


Measuring host plant selection and retention of *Halyomorpha halys* by a trap crop

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Abstract

Trap cropping may exploit a pest's dispersal and host selection behavior in order to protect a desired crop. Here, we used a combination of visual sampling, immunomarking, and harmonic radar to assess host plant selection and retention time of the highly mobile and invasive *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), as it moves within and between a polyculture trap crop of sorghum and sunflower, and a bell pepper cash crop. Visual sampling demonstrated no significant differences in *H. halys* densities across crops, whereas dislodging stink bugs to collect for protein analysis revealed ca. 4× more bugs in the trap crop plants than in the peppers. In total 145 *H. halys* were collected and of these 6% were doubly marked with proteins, demonstrating that minimal movement occurred between the two planting systems. Tracking tagged *H. halys* with harmonic radar revealed that the trap crop retained adult *H. halys* within the plots 1.5× longer and reduced their movement by nearly half compared with bugs released in the pepper cash crop. The data suggest the trap crop of sunflower plus sorghum has the potential to attract and arrest the invasive *H. halys*, demonstrating that trap cropping may operate as an effective management tool.

Introduction

The dispersal behavior of insect pests is the basis for their spatial and temporal distribution and is a determining factor in the severity of their pest status in an agricultural landscape. A number of factors influence insect dispersal behavior, including reproduction, competition, environmental hazards, chemical ecology, or hosts and dietary requirements (Bowler & Benton, 2005; Mazzi & Dorn, 2012). Whereas generalist herbivores utilize a variety of different host plants, many specialist insect pests exhibit marked preferences for specific hosts and plant growth stages (Cates, 1980; Bernays & Minkenberg, 1997; Panizzi, 1997; Rice et al., 2014). Consequently, these preferences may result in the movement of insect pest populations to crop fields composed of preferred host plants or stages. The suitability of the crop habitat for the development,

survival, and population increase of the pest determines the future spatial and temporal dynamics of insect pests (Stinner et al., 1983; Kennedy & Storer, 2000; Venugopal et al., 2015a). Concentrations of generalist pests within a preferred crop could serve as a potential reservoir of pests capable of dispersing to other crops within the agricultural landscape (Kennedy & Storer, 2000; Tschardt & Brandl, 2004; Shelton & Badenes-Perez, 2006; Mazzi & Dorn, 2012; Sivakoff et al., 2013). Thus, understanding a pest's dispersal behavior and relationship with its hosts is necessary to develop effective behavior-based cultural pest management tactics.

One tactic that exploits a pest's dispersal behavior and host plant selection, and that has been used for centuries, is trap cropping: growing a highly attractive host plant ('trap crop') to preferentially attract or intercept pests before they colonize the cash crop (Hokkanen, 1991). The effectiveness of trap cropping as a management method depends on the targeted insect pest's mobility during its host-finding stage and its preference for the trap crop plants (Shelton & Badenes-Perez, 2006). Trap cropping without additional management inputs (e.g., insecticide

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application) has frequently been ineffective at managing the target pest (Shelton & Badenes-Perez, 2006), potentially due to the trap crop's failure to adequately attract or retain the pest (Holden et al., 2012). Although these factors are recognized as key in designing efficacious trap cropping systems, the trap cropping literature is biased towards experiments assessing only the attractiveness of candidate trap plants (Shelton & Badenes-Perez, 2006), with no apparent studies explicitly measuring trap crop retention of target pests (Holden et al., 2012).

The study described herein investigated pest dispersal within a trap cropping system through its attraction and retention of the generalist herbivore *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), the brown marmorated stink bug, an invasive pest that uses more than 100 reported host plants, including many economically important fruit and vegetable crops species (Bergmann et al., 2015). Highly mobile during both nymphal (Lee et al., 2014) and adult stages (Lee & Leskey, 2015; Wiman et al., 2015), the pest may utilize multiple host plants throughout the season. Host-switching behavior leading to a mixed diet is thought to be important for *H. halys* due to differential survivorship on individual host plants (Nielsen & Hamilton, 2009; Acebes-Doria et al., 2016). We studied a polyculture trap crop composed of sunflowers and sorghum, recently reported to be highly attractive hosts for stink bugs (Holden et al., 2012; Nielsen et al., 2016), that was planted in a border around a bell pepper cash crop.

To better understand how *H. halys* used the trap and cash crops spatially and temporally, we employed immunomarking and harmonic radar tracking techniques. Marking insects in situ with a field-applied protein marker minimally impacts dispersal over space and time (Hagler et al., 1992). Insects are 'marked' with a unique protein, either by direct contact during application or by picking up the protein via contact with previously marked surfaces, and subsequently analyzed for the specific protein by an enzyme-linked immunosorbent assay (ELISA) (Jones et al., 2006; Hagler et al., 2014). Using highly sensitive, inexpensive, and ecologically safe markers, this method has been used to study the natural dispersal and movement patterns of a variety of natural enemies (Horton et al., 2009; Swezey et al., 2014) and herbivores (Sivakoff et al., 2012; Reisig et al., 2013; Swezey et al., 2013). The use of harmonic radar permits continuous tracking of tagged insects through echo-location of a transmitted wave reflected from the tag with a rectifier circuit (Chapman et al., 2011) and has been shown to be an effective method for tracking *H. halys* (Lee et al., 2013; Morrison et al., 2016). Using both tracking approaches, we assessed whether the polyculture trap crop acts as a population sink

for *H. halys* or temporarily arrests the pest and serves as a pest reservoir with the potential for dispersal to the cash crop.

