

Horticultural Entomology

Trap Cropping Harlequin Bug: Distance of Separation Influences Female Movement and Oviposition

Alexander D. Bier,¹ Anna K. Wallingford², Ariela I. Haber,^{1,○} Megan V. Herlihy-Adams,¹ and Donald C. Weber^{1,3,○}

¹Invasive Insect Biocontrol and Behavior Laboratory, USDA Agricultural Research Service, Beltsville, MD 20705, USA, ²Cooperative Extension IPM, University of New Hampshire, Durham, NH 03824, USA, and ³Corresponding author, e-mail: don.weber@usda.gov

Subject Editor: Jana Lee

Received 1 October 2020; Editorial decision 29 January 2021

Abstract

To evaluate perimeter trap crops for management of harlequin bug, *Murgantia histrionica* (Hahn), we undertook greenhouse and field experiments with mustard greens as trap crop for a collard cash crop. We confirmed that harlequin bugs prefer to immigrate to and reside on mustard. Females, however, in greenhouse cage experiments, ‘commuted’ to collards to lay their eggs. In separate spring and fall field plantings, using replicated 12 m by 12 m collard plots in 1-ha fields, we tested mustard planted as an adjacent perimeter trap crop, or a perimeter trap crop separated by 2 unplanted rows (2.3 m), or with no trap crop. Adults accumulated on the spring mustard crop but overall numbers remained low, with all collards sustaining <1% leaves damaged. In the fall, the separation of 2.3 m reduced oviposition on collards fourfold, and feeding damage approximately 2.5-fold, compared to collards with an adjacent trap crop. Fall control plots with no border trap crop showed even lower foliar damage; likely result of preferential immigration of harlequin bugs to mustard at the field scale, resulting in fewer bugs near the control treatment plots. Thus, the spatial arrangement of the mustard trap crop, and its separation from the cash crop, influences pest abundance and damage. A separated mustard border can reduce bug movement including female commuting and egg-laying, thus better protecting the collard cash crop. Future research should address reduction in area of trap crops, deployment of semiochemicals, and possible changes in timing, to promote trap cropping that is practical for grower implementation.

Key words: cole crops, cultural control, commuting, Brassicaceae, crucifer

Harlequin bug, *Murgantia histrionica* (Hahn) (Hemiptera: Pentatomidae) is a major economic pest of cruciferous crops in the southern United States. Damage takes the form of white splotches, yellowing, distorted or stunted growth, and wilting on mustard-family crops. The feeding causes unmarketable crops and reduces yield, resulting in economic losses (Ludwig and Kok 2001, Wallingford et al. 2011). Broad-spectrum insecticides are commonly used to control harlequin bug populations (Rogers and Howell 1973, McLeod 2005, Walgenbach and Schoof 2005, Kuhar and Doughty 2009). However, in light of the adverse environmental consequences and the potential for pests to develop resistance to broad-spectrum insecticides, alternative harlequin bug management strategies are needed.

Trap crops are ‘plant stands that are, *per se* or via manipulation, deployed to attract, divert, intercept, and/or retain targeted insects or the pathogens they vector in order to reduce damage

to the main crop’ (Shelton and Badenes-Pérez 2006). Perimeter trap cropping is a cultural control method in which a crop that is highly attractive to the pest borders the cash crop, so that the pest colonizes the border crop instead of the cash crop (Adler and Hazzard 2009). Only a limited number of trap cropping systems have enjoyed commercial adoption, because of their knowledge-intensive nature, as well as costs in terms of land and labor required for maintenance (Shelton and Badenes-Pérez 2006). However, desire to minimize chemical control and its environmental consequences, or to deploy only organic certified practices, has renewed interest in trap cropping (Hokkanen 1991, Shelton and Badenes-Pérez 2006, Balusu et al. 2015). Trap crops of mustard (*Brassica juncea* L. (Brassicales: Brassicaceae)) have been demonstrated to attract and retain invading harlequin bug aggregations, thereby avoiding injury to nearby cash crops (*Brassica oleracea* L.; Ludwig and Kok 1998, Wallingford et al. 2013).

While trap crops can be an effective approach to divert harlequin bug from cultivated *Brassica* crops, these additional crops take up valuable crop space, and careful management of pest aggregations occurring on trap crops is often necessary. Minimizing such costs will require a better understanding of harlequin bug colonization dynamics in *Brassica* trap cropping systems.

To help develop a trap cropping strategy for harlequin bug that optimizes crop space, the current study investigated the role of distance between trap crop (mustard) and cash crop (collard) in harlequin bug colonization. We conducted choice tests in a greenhouse to assess its preferences for mustard versus collard at different distances of separation between these two crops. We also conducted tests in the field during both spring and fall to assess the efficacy of a border trap crop at different distances from the cash crop.

Materials and Methods

Plants and Insects

Collard (*B. oleracea* L., var. *acephala*, cv. Champion), and mustard greens (*B. juncea* L., cv. Southern Giant Curled) (both from Johnny's Seeds, Albion, ME) were used for all greenhouse and field experiments. Greenhouse plants for experiments and for field transplantation were grown in peat-based potting medium (Promix BX, Premier Tech Horticulture, Rivière-du-Loup, Québec, Canada), fertilized with slow-release fertilizer (Osmocote Pro, Scotts Miracle-Gro, Marysville, OH) in greenhouses (20–25°C). In the greenhouse experiments, collard and mustard plants each had >6 true leaves before use. For field experiments, the transplanted plants developed within the greenhouse for 4–5 wk prior to transplant into the field. Mustards were direct-seeded instead in the fall to establish a solid stand of trap crop.

