Association Between *Bagrada hilaris* Density and Feeding Damage in Broccoli: Implications for Pest Management

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ABSTRACT

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Bagrada hilaris (Burmeister) (Hemiptera:Pentatomidae) has become a serious economic pest of brassica crops in Arizona and southern California. Feeding damage on broccoli can result in excessive seedling mortality and, on established plants, in malformed plants which are not commercially marketable. Although management tactics have been developed for this new pest, a reliable sampling technique for estimating adult density is lacking. Here, we analyzed data from 18 insecticide trials conducted from 2011 to 2014 in plots planted to broccoli to determine if estimates of fresh feeding damage on broccoli plants can be used to forecast *B. hilaris* densities. The proportion of plants with fresh feeding damage in a plot was significantly affected by year, trial nested within year, number of insecticides applied, and time after insecticide application. Average density of *B. hilaris* adults per plant in a plot was significantly affected by year, trial nested within year, and number of insecticides applied, while time after insecticide application had a marginal effect on population density. Across plots and years, the proportion of plants with fresh feeding damage was strongly and positively associated with the average number of *B. hilaris* adults per plant. Our results suggest that sampling young plants for signs of fresh feeding damage will provide a reliable and accurate sampling technique for monitoring *B. hilaris* in broccoli fields. We recommend that growers and pest control advisers focus their monitoring efforts on sampling broccoli plants for the presence of fresh feeding damage, rather than adults alone, when making pest management decisions.

INTRODUCTION

The invasive stink bug *Bagrada hilaris* (Burmeister), known as bagrada bug or painted bug, was first identified in North America in Los Angeles, CA, in 2008, and has since spread throughout the southwestern United States and into parts of New Mexico, Texas, Hawaii, and northern Mexico (Matsunaga 2014; Palumbo and Natwick 2010; Reed et al. 2013; Sánchez-Peña 2014). *B. hilaris* is now considered a serious economic pest of fresh-market brassica vegetable crops grown during the fall and winter in the desert growing regions of Arizona and southern California (Palumbo 2015). Direct-seeded broccoli, *Brassica oleracea* L. var. *italica* Plenck, is particularly susceptible to *B. hilaris* feeding damage during crop establishment because highly mobile adults migrating into newly planted fields can rapidly injure and destroy seedling plants (Huang et al. 2014b; Reed et al. 2013). Foliage feeding by adults on established plants can further reduce yields by severely stunting plant growth, or damaging terminal growing points leading to malformed or multi-headed plants that are not commercially marketable (Huang et al. 2014a; Palumbo and Natwick 2010; Reed et al. 2013). Growers estimated that *B. hilaris* infested more than 80% of the acreage in Arizona and southern California from 2010 to 2014, resulting on average in more than 10% stand losses and plant injury to direct-seeded broccoli crops (Palumbo 2015). Consequently, preventing excessive feeding damage on newly established crops is critical to economic broccoli production (Palumbo and Natwick 2010; Reed et al. 2013).

Growers currently rely on preventative spray applications of pyrethroid insecticides to manage *B. hilaris* infestations (Palumbo 2015; Palumbo et al. 2015). Ideally, broccoli fields should be monitored regularly for *B. hilaris* before making control decisions. Failure to obtain reliable estimates of adult density on young plants prior to treatment can result in unacceptable plant damage or improper insecticide use. However, a reliable sampling technique for determining adult *B. hilaris* density is lacking. Accurately estimating adult density on broccoli can be difficult because adult numbers in fields tend to fluctuate throughout the day. Research has indicated that there is a clear positive association between daily temperature and population density of *B. hilaris* on broccoli (Huang et al. 2013). Those studies consistently showed that adult numbers are very low in the cooler hours of the morning (0600 to 0900 h) and peak in mid to late afternoon (1200 to 1600 h), when temperatures are high. Reasons for such daily fluctuations are unclear, but one possibility is that *B. hilaris* individuals hide in soil cracks when temperature declines. Another possibility is that adults are most mobile and show highest migration into broccoli fields when temperatures are high (Huang et al. 2013). Consequently, University of Arizona IPM guidelines currently recommend monitoring for *B. hilaris* in broccoli crops from mid-morning to late afternoon when ambient temperatures are at or above 30°C (Palumbo 2014). However, this can be problematic for pest control advisers (PCAs) that scout large numbers of broccoli acres because they generally begin scouting fields at dawn, at which time sampling would likely result in inaccurate, low estimates of *B. hilaris* density.

