Effects of Induced Starvation on *Bagrada hilaris* (Hemiptera: Pentatomidae) Survival[1]

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Abstract *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae) is a serious pest of cruciferous crops, including leafy crucifers grown in enclosed greenhouses in the Central Coast of California. These enclosed greenhouses face season-long risk of feeding injury to plantings of cruciferous crops. Understanding the survival ability of *B. hilaris* would help develop crop-free periods as a cultural tactic for *B. hilaris* control. A series of trials was conducted with 2nd–3rd and 4th–5th instar nymphs and male and female adults to determine how well they survive when they had full, partial, or no access to food and water sources. The treatments for the experiment were: (a) soil plus food (a piece of broccoli crown); (b) soil plus water; (c) soil-only; and (d) empty (no soil, water, and food). *Bagrada hilaris* were individually released into 20-ml ventilated scintillation vials that were deployed outside by burying them in the ground with only the ventilated top exposed for multiple days. Results show that *B. hilaris* survived between 3 and 6 d after deployment without access to food and water. In all experiments, survival of *B. hilaris* in the food or water treatments was significantly greater than in the soil-only and empty treatments on final day of the trial. *Bagrada hilaris* in the soil-only treatment had significantly lower number of live *B. hilaris* than in the empty treatment on the days before the final day of the experiment. The survival of *B. hilaris* that had access to food was not significantly different from those that had access to water.

Key Words enclosed greenhouses, Asian cruciferous vegetable, painted bug, bagrada bug, Central Coast of California

Bagrada hilaris (Burmeister) (Hemiptera: Pentatomidae) is an invasive stink bug species that preferentially feeds on cruciferous crops (Family: Brassicaceae) (Reed et al. 2013, Huang et al. 2014a). It is native to southern Africa, Middle East, and Asia (Reed et al. 2013). It was first detected in North America in Los Angeles Co., CA, in 2008 (Palumbo and Natwick 2010). Since then, it has been detected or established in several counties in California, including in the northern regions of Central Coast (Joseph 2014). The major crops damaged by *B. hilaris* are broccoli (*Brassica oleracea* L. var. *italic* Plenck), cauliflower (*B. oleracea* L. var. *botrytis*), turnip (*B. rapa* L. var. *rapa* L.), kale (*B. oleracea* L. var. *acephala*), choi (*B. rapa* L. var. *chinensis*), arugula (*Eruca sativa* Mill.), and mizuna (*B. rapa* L. var. *nipposinica*), and several Asian leafy cruciferous crops.

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Although *B. hilaris* can feed on all developmental stages of cruciferous plants, feeding on young seedling stages is most injurious (Reed et al. 2013). *Bagrada 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 (Huang et al. 2014b, Reed et al. 2013). Feeding injury on leaves appears as “starbursts” on leaf surfaces, and with time, starburst damage changes into necrotic tissue. Even when primarily cosmetic, this feeding injury on leaves can render leafy cruciferous crops such as choi, arugula, mizuna, kale, and several Asian greens 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, known as “blind head” (Palumbo and Natwick 2010). On some plants*, B. hilaris* feeding also stimulates the growth of multiple secondary shoots, which causes plants to produce unmarketable and undersized broccoli and cauliflower heads.

In the Central Coast of California, several Asian leafy cruciferous crops are grown in enclosed greenhouses, but these crops are still vulnerable to damage by *B. hilaris*. These crops include Chinese broccoli or gai-lan (*Brassica oleracea* L. var. *alboglabra* Bailey) and Chinese flowering cabbages or choy sums (*Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen and Lee) (Cantwell et al. 1996). In the Central Coast of California, large populations of *B. hilaris* begin to appear in crops beginning in mid-July and continuing through late November. However, there have also been isolated reports of high numbers of *B. hilaris* in enclosed greenhouses during late spring and early summer (April–June). Greenhouses increase temperatures for faster plant growth and shorter time to harvest. However, these environmental conditions appear conducive to the buildup of high populations inside greenhouses early in the year, before issues in the field become apparent. When these greenhouses are infested with *B. hilaris*, growers depend primarily on organically approved insecticides to control insect pests. Although organically approved insecticides provide a certain degree of *B. hilaris* control (Palumbo et al. 2015, S.V.J. unpubl. data), the levels of control are often unsatisfactory and the quality of the produce does not or barely meets the standards set by markets for leafy greens.