Harlequin bugs (F1 or F2 of field collections in Beltsville, MD) were reared on store-bought organic mustard and collard greens (Mom's Organic Market, College Park, MD), with potted collards and mustard greens (grown in our greenhouses as described above) used as a supplemental food source, in Popup Rearing and Observation Cages (0.3-m cube, BioQuip Products, Rancho Dominguez, CA).

Greenhouse Choice Tests

We conducted choice tests in a greenhouse to assess preferences of harlequin bugs for different host plants (collard vs mustard) and to determine whether the distance between these two host plants played a role in harlequin bug preference. We laid out screen cages (Observation Cages 0.61 m × 0.61 m × 0.91 m) horizontally on greenhouse benches. Into each cage we placed a black corrugated plastic sheet (0.004 m × 0.61 m × 0.91 m, CorrugatedPlastics.net, Hillsborough, NJ) with three slits for varying distances (0.20, 0.45, and 0.70 m from the cage end, centered within its length) to hold the stems of the potted plants (watered daily) while creating a measured platform arena for the bugs to move between plants (Fig. 1). Each cage contained one mustard plant at 0.20 m and one collard plant at either of two distances (0.45 or 0.70 m); the resulting 25 and 50 cm distances are referred to as 'adjacent' and 'separated', respectively. Generally, these distances reflected overlapping (but not touching) plant canopies, and those with 20–30 cm separation of the two plant canopies.

We conducted a separate experiment at each of two densities: a low density (one male and one female) and a high density (10 males and 10 females), and the experiment was replicated three times (on three different weeks) at each density. For each experiment, males

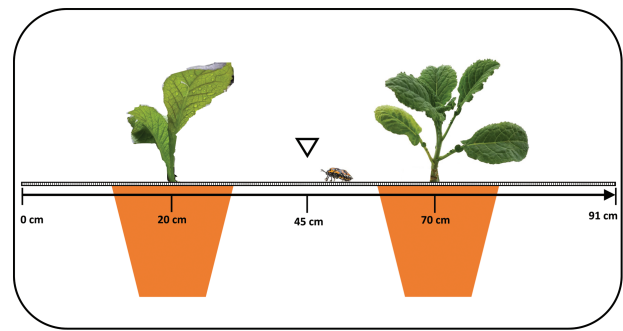


Fig. 1. Greenhouse experimental arena setup, for low and high densities. The mustard plant at 20 cm and the collard plant placed at either 45 or 70 cm with the stems coming up out of the board through the triangular cutout at those respective distances of 25 or 50 cm, 'adjacent' and 'separated', respectively.

and females were randomly selected from the small mixed-sex dorms they were reared in, and released mid-morning at the halfway mark between the collard and the mustard plant. At 24-, 48-, and 72-h post-release, with as little disruption as possible to the plants and the bugs, we noted the sex and location of each of the bugs. We counted insects found on neither plant as 'off-plant'. Counting and collection of egg masses occurred at the 72-h mark. We did not reuse any bugs for the experiments.

Field Trap Cropping Experiments

Experimental design

We conducted two field trap cropping experiments in 2019. We transplanted both mustard and collard plants for the spring experiment on 23 April into a 1-ha field on Beltsville Agricultural Research Center (BARC) South Farm, College Park, Prince George's County, MD, United States (latitude 39.0166, longitude -76.9419; field SG9). The fall planting was in a 1-ha field (latitude 39.0173, longitude -76.9426; field SG8) to the north of the spring experiment; mustards were direct-seeded on 14 August and collards transplanted on 29 August. Transplants were planted into the fields 4–5 wk from sowing for both experiments, and all had 3–6 leaves at time of transplanting. For the spring experiment only, to supplement overwintering bug populations, we released a total of 803 mixed-sex adult bugs (collected within 3 km) on 21 May and 27 June 2019 into three fallow fields between 80 and 150 m distance from the experimental field. We did not conduct any releases for the fall experiment, as there was already a large harlequin bug population present.

To test the effect of a mustard perimeter as a trap crop, three replicates of each treatment (control: collard only; adjacent: collard with an adjacent border of mustard: 0.76 m from the collard; and separated: collard with a border of mustard 2.3 m from the collard) were planted in a Latin square design in 1-ha field on BARC South Farm (Fig. 2 shows an overview). Each plot of collards was 12 m × 12 m and comprised 16 rows spaced 0.76 m apart with 30 plants per row spaced 0.41 m apart. We transplanted the mustard in the adjacent plots 0.76 m from the collard plots into two rows 0.76 m apart, with 37 plants per row also spaced 0.41 m apart. We planted the mustard in the 'separated' plots 2.3 m from the collard plots. For both experiments, we planted the mustard in two rows 0.76 m apart, with 45 plants per row also spaced 0.41 m apart in the spring, or seeded directly at rate of 6.7 kg/ha in the fall planting. The proportion of the total plot area used for trap crop for both seasons was 36.0% with the adjacent mustard planting, and 30.6% of total



Fig. 2. Fall 2019 aerial view showing the three treatments in a Latin square. The middle three plots shown in the picture are examples of the adjacent, control, and separated treatment plots, respectively, top to bottom. The Spring 2019 planting is partially visible in the upper right corner with the same design.

area for the separated trap crop layout. Mowers, trimmers, and hand weeding were employed weekly to suppress weeds.

