

Host selection behavior of *Bagrada hilaris* (Hemiptera: Pentatomidae) on commercial cruciferous host plants



Ta-I. Huang^a, Darcy A. Reed^b, Thomas M. Perring^b, John C. Palumbo^{a,*}

^aYuma Agricultural Center, Department of Entomology, University of Arizona, 6425 W. 8th St, Yuma, AZ 85364, USA

^bDepartment of Entomology, University of California, Riverside, 900 University Ave., Riverside, CA 92521, USA

ARTICLE INFO

Article history:

Received 11 September 2013

Received in revised form

15 January 2014

Accepted 19 January 2014

Keywords:

Host preference

Stink bug

Bagrada hilaris

Pest management

Cruciferous

ABSTRACT

A series of host-choice tests were conducted under greenhouse conditions to evaluate the host selection behavior of the Bagrada bug, *Bagrada hilaris* on commercial cruciferous seedlings. In addition, a separate choice test was conducted to investigate the selection behavior of *B. hilaris* adults for broccoli plants of various growth stages: cotyledon, 1-leaf, 2-leaf, and 4-leaf plant stages. In comparing host selection among the commercial seedlings, observations on host attractiveness, host acceptance and host susceptibility of the cruciferous cultivars to *B. hilaris* adults were measured by recording numbers of adults per plant, the time at which feeding damage was first observed, and the time plant mortality occurred for each cultivar, respectively. Results showed that significantly more adult *B. hilaris* were attracted to a commercial radish cultivar than all other hosts, followed by red and green cabbage. Measurements of host acceptance varied among the cruciferous cultivars, however in terms of feeding damage, alyssum, arugula and broccoli appeared to be relatively less acceptable hosts for *B. hilaris*. Similarly, all host plants were susceptible to *B. hilaris* feeding damage and plant mortality varied among cultivars. In the broccoli growth-stage trials, experiments were designed in a similar fashion except that the experiment was conducted in plastic cages. A greater number of *B. hilaris* adults were attracted to 4-leaf-stage broccoli than other growth stages, but feeding damage was observed more quickly on cotyledon-stage plants. The implications of these findings for developing alternative pest management approaches for *B. hilaris* in commercial cruciferous crops are discussed.

© 2014 The Authors. Published by Elsevier Ltd. Open access under [CC BY-NC-ND license](http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Bagrada bug, *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae), is an invasive stink bug species that is native to Africa, India, and Asia (Howard, 1906). It was first discovered in North America in Los Angeles, CA, in 2008 and has recently become established in the desert southwest of the United States (http://cirs.ucr.edu/bagrada_bug.html). It is now considered a serious economic pest of a variety of cruciferous vegetable crops grown during fall and winter months in the agricultural valleys of Arizona and southern California (Palumbo and Natwick, 2010). A recent survey of growers from Yuma, AZ and the Imperial Valley, CA estimated

that greater than 90 percent of broccoli acreage planted in 2010 and 2011 was infested with *B. hilaris* at some point in the growing season, and on average, this resulted in stand losses and plant injury exceeding 5% and 10% in cauliflower and broccoli crops, respectively (<http://cals.arizona.edu/crops/vegetables/advisories/more/insect83.html>). The potential economic impact of *B. hilaris* on the western vegetable industry could be significant considering that the production of cruciferous crops in Arizona and California was collectively valued at over \$1 billion in 2011 (CDFA, 2012; USDA NASS, 2012).

Bagrada hilaris was reported as a pest of oilseeds and vegetables in India, particularly of cauliflower (Verma et al., 1993; Panizzi, 1997) and mustard (Joshi et al., 1989; Lal and Singh, 1993; Vekarta and Patel, 1999). It was also reported on wheat (Rawat and Singh, 1980) and maize (Rizvi et al., 1986) in the Old World. In the Western Hemisphere, *B. hilaris* has been reported to feed on broccoli, cauliflower, cabbage and other cruciferous vegetable crops (Palumbo and Natwick, 2010; Huang et al., 2013) as well as various grasses, flowers and legumes (Reed et al., 2013). To date, host preferences of *B. hilaris* on cruciferous vegetables are not well

* Corresponding author.

E-mail addresses: jpalumbo@ag.arizona.edu, jpalumbo@cals.arizona.edu (J.C. Palumbo).

understood. However, studies of host selection behavior in related phytophagous pentatomids have been reported in the harlequin bug, *Murgantia histrionica* (Hahn) (Sullivan and Brett, 1974; Wallingford et al., 2013) and rice stink bug, *Oebalus pugnax* (F.) (Naresh and Smith, 1984); as well as phenological-stage preference in southern green stink bug, *Nezara viridula* (L.), brown stink bug, *Euschistus servus* (Say) (Huang and Toews, 2012), and redbanded stink bug, *Piezodorus guildinii* Westwood (Molina and Trumper, 2012).

Seedling crops are thought to be highly susceptible to direct feeding damage by *B. hiliaris* on cotyledons, newly emerged leaves and apical meristems (Palumbo and Natwick, 2010). Excessive feeding damage to apical meristems can result in destruction of the terminal growing points leading to either adventitious bud break (e.g., cabbage plants with multiple, unmarketable heads), or plants with no reproductive head being formed (e.g., broccoli with no crowns) (Palumbo and Natwick, 2010). Numerous cruciferous cultivars are commercially grown in the agricultural valleys of Arizona and southern California including broccoli, cauliflower, and cabbage types that produce marketable crowns or heads, as well as a number of leafy cultivars such as kale, mustards and arugula used for salads. Additionally, radish is commonly grown for its small bulbs. Currently, vegetable growers in Arizona and California rely heavily on frequent insecticide applications to control *B. hiliaris* adult infestations on seedling cruciferous crops (<http://cals.arizona.edu/crops/vegetables/advisories/more/insect83.html>; Palumbo, 2012a, 2012b). Alternative management tactics for *B. hiliaris* have not yet been developed, but will be important if economic production of cruciferous vegetables is to be sustainable.