Materials and methods

Field layout and design

Trials were carried out on a farm in Inwood, WV, USA (Redbud Farm), and at the Rutgers Agricultural Research and Extension Center, in Bridgeton, NJ, USA. There were two plot types: bell peppers surrounded by a trap crop and bell peppers without a trap crop (control). Bell peppers were planted on 29 May and 1 June 2014, in NJ and WV, respectively, as single-row spacing on black plastic in land that was USDA-certified organic or in transition. These plots consisted of 7.6×7.6 m plantings of bell peppers (*Capsicum annuum* L. var. Aristotle; Seminis Vegetable Seeds, St. Louis, MO, USA) arranged in five rows of 100 transplants spaced 38.1 to 45.7 cm within each row. In the trap crop plots, open-pollinated sunflower (*Helianthus annuus* L., Asteraceae) seed mix (#2160SG.36; Johnny's Select Seed, Winslow, ME, USA) and grain sorghum (*Sorghum bicolor* L. Moench var. 65B3cnv, Poaceae; Blue River Hybrids, Ames, IA, USA) were planted in a square around the perimeter of the peppers, 1.5 m away from the black plastic rows in a 1.22 m strip. Each trap crop species was planted in a 0.61 m wide margin, with at least two rows in the strip (Figure 1). Sunflowers were seeded at a rate of 11.2 kg ha^{-1} (seeds spaced 15.2 cm apart within the row), whereas the sorghum was seeded at a rate of 56 kg ha^{-1} (seeds spaced 7.6 cm apart). The study examined a total of four replicates (each consisting of paired control and trap crop plots spaced at least 10 m apart) with either mown turf or a combination of basil, watermelons, and strawberries planted between replicates.

Movement tracking with protein marking

In-situ protein marking. Two unique protein marking solutions were applied to the trap crop plots at times representing a mid- and late-season sampling period (21 July and 28 August 2014 in NJ, and 23 July and 18 August 2014 in WV), corresponding with the most attractive phenological growth stages (flowering and seed set) for sunflower and sorghum, respectively, and with the majority of pepper plants setting fruit (Nielsen et al., 2016). Protein marking solutions were a 5% liquid egg white solution (AllWhites; Michael Foods, Minnetonka, MN, USA) and a 20% milk solution [Provident Pantry powdered milk in NJ (Emergency Essentials, Orem, UT, USA) and Nature's Place Organic in WV (DZA Brands, Salisbury, NC, USA)], both diluted in tap water (Jones et al., 2006, 2011). Additionally, 0.3 g l^{-1} of sodium

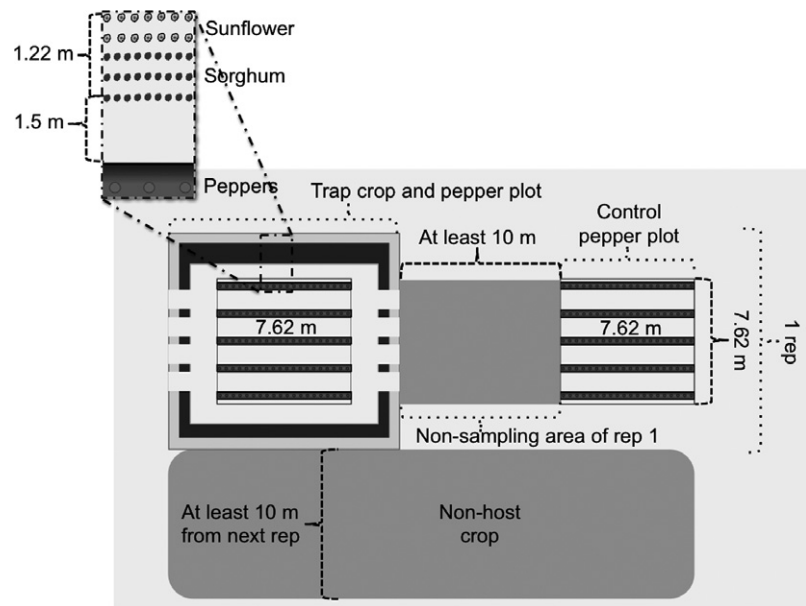


Figure 1 Trap crop and control plot layout.

ethylenediamine tetra acetate (EDTA; Thermo Fisher Scientific, Waltham, MA, USA) and a surfactant [1 300 ppm of Silwet L-77 in NJ (Momentive Performance Materials, Columbus, OH, USA) and 7.8 ml l⁻¹ Natural Wet in WV (SaferGro Laboratories, Ventura, CA, USA)], were added to the marking solutions to reduce water hardness and enhance the distribution and residual time of the solutions (Jones et al., 2006).

The protein solutions were applied to the plots with a 56.8 l spot sprayer with a 12 V, 0.13 l s⁻¹ pump (Fimco Industries, North Sioux City, SD, USA) at a rate of 935 l ha⁻¹. Using the hand wand, the protein solutions were slowly applied to each plant by spraying from the base to the tip of each plant, covering all surfaces from the bottom to the top, particularly of plants with flowering or fruiting bodies (generally where stink bugs are found). The egg white solution was only applied to the trap crop around the plot perimeter, whereas the milk solution was applied to the peppers within the plot interior. To limit drift of proteins between the two areas, a 1.8 × 3 m plastic sheet with a 2.54-cm polyvinyl chloride (PVC) pipe frame was held as a barrier between the trap crop and peppers during application.

Leaf sampling. To assess the uniformity of protein spraying prior to application and again after the solution had air-dried, we collected eight leaves haphazardly from the trap crop and pepper plants with no preference for plant section for each of the four plots in each state. In the laboratory, a 7-mm-diameter leaf disc was removed with a cork borer (cleaned after each use) from each of the eight leaves from each crop and each plot. Using clean forceps,

the leaf discs were placed in separate 1.5-ml microcentrifuge tubes and frozen for later protein analysis. Leaves for each protein (egg white and milk) were pooled separately for each crop (trap crop and pepper) and state across sampling dates and replicated by plot. The percentages of leaf samples per location that were marked with only egg white, only milk, or both proteins were compared separately against zero using a one sample

t-test. Statistical analyses were performed with SPSS v.20.0 (IBM, Armonk, NY, USA). For this and all subsequent analyses, $\alpha = 0.05$.