Sampling

We collected data weekly in the spring from 7 May to 17 July 2019 and weekly in the fall from 12 September to 8 October 2019. To ensure representative sampling across every plot, each collard section was divided into nine quadrants based on distance from the edge of the collards and cardinal direction: four rectangular quadrants along the perimeter of the collards on the north, east, south, and west sides of the plot ('outer'); four rectangular quadrants inside of the perimeter on the north, east, south, and west sides of the plot ('inner'); and a square-shaped quadrant in the center of the plot ('center'). Each mustard section was divided into four quadrants: north, east, south, and west. We randomly selected sample plants within each quadrant using a coordinate system based on row number and distance along the rows. We sampled four collard plants from each outer quadrant and the center quadrant and three collard plants from each inner quadrant for a total of 32 collard samples per plot. To sample mustards in the spring, we randomly selected four plants per quadrant (16 samples total). In the fall, we also took four samples per quadrant, but because the mustard trap crop was direct-seeded rather than transplanted as individual plants, each sample was a 0.41 row-meter section instead of a single plant. Three to four people conducted each sampling, so samples within each quadrant were divided as evenly as possible among samplers to avoid sampler bias. For each sample, we recorded the number of harlequin bug adult males, adult females, nymphs, and egg masses on the plant. We also recorded the total number of leaves and number of leaves exhibiting harlequin bug damage (Supp Figs. 1 and 2 [online only]).

Statistical Analysis

Greenhouse choice tests

Contingency tests (Fisher's exact tests or chi-square tests, depending on sample size; Lowry 2020) and binomial confidence intervals (CIs) were used to determine whether distribution of egg masses, females, and males differed in their occurrence on collard and mustard plants, and in the case of the adults, off the plants (not on either collard or mustard). Since low- and high-density trials were not performed

simultaneously, analyses were kept separate by density. For each density, location of egg masses, females, and males was compared as to adjacent versus separated distance by contingency tests, and the results pooled if no treatment effect was discovered. Locations of egg, male, and female were then tested as to difference in location (collard vs mustard) using a 3×2 Exact test, and if significant ($P < 0.01$), preplanned 2×2 tests of egg mass versus female, and 3×2 tests (including the 'off-plant' location) of female versus male, were used to determine if location patterns differed between the two. In all cases, the distribution on plants (collard vs mustard) was tested as to conformity with the null hypothesis of equal numbers observed on each host plant, and if the null hypothesis was rejected, we calculated the 95% binomial CI for the ratio of preferred:nonpreferred plant (Pezzullo 2009).

Field trap cropping experiment

We averaged the number of males, females, nymphs, egg masses, and damaged collard leaves per plant in each plot at each sampling date. For analyses of males, females, nymphs, and egg masses, our dependent variables were the cumulative average counts (summed across all sampling dates) of each life stage, respectively. Cumulative pest numbers are often used for estimation and analysis of crop impact for a variety of field and vegetable crops and their pests (Reisig and Godfrey 2014, Koch et al. 2016, Zobel et al. 2016, Haar et al. 2019). For the analysis of collard damage, our dependent variable was the number of leaves damaged at the last sampling, which approximated harvest-time. We conducted separate analyses for the spring and fall experiments.

Effect of trap crop border on collard crop.

To determine whether there were differences in numbers of males, females, nymphs, egg masses, and number of leaves damaged in collards among adjacent, separated, and control plots, we conducted general linear mixed models using Proc Mixed in SAS (SAS Institute 2018). Trap crop proximity (adjacent, separated, or none) was the main effect. Block (1–3) was also included as a random effect.

Bug density by host plant.

To quantify differences between host plants (collard vs mustard) and trap crop proximity (adjacent vs separated), we conducted separate analyses on numbers of males, females, nymphs, and egg masses using SAS Proc Mixed. We conducted these as split plot designs with trap crop proximity as the whole-plot effect and host plant as the subplot effect. Block and block * trap crop proximity were included as random effects.

Results

Greenhouse Choice Tests

At low (1:1) bug density, we found no differences by host plant between adjacent and separated distance treatments in the pattern of egg masses (2×2 exact test, $P = 0.62$, overall $n = 28$), females (2×3 exact test, $P = 0.64$, $n = 72$), or males (2×3 exact test, $P = 0.59$, $n = 72$). Therefore, the adjacent and separated treatments were pooled for the comparison of egg mass, female, and male distributions, which differed strongly (2×3 exact test, $P < 0.0001$; $n = 142$ excluding 'off-plant'). We found 4.6 times more egg masses on collard, compared to mustard plants (95% binomial CI 1.7, 15.5; $P = 0.0009$, $n = 28$; Fig. 3), whereas the majority of females and males were found on mustard, 1.54 and 2.12 times more, respectively. For females, this difference between hosts was not statistically significant ($P = 0.12$, $n = 61$ with additional 15.3%