Crop-free periods are a potential cultural tactic that growers could use for *B. hilaris* management within enclosed greenhouses. More biological information on *B. hilaris* survival is needed to develop this tactic. Knowing how well *B. hilaris* survive without access to food and water will help determine if a crop-free period might be an option for *B. hilaris* management and which factors will influence its success. It has been shown that *B. hilaris* adults can live approximately 28 d when feeding on cruciferous hosts and actively reproducing (Colazza et al. 2004, Gunn 1918, Singh and Malik 1993). Their longevity can increase to 44–96 d when feeding only on noncruciferous hosts, during which time they do not reproduce (Hutson 1935). *Bagrada hilaris* can survive for extended periods if cruciferous, or even non-cruciferous, hosts are available because both can supply a degree of nutrition and, importantly, water. This is problematic because *B. hilaris* can complete a generation in 41 d at 24°C and $<$21 d at 35°C (Reed et al. 2013). Temperatures in enclosed greenhouses are typically higher than outside, helping *B. hilaris* population increase in even 3 weeks. The primary objective of this study was to determine the ability of nymph and adult *B. hilaris* to survive when deprived of food or water. This

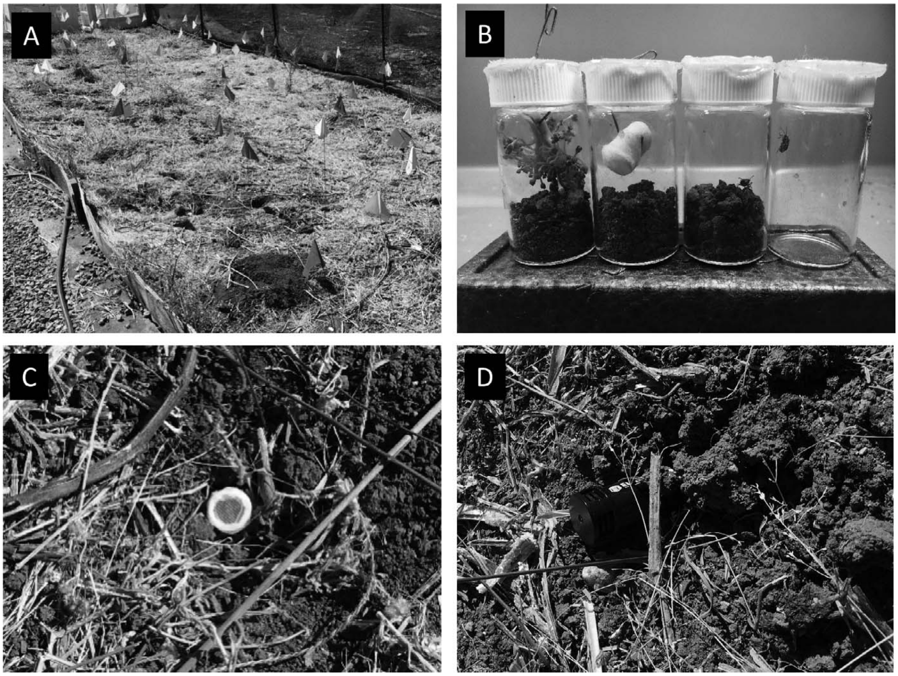

Fig. 1. (A) The site where the experiment was conducted; (B) treatments: food + soil, water + soil, soil only, and empty; (C) vial deployed in the ground with the ventilated top facing out; and (D) the temperature data logger deployed in the ground.

information will help develop crop-free periods as a viable cultural control tactic for *B hilaris* in enclosed greenhouses.