An understanding of the host preferences of *B. hiliaris* adults may allow for the development of non-chemical alternatives such as trap cropping (Shelton and Badenes-Perez, 2006), as well as provide an useful information on its chemical ecology that could be used in developing integrated pest management programs (Metcalfe and Kogan, 1987). For instance, plant volatiles such as glucosinolate-derived volatiles in crucifers play the functions of plant defense or herbivore attraction (Rohloff and Bones, 2005). Non-volatiles such as phytoalexins and phytoanticipins produced after glucosinolate hydrolysis by myrosinases play crucial ecological roles in protecting plants against various pests (Ahuja et al., 2010). Therefore, the main objectives in our study were to investigate the host selection behavior of adult *B. hiliaris* on the cotyledon stage seedling plants of commercial crucifers, and to evaluate whether the adults have a preference among phenological growth stages of young broccoli plants.

2. Materials and methods

2.1. Plant preparation and insect colony

Eleven commercial cruciferous vegetable cultivars were selected for evaluation in the host preference tests: arugula/rocket, broccoli, green cabbage, red cabbage, napa cabbage, cauliflower, kale, Kohlrabi, green mustard, red mustard, and radish (species listed in Table 1). These cultivars were chosen based on their commercial importance and all are widely grown in southwest desert area. In addition, two non-crop cruciferous plant species, sweet alyssum and stock (species listed in Table 1), were included in the host selection tests as they are popular ornamental landscape species. Infestations of *B. hiliaris* and associated feeding damage have been reported on all of these cultivars in the Yuma Valley, AZ and Coachella Valley, CA. All plant species used in the tests were direct-seeded into 5 × 5 cm² pots for germination with a commercial-grade potting soil (Miracle-Gro[®]) and irrigated daily in the greenhouse. All adult insects used for preference studies were

Table 1

Host plant species used in the *B. hiliaris* host preference tests.

Host species	Common name	Cultivar
<i>Brassica oleracea</i> var. <i>italica</i> Plenck	Broccoli	Emerald Crown
<i>Brassica oleracea</i> var. <i>capitata</i> L.	Green Cabbage	Gazelle F1
<i>Brassica oleracea</i> var. <i>capitata</i> L.	Red Cabbage	Ruby Perfection F1
<i>Brassica oleracea</i> var. <i>botrytis</i> L.	Cauliflower	Ponderet F1
<i>Brassica oleracea</i> var. <i>acephala</i> DC.	Kale	Winterbor F1
<i>Brassica oleracea</i> var. <i>gongylodes</i> L.	Kohlrabi	Winner F1
<i>Brassica rapa</i> var. <i>pekinensis</i> (Lour.)	Napa Cabbage	Minute F1
<i>Brassica juncea</i> var. <i>rugosa</i> (Roxb.)	Green Mustard	Green Wave
<i>Brassica juncea</i> var. <i>rugosa</i> (Roxb.)	Red Mustard	Red Giant
<i>Eruca sativa</i> Mill.	Arugula	Sylvetta OG
<i>Raphanus sativus</i> var. <i>sativus</i> L.	Radish	Rover F1
<i>Lobularia maritime</i> L. (Desv.)	Sweet Alyssum	Carpet of Snow
<i>Matthiola incana</i> (L.) W.T. Aiton	Stock	Quartet Mix

obtained from a *B. hiliaris* colony maintained at the Yuma Agricultural Center where all life stages were provided with organic broccoli heads (Earthbound Farm, Salina, CA) and occasionally supplemented with dry alyssum plants. Mating pairs (sexually mature) were selected from the colony and starved for 24 h before being exposed to host plants in the experiments. Insects were used only once for each replicate.

2.2. Study arena and environment

All preference studies were carried out in an air-conditioned greenhouse at the Yuma Agricultural Center located in Yuma, AZ. Average ambient temperatures in the greenhouse were between 26.8 ± 6.5 °C (September) and 22.7 ± 7.8 °C (October) in fall 2012 studies with a photoperiod of 12:12 (L:D) h. In spring 2013 studies, average temperatures were between 21.4 ± 7.9 °C (April) and 24.7 ± 4.3 °C (May) with a photoperiod of 14:10 (L:D) h. A 6.15 L plastic container, 30 cm in diameter (Progressive[®]), was used as the study arena. Each container with plants were placed in a white mesh cage (BugDorm, BioQuip, Rancho Dominguez, CA) (Dimensions: L60 × W60 × H60 cm) for the host-preference test. Plants were placed in a hand-built plastic container (Dimensions: L56 × W38 × H61 cm) for the growth-stage preference test. After transplanting, plants were allowed to grow for 24 h in a similar location in the greenhouse before insects were released. Temperatures were monitored hourly using a data logger (HOBO Pendant, Onset Computer Corp., Cape Cod, MA) placed inside the BugDorm.