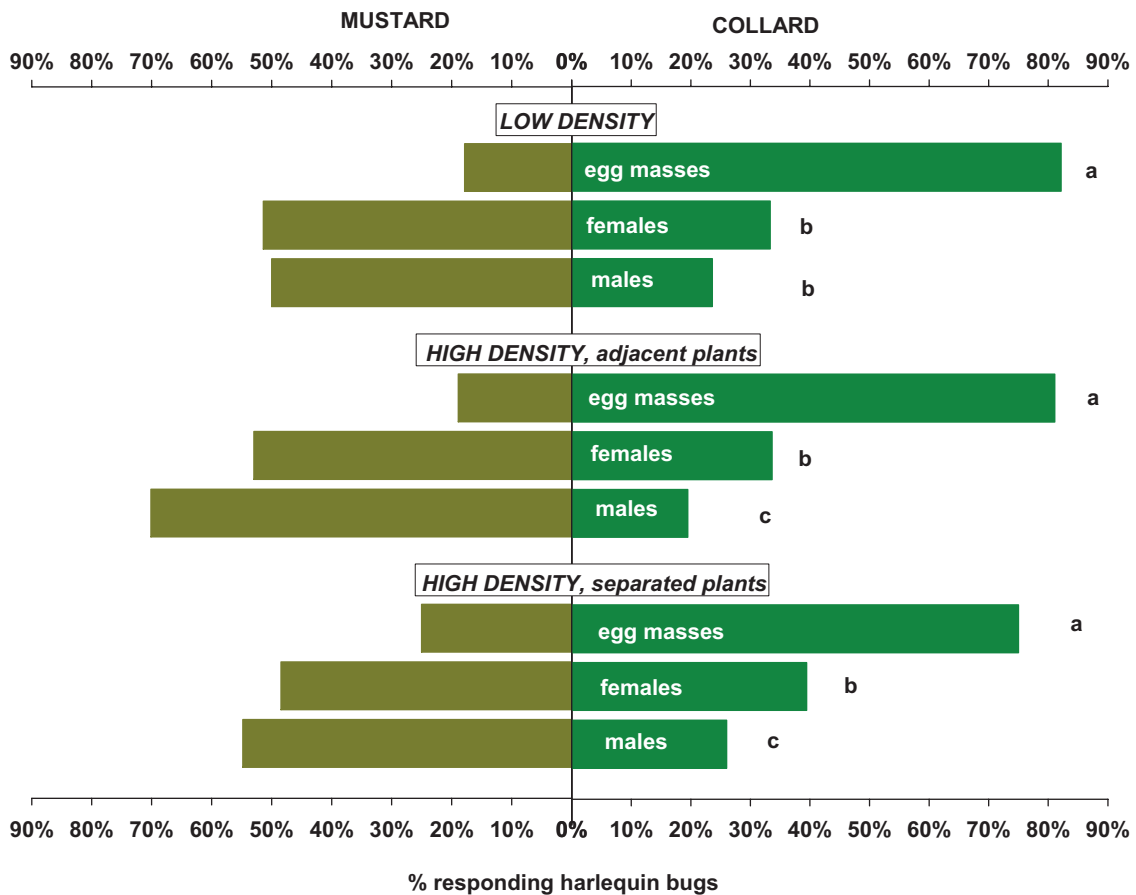


Fig. 3. Counts of adults and egg masses observed on host plants in greenhouse cages with 1 pair (low density) or 10 pairs (high density) of harlequin bugs on one potted mustard and one potted collard plant at either 25 cm distance (adjacent) or 50 cm distance (separated). Adjacent and separated treatments are pooled for low density, because proportions did not differ by distance (see text for details). Life stages with the same following letter within each trial do not differ in their distribution on the host plants (Fisher's exact test, Lowry 2020).

off-plant); however, for males, the difference was statistically significant (binomial CI 1.2, 4.0; $P = 0.013$, $n = 53$ with 26.4% off-plant). Female and egg mass distribution differed (2×2 exact test, $P = 0.0002$, $n = 89$), but female and male distribution did not (2×2 exact test, $P = 0.44$, $n = 114$).

At high (10:10) density, we found differences in the distribution of males between adjacent and separated distance treatments ($2 \times 3 \chi^2 = 17.1$, $P = 0.0002$, $n = 631$); there was a significant difference between plant hosts (2×2 exact test, $P = 0.006$, $n = 537$) and for proportion on either plant versus off-plant (2×2 exact test, $P = 0.002$, $n = 631$). Males were off-plant in a larger proportion of observations for the separated treatment, and in larger proportion on mustard plants for the adjacent treatment. Because of these differences in male distribution, adjacent and separate distance treatments were analyzed separately for the high-density experiments, even though egg mass and female distribution did not differ between adjacent and separated distances (egg mass: 2×2 exact test, $P = 0.43$, $n = 146$; females: $2 \times 3 \chi^2 = 2.3$, $P = 0.31$, $n = 643$).

For both high-density treatments, with adjacent and separated plants, egg masses were disproportionately laid on collard (Fig. 3), yet both females and males were significantly more likely to be observed on mustard plants, with males more disproportionately on mustard than females (as indicated by letter separations in Fig. 3). The number of egg masses on collard was 4.29 and 3.00 times more, respectively, than on mustard, for adjacent and separated plants

(adjacent CI 2.4, 8.3; $P < 0.0001$; $n = 74$; separated CI 1.7, 5.4; $P < 0.0001$; $n = 72$); these proportions differed from those of the females (adjacent and separated 2×2 exact tests, both $P < 0.0001$, $n = 352$ and $n = 355$, respectively), which were found 1.57 times more on mustard than collard in the adjacent treatment (95% CI 1.2, 2.0; $P = 0.0002$, $n = 278$, 13.4% off-plant), but not significantly more on mustard than collard in the separated treatment (1.23 times more on mustard, $P = 0.096$, $n = 283$, 12.1% off-plant). Males were found 3.60 and 2.11 times more on mustard than on collard for adjacent and separated treatments, respectively (adjacent CI 2.7, 4.9; $P < 0.0001$, $n = 276$, 10.4% off-plant; separated CI 1.6, 2.8; $P < 0.0001$; $n = 261$; 19.2% off-plant). Male distribution was more strongly on mustard plants than were females for both adjacent and separated treatments (adjacent 2×2 exact test, $P < 0.0001$, $n = 554$; separated 2×2 exact test, $P = 0.003$, $n = 544$).

Field Experiments

Effect of trap crop border on collard crop

In spring, few harlequin bugs or egg masses were present on collard plants (Table 1; Fig. 4A; Supp Fig. 3 [online only]). There were no statistically significant differences among trap crop treatments for collard plant infestation (males: $F_{2,4} = 1.11$, $P = 0.41$; females: $F_{2,4} = 0.86$, $P = 0.49$; nymphs: $F_{2,4} = 0.30$, $P = 0.76$; egg masses: $F_{2,4} = 0.85$, $P = 0.49$; damage: $F_{2,4} = 1.43$, $P = 0.34$).

