 ± 0.6bc	0.4 ± 0.2ab	0.4 ± 0.2a
'Shungiku'[j]	1.4 ± 0.5a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	2.2 ± 0.2a	0.0 ± 0.0c	0.0 ± 0.0b	0.0 ± 0.0a
$F_{df1,df2}$; P	$F_{8, 56} = 1.4$; $P = 0.208$	$F_{8, 37} = 1.3$; $P = 0.259$	$F_{8, 42} = 1.0$; $P = 0.439$	$F_{8, 43} = 1.6$; $P = 0.154$	$F_{8, 133} = 1.7$; $P = 0.117$	$F_{8, 103} = 6.5$; $P < 0.001$	$F_{8, 124} = 2.7$; $P = 0.008$	$F_{8, 124} = 1.6$; $P = 0.138$

Values followed by the same letter within a host type are not significantly different (Tukey's HSD Test, $\alpha = 0.05$)

[a]*Eruca sativa*

[b]*B. juncea* var. *nipposinica*

[c]*B. oleracea*

[d]*B. rapa* var. *chinensis*

[e]Various species of *Brassica* spp.

[f]*B. rapa*

[g]*B. rapa* var. *narinosa*

[h]*B. rapa* var. *perviridis*

[i]*B. juncea*

[j]*Glebionis coronaria*

seeds, as well as a non-host (lettuce), was evaluated in a no-choice setting. Next, feeding preference was assessed in a choice setting. For the crop type experiments, patterns of damage varied widely among the tested hosts, including among only cruciferous crops. In the crop variety experiments, difference in damage was evident among varieties, although in some cases, there were no varietal differences. In the no-choice assays, *B. hilaris* fed on all cruciferous hosts, although germinating seeds of arugula, turnip, and mizuna were most affected by feeding damage such as leaf distortion and seedling deformity or mortality. This suggests that *B. hilaris* is capable of feeding on germinating stages of all cruciferous hosts that we tested if they can physically access the seeds. Interestingly, the choice assay results also show that arugula, turnip, and mizuna had more feeding injury than other hosts, suggesting that germinating seeds of these hosts were most preferred by *B. hilaris*, although patterns could also be driven by susceptibility to injury. Previous research on host preference used seedlings (Makwali et al. 2002; Huang et al. 2014a) that were already past the germination stage. With seedlings, *B. hilaris* adults preferred seedlings (cotyledon-stage) of radish and red and green cabbage over sweet alyssum, arugula, and broccoli plants (Huang et al. 2014a). Radish was not used in our study because this crop is not grown extensively in the Central Coast of California. By describing the susceptibilities of germinating seeds of various crop types and varieties, our results expand the information available on crop susceptibility to include the time period when seeds first begin germinating.

It is unclear why certain crop types or varieties suffered more damage than others (e.g., arugula, turnip, and mizuna in the crop type experiment). First, these species/varieties may germinate more quickly than others. The seeds in this study were evaluated after 7 days to provide sufficient time for all seeds to germinate. It is possible that early germinating seeds are more vulnerable to *B. hilaris* feeding than slowly germinating seeds. Second, it is also possible that *B. hilaris* is more attracted to germinating seeds of certain hosts. This could be related to preference of varying levels and blends of volatiles released. Germinating cauliflower seeds release volatile chemicals such as cyanides and isothiocyanates after hydrolysis of glucosinolates (Valette et al. 2006). The strength of the volatiles released varies across cruciferous species and varieties (Valette et al. 2006; Hong and Kim 2013). Variation in defensive chemical content can be important for feeding by herbivores on cruciferous host. For two specialist herbivores, the flea beetle, *Phyllotreta cruciferae* (Goeze) and diamondback moth, *Plutella xylostella* (L.), feeding was greatest when glucosinolate levels in *B. rapa* (syn. *campestris*) (L.) were intermediate (Siemens and Mitchell-olds 1996). It is likely that *B. hilaris* are similarly responding to chemical compounds released by germinating seeds of all of the tested cruciferous hosts and that this affects preference. Moisture content in the seeds may further affect the type and amount of volatiles released from the seeds, which could influence *B. hilaris* behavior and feeding damage (Jorgensen 2001; Hudaib et al. 2010).

