N O T E

Damage by *Bagrada hilaris* (Hemiptera: Pentatomidae) Adults on Germinating Stages of Arugula Seed in a Choice Test[1]

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Bagrada hilaris (Burmeister) (Hemiptera: Pentatomidae) is a serious invasive pest of cruciferous crops (Family: Brassicaceae) (Palumbo and Natwick 2010, Plant Health Progr. doi:10.1094/PHP-2010-0621-01-BR). It was first detected in North America in Los Angeles Co., CA, in 2008 (Palumbo and Natwick 2010) and has been a widespread pest in the central coastal area of California since 2012 (Joseph 2014, J. Entomol. Sci. 49: 318–321). This stink bug threatens both heading and salad cruciferous crops. In recent years, annual economic losses in the region to *B. hilaris* are estimated in the several millions of U.S. dollars (S.V.J. pers. obs.). Feeding by *B. hilaris* on young cruciferous plants causes severe stunting and even plant mortality (Joseph et al. 2017, Arthropod-Plant Interact. DOI 10.1007/s11829-017-9501-0). Also, *B. hilaris* feeding kills the apical meristem of young seedlings, causing plants to not produce a head (Palumbo and Natwick 2010) or produce many secondary shoots, resulting in multiple unmarketable and undersized heads. Of the cruciferous salad crops, some of the most common in the central coast are arugula (*Eruca sativa* Miller), choi (*B. rapa* L. var. *chinensis*), and mizuna (*B. rapa* L. *nipposinica*). *Bagrada hilaris* can substantially damage both new and mature plantings of these crops (Joseph et al. 2017; S.V.J. pers. obs.).

Although *B. hilaris* can seriously injure both direct-seeded and transplanted cruciferous crops, their threat to direct-seeded crops is more pronounced as they have the opportunity to feed on germinating seeds and young seedlings. These plant stages are especially vulnerable to damage. More than 95% of broccoli (*Brassica oleracea* var. *italica* Plenck) and all cruciferous salad crops are direct-seeded in the Salinas Valley. Large numbers of adults and nymphs were observed in cracks and crevices in the soil of production beds when direct-seeded crops are first germinating and emerging from the soil. These high populations in soil can

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inflict substantial damage (S.V.J. pers. obs.). For cruciferous crops, nonpelleted seeds are typically planted into bare ground containing little to no moisture and remain in the soil for 2 to 3 d before fields are sprinkler irrigated and germination begins. While *B. hilaris* will undoubtedly feed on seedlings, it is unclear if they are likely to damage seeds before they soak up water. Once seeds imbibe water, it is unclear how susceptible these seeds are to damage, as well as which stages of germinating seeds *B. hilaris* prefer. This information is critical for determining precisely when newly planted fields are susceptible and for determining when to apply insecticides. Most effective insecticides are contact poisons and, therefore, are only effective when the pest is present (Palumbo et al. 2015, J. Econ. Entomol. 108: 672–682). The objective of this study was to determine the combined preference of *B. hilaris* to the initial stages of seed germination and susceptibility of these stages for a cruciferous salad crop (arugula) through a choice study.

For the study, *B. hilaris* adults were collected from broccoli fields in Salinas and Chualar, CA, and then maintained in a controlled environmental chamber at 21 ± 1°C; 45% relative humidity; and 16:8 (L:D) photoperiod in mesh cages. Two- to 5-week-old potted broccoli plants were provided as a food source, and plants were changed weekly. All experiments were conducted in the same environmental chamber and under the same conditions. The arugula variety used in this study was 'Astro' (Snow Seeds Co., Salinas, CA). Seeds were not treated with any pesticides.