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A limited number of studies have described monitoring and sampling techniques for *B. hilaris* populations on brassica crops (Ahuja et al. 2008; Joseph 2014; Mari and Lohar 2010; Srivastava and Srivastava 2012; Verma et al. 1993), but none of these techniques are appropriate for estimating adult density on broccoli plants under Arizona growing conditions. One sampling approach that has not been previously considered is measuring plant damage as an indicator of *B. hilaris* abundance in fields. *B. hilaris* exhibits a lacerate-and-flush feeding method similar to other piercing/sucking Heteropterans that results in characteristic “starburst-shaped” lesions on cotyledons and foliage of young broccoli plants (Palumbo and Natwick 2010; Reed et al 2013). Initially, the lesions appear on the damaged leaf surface as pale green, irregular-shaped spots (3 to 5 mm in diameter). The lesions remain in this condition for about 24 h, but soon thereafter become chlorotic and appear white. These symptoms are consistent with recent studies indicating that feeding by *B. hilaris* can significantly reduce chlorophyll content in broccoli leaf tissue (Huang et al. 2014a). Accordingly, we hypothesized that broccoli plants with recent feeding damage could provide an integrative measure (i.e., over the last 24 h) of adult *B. hilaris* population density in fields. This study was undertaken to determine if estimates of fresh feeding damage on young broccoli plants can be used to forecast *B. hilaris* densities under experimental field conditions.

EXPERIMENTAL APPROACH AND STATISTICAL ANALYSIS

To evaluate the strength of the association between proportion of plants with fresh feeding damage and population density of *B. hilaris*, we analyzed data from 18 insecticide trials conducted from 2011 to 2014 in plots planted to broccoli ‘Emerald crown’ at the University of Arizona, Yuma Agricultural Center located in Yuma, AZ (e.g., Palumbo 2011, 2012; Palumbo et al. 2013). In each insecticide trial, broccoli was direct seeded into double-row beds on 106.7-cm centers in early September in plots that were two beds wide by 12.2 m long and bordered by two untreated beds. Each plot contained approximately 320 plants. For each trial, we used data from six plots treated with a highly effective pyrethroid insecticide (positive control) and six plots not treated with an insecticide (negative control). Bifenthrin (Brigade 2F, FMC Corporation, Philadelphia, PA) was the pyrethroid insecticide used in these studies and has consistently been the most efficacious treatment against *B. hilaris* adults in experimental trials and commercial broccoli fields (Palumbo 2011; Palumbo 2012; Palumbo et al. 2013; Palumbo 2015; Palumbo et al. 2015). In each trial, three plots were treated once and three plots were treated twice at the onset of the experiment. The spray interval between applications for plots that were treated twice was 6 to 7 days after the first application. One, three, and five days after spraying the treated plots twice, the proportion of plants with fresh feeding damage and average number of *B. hilaris* adults per plant was estimated in each plot by sampling 20 arbitrarily selected plants. Sampling of *B. hilaris* was conducted by carefully examining whole plants (1 to 4 leaf stage) for the presence of live adults on leaves, petioles, and stems, as well as on the soil surface beneath each plant. Sampling for feeding damage was conducted by inspecting the terminal growth and young leaves on the same 20 plants for at least one light green starburst-shaped lesion. The number of plants showing damage in each plot was recorded. Sampling in each trial was conducted at 1200 to 1400 h.

We used multiple regression to evaluate factors affecting extent of feeding damage by, and population density of, *B. hilaris.* Experimental units in these analyses were the plot, and the response variable was the proportion of plants with fresh feeding damage or the average number of *B. hilaris* adults per plant. The explanatory variables were year, trial nested within year, number of insecticides applied per plot (0, 1, or 2), number of days after treatment (1, 3, or 5), and the interaction between number of insecticides applied and number of days elapsed after treatment. To evaluate if feeding damage reflects population density of *B. hilaris*, we pooled data over the four years and used simple linear regression to assess the association between the proportion of plants with fresh feeding damage (explanatory variable) and the average number of *B. hilaris* adults per plant (response variable). All statistical analyses were performed in JMP version 11.0 (SAS Institute Inc., Cary, NC).

ASSOCIATION BETWEEN FEEDING DAMAGE AND ADULT DENSITY

The proportion of plants with fresh feeding damage was significantly affected by year, trial nested within year, number of insecticides applied, and time after insecticide application, but the interaction between number of insecticides applied and time after insecticide application was not significant (Table 1). Similarly, average density of *B. hilaris* per plant was significantly affected by year, trial nested within year, and number of insecticides applied (Table 1). Time after insecticide application had a marginal effect on population density, while the interaction between number of insecticides applied and time after insecticide application was not significant (Table 1).