Materials and Methods

The experiments were conducted in a 3.6 × 10-m plot of bare ground at the University of California Cooperative Extension office in Salinas, CA, in 2016 (Fig. 1A). The *B. hilaris* used for the experiments were obtained from a laboratory colony. Adults for the laboratory colony were collected in 2015 from a broccoli field in San Lucas, CA. The insects were maintained in plastic containers of various sizes: 19.4 × 13.5 × 10.9 cm (L × W × H; 1.6 L); 24.4 × 18.2 × 15.8 cm (3 L); and 34.0 × 20.1 × 12.4 cm (5 L) (Really Useful Boxes, Normanton, West Yorkshire, United Kingdom), and 19.4 × 16.5 × 11.4 cm (small size) and 33.3 × 19.4 × 11.4 cm (large size) (Sterilite®, Townsend, MA). A fresh piece of organically grown broccoli head (approximately 20 g per container) was added every day as food source. Broccoli heads were purchased from the local produce market and were stored in refrigerator at 4°C. To allow air circulation, a single, large rectangular hole was cut in the lid and the hole was covered by "no-see-um" fabric mesh (Catalog No. 7250NSW, BioQuip, Rancho Dominguez, CA) which was weaved systematically forming a triangular shape (0.4 mm at base and 0.6 mm high). The rearing containers with *B. hilaris* were placed on a laboratory bench under continuous light provided by incandescent bulbs. The heat generated by the incandescent lamps maintained air temperature at approximately 39°C and relative humidity remained at approximately 20% in the containers.

The treatments for the experiment were (a) soil plus food; (b) soil plus water; (c) soil only; and (d) empty (no soil, water, or food; Fig. 1B). Soil was either included or excluded in the soil only and empty treatments to test for how presence of a substrate affects mortality. In the field, *B. hilaris* hide in the cracks and crevices in the soil when air temperatures are cooler (15–20°C; Reed et al. 2013). Trials were conducted in 20-ml scintillation vials (Wheaton Science Products, Millville, NJ). The polypropylene top of each vial was modified with a circular cut (approximately 1.5-cm diameter) and "no-see-um" nylon fabric mesh was glued along the cut to allow air flow. One *B. hilaris* was added to each vial. Ten replicates of each treatment were arranged in a completely randomized design in the field, with each vial constituting a replicate. Soil (Clear Lake Clay) was collected from a field in Salinas and oven-dried for 24 h before use in the experiments. Large soil aggregates were pulverized before oven-drying. Ten grams of pulverized soil was added to a scintillation vial for all treatments except the empty treatment. In the food treatment, a piece of organic broccoli (approximately 1 g) provided both water and nutrition to the *B. hilaris*. For the soil plus water treatment, a 2-cm-long dental wick (Tidi® products, Neenah, WI) dipped in tap water for 6 s provided moisture. Both broccoli and the water-soaked dental wick were suspended from the vial top. A 5-cm-long copper wire was twisted to form hooks at both ends and dangled from the "no-see-um" fabric at the top and held the broccoli or dental wick at the other end. All vials were buried vertically into the soil, with only the ventilated top exposed (Fig. 1C). The experiment was conducted with early-instar nymphs (2nd–3rd instars), late-instar nymphs (4th–5th instars), and both male and female adults. Two trials, with 10 replications per treatments each time, were conducted for each category of nymphs and adults (insect stage). The focus of this experiment was only to determine how well *B. hilaris* life stages survive when deprived of food and water. The trials were, therefore, terminated when the number of live *B. hilaris* reached 1 or 0 across the soil-only and empty treatments. The duration of the individual trials could vary among and within life stages. For 2nd–3rd instars, the first and second trials were deployed on 6 and 19 July, and terminated on 11 and 22 July, respectively. The first and second trials for 4th–5th instars were deployed on 18 and 25 July, and terminated on 24 July and 1 August. Trials for females were deployed on 1 and 10 August and terminated on 7 and 14 August for the first and second trials. For males, the first and second trials were deployed on 19 and 25 July and terminated on 23 and 30 July. Stages of nymphs were determined using the description in Taylor et al. (2015). The males and females were separated while they were copulating. Females are larger than males and this attribute helped to sort by sex for the experiments.

To measure the temperature of the soil at the surface, a temperature logger that logged temperature at 60-min intervals (EL-USB-2, Lascar Electronics, Inc., Erie, PA) was placed near soil surface where experiments were conducted (Fig. 1D). The temperature logger was at an approximately 15° angle from horizontal with the probe part covered by soil. Air temperature for the local area was obtained from

National Oceanic and Atmospheric Administration's Climate Data Online archive of temperature data. The daily minimum, maximum, and average temperatures were obtained for the Salinas Municipal Airport Station (GHCND:USW00023233; 36.6636°, −121.6081°).