Insect sampling. Prior to protein application, *H. halys* adults were collected from other areas of the farms as negative controls. Additionally, within 1 week prior to protein application or within 1 week after protein application (17 July and 27 August 2014 in NJ, and 22 July and 24 August 2014 in WV) with the crops at the same phenological stages mentioned above, we measured the density of *H. halys* adults and nymphs within the trap crop perimeter, trap-cropped peppers, and the control pepper plots. Within the trap crops we visually assessed stink bug abundance by searching for bugs under the leaves and down in the canopies along two 1-m-long sampling sites for each of the four sides of the trap crop, for each replicate plot. We also sampled the trap-cropped peppers and the control plot peppers by visually assessing stink bug abundance on 10 arbitrarily selected, sequentially placed plants for each of the five rows of peppers.

To measure source-sink dynamics of *H. halys* within the trap crop perimeter and trap-cropped peppers, stink

bugs were sampled 2 h after protein application, and on a daily basis between 4 and 7 days after application at the sample sites above. Stink bugs were collected by gently agitating the plants to dislodge bugs onto a flat, 20.3 × 30.5 cm sticky sheet of cardstock coated with a thin layer of Tangle-Trap (Tanglefoot, Contech Enterprises, Victoria, BC, Canada). Collected *H. halys* adults were immediately removed from the sticky sheet with a clean, wooden toothpick (to reduce potential protein contamination), and placed in individual 1.5-ml microcentrifuge tubes and frozen for protein analysis.

Stink bug densities observed during visual sampling were combined separately for each crop (trap crop and pepper) and state across sampling dates, replicated by plot, and compared using a one-way ANOVA, blocking by state. This was repeated for the abundance of *H. halys* collected within the trap crop and trap-cropped peppers for the stink bugs collected for ELISA analyses.

Protein assessment. Separate immunoassays were performed as indirect ELISA to detect egg white or milk protein markers coating the stink bugs and leaf samples, following the methods described in Blaauw et al. (2016). Commercially available antibodies for chicken egg albumin were used, such as rabbit anti-egg (C6534; Sigma-Aldrich, St. Louis, MO, USA) and bovine casein, rabbit anti-casein (bs-0813R; Bioss, Woburn, MA, USA). The secondary antibodies used for both the egg white and milk assays were peroxidase conjugated (31503; Pierce Biotechnology, Rockford, IL, USA) donkey anti-rabbit IgG (H + L) (SAB3700926; Sigma-Aldrich). For detailed methods on the ELISA procedure, please see the supporting information.

The optical density (OD) for each sample was measured with a Synergy 4 microplate reader (BioTek Instruments, Winooski, VT, USA) at 450 nm, using 490 nm as the reference standard. All readings were controlled (blanked) using wells with tris-buffered saline (TBS) + EDTA extraction buffer and additionally wells containing deionized water with no antigen present. Stink bug and leaf samples were scored positive for the presence of the protein marker if the ELISA OD reading was four standard deviations greater than the mean negative control (TBS + EDTA) result (Jones et al., 2011).

The densities of positively marked stink bugs were summed separately for each crop and sample period to calculate the percentage of bugs marked with only egg white, only milk, or both proteins within the trap crop or peppers. We compared the percentages (arcsine \sqrt{x} -transformed) of positively marked specimens between the trap crop and peppers with a one-way ANOVA, blocked by state as the random factor, Tukey's HSD was used for

pairwise comparisons of percentages of marked bugs for each protein. Statistical analyses were performed with SPSS v.20.0.

Retention assessment with harmonic radar

Stink bug adults for harmonic radar. Source populations of *H. halys* were collected from Jefferson County, WV, in 2014. These were foraging overwintering and F1-generation adults found on preferred hosts that had been baited with the *H. halys* aggregation pheromone (10,11-epoxy-1-bisabolene-3-ol) (Khrimian et al., 2014; Leskey et al., 2015) in combination with a synergist (methyl decatrienoate) (Weber et al., 2014). Prior to tagging and use in experiments, *H. halys* adults were held in a 1.8-m² semi-field cage with a mixed variety of potted tomatoes, winter squash, okra, and swiss chard ad libitum under ambient light and temperature conditions.

Reliability of harmonic radar in vegetable production. In order to establish the baseline reliability of using harmonic radar to track tagged *H. halys* adults within sunflowers, sorghum, and pepper plants, we tested the researchers' ability to find dead, pinned *H. halys* specimen within the plots. The stink bugs were tagged with diodes affixed to copper wires (see Colpitts & Boiteau, 2004) and reinforced with cyanoacrylate glue on their pronotum (Lee et al., 2013). For a detailed explanation of our methods and results for testing the reliability of harmonic radar in the trap crop system, please see the supporting information.

Retention capacity with harmonic radar. In order to track *H. halys* movement between the cash crop and trap crop, a marine harmonic radar device was used. Adults from wild foraging populations that had been collected (described above) were tagged as stated previously. Along with the tag, adults were marked with a unique dab of color from an oil-based paint marker (DecoColor; Uchida of America Corps, Torrance, CA, USA) corresponding to the treatment to which it was assigned. Adults were tagged and marked in the 24-h preceding deployment, and confined in a small plastic container (11.5 cm diameter, 8 cm high) with a moistened water wick, but without food in order to induce foraging behavior. Before sunrise, two adults were released per plot, one on a randomly selected side of the trap crop, and another in the center of the plot in the cash crop for a total of eight adults per time release. Adults were tracked with the harmonic radar at 1, 3, 6, and 24 h after release. At each time point, the distance of the adult from its corresponding release point, its habitat location (e.g., peppers, sorghum, sunflower, or outside the plot), and the time it was retained in the habitat in which it

was released was recorded. When released adults could not be located within the plot, they were marked as 'outside', and the distance moved was recorded as the maximum distance from the last known location to the farthest edge of the plot. The sampling season was split into three periods: early (16 June – 15 July 2014), mid (16 July – 15 August 2014), and late (16 August – 15 September 2014). There were 3–4 releases per time period, with a total of 80 adults tracked over the course of the experiment. During at least two releases per sampling period, the phenological stage of each crop was also assessed. The dominant phenological stage for peppers, sunflower, and sorghum was evaluated by assessing the stage of 20 pepper plants or 1 m row of the trap crop per plot, following descriptions by De Coss-Romero & Peña (1998), Besancon et al. (2014), and Lancashire et al. (1991).