2.3. Host-Selection choice tests among 12 cruciferous cultivars

Six trials of choice tests were conducted in fall 2012 to examine the host preference of *B. hiliaris* and the associated host plant responses to *B. hiliaris* feeding. In the first 3 trials, 2 cotyledon-stage plants from each tested host species (arugula, sweet alyssum, broccoli, green cabbage, red cabbage, napa cabbage, cauliflower, kale, kohlrabi, green mustard, red mustard, and radish) were transplanted into each arena in a circular arrangement along the perimeter of the arena (Fig. 1a). Each host was positioned approximately 5 cm away from adjacent host and from the outer edge of arena, and 5.75 cm away from the center Petri dish. For each of the 12 hosts, pair cotyledon-stage plants were placed about 1 cm apart from each other. The planting sequence and position of each host in the arena were randomly arranged. Additional soil was added to the arena to even the surface after transplanting. Six mating pairs of *B. hiliaris* were then released from a Petri dish (8.5 cm) that was placed in the center of the arena at 10:00 am on the following day. Host selection behavior was examined by measuring the following variables: *host attractiveness* (the number of *B. hiliaris* observed on

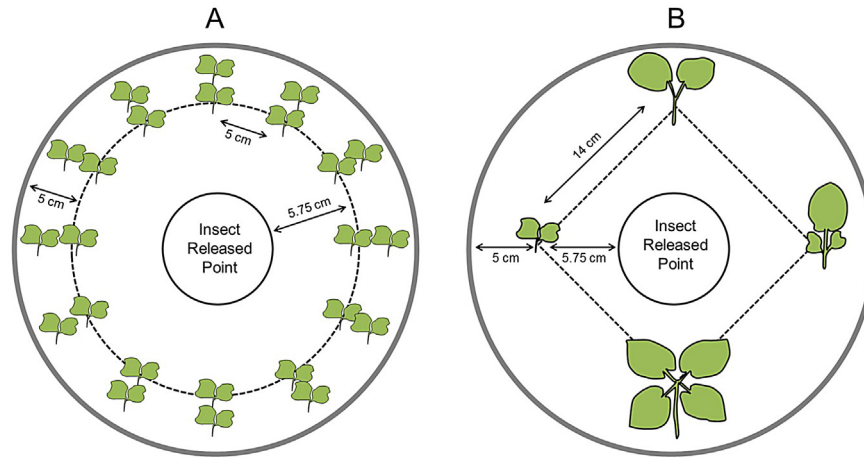


Fig. 1. Diagrams of multiple-choice experimental arenas to test *B. hiliaris* host preference (A) and plant stage preference (B).

each host); *host acceptance* (the time at which the first feeding damage was observed); and *host susceptibility* (the time at which plant mortality occurred) (Schoonhoven et al., 2005). All measurements were recorded at 0.5 h (10:30 am), 1 h (11:00 am), 2 h (12:00 pm), 4 h (2:00 pm), 8 h (6:00 pm), 12 h (10:00 pm), 24 h (10:00 am), and 48 h (10:00 am) after the release of the mating pairs of *B. hiliaris* into the arena. Observations of adults and plant damage/mortality were made through the transparent mesh outside the screened cage. In the last 3 trials, green mustard was replaced by stock, while the remaining 11 host plants remained the same. Experiments were evaluated the same way as described above. In each trial, 4 arenas were evaluated for a total of 24 replicates for the entire experiment. Nighttime observations (12 h) were conducted with the assistance of a fluorescent head lamp as described in Huang et al. (2013). Symptoms of feeding damage were characterized as small visible white blotches on the cotyledons (Palumbo and Natwick, 2010). Plant mortality was determined when both sides of the cotyledon were completely wilted/desiccated and there was no sign of an actively growing apical meristem. Experiments were analyzed as a randomized complete block design with each separated trial considered a block and each arena within the block was a replicate. Since ‘green mustard’ was replaced by

‘stock’ in the last 3 host-preference trials, data for the last 3 trials were analyzed separately from those of the first 3 trials. Analysis of variance ANOVA (PROC GLIMMIX; SAS Institute, 2009) was performed to test the effects of host species on attractiveness, host acceptance, and host susceptibility. Treatment means were separated with LSMEANS test at $P < 0.05$. Adjustments for multiple comparisons were made according to Bonferroni correction (SAS Institute, 2009). The raw data are presented in the tables, but these data were subjected to logarithmic transformation (Zar, 1999) and arcsine transformation (percentage only) before analyses.

2.4. Growth stage-selection choice tests on broccoli

Six trials of choice tests were conducted in spring 2013 to examine *B. hiliaris* host selection behavior among growth stages of young broccoli plants. Broccoli was chosen among all the cultivars for these tests because it is the most economically important cruciferous crop grown in Arizona and California for both conventional and organic production (<http://www.nass.usda.gov>). In each trial, four growth stages of young broccoli plants (cotyledon, 1-leaf, 2-leaf, and 4-leaf) were transplanted into the arena in a square arrangement (Fig. 1b). Plants were positioned approximately 14 cm

Table 2

Mean total number (\pm SE) of *B. hiliaris* adults observed per host, time (\pm SE) to observation of first feeding damage and of plant mortality in a host-preference test in greenhouse. Experiments were conducted in fall 2012 with 12 replicates.

Host plant	Host Attractiveness ^a	Host Acceptance ^b	% Plants with feeding damage (n = 12)	Host Susceptibility ^c	% Plant mortality (n = 12)
Alyssum	3.3 \pm 0.9def	7.6 \pm 1.8abc	100	20.0 \pm 4.0	25.0
Arugula	1.1 \pm 0.5g	12.0 \pm 4.3a	33.3	8.0	8.3
Broccoli	2.9 \pm 0.5def	7.8 \pm 1.3 ab	83.3	12.0 \pm 4.0	33.3
Cabbage Green	4.9 \pm 0.9bcd	3.4 \pm 1.0d	100	7.2 \pm 0.8	41.7
Cabbage Red	6.8 \pm 1.8b	2.7 \pm 0.8d	100	5.5 \pm 1.5	33.3
Cabbage Napa	1.6 \pm 0.5 fg	5.4 \pm 1.2bcd	58.3	–	0.0
Cauliflower	3.8 \pm 0.9cde	4.1 \pm 1.1d	100	9.3 \pm 1.9	75.0
Kale	5.3 \pm 0.7bc	5.2 \pm 0.9bcd	100	17.6 \pm 8.5	41.7
Kohlrabi	2.4 \pm 0.5efg	5.6 \pm 1.4bcd	66.7	12.0 \pm 6.1	25.0
Mustard Green	2.3 \pm 0.6efg	5.5 \pm 2.4bcd	75.0	12.8 \pm 4.6	41.7
Mustard Red	2.4 \pm 0.6efg	4.4 \pm 0.8cd	91.7	9.3 \pm 3.0	50.0
Radish	9.7 \pm 1.3a	3.3 \pm 0.5d	100	16.8 \pm 4.1	83.3
F	10.60	2.74		1.12	
P	<0.0001	0.0036		0.373	

^a Total numbers of adults per host plant.