For each insect stage, trial, and day, the effect of treatment on the likelihood insects were alive or dead was analyzed separately using nominal logistic regression (JMP 12.01; SAS Institute 2015). Analyses for each insect stage were conducted separately because trials were conducted separately and under varied environmental conditions. When there was a significant treatment effect for a given observation day, starvation treatments were compared by examining the odds ratio between two treatments, that is, probabilities of finding live individuals were compared between two treatments by examining a chi-square value of odds ratio at $\alpha = 0.05$.

Results

In the first trial with young nymphs, significantly fewer *B. hilaris* survived in the soil-only and empty treatments than in the food and water treatments by the fourth day of deployment (Table 1; Fig. 2A). In the second trial with young nymphs, significantly fewer *B. hilaris* were alive by the second day of deployment in the soil-only and empty treatments than in the food and water treatments (Table 1; Fig. 2B). By the third day, all individuals were dead in the soil-only and empty treatments. Significantly fewer *B. hilaris* survived in treatments with only water than with food. None of the *B. hilaris* died in the food treatment.

In the first trial with older nymphs, significantly fewer *B. hilaris* had survived in the soil-only treatment than in the food and water treatments on the third day (Table 1; Fig. 3A). At the fourth day, the number of live *B. hilaris* was significantly lower in the soil-only treatment than in the other treatments. None of the *B. hilaris* died in the food treatment. At the fifth and sixth days, there was no significant difference between soil-only and empty treatments. In the second trial with older nymphs, significantly fewer *B. hilaris* survived in the soil-only treatment than in the food treatment at third and fourth days of deployment (Table 1; Fig. 3B) and there was no significant difference between empty and soil-only treatments. At the seventh day of deployment, significantly fewer *B. hilaris* nymphs were alive in both the soil-only and empty treatments than in the food and water treatments.

In the first trial with adult female *B. hilaris*, significantly fewer insects were alive in the soil-only treatment than in the water and food treatments at the fifth day of deployment (Table 1; Fig. 4A) and there was no significant difference between empty and soil-only treatments. At the sixth day, the number of females alive was significantly lower in the soil-only and empty treatments than in the food and water treatments. In the second trial, the number of live females was significantly lower in both the soil-only and empty treatments than in the food treatment at third day after deployment (Table 1; Fig. 4B). At the fourth day, none of the females survived in the soil-only and empty treatments, while insects were still alive in the water and food treatments.

In the first trial with adult males, fewer *B. hilaris* survived in both the soil-only and empty treatments than in the water and food treatments at the second day of