Two separate ANOVAs were used with Tukey's HSD for pairwise comparisons, one for each response variable (retention time and distance moved from release point), each with nearly the same model. Each model contained the release habitat (pepper or trap crop), sampling period (flowering, fruiting, or post-harvest), and sex (male or female) as explanatory variables, and release date as a random blocking variable to control for abiotic variables. The second-order interaction between sampling period and release habitat was also included on the basis that different habitats have varying retention capacities (e.g., Boiteau & Mackinley, 2015), and that phenology of crops often impacts the abundance of *H. halys* (Martinson et al., 2015). In addition, the model examining distance moved

also contained the time since release (1, 3, 6, or 24 h after release) as an additional explanatory variable. Residuals for both models met assumptions of normality and requirements for homoscedasticity. Finally, in order to rule out the possibility that the patterns of where adults were found at the end of the experiment arose solely as a reflection of the surface area of each of the habitats in the plots, a χ^2 analysis was performed. In particular, the observed frequencies of tagged adults in each habitat were compared to the null hypothesis of frequencies for where the adults would be expected to be located at the end of the experiment based on the surface area of the trap-cropped peppers (71.9%) and trap crop (28.1%) and calculated from the total number of released adults. Analyses were performed in R v.3.1 (R Core Development Team, 2015).

Results

Movement tracking with protein-marking

The ELISA analysis of the 128 total leaf samples collected from both the trap crop and pepper plantings revealed that the majority of the leaves from the trap crop and those collected from the peppers were successfully marked with their respective protein marker (Table 1). Marking efficacy of both crops was high, with over 90% of leaf samples testing positive for the appropriate protein marker. Some sample contamination, as measured by the presence of both proteins on a leaf, did occur perhaps from drift during application. The percentage of these doubly-marked

Table 1 Mean (\pm SD) optical density for all samples that tested positive for the protein marker and mean (\pm SEM) percentage of leaves marked positive with egg white, milk, or both protein marker solutions collected from the trap-cropped peppers or the trap crop plants, and compared to zero with a one-sample t-test. In total 128 leaves were collected per crop

Protein ¹	Crop leaves	n positive	Optical density	% marked positive	T (d.f. = 15)	P
Egg white	Pepper	1	0.271	0.8 \pm 0.8	1	0.33
	Trap crop	126	0.310 \pm 0.034	98.4 \pm 1.1	92.2	<0.001
Milk	Pepper	118	0.248 \pm 0.059	92.2 \pm 3.7	24.5	<0.001
	Trap crop	0	–	0	–	–
Egg white + milk	Pepper	12	Egg: 0.258 \pm 0.010 Milk: 0.218 \pm 0.062	2.3 \pm 1.3	1.8	0.082
	Trap crop	1	Egg: 0.299 Milk: 0.184	0.8 \pm 0.8	1	0.33
Egg white	– control ²	0 ³	0.067 \pm 0.062			
	+ control ²	42	0.313 \pm 0.021			
Milk	– control ²	0 ³	0.047 \pm 0.046			
	+ control ²	42	0.257 \pm 0.028			

¹Trap crop was marked with only egg white and the peppers with only milk.

²The '– control' is TBS + EDTA to determine the baseline optical density threshold for no protein marking, whereas the '+ control' is the protein marking solution used to determine whether the ELISA worked. See supporting information for more details.

³42 samples tested; none were positive.

leaves was not significantly greater than zero and thus contamination was not considered to be an important issue here (Table 1).

Comparing *H. halys* densities across the age (nymph or adult) and crop factors, established there was no effect of age ($F_{1,36} = 1.96$, $P = 0.20$) or interaction between crop and age ($F_{2,36} = 0.14$, $P = 0.87$). Similarly, there was no effect of age ($F_{1,24} = 0.34$, $P = 0.56$) or interaction between crop and age ($F_{1,24} = 0.01$, $P = 0.93$) for densities of *H. halys* collected for ELISA analysis, and thus nymph and adult abundances were combined for the analyses and results.

Through visual sampling, a total of six *H. halys* nymphs and 19 adults were observed during the four sampling periods. Although numerically nearly twice as many stink bugs were observed within the trap crop than on either the trap-cropped peppers or the control peppers, the low observation rate resulted in no significant difference of stink bug densities across the crops ($F_{2,20} = 2.51$, $P = 0.11$; Figure 2A). In total 145 *H. halys* were collected