In fall, numbers of adult male and female harlequin bugs on collards were also relatively low (Table 2; Fig. 4B; Supp Fig. 4 [online only]), and differences among trap crop treatments were nonsignificant (males: $F_{2,4} = 3.78$, $P = 0.12$; females: $F_{2,4} = 1.91$, $P = 0.26$). However, collards in adjacent plots had over six times as many nymphs as collards in separated plots, and over 22 times as many nymphs as collards on control plots ($F_{2,4} = 5.28$, $P = 0.08$; Table 2). Egg masses on collards exhibited a similar pattern as nymphs. Collards in adjacent plots had over three times as many egg masses as collards in separated plots, and over 27 times as many egg masses as collards in control plots ($F_{2,4} = 4.85$, $P = 0.09$; Table 2). In addition, collards in adjacent plots exhibited harlequin bug damage on over twice as many leaves as in separated plots and on over seven times as many leaves as in control plots ($F_{2,4} = 6.13$, $P = 0.06$; Table 2).

Bug density by host plant

In spring, there were over 41 times as many male harlequin bugs on mustards as on collards ($F_{1,8} = 46.93$, $P < 0.0001$; Table 3; Fig. 4A), regardless of the separation between the mustard border and

collard plot (trap crop treatment * host plant interaction: $F_{1,8} = 1.41$, $P = 0.27$). Over 32 times as many females were present on mustard as on collard ($F_{1,8} = 41.38$, $P = 0.0002$; Table 3), and this difference was also independent of the border separation (trap crop treatment * host plant interaction: $F_{1,8} = 0.57$, $P = 0.47$). There were over four times as many nymphs on mustard as on collard ($F_{1,8} = 18.85$, $P = 0.01$; Table 3), regardless of border separation (trap crop treatment * host plant interaction: $F_{1,8} = 1.63$, $P = 0.27$). The number of egg masses in spring did not differ by host plant ($F_{1,6} = 2.82$, $P = 0.14$).

In fall, there were over 46 times as many males on mustards as on collards ($F_{1,6} = 10.27$, $P = 0.02$; Table 4; Fig. 4B), whether the mustard border was separated or adjacent to the collard plot (trap crop treatment * host plant interaction: $F_{1,6} = 0.01$, $P = 0.91$). Over 11 times as many females were present on mustard as on collard ($F_{1,6} = 8.90$, $P = 0.02$; Table 4), regardless of whether the mustard border was adjacent or separated (trap crop treatment * host plant interaction: $F_{1,6} = 0.00$, $P = 0.95$). Conversely, nymphal density was greater on collard than on mustard ($F_{1,4} = 5.92$, $P = 0.07$), and this

Table 1. Spring field experiment least squares mean (and 95% confidence limits) cumulative numbers of males, females, nymphs, and egg masses; and number of damaged leaves in collards at the last sampling in plots with no mustard border (control), an adjacent mustard border, and a separated mustard border

| | Least squares mean number per plant | | |
|----------------|-------------------------------------|--------------------|--------------------|
| | Control | Adjacent | Separated |
| Males | 0.32 (-0.12, 0.77) | 0.04 (-0.40, 0.49) | 0.02 (-0.42, 0.47) |
| Females | 0.30 (-0.16, 0.77) | 0.04 (-0.42, 0.51) | 0.05 (-0.41, 0.52) |
| Nymphs | 0.05 (-0.09, 0.19) | 0.09 (-0.05, 0.23) | 0.04 (-0.10, 0.18) |
| Egg masses | 0.24 (-0.05, 0.53) | 0.08 (-0.21, 0.37) | 0.06 (-0.23, 0.35) |
| Damaged leaves | 0.70 (0.02, 1.38) | 0.29 (-0.39, 0.98) | 0.13 (-0.56, 0.81) |