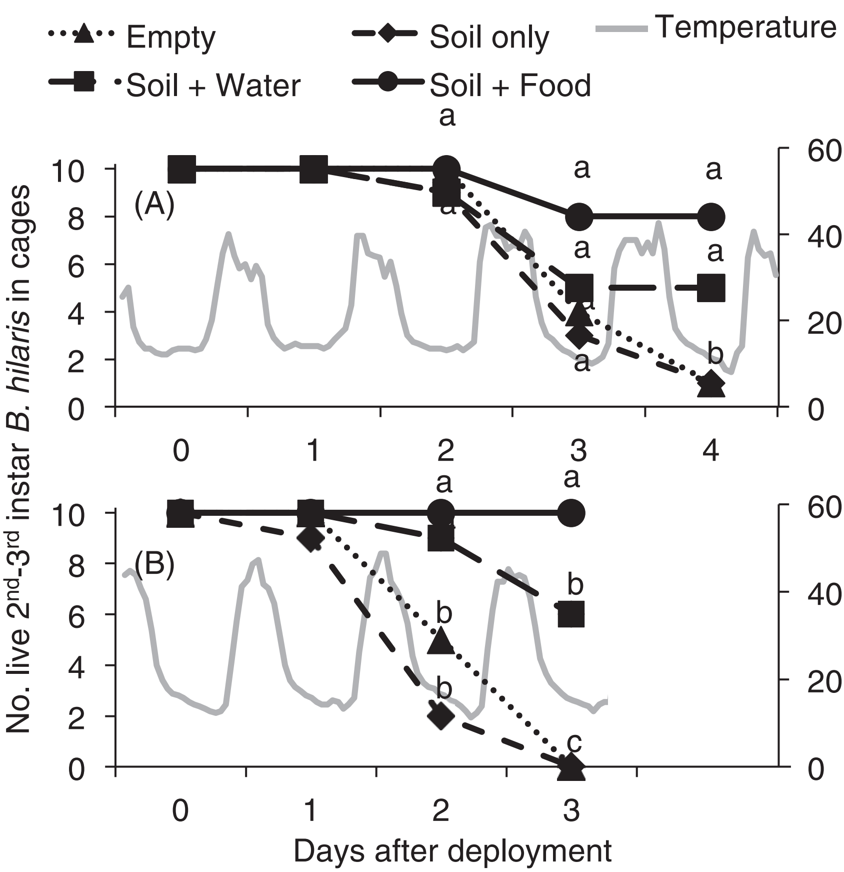

Fig. 2. Number of live 2nd–3rd instar *B. hilaris* (mean ± SE) in each starvation treatment in (A) trial 1, and (B) trial 2. Symbols with the same letters within each day postdeployment are not significantly different based on a comparison of χ^2 of odds ratio, $\alpha = 0.05$. Soil surface temperature is presented as a gray line. Area air temperature for trial 1: minimum range, 11–13°C; maximum range, 19–24°C; average, 16°C. For trial 2: minimum range, 11–13°C; maximum range, 22–24°C; average, 17°C.

deployment (Table 1; Fig. 5A). By the third day, none of the males survived in the soil-only and empty treatments, but a few males were alive in the other two treatments. In the second trial with males, all the males had died at the third day in the soil-only treatment and was significantly lower than in the other treatments (Table 1; Fig. 5B). At the fourth day, the number of live males in the empty treatment was not significantly different from soil-only and food treatments.

The temperatures in the ground fluctuated between <16 and >40°C. In the experiments with female *B. hilaris*, temperatures lower than 16°C were not recorded. The specific ground temperatures for each experiment are presented in Figs. 2–5.

Table 1. The effects of starvation treatments on the survival of various stages of *B. hilaris* in the unmanaged site in Salinas, CA.

B. hilaris stage/sex	Trial no.	Days after deployment	χ^2	df	*P*
2nd–3rd instar	1	1	—*	—	—
		2	2.8	3	0.411
		3	5.9	3	0.116
		4	16.1	3	0.001
	2	1	2.8	3	0.415
		2	21.4	3	$<$0.001
		3	40.4	3	$<$0.001
4th–5th instar	1	1	5.1	3	0.165
		2	6.6	3	0.088
		3	15.7	3	0.001
		4	21.1	3	$<$0.001
		5	11.6	3	0.009
		6	15.2	3	0.002
	2	1	5.1	3	1.165
		2	7.1	3	0.068
		3	8.5	3	0.037
		4	11.8	3	0.008
		5	13.4	3	0.004
		6	14.4	3	0.002
		7	19.3	3	0.001
Female	1	1	1.8	3	0.614
		2	0.4	3	0.942
		3	0.3	3	0.955
		4	3.9	3	0.289
		5	13.1	3	0.004
		6	15	3	0.002
	2	1	0.6	3	0.889
		2	1.86	3	0.599
		3	10.9	3	0.012
		4	32.9	3	$<$0.001

Table 1. Continued.

***B. hilaris* stage/sex**	**Trial no.**	**Days after deployment**	χ^2	**df**	***P***
Male	1	1	2.9	3	0.042
		2	21.4	3	<0.001
		3	40.4	3	<0.001
	2	1	7.8	3	0.049
		2	8.1	3	0.044
		3	12.1	3	0.007
		4	11.1	3	0.011
		5	17.2	3	<0.001

* None of the individuals died on this observation date.

Discussion

In our experiment, *B. hilaris* life stages survived on average 4.6 d without access to food or water. When *B. hilaris* had access to food and water, mortality was very low. This suggests that if a population of *B. hilaris* has no access to food and water and is trapped in the enclosed greenhouse, the population can crash within a week if temperatures are similar to those in our experiment. It must also be considered that *B. hilaris* may lay eggs after food and water are removed. Once they hatch, the nymphs might live for a few more days, although they are very susceptible to desiccation. Therefore, a crop-free period of 2 weeks should provide sufficient time for trapped *B. hilaris* to die in the enclosed greenhouse. Previous research has shown that *B. hilaris* individuals starved for 2 or 3 d tend to walk longer distances and at faster paces than nonstarved individuals (S.V.J. unpubl. data). This suggests that they would seek out food and water sources within the enclosed greenhouse and that some individuals could leave the greenhouse through any vents or gaps if present.