In the choice experiment, single adult *B. hilaris* were given access to four different treatments: (1) nonwatered seed, (2) watered seed (0 d old), (3) 1-d-old seedling, and (4) 2-d-old seedling. All experiments were conducted in plastic tool-organizer boxes containing a number of individual open-top plastic compartments (Stanley Tools, Towson, MD), each of which served as a replicate. The individual compartments were secured with a plastic lid into which 12 2-mm-diameter holes were drilled to permit airflow. Seeds (three per treatment) were placed in four plastic vial lids (1.5 cm diam.) that had been inverted and glued to a 5.3 × 3.8–cm piece of cardboard covering the bottom of the compartment. Lids were lined with a 2 × 2–cm piece of paper towel. Seeds were watered by moistening the paper towel at the appropriate time. Treatments were randomly assigned to compartments, and different seed stages were randomly arranged within compartments. Seeds were exposed to insects for 24 or 48 h. After the exposure period, the introduced *B. hilaris* was removed (Day 1 or 2). For the three watered treatments, seeds were allowed to continue to germinate until Day 7, at which point injury symptoms were evaluated. Thus, seedlings were evaluated at Day 7 for watered (0 d old) seed treatment, at Day 8 for 1-d-old seedling treatment, and at Day 9 for 2-d seedling treatment. The treatment that had not been watered was watered on Day 7, allowed to germinate for 7 d, and then evaluated (Day 14). For all treatments, the paper towel was moistened three times with 0.5 ml of distilled water while seeds were germinating. At evaluation, the seedlings possessed at most two cotyledons, but no true leaves. Using a dissecting microscope at 10× magnification, the number of germinated seeds, seedlings with any *B. hilaris* feeding injury on leaves, discrete feeding injury sites on leaves, and distorted leaves (wherein feeding sites were inseparable) were determined. The seeds were considered germinated if the radicle had emerged from the seed coat. Noncracked seeds and seeds with a cracked seed coat but no visible radicle were grouped as nongerminated seeds. All discrete discolored spots caused by *B. hilaris* feeding on intact leaves were called injury sites and counted.

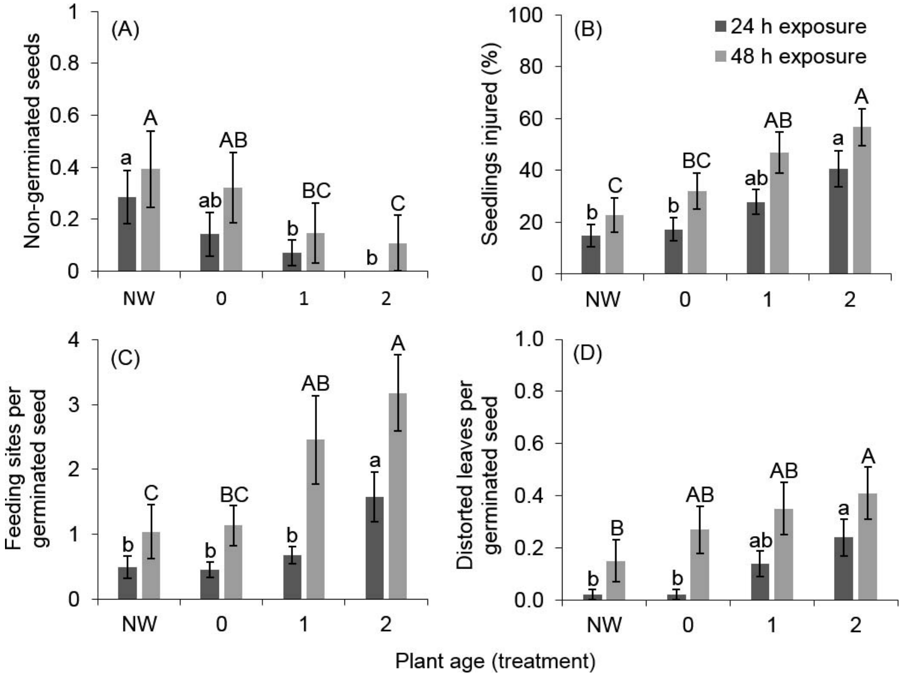

Fig. 1. Mean (±SE) (A) nongerminated seeds, (B) percentage of injured seedlings, (C) feeding injury sites, and (D) leaves distorted by feeding injury per seed on various stages of arugula seedlings. Abbreviation: NW = non-water. Bars of the same fill color with the same letters are not significantly different (least significant difference test, $\alpha = 0.05$).