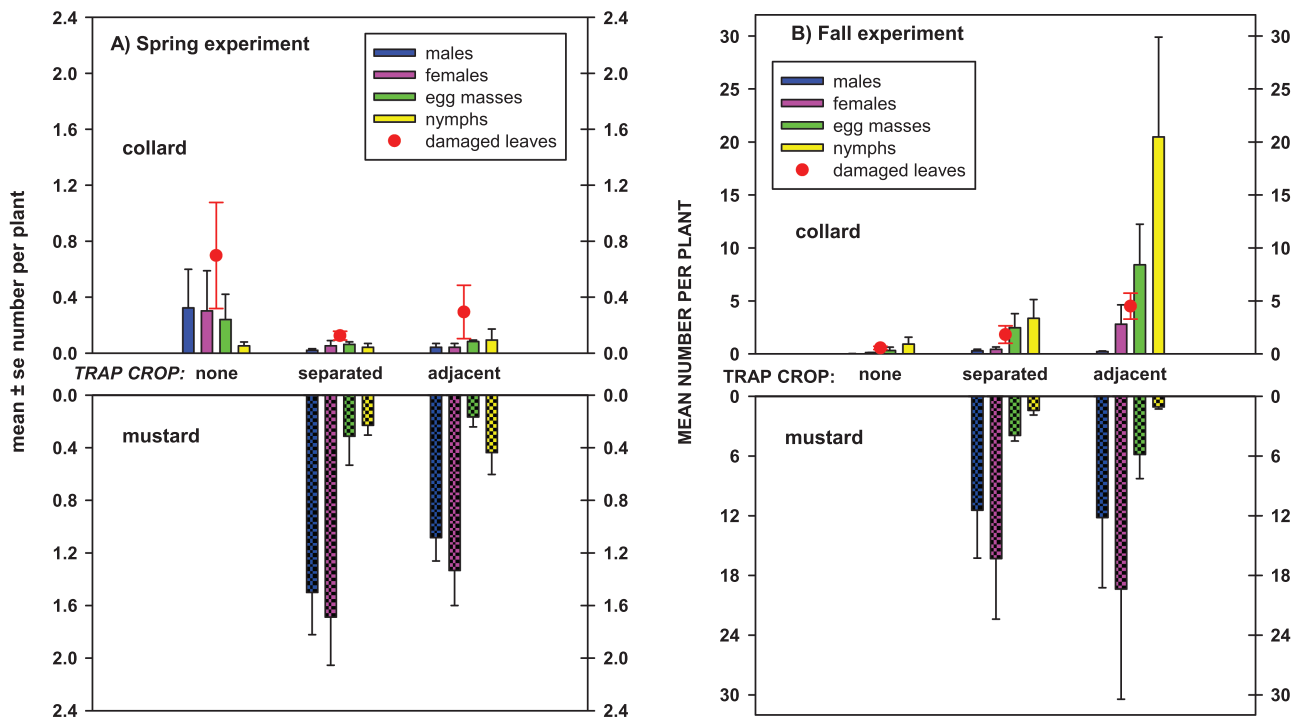


Fig. 4. Mean cumulative numbers per plant of harlequin bug males, females, egg masses, and nymphs; and mean number of damaged leaves in collard cash crop at the final sampling, Spring 2019 (A) and Fall 2019 (B), Beltsville, MD. Each experiment had three treatments, with mustard trap crops as: 1) 'none', absent, 2) 'adjacent' in next row (0.8 m) to collard crop, and 3) 'separated' with 2.3 m separation from collard crop.

Table 2. Fall field experiment least squares mean (and 95% confidence limits) cumulative numbers of males, females, nymphs, egg masses; and number of damaged leaves in collards at the last sampling in plots with no mustard border (control), an adjacent mustard border, and a separated mustard border

| | Least squares mean number per plant | | |
|----------------|-------------------------------------|---------------------|----------------------|
| | Control | Adjacent | Separated |
| Males | 0.02 (-0.22, 0.27) | 0.22 (-0.03, 0.46) | 0.29 (0.04, 0.53) |
| Females | 0.13 (-2.82, 3.07) | 2.80 (-0.14, 5.74) | 0.43 (-2.52, 3.37) |
| Nymphs | 1.07 (-14.62, 16.77) | 23.92 (8.22, 39.61) | 3.92 (-11.78, 19.61) |
| Egg masses | 0.36 (-6.96, 7.67) | 9.72 (2.40, 17.03) | 2.86 (-4.46, 10.17) |
| Damaged leaves | 0.56 (-1.81, 2.94) | 4.50 (2.13, 6.88) | 1.82 (-0.55, 4.20) |

Table 3. Spring field experiment least squares mean (and 95% confidence limits) cumulative numbers of males, females, nymphs, and egg masses per plant on collards versus mustards in plots with adjacent and separated mustard borders

| | Least squares mean number per plant | | | |
|------------|-------------------------------------|--------------------|-----------------------------|--------------------|
| | Plots with adjacent border | | Plots with separated border | |
| | Collard | Mustard | Collard | Mustard |
| Males | 0.04 (-0.38, 0.47) | 1.08 (0.66, 1.51) | 0.02 (-0.40, 0.45) | 1.50 (1.08, 1.92) |
| Females | 0.04 (-0.48, 0.57) | 1.33 (0.81, 1.86) | 0.05 (-0.47, 0.58) | 1.69 (1.16, 2.21) |
| Nymphs | 0.09 (-0.15, 0.34) | 0.44 (0.19, 0.68) | 0.04 (-0.21, 0.29) | 0.23 (-0.02, 0.48) |
| Egg masses | 0.08 (-0.20, 0.36) | 0.17 (-0.11, 0.45) | 0.06 (-0.22, 0.34) | 0.31 (0.03, 0.59) |

difference was greater in plots with adjacent borders than in plots with separated borders (trap crop treatment * host plant interaction: $F_{1,4} = 5.94$, $P = 0.07$; Table 4). Over five times as many egg masses were present on collard than on mustard ($F_{1,6} = 6.60$, $P = 0.04$; Table 4), regardless of whether the border was adjacent or separated (trap crop treatment * host plant interaction: $F_{1,6} = 3.36$, $P = 0.12$).

Discussion

Differential host plant preference has been well documented for harlequin bug, and often reported in terms of where adults and nymphs occur on crops in the field (Sullivan and Brett 1974; Ludwig and Kok 2001; Wallingford et al. 2011, 2013). Here we found that, while female harlequin bugs are observed on mustard plants more frequently than on collard plants, they prefer to lay their eggs on collard in greenhouse experiments. We presume that females leave the preferred host plant in order to oviposit, then return to that host plant. In other words, gravid females will ‘commute’ for the purpose of oviposition. Reasons for this commuting behavior are unclear, but may be related to better survival of young nymphs on collard (J. Ononogbo, unpublished data). Regardless of the reason, it is important to discourage this behavior when using a trap crop for pest management, by promoting retention (arrestment) and/or mortality of females on the trap crop, by physical separation or by other tactics. We also observed evidence of this commuting behavior in our fall field experiment. We observed greater numbers of egg masses and nymphs on cash crops with adjacent trap crops compared to cash crop with a separated trap crop (Fig. 4B). Furthermore, we found that separation of the border trap crop by only two rows’ distance (2.3 m instead of 0.8 m adjacent row spacing) afforded a significant level of protection to the collard cash crop: a greater than 2.5-fold reduction in leaves injured. Notably, fall collard plots with no trap crop showed even lower foliar damage. Likely this is a result of initial colonization of immigrating harlequin bugs overwhelmingly to mustard at the field (hectare) scale, resulting in fewer bugs