These results show that spraying of the pyrethroid significantly reduced the proportion of plants with fresh feeding damage and population density of *B. hilaris* in the treated plots (Table 2). Furthermore, the proportion of plants with fresh feeding damage increased in plots treated or not with insecticides as time elapsed after application of insecticides, while the increase in population density as time elapsed after spraying was more marginal (Table 3).

These trials generated significant variation in the proportion of plants with feeding damage and population density of *B. hilaris* across the experimental plots evaluated during the four years. The association between proportion of plants with fresh feeding

TABLE 1
Results of multiple regression analyses evaluating effects of year, trial nested within year, number of insecticides applied per plot, number of days elapsed after treatment, and the interaction between the last two factors on proportion of plants with fresh feeding damages and average number of *B. hilaris* adults per plant. Coefficient of determination (R^2) for each model is reported.

Effect	F	Df	P
Proportion of plants with fresh feeding damage (R^2 = 72.5%)			
Year	17.19	3, 163	< 0.0001
Trial [year]	4.79	14, 163	< 0.0001
Number of insecticides	143.49	2, 163	< 0.0001
Days after treatment	6.37	2, 163	0.0022
Insecticides × days after treatment	0.25	4, 163	0.91
Average number of B. hilaris adults per plant (R^2 = 66.9%)			
Year	14.75	3, 163	< 0.0001
Trial [year]	3.37	14, 163	< 0.0001
Number of insecticides	115.02	2, 163	< 0.0001
Days after treatment	2.46	2, 163	0.089
Insecticides × days after treatment	0.14	4, 163	0.97

damage and average number of *B. hilaris* adults per plant was significant (N = 64 plots measured three times, df = 190, slope = 15.6, $P < 0.0001$; Fig. 1). Proportion of plants with fresh feeding damage was strongly associated with population density of *B. hilaris*, as 82% of the variation in adult density was explained by variation in feeding damage (R^2 = 82.5%).

IMPLICATIONS FOR PEST MANAGEMENT

The strong positive association between proportion of damaged plants and numbers of *B. hilaris* adults per plant shown in this study indicates that examining plants for fresh feeding lesions will provide an accurate measure of *B. hilaris* density in broccoli fields. Using fresh feeding damage as an estimate of adult density is a convenient sampling technique as well; plants can be sampled regardless of time of day. Furthermore, an important advantage to using this approach is that it provides a more rapid method of monitoring broccoli fields. Fresh feeding lesions on the foliage of plants (Figs. 2 and 3) can be detected more easily and quickly than *B. hilaris* adults hiding underneath leaves and on stems, at the base of plants, or in soil cracks. We are confident that this will allow growers and PCAs to more cost-efficiently sample fields. They are now encouraged to sample plants regularly for the presence of fresh feeding lesions on cotyledons and young leaves when monitoring the *B. hilaris* infestations in fields, particularly during early morning hours when temperatures are cool. During warmer times of the day, *B. hilaris* adults can often be found on, or adjacent to, plants with recent feeding lesions.

It is important to note that this sampling technique may not be capable of detecting *B. hilaris* feeding lesions on direct-seeded broccoli during early stand establishment (3 to 4 days after seedling emergence). Adults will often move into new fields immediately following emergence of the hypocotyl and cotyeldons and begin feeding on the apical meristem (Huang et al. 2013). On broccoli plants, feeding by *B. hilaris* on the terminal growing point leaves no visible sign of damage until later in plant growth when the plant either produces multiple unmarketable crowns, or 'blind' plants where no crown is produced (Palumbo and Natwick 2010; Reed et al. 2013). Consequently, growers and PCAs prophylactically treat fields with foliar insecticides for the first week following emergence to prevent injury to the apical meristem (Palumbo 2015). After stand establishment (7 days after seedling emergence), PCAs can begin to monitor for fresh feeding lesions on the established cotyledons and young leaves.