Populations of *B. hilaris* develop on cruciferous weed species (Reed et al. 2013). In the Central Coast of California, these species include shortpod mustard (*Hirschfeldia incana* (L.) Lagreze-Fossat) and perennial pepperweed (*Lepidium latifolium* L.). They are common in the agricultural landscape, including in areas immediately adjacent to the crop fields or in unmanaged lands (Zalom et al. 2012, I.M.G. unpubl. data). When growers establish a new cruciferous crop with seeds or seedlings, the risk of *B. hilaris* infestation to the newly planted crop is a concern. Declining quality of the weed hosts likely triggers *B. hilaris* movement to find healthy plant hosts. *Bagrada hilaris* can survive feeding on even noncruciferous hosts in the absence of cruciferous hosts (Hutson 1935). Our results show that dispersing *B. hilaris* need to find a food or water source such as cruciferous or noncruciferous hosts within a week. Even if older nymphs are able to survive longer than adults,

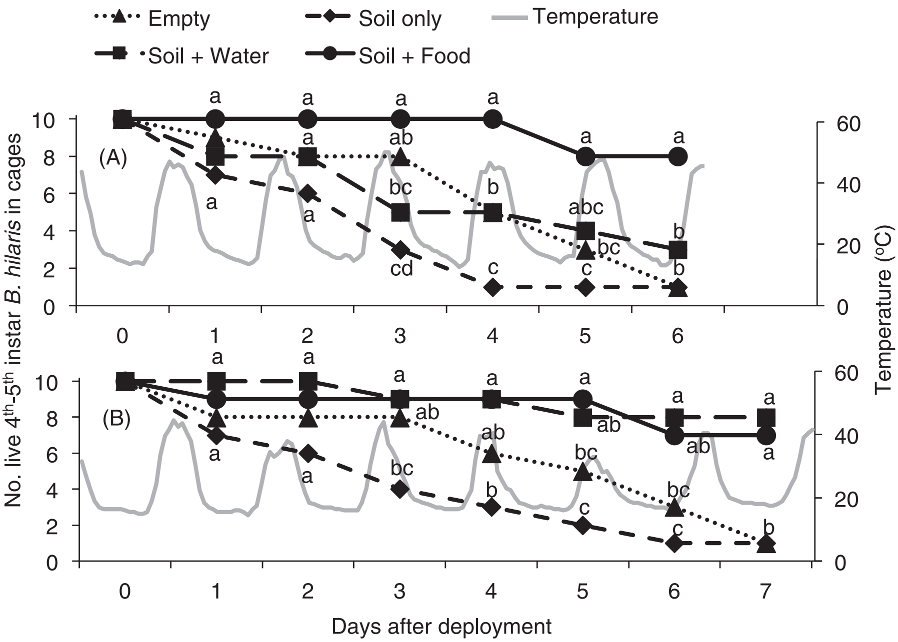

Fig. 3. Number of live 4th–5th instar (mean ± SE) *B. hilaris* in various starvation treatments in (A) trial 1, and (B) trial 2. Symbols with the same letters within each day postdeployment are not significantly different based on a comparison of χ^2 of odds ratio, $\alpha = 0.05$. Soil surface temperature is presented as a gray line. Area air temperature for trial 1: minimum range, 11–14°C; maximum range, 21–24°C; average, 17°C. For trial 2: minimum range, 12–15°C; maximum range, 18–23°C; average, 15°C.

nymphs likely experience a greater risk of death than adults because they are restricted to walking and cannot fly longer distances.