near the control treatments at the plot (<0.1 ha) scale. Both fall and spring results confirmed that adult harlequin bugs prefer mustard over collard, but spring densities were too low to show any differences based on presence and separation of the trap crop, whereas at the higher fall densities, both the adult preference for mustard, and the separation of this trap crop from the corresponding encircled collards, had impact on pest populations and crop damage. The direct seeding of the mustard trap crop in the fall resulted in dense walls of mustard that maintained their foliage through harvest-time, whereas in spring, the mustard plants flowered and then senesced in the last 2 wk before the final sampling, likely making them less attractive for adults.

English-Loeb and Collier (1987) found that adult harlequin bugs make frequent short-distance movements within their native habitat of bladderpod (*Isomeris arborea* (Brassicales: Capparaceae)) shrub, which also had abundant *Brassica* spp. plants, in southern California. Marked females moved more frequently than males, a trend possibly attributable to oviposition. Females departed more quickly from lower-quality plants, those with lower numbers of capsules and racemes, which supported poorer nymphal survival. Females and males moved an average of 4.3 and 3.8 m per day, respectively (English-Loeb and Collier 1987). In an agricultural setting in Maryland, Cabrera Walsh et al. (2016), using marked bugs, found that ~15% of bugs departed high-quality collard plants per day, regardless of presence or absence of a synthetic aggregation pheromone lure. Marked bugs were found a mean of 30 m distant from their original plant, ‘suggesting that migration was not necessarily toward the closest available hosts’. Taken together, the two studies show that harlequin bugs move frequently over distances that easily exceed the maximum distance of separation in our trap crop field experiments.

Trap crops in the family Brassicaceae have frequently been employed experimentally, mostly to protect *Brassica* crops against specialist pests (Badenes-Pérez 2019). The most frequent target pests are diamondback moth (*Plutella xylostella* L. (Lepidoptera: Plutellidae))

Table 4. Fall field experiment least squares mean (and 95% confidence limits) cumulative numbers of males, females, nymphs, and egg masses per plant on collards versus mustards in plots with adjacent and separated mustard borders

| | Least squares mean number per plant | | | |
|------------|-------------------------------------|---------------------|-----------------------------|---------------------|
| | Plots with adjacent border | | Plots with separated border | |
| | Collard | Mustard | Collard | Mustard |
| Males | 0.22 (-10.04, 10.48) | 12.18 (1.93, 22.44) | 0.29 (-9.97, 10.55) | 11.45 (1.19, 21.71) |
| Females | 2.8 (-12.47, 18.08) | 19.37 (4.09, 34.65) | 0.43 (-14.85, 15.70) | 16.30 (1.03, 31.58) |
| Nymphs | 23.92 (11.70, 36.13) | 5.85 (-6.36, 18.07) | 3.92 (-8.30, 16.13) | 3.93 (-8.28, 16.15) |
| Egg masses | 9.72 (4.21, 15.22) | 1.08 (-4.42, 6.58) | 2.86 (-2.64, 8.36) | 1.41 (-4.09, 6.91) |

and other leaf-feeding Lepidoptera, pollen beetle (*Meligethes aeneus* F. (Coleoptera: Nitidulidae)), flea beetles (*Phyllotreta* and *Psylliodes* (Coleoptera: Chrysomelidae)), and Heteroptera, including harlequin bug and other pentatomid species (Table 1 in [Badenes-Pérez 2019](#)). Research on trap crops for diamondback moth and for pollen beetle has been most intensive, reflecting their pesticide resistance due to reliance on chemical control as the primary control tactic; however, trap cropping has not been adopted on significant acreage for economic reasons ([Skellern and Cook 2018](#), [Sherbrooke et al. 2020](#)).

Perimeter trap cropping ([Adler and Hazzard 2009](#)) has been shown to be an effective trap crop configuration for several systems, reflecting both the border-focused nature of pest immigration as well as its directional variability, depending on weather and source of insects. However, trap cropping by definition involves substituting area that would or could otherwise be devoted to the main cash crop, to a trap crop which is presumably worthless (or worth less) to harvest. Increasing harvest value of the trap crop, essentially making it another cash crop, would reduce the economic cost of its implementation, but this is often not possible. [Hokkanen \(1991\)](#) recommended 10% as a reasonable starting point for area dedicated to a trap crop; [Sherbrooke et al. \(2020\)](#) tested 10 or 20% trap crops (*B. juncea* cv. G-type, or *B. vulgaris* cv. Green Wave), for broccoli in Arizona, to manage diamondback moth. The authors considered this to be a feasible area and configuration for a broccoli field, but the interplanted trap crop was not successful in terms of reducing pest infestation on the broccoli cash crop. [Wallingford et al. \(2013\)](#) used 20% of the crop area, a single unseparated trap crop row of *B. juncea* cv. Southern Curled Giant on two sides of eight rows of the collard (cv. Champion) cash crop, to successfully protect it from harlequin bug damage in two separate locations in coastal Virginia, compared to collards without a trap crop. In contrast, [Ludwig and Kok \(1998\)](#) employed a large noncrop perimeter area of 62.5% (with only 27 m² broccoli out of a total 72 m² for each plot) for harlequin bug trap cropping, using adjacent rape (*Brassica napus* cv. Dwarf Essex) or mustard (*B. juncea* cv. Southern Giant Curled) in southwestern Virginia. Although their trap crops were more attractive than the cash crop, and somewhat effective at preventing broccoli infestation at low bug populations, senescence of the mustard crop, especially when transplanted and in the spring season, and/or higher bug and flea beetle populations, proved problematic. [Bender et al. \(1999\)](#) found that an unseparated perimeter crop of two separate seedings of *B. juncea* (cv. not stated; 62.8% was trap-crop area) partially protected plots of cabbage from harlequin bugs in western Texas, but failed to do so for Lepidoptera, the target pests.