^b Time at which feeding damage was first observed (h); hours shown apply only to those plants in which damage or mortality occurred.

^c Time at which plant mortality first occurred (h); hours shown apply only to those plants in which damage or mortality occurred. Means in columns followed by the same letters are not significantly different ($P < 0.05$, LSMEANS test).

Table 3
Mean total number (\pm SE) of *B. hiliaris* adults observed per host, time (\pm SE) to observation of first feeding damage and of plant mortality in a host-preference test in greenhouse. Experiments were conducted in fall 2012 with 12 replicates.

Host plant	Host attractiveness ^a	Host acceptance ^b	% Plants with feeding damage (n = 12)	Host susceptibility ^c	% Plant mortality (n = 12)
Alyssum	3.3 \pm 0.7cde	11.9 \pm 5.2abc	83.3	–	0
Arugula	3.0 \pm 0.8de	8.5 \pm 1.9abc	100	29.0 \pm 11.0abc	33.3
Broccoli	5.8 \pm 0.7b	9.6 \pm 2.6abc	100	26.4 \pm 5.9abc	41.7
Cabbage Green	5.7 \pm 1.1b	3.9 \pm 0.7cd	91.7	7.8 \pm 1.4d	41.7
Cabbage Red	6.4 \pm 1.1b	4.8 \pm 1.2cd	100	22.0 \pm 7.1abc	58.3
Cabbage Napa	2.1 \pm 0.4de	11.7 \pm 2.3a	100	16.0 \pm 4.0bcd	25
Cauliflower	4.9 \pm 0.6bc	3.3 \pm 0.9d	100	9.6 \pm 4.3d	83.3
Kale	5.5 \pm 0.6b	6.5 \pm 0.9abcd	100	39.0 \pm 9.0a	33.3
Kohlrabi	2.1 \pm 0.4de	10.4 \pm 2.5 ab	100	20.0 \pm 6.5bcd	50
Mustard Red	3.3 \pm 0.4cd	5.6 \pm 1.0bcd	100	22.0 \pm 4.9bc	91.7
Stock	1.8 \pm 0.7e	14.6 \pm 6.2a	58.3	29.7 \pm 6.9 ab	58.3
Radish	9.7 \pm 1.2a	4.2 \pm 0.7cd	100	11.4 \pm 1.6cd	83.3
F	10.14	2.74		3.07	
P	<0.0001	0.0033		0.0034	

^a Total numbers of adults per host plant.

^b Time at which feeding damage was first observed (h); hours shown apply only to those plants in which damage or mortality occurred.

^c Time at which plant mortality first occurred (h); hours shown apply only to those plants in which damage or mortality occurred. Means in columns followed by the same letters are not significantly different ($P < 0.05$, LSMEANS test).

away from each other and 5 cm away from the edge of arena. The planting sequence and position of each plant in the square were randomly assigned. Soil was added to even the surface after transplanting. Six mating pairs of *B. hiliaris* were then released from the Petri dish placed in the center of the arena at 10:00 am the next day. Observations were made through the transparent mesh outside the plastic container. Host attractiveness, host acceptance and host susceptibility were recorded as described above. In each trial, 4 arenas were evaluated for a total of 24 replicates in this study. Data were analyzed using analysis of variance ANOVA (PROC GLIMMIX; SAS Institute, 2009) followed the same fashion described above.

3. Results

3.1. Host selection by adult *B. hiliaris* among crucifers

The overall number of *B. hiliaris* observed across 48 h was significantly different among hosts in both first and last 3 trials with no block effects (Tables 2 and 3). Radish was the most attractive host for *B. hiliaris* in the first 3 trials, followed by red cabbage (Table 2). In the last 3 trials, more *B. hiliaris* were observed on radish than any other host. Numbers of individuals observed in broccoli, green and red cabbage, cauliflower, and kale were not significantly different from each other, but higher than on the other cultivars (Table 3). There were significant differences among cultivars in host acceptance in the first 3 trials ($F = 2.74$; $df = 11, 107$;

$P = 0.0036$) and the last 3 trials ($F = 2.74$; $df = 11, 122$; $P = 0.0033$) without block effects (Tables 2 and 3). In the first 3 trials, feeding damage was first observed on green and red cabbage, cauliflower, and radish significantly sooner than on arugula, broccoli, and alyssum, but did not differ from Kohlrabi, napa cabbage, kale, green and red mustard (Table 2). In the last 3 trials, feeding damage was first observed in cauliflower but overall the average time first feeding damage was observed did not differ significantly from kale, red mustard, radish, green and red cabbage (Table 3). When examining host susceptibility, plant mortality was variable and there was no significant difference among the cultivar hosts in the first 3 trials ($P = 0.3733$). However, there were significant differences among hosts in the last 3 trials ($F = 3.07$; $df = 10, 59$; $P = 0.0034$) without block effects. Plant mortality in the green cabbage and cauliflower occurred sooner than in all other hosts except for Kohlrabi, napa cabbage, and radish (Tables 2 and 3).