When food deprived, *B. hilaris* survive solely on their stored energy reserves, and their survival depends on effectively exploiting these reserves before finding a new source of food. A combination of several factors likely influences how these stored reserves are used up, including movement activity, exposure to extreme temperatures, and exposure to solar radiation. First, movement such as walking and flight would easily exhaust the energy reserves. When bugs are deprived of food, these activities are likely to be more pronounced (S.V.J. unpubl. data). In this study, however, *B. hilaris* were enclosed in glass vials for the duration of the experiment, which may have reduced movement and increased survival compared with less controlled conditions. Our results show that *B. hilaris* survival tended to be lower in the soil-only treatment than in the empty treatment. Perhaps, the greater mortality was caused by quicker loss of stored reserves because of movement searching for food within the loose soil in the vial. In the empty treatment, it is likely that the smooth surface of the glass vial (with no soil) helped reduce movement and

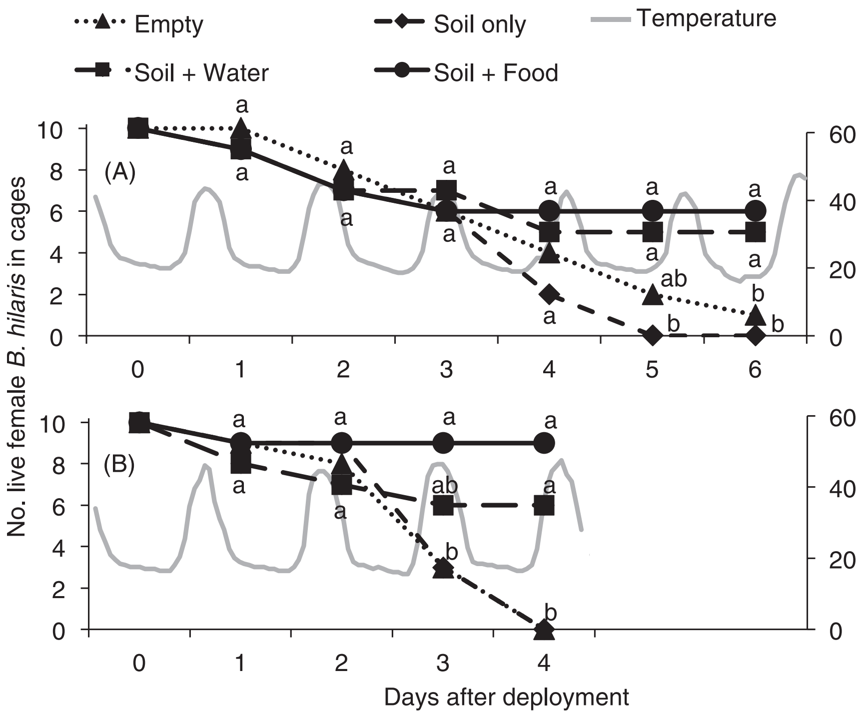

Fig. 4. Number of live female *B. hilaris* (mean ± SE) in various starvation treatments in (A) trial 1, and (B) trial 2. Symbols with the same letters within each day postdeployment are not significantly different based on a comparison of χ^2 of odds ratio, $\alpha = 0.05$. Soil surface temperature is presented as a gray line. Area air temperature for trial 1: minimum range, 12–15°C; maximum range, 19–21°C; average, 16°C. For trial 2: minimum range, 12–13°C; maximum range, 20–24°C; average, 16°C.

conserve energy reserves for little longer than in the soil-only treatment. Second, previous research has shown that exposure to temperatures <16°C and >40°C could affect development of nymphs (Deep et al. 2014) and that high relative humidity has negative effects on their survival (Huang et al. 2013, Nagar et al. 2011). Finally, in the greenhouse, solar radiation is likely lower than outside, which could influence survival. In addition, *B. hilaris* may be able to find shaded spots in a greenhouse. In this study, although trials were conducted outside, which exposed the insects to environmental conditions, *B. hilaris* were introduced in glass vials with nylon screen on the top which might have reduced the intensity of prevailing extreme conditions such as solar radiation and high daytime temperatures.