Leaves with completely discolored and dead tissue that were distorted and misshapen were called distorted. Experiments for the two exposure lengths were conducted separately and in batches. Each batch consisted of 14 replications and was repeated twice for a total of 28 replications for each watering treatment by exposure length combination.

For data analysis, the number of injured seedlings was expressed as a proportion of total seedlings, and the total number of feeding injury sites on the seedlings was expressed as number of injury sites per seedling. The number of nongerminated seeds, number of feeding sites per seedling, and fresh weight of the seedlings were log-transformed (ln[x +1]) to establish homogeneity of variance. The proportion of total seedlings injured was arcsine square root transformed. Each exposure length (24 or 48 h) was analyzed separately with analysis of variance using the general linear model (PROC GLM) procedure in SAS (SAS Institute 2012, Version 9.3, SAS Institute Inc., Cary, NC). Means were separated using the least significant difference method ($\alpha = 0.05$).

In the 24-h exposure experiment, the nonwatered treatment had the fewest seeds germinate, while all of the 2-d-old seedlings did so successfully ($F_{3,\ 81} = 2.8$; $P = 0.038$; Fig. 1A). The 0-d-old seeds and 1-d-old seedlings were intermediate for germination rate. Compared to the nonwatered seeds and 0-d-old seeds and, the 2-d-old seedlings were more damaged (Fig. 1). They were more likely to be injured ($F_{3,\ 81} = 5.2$; $P = 0.002$; Fig. 1B), had more feeding injury sites ($F_{3,\ 81} = 4.9$; $P = 0.004$; Fig. 1C), and had more distorted leaves ($F_{3,\ 81} = 5.1$; $P = 0.003$; Fig. 1D). Values for the 1-d-old seedlings fell between those of the other treatments, but were not significantly different from the other treatments.

In the 48-h exposure experiment, the overall patterns among treatments were similar to the 24-h exposure. The number of nongerminated seeds was significantly higher in the nonwatered than 2-d-old seedlings treatment ($F_{3,\ 80} = 3.3$; $P = 0.025$; Fig. 1A). Compared to nonwatered seeds and 0-d-old seedlings, the 2-d-old seedlings were significantly injured, but not significantly more compared to 1-d-old seedlings ($F_{3,\ 76} = 3.9$; $P = 0.011$; Fig. 1B). Similarly, greater numbers of feeding sites were observed for 2-d-old or 1-d-old seedling treatment than for both the 0-d-old and nonwatered seeds ($F_{3,\ 77} = 6.0$; $P = 0.001$; Fig. 1C). The pattern for distorted leaves in 48-h exposure was similar to the pattern in the 24-h exposure ($F_{3,\ 77} = 3.1$; $P = 0.030$; Fig. 1D).

The results show that 2-d-old germinating seeds were more attractive to and more likely to be damaged by *B. hilaris* adults than younger seeds. Nevertheless, nonwatered seeds could still be damaged. This demonstrates that planted, but nonwatered, seeds are least likely to be damaged by *B. hilaris*, with increasing susceptibility a couple of days after irrigation as cotyledons develop. Based on the 24-h experiment, damage to watered seeds is similar to damage to nonwatered seeds between 0 and 24 h postwatering. This suggests that insecticide should probably be applied a day after planting the seed. In the central California coastal area, seeded crops are sprinkler irrigated following planting until stand establishment. Perhaps, insecticide could be delivered through initial watering or delayed by a day if *B. hilaris* are abundant in the weed hosts surrounding the field or adjacent crop fields. Seedlings at germination stages are vulnerable to *B. hilaris* as they can quickly move into newly planted direct-seeded crop.

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