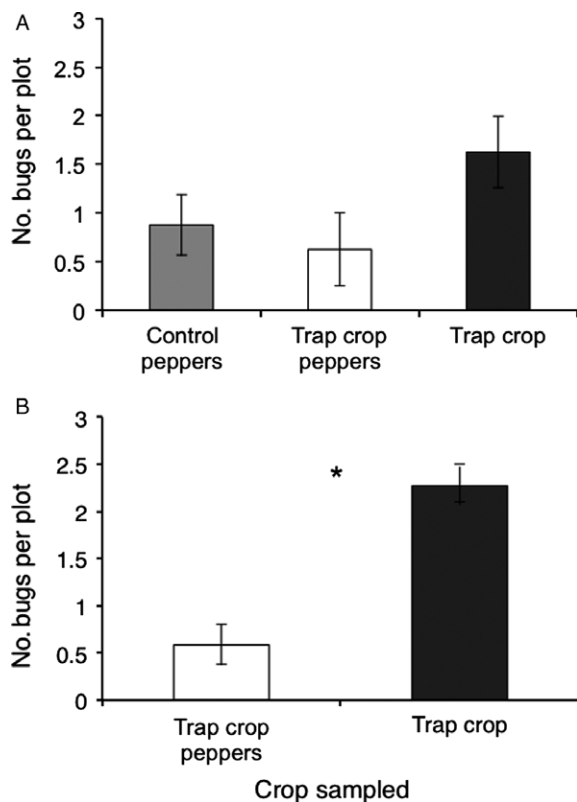


Figure 2 Mean (\pm SEM) density of *Halyomorpha halys* (A) observed within control pepper, trap-cropped pepper, or trap crop plots, and (B) collected from trap-cropped pepper or trap crop plots for use in ELISA analyses in West Virginia and New Jersey, USA, during 2014. The asterisk indicates a significant difference (ANOVA: $P < 0.05$).

via plant agitation across all sampling sites and crops, with 28 collected from the peppers and 117 from the trap crop. Of these, 63 were nymphs and 82 were adults. With all life stages combined, there were significantly fewer *H. halys* collected from the trap-cropped peppers with nearly 4 \times more bugs collected from the trap crops ($F_{2,20} = 2.51$, $P = 0.11$; Figure 2B). This demonstrates that although a portion of *H. halys* ignored the trap crop to make it to the cash crop, a trap crop composed of sunflowers and sorghum is more attractive to (most) *H. halys*.

Assessing the distribution and movement of collected *H. halys* utilizing immunomarking and ELISAs revealed that of the 145 *H. halys* that were collected, 79.3% of bugs tested positive for proteins. A significantly higher percentage of stink bugs were marked only with egg white from the trap crop compared to the peppers (Table 2). Although fewer bugs overall were marked with only milk, there was a significantly higher percentage of *H. halys* collected from the trap-cropped peppers that were marked with milk, because no bugs collected from the trap crop tested positive for only milk (Table 2). Conversely, eight bugs were collected from both the trap-cropped peppers and from the trap crops that were marked with both proteins. In a small unreplicated study, 85% tested positive for egg protein and 60% for milk protein of the 10 adult *H. halys* per treatment allowed to walk across a pepper plant treated with either an egg white or milk solution (same as field applied solutions) for 10 min (BR Blaauw, unpubl.). This established the potential for *H. halys* to be marked either directly or to a lesser extent through direct contact with a previously marked leaf. Thus, the doubly-marked bugs demonstrated movement between the two planting systems, but there was no significant difference in the percentages of all bugs marked with both proteins (Table 2). Analyzing the marked insects across proteins and crops revealed differences amongst marking percentages ($F_{5,41} = 40.3$, $P < 0.0001$), with higher percentage of bugs marked with only egg collected from the trap crop compared to any other protein or crop combination (Figure 3). Subsequently, the percentage of bugs marked with only milk collected from the trap-cropped peppers was higher than the percentages of bugs marked with both proteins from either crop (Figure 3). The percentages of bugs marked with both proteins collected from the trap-cropped peppers and those from the trap crop were not significantly different from each other or from zero (Figure 3), revealing that movement of *H. halys* from the trap crops to the peppers and vice versa is statistically negligible.

Retention assessment with harmonic radar

The phenology of the crops was assessed throughout the three sampling periods with the early stage dominated by

Table 2 Mean (\pm SD) optical density for all samples that tested positive for the protein marker and mean (\pm SEM) percentage of *Halyomorpha halys* adults per sampling site marked positive with egg white, milk, or both protein marker solutions collected from the trap-cropped peppers or the trap crops, analyzed with an ANOVA. Total number of bugs collected was 145

Protein ¹	Crop sampled	n positive	Optical density	% marked positive	F _{1,13}	P
Egg white	Pepper	0	–	–	66.83	<0.0001
	Trap crop	98	0.238 \pm 0.048	83.4 \pm 6.1		
Milk	Pepper	9	0.149 \pm 0.033	26.5 \pm 4.6	22.56	0.0003
	Trap crop	0	–	–		
Egg white + milk	Pepper	4	Egg: 0.222 \pm 0.056 Milk: 0.111 \pm 0.016	10.9 \pm 4.6	0.41	0.54
	Trap crop	4	Egg: 0.159 \pm 0.009 Milk: 0.122 \pm 0.034	6.4 \pm 4.8		
Egg white	– control ²	0 ³	0.056 \pm 0.011			
	+ control ²	64	0.280 \pm 0.049			
Milk	– control ²	0 ³	0.038 \pm 0.006			
	+ control ²	64	0.117 \pm 0.035			

¹Trap crop was marked with only egg white and the peppers with only milk.

²The ‘– control’ is TBS + EDTA to determine the baseline optical density threshold for no protein marking, whereas the ‘+ control’ is the protein marking solution used to determine whether the ELISA worked. See supporting information for more details.

³64 samples tested; none were positive.

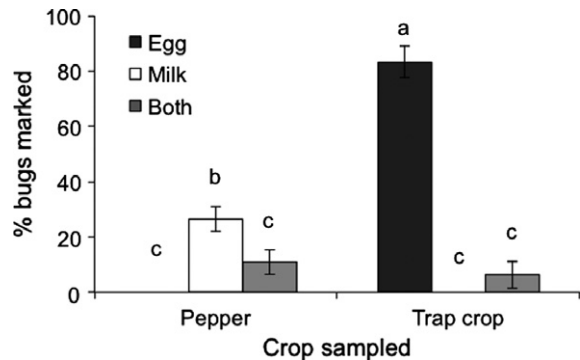


Figure 3 Mean (\pm SEM) percentage of *Halyomorpha halys* per plot marked positive with egg white, milk, or both protein marker solutions compared across crops. Means capped with a different letter are significantly different (Tukey's HSD: $P < 0.05$).

the flowering stage for peppers, and the vegetative stages dominating the two trap crop plants (Table 3). The pepper crop was primarily in the early stages of fruiting during the mid-sample period, whereas the sorghum plants were still vegetative and the sunflowers were flowering and beginning to set fruit, which corresponds to when *H. halys* are most attracted to sunflower (Table 3) (Nielsen et al., 2016). During the late sampling period, most of the pepper plants were at the late fruiting stage with sunflower senescing and sorghum at its attractive stage, seed filling (Table 3) (Nielsen et al., 2016).