Survival of *B. hilaris* during a host-free period in an enclosed greenhouse will depend on the availability of water. Even when deprived of their cruciferous hosts, *B. hilaris* can survive beyond a week when they have access to a water source, and any noncruciferous living plant can serve this purpose (Hutson 1935). This is in agreement with our results. Within the time frame of our experiment, survival of *B.*

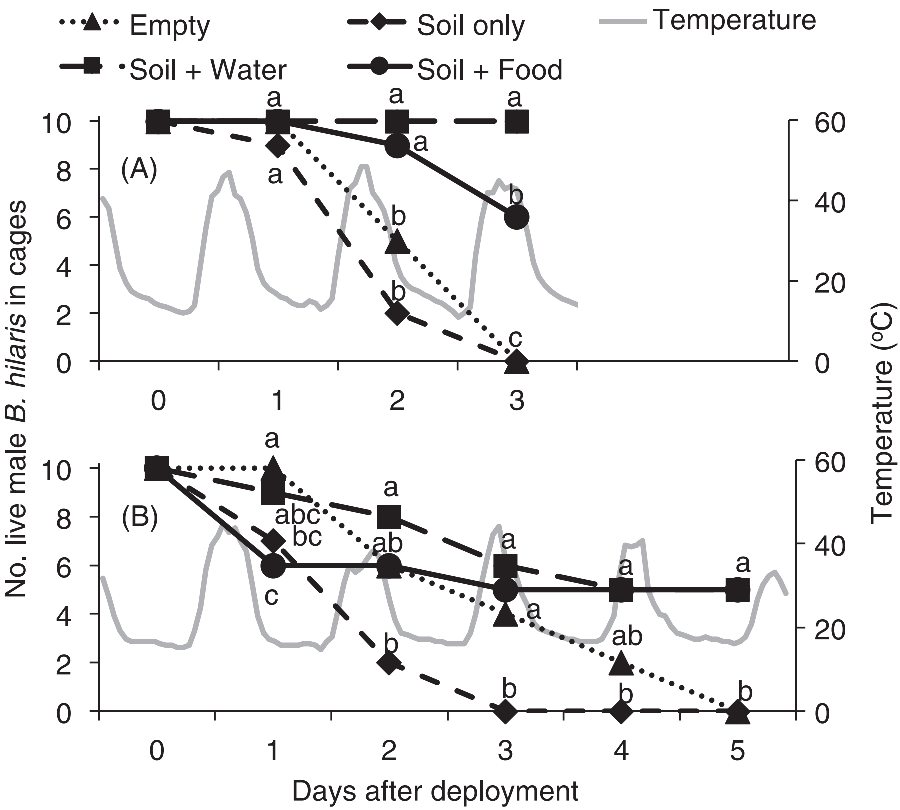

Fig. 5. Number of live male *B. hilaris* (mean ± SE) in various starvation treatments in (A) trial 1, and (B) trial 2. Symbols with the same letters within each day postdeployment are not significantly different based on a comparison of χ^2 of odds ratio, $\alpha = 0.05$. Soil surface temperature is presented as a gray line. Area air temperature for trial 1: minimum range, 14–15°C; maximum range, 20–22°C; average, 16°C. For trial 2: minimum range, 12–15°C; maximum range, 21–24°C; average, 17°C.

hilaris individuals that have access only to water was not different from those that had access to food. At a given time, several noncruciferous crops such as Chinese chives (*Allium tuberosum* Rottler ex Sprengel) or cucurbit crops are grown in greenhouses (Cantwell et al. 1996). *Bagrada hilaris* could persist by feeding on these crops and remain unnoticed until cruciferous crops become available, at which point they could damage plants and grow their populations. *Bagrada hilaris* could also survive if they have access to any nonplant water source. In an enclosed greenhouse, *B. hilaris* may be able to easily find moist surfaces or water-soaked soil due to a leaky faucet or a faulty sprinkler system. To limit the survival of *B. hilaris* between plantings of cruciferous crops, both incidental water and non–host plants will need to be avoided to prevent availability of water.

In conclusion, our results show that *B. hilaris* population can crash in 2 weeks if existing *B. hilaris* life stages and newly emerged nymphs do not have access to food or water by implementing a crop-free period. Unfortunately, *B. hilaris* do not need to find and feed on cruciferous plants to survive. Instead, they can survive for a period of time with only a water source, which could take the form of a noncruciferous plant. Typically, enclosed greenhouse growers produce Asian vegetables year-round and, thus, plant cruciferous or other leafy crops back-to-back or treat them as perennial crops and harvest foliage for an extended period. Securing the enclosed greenhouse adequately will be necessary to prevent influx of *B. hilaris* individuals during the crop-free period. Moreover, this cultural control tactic could be integrated with other tactics such as vacuuming or insecticide spray to improve sustainable management of *B. hilaris* in enclosed greenhouses.

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