In our experiments, we configured the trap crop as a perimeter trap crop, with 36% of the total area dedicated to the 'adjacent' configuration of the mustard planting, and 55.6% of total area for the 'separated' trap crop layout, including both the mustard perimeter (55% of non-cash-crop area) and bare ground (45% of

non-cash-crop area). For high-value vegetable farms with limited land area, this would not be an economically viable cultural control, as currently configured. The economic disadvantage of such extensive trap crops has been pointed out by [Sherbrooke et al. \(2020\)](#) and several earlier authors.

Several modifications could minimize the area and the economic return lost to the trap crop, while simultaneously increasing its effectiveness. Semiochemically assisted trap cropping ([Shelton and Badenes-Pérez 2006](#)) could potentially 'supercharge' a small area of trap plants, making possible a drastic reduction in the trap-crop area. Plant or insect volatile semiochemicals might alter both initial colonization or subsequent movement by harlequin bug adults and nymphs. In this regard, the male-produced aggregation pheromone, murgantiol, available in mixed synthetic form commercially, is known to be highly attractive to adults and nymphs ([Khrimian et al. 2014](#), [Weber et al. 2014](#)). Its influence is so strong that it can shift preference to nonhost plants, and synthetic isothiocyanates (mustard-produced volatiles) can add to this effect ([Thrift et al. 2018](#)). Though highly attractive to harlequin bug, pheromone lures do not increase retention of adults, at least not on individual plants ([Cabrera Walsh et al. 2016](#)); therefore, pest populations would have to be monitored closely for vicinity effects ([Wallingford et al. 2018](#)), and controlled as necessary.

Researchers have attempted to develop dead-end trap crops, thus precluding subsequent pest reproduction and emigration to the cash crop ([Shelton and Nault 2004](#), [Badenes-Pérez et al. 2014](#), [Veromann et al. 2014](#)). [Badenes-Pérez et al. 2014](#) chose *Barbarea vulgaris* as a dead-end species for diamondback moth. A trap crop could also be engineered to be toxic (e.g., with RNAi against harlequin bugs, [Howell et al. 2020](#)), or treated with systemic or repeated insecticide applications. Suppression of pest populations on the trap crop could also entail plantings of insectary plants to support predators and/or parasitoids, either as combined insectary-trap crops ([Badenes-Pérez et al. 2017](#)), or as separate plantings (cash, trap, and insectary crops; [Shrestha et al. 2019](#)).

Even if the trap crop does not kill the adults or limit their reproduction, maintenance of a high-quality trap crop, possibly by multiple plantings ([Bender et al. 1999](#)) or by species polyculture (as [Parker et al. \(2016\)](#) used to protect a broccoli crop from flea beetles) can promote retention of adults and immature stages and serve as an egg sink. This would address concerns about senescence or loss of attractiveness, as occurred with single-species mustard trap crops in [Ludwig and Kok \(1998\)](#) and single-species turnip trap crops for yellowmargined leaf beetle (*Microtheca ochroloma*, Coleoptera: Chrysomelidae) ([Balusu et al. 2015](#)).

Changing the timing of the trap crop, such that its removal of immigrating pests is largely achieved early in the cash crop season, recognizes that trap cropping involves successful use of both time and space during the growing season. Early students of harlequin

bug (e.g., Chittenden 1920, White and Brannon 1939) advocated early-season planting of trap crops such as mustards, kale, or turnips, before main crops, with subsequent hand-picking, treatment, or destruction of the trap crop. These same two authors also advocated late-season trap crops to prevent overwintering of bugs. Modern extension advice (e.g., Knox 2012, Cranshaw 2018) echoes the recommendation for early planting of attractive trap crops for harlequin bug.

Finally, the cash crop could be made less attractive by deploying 'push' tactics in addition to the 'pull' tactics employed here, in order to repel, deter, or prevent colonization. These could involve cultivar choice, antifeedants, or repellent interplantings, and barriers to crop colonization (Eigenbrode et al. 2016). Tactics that might deter permanent or temporary immigration from the trap crop include physical separation, as in our study, or physical barriers including killed or living nonhost plants.

Expected success of trap cropping depends on multiple factors including cash crop and trap crop spatial arrangement and timing, attractiveness and arrestment, for multiple pests, over a possibly unpredictable period of time preceding cash crop harvest, as well as variable conditions affecting crop quality, pest numbers, and movement. With this study we have evaluated the impact of physical separation on behavior of harlequin bug, a key pest of cole crops. The tendency of females to commute to the cash crop from the trap crop (and back again) is reduced by this separation, but this effect remains to be evaluated under a range of conditions before grower adoption can be recommended. Nonetheless, commuting is yet another reason that understanding and managing aggregations on trap crops is a critical aspect to successful behavioral pest management.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

Acknowledgments

Many thanks to Zoey Lake, Kayla Pasteur, Rubi Del Mar Santiago, and Taissae Sanchez Medina for the intensive field counts and assistance with the lab assays. Additional thanks go to our BARC West Research Farm Services (George Meyers, Jen Showalter, John Bouma, and Stewart Macmaster) for the planting and maintaining of the fields.

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