3.2. Selection by adult *B. hiliaris* among broccoli growth stages

The extended leaf height, the distance from the soil surface to the tip of the tallest extended leaf of each stage used in our experiment, was 2.22 ± 0.08 , 3.85 ± 0.11 , 7.20 ± 0.13 , and 9.59 ± 0.24 cm in cotyledon, 1-leaf, 2-leaf, and 4-leaf stage plants, respectively. Overall, when summed across observation intervals, significantly more *B. hiliaris* adults were observed on 4-leaf stage plants ($F = 37.52$; $df = 3, 87$; $P < 0.0001$), but there was a block effect ($F = 5.49$; $df = 5, 87$; $P = 0.0002$) (Table 4). Further analyses of

Table 4
Mean total number (\pm SE) of *B. hiliaris* observed per plant stage, and mean time (\pm SE) to observation of feeding damage and of plant mortality in young broccoli plants in a growth-stage preference test in greenhouse. Experiments were conducted in spring 2013 with 24 replicates.

Host plant	Host attractiveness ^a	Host acceptance ^b	% Plants with feeding damage (n = 12)	Host susceptibility ^c	% Plant mortality (n = 12)
Cotyledon	5.3 \pm 0.8c	3.7 \pm 0.8b	100	19.2 \pm 2.9	54.2
1-leaf	8.5 \pm 1.6b	13.2 \pm 3.7a	95.8	21.3 \pm 3.7	45.8
2-leaf	9.8 \pm 1.9b	10.4 \pm 2.8a	95.8	–	0.0
4-leaf	24.9 \pm 2.0a	7.8 \pm 1.0a	100	24	4.2
F	37.52	3.32		0.23	
P	<0.0001	0.0236		0.8006	

^a Total numbers of adults per host plant.

^b Time at which feeding damage was first observed (h); hours shown apply only to those plants in which damage or mortality occurred.

^c Time at which plant mortality first occurred (h); hours shown apply only to those plants in which damage or mortality occurred. Means in columns followed by the same letters are not significantly different ($P < 0.05$, LSMEANS test).

the block effect revealed that 4-leaf stage plants attracted the most *B. hiliaris* adults in trial 1, 2, 3 and 6, but did not differ from the 2-leaf stages in trial 4 and 5. In terms of host acceptance, cotyledon-stage plants had the shortest time to appearance of feeding damage among all stages ($F = 3.32$; $df = 3, 85$; $P = 0.0236$), but there was a block effect ($F = 3.46$; $df = 5, 85$; $P = 0.0068$) (Table 4). Further analyses of the block effect revealed that the time required for the appearance of first feeding damage in cotyledon stage plants was not significantly different from other stages in trial 2 and 5. In addition, damage symptoms on 4-leaf plants were only observed on newer growing leaves (young leaves on node positions 1 and 2 from the apical meristem). Host susceptibility varied considerably, but there was no significant difference among growth stages ($F = 0.15$; $df = 2, 18$; $P = 0.8006$). Percent plant mortality ($n = 12$) was very low for the 2- and 4-leaf stage broccoli plants relative to the smaller seedling stages (Table 4).

4. Discussion

This study examined the host attractiveness, acceptance and susceptibility of *B. hiliaris* adults when given the choice of 12 cruciferous host plants. Results from these studies provide the first documented report of host selection behavior of *B. hiliaris* for important commercial cruciferous cultivars. Cotyledon-stage plants were selected for the host choice tests because it is believed that the seedling plant is highly attractive to *B. hiliaris* adults, as well as very susceptible to their feeding (Huang et al., 2013). Furthermore, field observations in the desert growing regions of the southwestern U.S. have shown that *B. hiliaris* adults often invade commercial fields when crops are beginning to emerge, causing serious damage to cotyledons, newly emerged leaves and apical meristems (Palumbo and Natwick, 2010; Huang et al., 2013). Because the leaf area of individual cotyledons of the host species differed, some variation in the size of cotyledons among the host cultivars was unavoidable. However, cotyledon size did not appear to affect host selection by *B. hiliaris* adults since plants with the largest cotyledons in our study, napa cabbage (~ 1.7 cm² leaf area), did not attract a greater number of adults than the cultivar with the smallest cotyledons, sweet alyssum (~ 0.2 cm² leaf area).

Preference tests were carried out in a circular/square arrangement design so that insects released from the center point had to travel the same distance to access plants among the 12 hosts/4 growth stages, respectively (Fig. 1). Similar designs have been used in other host-preference tests in the field for diamondback moth, *Plutella xylostella* (L.) (Badenes-Perez et al., 2004), in the greenhouse for cottonwood leaf beetles, *Chrysomela scripta* F. (Bingman and Hart, 1992), and in Petri dishes for two willow-feeding sawflies, *Nematus salicis* (L.) and *N. pavidus* Serville (Roininen and Tahvanainen, 1989). Unfortunately, we were not able to reliably evaluate ovipositional preferences in our studies due to the oviposition behavior of *B. hiliaris*. Unlike other pentatomids that oviposit eggs in a mass under leaves of host plants (Harris and Todd, 1981; Todd, 1989; Wallingford et al., 2013), *B. hiliaris* eggs are laid individually instead of in clusters, and they were often deposited on the Petri dish, plastic arena, mesh screen or on the soil in the arenas. Studies on the oviposition behavior and site selection of female *B. hiliaris* are needed before ovipositional preferences among hosts can be examined.

Green mustard was replaced in the last 3 trials in order to include stock in our choice test. Differences in host attractiveness and acceptance between red and green mustard were not significant in the first three trials, and the two cultivars used in this study appeared to be phenotypically identical at the cotyledon stage. Moreover, *B. hiliaris* has long been known to be attracted to stock (Lounsbury, 1898), which like sweet alyssum is readily infested in

ornamental landscapes and residential gardens throughout southern Arizona (J.C. Palumbo, unpublished data). These cultivars were also included in our study because of their potential as non-crop hosts for in-field insectaries and farmscape management (Chaney, 1998; Pease and Zalom, 2010). However, cotyledons of these two ornamental cultivars were among the least preferred by *B. hiliaris* in our study, and replacement of green mustard with stock did not affect the overall results in the two host-selection trials.