Fresh feeding damage on broccoli plants also provides an ideal sampling unit for developing pest management tactics. In other parts of the world where *B. hilaris* occurs, action thresholds used for initiating control on mustard (Daiber 1991), kale (Nyabuga 2008), and caper (Infantino et al. 2007) crops have been based on adult densities (e.g., three adults/plant on kale). Similarly, a nominal action threshold based on *B. hilaris* adult density had previously been established for broccoli grown in Arizona (one adult per 2 m; Palumbo 2014), but did not take into account the temperature-density relationships observed in our desert cropping system (Huang et al. 2013). Based on the results of this study, our nominal threshold has been modified and now triggers insecticide treatments when the number of broccoli plants with fresh lesions exceeds 5%. Development of a simple action threshold based on fresh feeding damage is currently being scientifically validated, and preliminary data suggests that maintaining *B. hilaris* feeding damage below 5% can significantly reduce unacceptable crop losses (J. Palumbo, *unpublished*).

Recent research has further demonstrated the importance of sampling for *B. hilaris* feeding damage on plants following insecticide applications, rather than solely for adult density (Palumbo et al. 2015). For example, pyrethroid insecticides significantly prevented *B. hilaris* feeding damage on treated broccoli plants as a result of rapid adult knockdown mortality. In contrast, foliar application of dinotefuran (a commonly used neonicotinoid insecticide) did not provide strong knockdown adult mortality, but did prevent feeding damage at levels

TABLE 2
Least squares means (LSM) and associated standard error (SE) of the proportion of plants with fresh feeding damage and number of *B. hilaris* adults per plant for plots treated with 0, 1, and 2 pyrethoids. LSMs were calculated after keeping explanatory variables included in multiple regression models (Table 1) at average value.

Variable	Number of insecticide applications	LSM	SE
Proportion of plants with feeding damage	0	0.28	0.01
	1	0.04	0.01
	2	0.02	0.02
Number of *B. hilaris* adults per plant	0	4.7	0.2
	1	0.7	0.3
	2	0.3	0.3

TABLE 3
Least squares means (LSM) and associated standard error (SE) of the proportion of plants with fresh feeding damage and number of *B. hilaris* adults per plant for plots sampled 1, 3 and 5 days after application (DAA) of a pyrethroid. LSMs were calculated after keeping explanatory variables included in multiple regression models (Table 1) at average value.

Variable	Time (DAA)	LSM	SE
Proportion of plants with feeding damage	1	0.08	0.01
	3	0.11	0.01
	5	0.15	0.01
Number of *B. hilaris* adults per plant	1	1.6	0.3
	3	1.8	0.3
	5	2.3	0.3

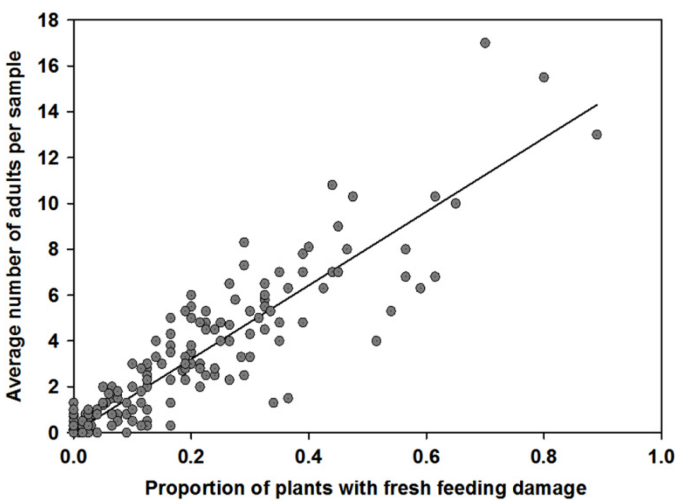

FIGURE 1

Association between proportion of plants with fresh feeding damage and average number of *B. hilaris* per sample (n = 20 plants) across plots treated or not with a pyrethroid.

FIGURE 2

Fresh (top) and old (bottom) *B. hilaris* feeding lesions on broccoli cotyledons.

FIGURE 3

Fresh feeding lesions (top) and old feeding lesions (bottom) on 2-leaf stage broccoli.

comparable to the pyrethroid. The absence of feeding lesions on treated plants was due to apparent antifeedant properties of dinotefuran (Palumbo et al. 2015). Thus, from a practical perspective, broccoli growers and PCAs are encouraged to focus their monitoring efforts on sampling plants for *B. hilaris* feeding lesions rather than adult density alone when assessing post-treatment insecticide performance or making other pest management decisions. From a research perspective, we clearly recognize the necessity of measuring fresh feeding damage when evaluating alternative *B. hilaris* management approaches that do not involve adult mortality (e.g., chemical or biological repellency, or antifeedant activity).

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