The overall model for retention time of *H. halys* adults released in either the trap crop or cash crop and tracked

with harmonic radar explained a significant portion of the variation in the data ($F_{7,72} = 3.83$, $P = 0.0014$), with both the release habitat ($F_{1,72} = 9.03$, $P = 0.0037$; Figure 4A) and the sampling period ($F_{2,72} = 5.45$, $P = 0.0062$; Figure 5A) affecting the retention time of tagged adult *H. halys*. Overall, the trap crop retained adults 1.5 \times longer compared to the peppers. The difference in the retention time between the two habitats was greatest when peppers were primarily in the flowering stage, where the retention time of the trap crop was over 4 \times greater compared to the cash crop (Figure 5A). The numerical advantage in retention remained for the trap crop throughout the season, though it was not statistically different from the cash crop in the latter two periods of the season (Figure 5A). The interaction between release habitat and phenology was not significant ($F_{2,72} = 0.541$, $P = 0.59$), and the sex of the adult did not affect the retention time on the hosts ($F_{1,72} = 1.63$, $P = 0.20$).

The overall model for the distance moved by adult *H. halys* from the release point in the trap or cash crop and tracked with harmonic radar was significant ($F_{9,278} = 15.62$, $P < 0.0001$), with both the release habitat ($F_{1,278} = 16.34$, $P < 0.0001$) and the sampling period ($F_{2,72} = 35.51$, $P < 0.0001$) affecting the distance that adult *H. halys* traveled after release. Specifically, adults released in the cash crop moved almost twice as far as those in the trap crops (Figure 4B). This pattern was most pronounced in the early, and least so in the mid-period (Figure 5B). There was no significant interaction between the release habitat and the phenology of the plants on the distance

Table 3 Summary of the dominant phenological stages for the cash crop (peppers) and two host plants comprising the trap crop in three sampling periods at Redbud Farm (Inwood, WV, USA) in 2014. Mean (\pm SEM) host plant growth stages are presented with number of tagged *Halyomorpha halys* adults released and relocated using harmonic radar

Sampling period	n bugs released ⁴	Host plants sampled					
		Peppers ¹		Sorghum ²		Sunflower ³	
		Mean \pm SEM	% found ⁵	Mean \pm SEM	% found ⁵	Mean \pm SEM	% found ⁵
Early	24	1.8 \pm 0.1	4.2 ⁶	4.0 \pm 0.0	0	59.5 \pm 0.5	33.3
Mid	32	2.7 \pm 0.1	28.1	5.1 \pm 0.1	0	66.3 \pm 1.0	53.1
Late	24	3.9 \pm 0.1	25	7.7 \pm 0.2	33.3	86.9 \pm 1.1	4.2

¹Pepper phenological stages are based on 1 = vegetative, 2 = blossoming, 3 = early fruiting, and 4 = late fruiting (after De Coss-Romero & Peña, 1998). Dominant phenological stage evaluated by assessing the stage of 20 pepper plants.

²Sorghum phenological stages according to Besancon et al. (2014). Dominant stage assessed by rating all the plants in a 1 m row of crop.

³Sunflower phenological stages according to species-specific BBCH (Lancashire et al., 1991). Dominant stage assessed by rating all the plants in a 1 m row of crop.

⁴Tagged *H. halys* adults released on plants in each sampling period and tracked with harmonic radar. Half of the adults were released on peppers, and half were released on sunflower.

⁵Of those tagged *H. halys* adults released during the sampling period, this percentage was found on plants of these phenological stage at the end of the harmonic radar trials.

⁶Note: most of tagged adults left the pepper plots, thus percentages within a sampling period do not add up to 100%. Those that remained in the cash crop were on stage 3 peppers.

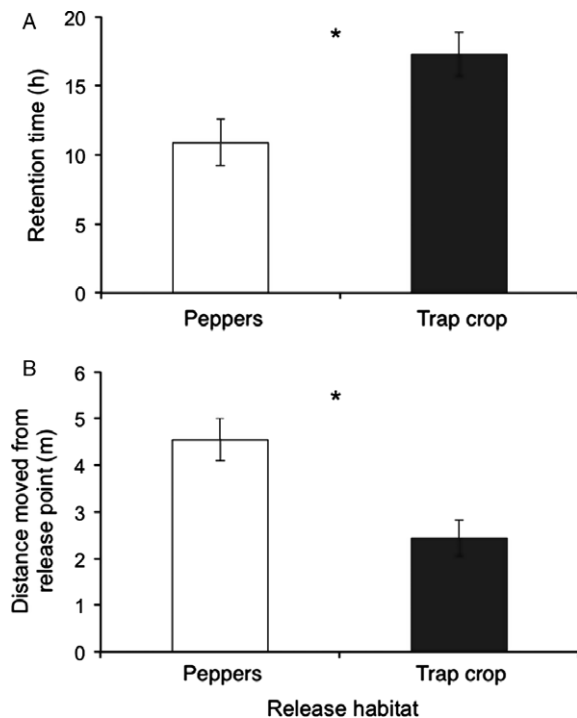


Figure 4 Retention capacity of micro-tagged *Halyomorpha halys* adults in either a cash (peppers) or trap (sunflower and sorghum) crop illustrated by mean (\pm SEM) (A) retention time (s), and (B) distance moved (m) from the release point in each habitat type over the course of the season in 2014 in West Virginia, USA. Asterisks indicate significant differences between the habitat types (Tukey's HSD: $P < 0.05$).

moved by adults ($F_{2,278} = 2.55$, $P = 0.080$). However, the time elapsed since the adult was released affected the distance moved ($F_{3,278} = 15.12$, $P < 0.0001$; Figure 6), with adults having moved greater distances by later sampling intervals. Adults at the 24-h sampling interval had moved over 4 \times the distance as those at the 1-h sampling interval, and individuals released in the trap crop had moved consistently shorter distances at each interval than those in the cash crop (Figure 6). The sex of the released adult did not affect the distance that the adult moved ($F_{1,278} = 0.768$, $P = 0.38$).