No single cruciferous host plant was overwhelmingly preferred by *B. hiliaris* in our studies, but the data clearly showed that cotyledon-stage seedlings of radish, red cabbage and green cabbage were the most consistently attractive and acceptable hosts during the 48-h exposure time. Plant mortality to *B. hiliaris* feeding varied among cultivars and appeared to be related to host selection and feeding damage. For example, plant mortality and feeding damage were low in arugula which was one of the least attractive hosts, whereas, radish was highly attractive and had high levels of feeding damage and plant mortality. These data are consistent with anecdotal reports by Arizona and California vegetable growers that radish and cabbage appear to be more heavily infested by *B. hiliaris* than other cruciferous crops they produce. Makwali et al. (2002) reported that a related radish plant species, *Raphanus raphanistrum* L., was highly attractive and susceptible to *Bagrada crucifera* Kirk. in laboratory conditions, and suggested that this annual weed could be exploited as a diversionary host to protect other cruciferous crops. In contrast, head cabbage and radish varieties showed levels of resistance to another related stink bug species, the harlequin bug, *M. histrionica*, whereas Chinese cabbage and mustard varieties were more susceptible (Sullivan and Brett, 1974). Another preference study with *M. histrionica* showed that this insect strongly preferred mustard over arugula, bean, collard, rapeseed, and rapini, suggesting that mustard could be an effective trap crop for reducing feeding injury in collard fields (Wallingford et al., 2013).

A greater number of *B. hiliaris* adults were attracted to the 4-leaf stage broccoli in the growth stage preference test. In contrast, cotyledon stage plants appeared to be more acceptable to *B. hiliaris* than the other plant stages based on the quicker time at which feeding damage was first observed. However, there were no differences in host susceptibility among the cotyledon and 1-, 2- and 4-leaf stage broccoli plants although a greater percentage of cotyledon and 1-leaf stage plant were killed by *B. hiliaris* feeding. This is consistent with results in Huang et al. (in press) that showed feeding damage on broccoli plants was particularly destructive to cotyledons, whereas 4-leaf stage plants were less vulnerable to *B. hiliaris* feeding. Feeding symptoms on the 4-leaf plants in this study were primarily localized on the newer growing leaves suggesting that younger leaf tissue is a more attractive food source than the older leaves. Moreover, it appears likely that cotyledon and 4-leaf-stage broccoli plants are similarly acceptable to *B. hiliaris*, but adults were more attracted to 4-leaf-stage plants due to their size and leaf architecture.

It is also possible that host attraction of *B. hiliaris* to cruciferous plants is dependent on the choice made by a conspecific adult due to its aggregative behavior. Under field conditions, it isn't unusual to find multiple mating pairs of *B. hiliaris* on a single plant, suggesting that host plant location could be facilitated by pheromone attraction (Huang et al., 2013). Several agriculturally important stink bug species are known to produce pheromones to attract conspecific males and females (Millar et al., 2002). De Pasquale et al. (2007) and Guarino et al. (2008) identified volatile and contact compounds from male *B. hiliaris* adults that were considered attractants for mating and/or aggregation. Thus, pheromone attraction, along with other age or cultivar specific plant volatiles, may play an important role in the host attraction and acceptance of

B. hiliaris in cruciferous crops. Further research is clearly needed to examine whether plant and insect volatiles can preferentially attract *B. hiliaris* and influence their host feeding activity.

Results from our studies have provided a better understanding of host-plant selection in *B. hiliaris* that may contribute to the development of alternative management strategies. In addition, *B. hiliaris* appears to have a preference for larger broccoli plants. When provided a choice, *B. hiliaris* was more attracted to older 4-leaf stage broccoli plants than younger plants and these older host plants were less susceptible to feeding and suffered relatively less plant mortality. This likely occurred due to the fact that older broccoli plants with greater total leaf tissue can tolerate more *B. hiliaris* feeding injury during the short 48 h feeding bouts (Huang et al., in press). Similarly, the results on host susceptibility among broccoli stages in this study are consistent with other field studies indicating that control of *B. hiliaris* in broccoli is more critical on seedling plants (cotyledon-2 lf stages) than older plant stages (4–6 leaf stage) (J.C. Palumbo, unpublished results).

The observations recorded in these studies suggest that the development of a trap cropping strategy for preventing *B. hiliaris* damage to commercial brassicas may be feasible. Results in Tables 2 and 3 clearly showed that radish and/or red and green cabbage appear to be good candidates for designing and evaluating a potential trap cropping strategy for reducing *B. hiliaris* feeding injury in direct-seeded crops such as arugula, broccoli, kohlrabi, and red/green mustard. When specifically compared to broccoli (the primary cruciferous crop produced in the southwestern U.S.), radish and red/green cabbage seedlings consistently harbored more adults in the host selection experiments (Tables 2 and 3). However, given that red and green cabbage were less susceptible to *B. hiliaris* feeding than radish, these cultivars may be better candidates as trap crops. It may also be possible that intercropping or transplanting older broccoli plants (i.e., 4-5 leaf stage) within direct-seeded broccoli fields could help protect emerging seedlings from *B. hiliaris*. Similarly, planting or transplanting an attractive preferred host species such as radish could potentially be used to divert *B. hiliaris* away from broccoli and other less-preferred cruciferous cultivars. If successful, this could reduce insecticide usage in management of *B. hiliaris*, or perhaps more importantly, contribute to a viable alternative for organic production which lacks effective control measures (Palumbo et al., 2013). Trap cropping using cruciferous plants has been shown to effectively reduce the population of *M. histrionica* (Bender et al., 1999; Ludwig and Kok, 1998), rape blossom beetle, *Meligethes aeneus* (Fabricius) (Hokkanen, 1991), as well as some lepidopteran pests (Luther et al., 1996; Smyth et al., 2003). Field studies examining the potential of radish and larger broccoli planted as trap crops for protecting seedling broccoli fields from *B. hiliaris* adults are currently underway in Arizona.