Our results show that germinating seeds of lettuce were not attractive to *B. hilaris*. Lettuce was included in this study because it is the major, high-value crop (US$1.4 billion) in the Salinas Valley of California (Monterey County Crop Report 2014). Our results dismiss the anecdotal reports that *B. hilaris* can damage germinating seeds of lettuce. Sweet alyssum was included because it is a commonly used insectary plant in the Central Coast of California which is direct seeded in beds interspersed with the main crop to enhance predation of aphids by syrphid flies (Bugg et al. 2008). Mature sweet alyssum is attractive to *B. hilaris* (Joseph 2014), but our results show that germinating stages of sweet alyssum was not very attractive. This suggests that at least when seeds are still germinating, sweet alyssum would likely not be useful as a trap crop.

Trap crops could provide an additional tool for reducing damage by *B. hilaris* to germinating crops, and our results can inform their development. Although leafy Asian crops, arugula, turnip, and mizuna appear to have relatively similar levels of attractiveness compared to each other, these crops could still be used as a trap crop when the leafy Asian crops are grown as main crops. Crops with the same level of attractiveness can be developed as a trap crop by manipulating the spatial and temporal planting of trap and main crops, thereby diverting or concentrating the incidence and colonization of the pest (Shelton and Badenes-Perez 2006). Early planting of arugula, turnip, and mizuna, possibly before the main crop, may trap invading *B. hilaris*, allow them to be managed, and reduce the impact on the main crop as it germinates. When heading crops such as broccoli or cauliflower are the main crop, leafy Asian crops could be developed as a trap crop because the germinating stages of broccoli and cauliflower were less attractive to *B. hilaris* relative to leafy Asian crops. These tactics would function better if the *B. hilaris* adults attracted to the trap crop are killed or removed via chemical controls (conventional or organic) or with mechanical controls, such as incorporation of the trap crop into the soil or vacuuming. Lettuce, or another non-preferred crop, could serve as a barrier crop in between the trap crop and the main crop, limiting movement of *B. hilaris* into the main crop. It is possible that planting a trap crop before the main crop could attract *B. hilaris* adults earlier to the field and increase damage to the main crop. Thus, this tactic needs to be carefully evaluated in the field to determine its utility for effective *B. hilaris* management.

Although all varieties of leafy Asian crops developed *B. hilaris* feeding injury symptoms after a week of *B. hilaris* exposure, certain varieties of arugula, kale, choi, and Asian greens were fed upon more and developed more symptoms of damage. Perhaps, these differences in feeding preferences could be utilized for management of *B. hilaris* feeding at the germination stages. Those highly preferred varieties could be potentially considered as a trap crop, whereas the less preferred varieties which suffer less feeding injury could be planted as the main crop. These differences in susceptibility or attractiveness to *B. hilaris* could be also be used to develop more resistant varieties for *B. hilaris* management. The arugula varieties 'Wild Rocket' and 'Spirit' were more attractive to *B. hilaris* than other varieties tested. On mizuna, the variety

'Beira F1' was more attractive to *B. hilaris* than 'Scarlet' and 'Starbor F1.' The choi varieties 'Tokyo Bekana,' 'Feng Qing Choi F1,' 'Joi Choi F1,' and 'Win-Win Choi F1' were most attractive. The leafy Asian greens varieties 'Carlton F1' was more attractive to *B. hilaris* than 'Yukina Savon,' 'Tatsoi OG,' 'Komatsuna Summerfest F1,' 'Red Rain F1,' and 'Shungiku.'

In conclusion, our results suggest that germinating stages of arugula, mizuna, choi, and turnip seeds are more attractive to *B. hilaris* than cauliflower or broccoli and may be utilized as a trap crop when seeds are first planted. Although the results did not provide a clear indication of *B. hilaris* feeding preference among arugula, mizuna, choi, and turnip in the choice tests, when planted early along the field edges, one or more of these hosts may diffuse *B. hilaris* pressure and may produce lower feeding injury on the main crop than if the main crop was planted alone. Sweet alyssum plants have been found to be attractive to *B. hilaris* in the field (Joseph 2014), but *B*. hilaris adults did not show any preference for germinating seeds of sweet alyssum in this study, suggesting that it would likely not be useful as a trap crop but also that new plantings should not be highly attractive. Similarly, lettuce was not preferred by *B. hilaris* adults when provided in no-choice and choice studies. Varieties of several cruciferous salad crops demonstrated a spectrum of preference. Our results demonstrate that when seeds first germinate, the susceptibility and attractiveness of different crop types and crop varieties can be useful for determining the threat of *B. hilaris* injury to newly planted fields and for developing effective management tactics.

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