Of the total number of adults released, 62.5% were recovered. A total of 18% of the adults released in the pepper crop switched to the trap crop by the end of the experiment, whereas none of the individuals switched from the trap crop to the pepper crop (Figure 7). Of the 50 bugs that were recovered in the experiment, 68% ended up in the trap crop, and 46% in the cash crop, which is contrary to the proportion expected were the adults to have proportioned themselves according to the surface area of each habitat ($\chi^2 = 876.8$, d.f. = 1, $P < 0.0001$; Figure 8).

Discussion

Studies on insect dispersal, especially those utilizing marked individuals, have focused on movement between resources or attraction to a host (Shelton & Badenes-Perez, 2006). Whereas the attraction of a host is paramount for the trap cropping technique to work, previous studies have

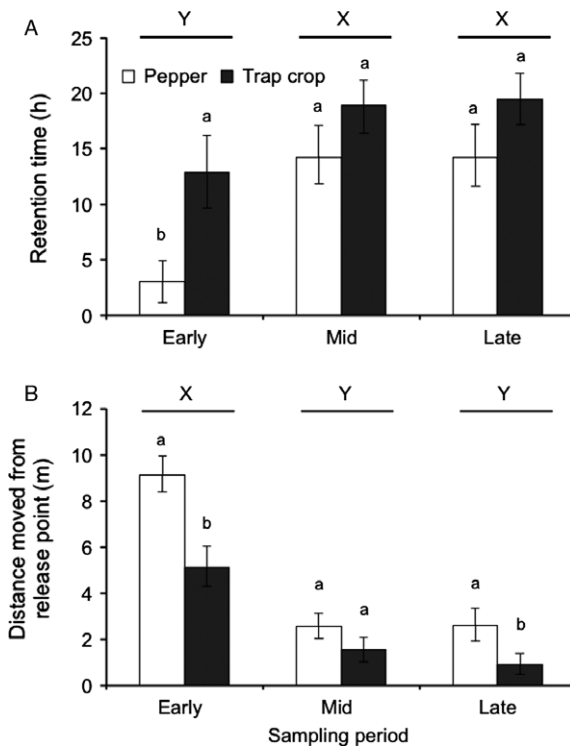


Figure 5 Retention capacity of micro-tagged *Halyomorpha halys* adults in either a cash (peppers) or trap (sunflower and sorghum) crop during different sampling periods in the season illustrated by mean (\pm SEM) (A) retention time (s), and (B) distance moved (m) from the release point in each habitat type in 2014 in West Virginia (USA). Means capped with different letters are significantly different within (a,b) and across (X,Y) the phenological stages (Tukey’s HSD: $P < 0.05$).

not explicitly measured trap crop retention of target pests, a crucial factor of trap crop effectiveness (Holden et al., 2012). Using a combination of visual sampling, immunomarking, and harmonic radar, we investigated the

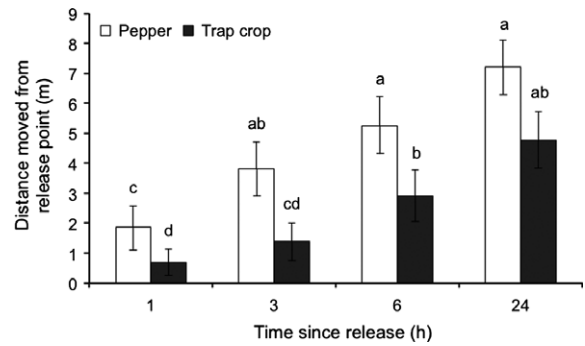
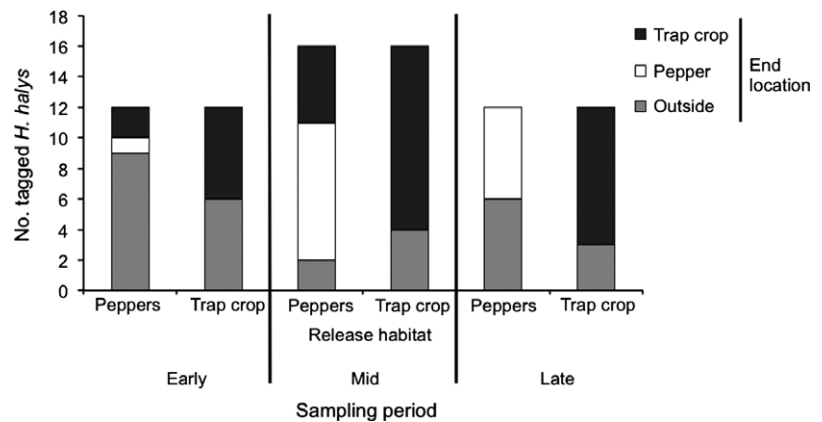


Figure 6 Mean (\pm SEM) distance moved (m) by micro-tagged *Halyomorpha halys* adults from their release point when released in a cash crop (peppers) or trap crop (sunflower and sorghum) at various sampling intervals over the course of a day. Means capped with the same letter are not significantly different (Tukey’s HSD: $P > 0.05$).

dispersal behavior of *H. halys*, and the subsequent attraction to and retention of trap crops for managing the pest in organic systems. Our results demonstrate that a trap crop comprised of sunflowers and grain sorghum both attracts and arrests the movement of *H. halys* during host finding, limiting their movement into – and even attracting them away from – a bell pepper cash crop.