Acknowledgments

The authors gratefully acknowledge Leo Chavez, Luis Ledesma, Javier Ruiz, and Gerardo Villegas for their assistance in growing broccoli and cauliflower plants and maintaining the bagrada bug colonies. John R. Ruberson (Kansas State University) provided a helpful review of an earlier manuscript draft. In addition, we gratefully acknowledge three anonymous reviewers for their constructive comments on an earlier draft. This research was funded in part by a grant from the USDA-NIFA, Western Region IPM Grants Program under award number 2011-34103-30851, and by a Specialty Crops Block Grant, USDA-AMS, administered by the Arizona Department of Agriculture under the award number SCRBP 11-02. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific

information and does not imply recommendation or endorsement by the University of Arizona, the University of California, or the Arizona Department of Agriculture.

References

- Ahuja, I., Rohloff, J., Bones, A.M., 2010. Defense mechanisms of Brassicaceae: implications for plant-insect interactions and potential for integrated pest management. A review. *Agron. Sustain. Dev.* 30, 311–348.
- Badenes-Perez, F.R., Shelton, A.M., Nault, B.A., 2004. Evaluation trap crops for diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *J. Econ. Entomol.* 97, 1365–1372.
- Bender, D.A., Morrison, W.P., Frisbie, R.E., 1999. Intercropping cabbage and Indian mustard for potential control of lepidopterous and other insects. *Hortscience* 34, 275–279.
- Bingman, B.R., Hart, E.R., 1992. Feeding and oviposition preferences of adult cottonwood leaf beetles (Coleoptera: Chrysomelidae) among *Populus* clones and leaf age classes. *Environ. Entomol.* 21, 508–517.
- CDA, 2012. California Agricultural Statistics Review. In: Vegetable and Melon Crops. California Department of Food and Agriculture, Sacramento CA, pp. 103–114.
- Chaney, W.E., 1998. Biological control of aphids in lettuce using in-field insectaries, pp. 73–84. In: Pickett, C.H., Bugg, R.L. (Eds.), *Enhancing Biological Control: Habitat Management to Promote Natural Enemies of Agricultural Pests*. Univ. Calif. Press, Berkeley, p. 422.
- De Pasquale, C., Guarino, S., Peri, E., Alonzo, G., Colazza, S., 2007. Investigation of cuticular hydrocarbons from *Bagrada hiliaris* genders by SPME/GC-MS. *Anal. Bioanal. Chem.* 389, 1259–1265.
- Guarino, S., Pasquale, C.D., Peri, E., Alonzo, G., Colazza, S., 2008. Role of volatile and contact pheromones in the mating behavior of *Bagrada hiliaris* (Heteroptera: Pentatomidae). *Eur. J. Entomol.* 105, 613–617.
- Harris, V.E., Todd, J.W., 1981. Rearing the southern green stink bug, *Nezara viridula*, with relevant aspects of its biology. *J. GA. Entomol. Soc.* 16, 203–209.
- Hokkanen, H.M.T., 1991. Trap cropping in pest management. *Annu. Rev. Entomol.* 36, 119–138.
- Howard, C.W., 1906. The bagrada bug (*Bagrada hiliaris*). *Transvaal Agric. J.* 5, 168–173.
- Huang, T., Reed, D.A., Perring, T.M., Palumbo, J.C., 2013. Diel activity and behavior of *Bagrada hiliaris* Burmeister (Hemiptera: Pentatomidae) on desert cole crops. *J. Econ. Entomol.* 106, 1726–1738.
- Huang, T., Reed, D.A., Perring, T.M., Palumbo, J.C., 2014. Feeding damage by *Bagrada hiliaris* (Hemiptera: Pentatomidae) and impact on growth and chlorophyll content of brassicaceous plant species. *Arthropod-Plant Interact.* (in press).
- Huang, T., Toews, M.D., 2012. Feeding preference and movement of *Nezara viridula* (L.) and *Euschistus servus* (Say) (Hemiptera: Pentatomidae) on individual cotton plants. *J. Econ. Entomol.* 105, 847–853.
- Joshi, M.L., Ahuja, D.B., Mathur, B.N., 1989. Loss in seed yield by insect pests and their occurrence on different dates of sowing in Indian mustard. *Indian J. Agri. Sci.* 59, 166–168.
- Lal, O.P., Singh, B., 1993. Outbreak of the painted bug, *Bagrada hiliaris* (Burm.) (Hemiptera: Pentatomidae) on mustard in northern India. *J. Entomol. Res.* 17, 155–157.
- Lounsbury, C.P., 1898. The Bagrada bug of cabbage and allied plants. *Agric. J. Cape Good Hope* 13, 101–105.
- Ludwig, S.W., Kok, L.T., 1998. Evaluation of trap crops to manage harlequin bugs, *Murgantia histrionica* (Hahn) on broccoli. *Crop Prot.* 17, 123–128.
- Luther, G.C., Valenzuela, H.R., Defrank, J., 1996. Impact of cruciferous trap crops on lepidopteran pests of cabbage in Hawaii. *Environ. Entomol.* 25, 39–47.
- Makwali, J.A., Wanjala, F.M.E., Khaemba, B.M., 2002. *Raphanus raphanistrum* L as a diversivorous host of *Brevicoryne brassicae* L and *Bagrada cruciferarum* Kirk. In: *Proceedings of the Horticulture Seminar on Sustainable Horticultural Production in the Tropics October 3rd to 6th 2001*. Jomo Kenyatta University of Agriculture and Technology, JKUAT, Juja, Kenya.
- Metcalfe, R.L., Kogan, M., 1987. Plant volatiles as insect attractants. *Crit. Rev. Plant Sci.* 5, 251–301.
- Millar, J.G., McBrien, H.L., Ho, H.Y., Rice, R.E., Cullen, E., Zalom, F.G., Üökl, A., 2002. Pentatomid bug pheromones in IPM: possible applications and limitations. *Int. Org. Biol. Control Bull.* 25, 241–250.
- Molina, G.A.R., Trumper, E.V., 2012. Selection of soybean pods by the stink bugs, *Nezara viridula* and *Piezodorus guildinii*. *J. Insect Sci.* 12, 104. <http://dx.doi.org/10.1673/031.012.10401>.
- Naresh, J.S., Smith, C.M., 1984. Feeding preference of the rice stink bug on annual grasses and sedges. *Entomol. Exp. Appl.* 35, 89–92.
- Palumbo, J.C., Natwick, E.T., 2010. The Bagrada bug (Hemiptera: Pentatomidae): a new invasive pest of cole crops in Arizona and California, Mar 2010. *Online. Plant Health Prog.* <http://dx.doi.org/10.1094/PHP-2010-0621-01-BR>.
- Palumbo, J.C., 2012a. Control of *Bagrada hiliaris* with insecticides in broccoli. *Arthropod Manage. Tests* 37, E8. <http://dx.doi.org/10.4182/amt.2012.E8>.
- Palumbo, J.C., 2012b. Control of *Bagrada hiliaris* with conventional and experimental insecticides on broccoli. *Arthropod Manage. Tests* 37, E9. <http://dx.doi.org/10.4182/amt.2012.E9>.
- Palumbo, J.C., Huang, T., Perring, T.C., Reed, D.A., Prabhaker, N., 2013. Control of *Bagrada hiliaris* broccoli with organically-approved insecticides, 2012. *Arthropod Manage. Tests, Online* 38, E 2 (in press).

- Panizzi, A.R., 1997. Wild hosts of Pentatomids: ecological significance and role in their pest status on crops. *Annu. Rev. Entomol.* 42, 99–122.
- Pease, C.G., Zalom, F.G., 2010. Influence of non-crop plants on stink bug (Hemiptera: Pentatomidae) and natural enemy abundance in tomatoes. *J. Appl. Entomol.* 134, 626–636.
- Rawat, R.R., Singh, O.P., 1980. India painted bug on wheat. *Plant Prot. Bull. Food Agric. Organ.* 20, 77–78.
- Reed, D.A., Palumbo, J.C., Perring, T.M., May, C., 2013. *Bagrada hilaris* (Burmeister), a new stink bug attacking cole crops in the southwestern United States. *J. Integr. Pest Manage. J. Integ. Pest Mngmt* 4 (3). <http://dx.doi.org/10.1603/IPM13007>.
- Rizvi, S.M.A., Singh, R., Singh, H.M., Singh, S.P., 1986. Painted bug, *Bagrada hilaris* (Burm.) appears in epidemic form on maize. *Narendra Deva J. Agri. Res.* 1, 173.
- Rohloff, J., Bones, A.M., 2005. Volatile profiling of *Arabidopsis thaliana* – putative olfactory compounds in plant communication. *Phytochemistry* 66, 1941–1955.
- Roininen, H., Tahvanainen, J., 1989. Host selection and larval performance of two willow-feeding sawflies. *Ecology* 70, 129–136.
- SAS Institute, 2009. SAS/stat User's Manual 9.2. SAS Institute, Cary, N.C.
- Schoonhoven, L.M., van Loon, J.J.A., Dicke, M., 2005. *Insect-plant Biology*, second ed. Oxford University Press., New York.
- Shelton, A.M., Badenes-Perez, F.R., 2006. Concepts and applications of trap cropping in pest management. *Annu. Rev. Entomol.* 51, 285–308.
- Sullivan, M.J., Brett, C.H., 1974. Resistance of commercial crucifers to the harlequin bug in the coastal plain North Carolina. *J. Econ. Entomol.* 67, 262–264.
- Smyth, R.R., Hoffman, M.P., Shelton, A.M., 2003. Effects of host plant phenology on oviposition preference of *Crocidolomia pavonana* (Lepidoptera: Pyralidae). *Environ. Entomol.* 32, 756–764.
- Todd, J.W., 1989. Ecology and behavior of *Nezara viridula*. *Annu. Rev. Entomol.* 34, 273–292.
- U.S. Department Agriculture, NASS, 2012. Arizona Agricultural Statistics, Vegetable Summary. United States Department of Agriculture, National Agricultural Statistics Service, Beltsville, MD. http://www.nass.usda.gov/Statistics_by_State/Arizona/Publications/Bulletin/11bul/main.
- Vekarta, M.V., Patel, G.M., 1999. Succession of important pests of mustard in North Gujrat. *Indian J. Entomol.* 61, 356–361.
- Verma, A.K., Patal, S.K., Bhalla, O.P., Sharma, K.C., 1993. Bioecology of painted bug (*Bagrada cruciferarum*) (Hemiptera: Pentatomidae) on seed crops of cauliflower (*Brassica oleracea* var *botrytis* subvar *cauliflora*). *Indian J. Agric. Sci.* 63, 676–678.
- Wallingford, A.K., Kuhar, T.P., Pfeiffer, D.G., Tholl, D.B., Freeman, J.H., Doughty, H.B., Schultz, P.B., 2013. Host plant preference of harlequin bug (Hemiptera: Pentatomidae), and evaluation of a trap cropping strategy for its control in collard. *J. Econ. Entomol.* 106, 283–288.
- Zar, J., 1999. *Biostatistical Analysis*. Prentice Hall, Upper Saddle River, NJ.