The efficacy of the trap crops appears to be largely dependent upon the phenology of the trap crop and the cash crop plants. When the pepper crop was primarily in the flowering stage, the tagged stink bugs were retained for a longer period within the trap crop than within the pepper plants. The strength of retention lessened once the pepper plants began fruiting even though the trap crop plants were at their most attractive growth stages. However, the numerical advantage in retention remained for the trap crop during subsequent samples, and most tagged *H. halys* that were released in the trap crops during the mid- and late-sampling periods remained on the trap crops, and none of those bugs moved to the pepper

Figure 7 The final location of micro-tagged *Halyomorpha halys* adults after a 24-h sampling period, based on whether it was originally released at the beginning, the middle, or the end of the sampling period, in the cash or the trap crop. Adults were recorded as being located in the trap crop (sunflower and sorghum), cash crop (peppers), or as outside the sampling area.



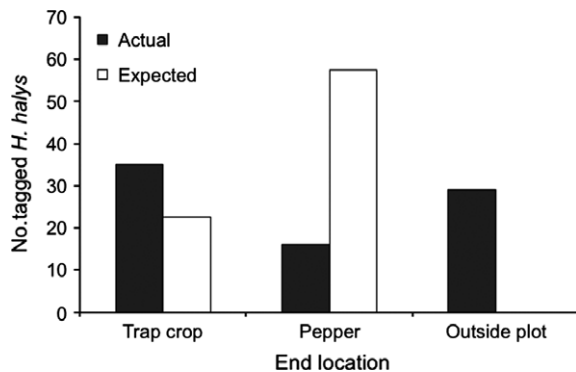


Figure 8 The final location of micro-tagged *Halyomorpha halys* adults after a 24-h sampling period according to where they were expected to be if partitioning in the habitat were simply according to the area of cash crop and trap crop in each plot, or where they actually were observed. The two distributions were different (χ^2 -test: $P < 0.05$).

cash crop. Looking at both marking techniques together, our results suggest that the trap crop was very attractive to *H. halys*, but due to lower population densities than expected during the sampling periods, our assessment of retention time and dispersal were limited. However, the trap crop seems to have a significantly greater retention capacity compared with the bell pepper cash crop, with the trap crop arresting dispersal and host-finding behaviors of *H. halys*. Optimizing a trap cropping system that is attractive and retains the target pest throughout the season, essentially outcompeting the cash crop, is crucial for an effective management tactic.

The strong capacity for dispersal and its polyphagous behavior has aided in *H. halys* emerging as a key pest of many fruit and vegetable crops in the mid-Atlantic region of the USA (Leskey et al., 2012a; Lee et al., 2014; Wiman et al., 2015). Additionally, as a highly mobile insect, *H. halys* individuals exhibit a strong edge effect in multiple cropping systems, attacking the crop frequently throughout the growing season as it disperses from the surrounding habitats (Venugopal et al., 2014, 2015b; Basnet et al., 2015; Blaauw et al., 2016). Consequently, growers currently rely heavily on broad-spectrum insecticides to manage *H. halys*, which has caused numerous growers to abandon integrated pest management strategies and return to calendar-based chemical management of pests (Leskey et al., 2012b). The same behaviors that make *H. halys* such an effective pest also make it a great candidate for management tactics that exploit its dispersal abilities and polyphagous habits, such as trap cropping. Our results agree with the suggestion from Nielsen et al. (2016) that the combination of open-pollinated sunflower mix and grain sorghum are attractive hosts for stink bugs.

Due to the sampling procedure we are unable to determine whether *H. halys* marked with only milk protein collected from the trap-cropped peppers were already on the pepper plants and were marked directly during protein application or if they immigrated directly into the pepper plot ignoring the trap crop. Additionally, the ELISA for bovine casein protein is less sensitive than that of egg albumin protein, and combined with the potential for removing protein from marked bugs due to the adhesive in the collection process (Jones et al., 2011), we may have underestimated the number of bugs marked positive with milk. As a result, we may have underestimated the percentage of bugs that had moved to the trap crops after visiting the peppers from the ELISAs compared with the switching rate observed by the harmonic radar trials.

Without a thorough understanding of the behavior and ecology of the pest and its interactions with its hosts, the chances for developing a successful method that exploits dispersal behavior are low, and the ability to modify and refine the method to enhance its efficacy for alternative pest management is limited. A comprehensive understanding of the behaviors that a pest exhibits during dispersal and host choice may assist in the development of synergistic semiochemicals that enhance behavior-based management strategies. For example, taking advantage of the attraction of *H. halys* to a trap crop along with its perimeter-driven dispersal behavior may create a non-cash crop area for a spatially precise insecticide application, thereby reducing the amount of insecticide applied to the cash crop. Similar to *H. halys* management in soybean (Leskey et al., 2012a) and peaches (Blaauw et al., 2015), exploiting this behavior through spatially precise perimeter-focused applications of insecticides is a tactic that can improve *H. halys* management while reducing insecticide inputs (Blaauw et al., 2015). Another example includes the combination of trap crop attraction with a common behavioral mechanism, such as an aggregation pheromone or food odors, which may enhance retention time and decrease *H. halys* movement to the cash crop (Morrison et al., 2016). The data presented here suggest that the previously suggested hosts, sunflower and sorghum, have the potential to not only attract the invasive *H. halys*, but also arrest the host-finding movement of the bugs, demonstrating that these plants have the potential to retain individuals for an extended period of time, and thus operate as an effective management tool. However, further research is needed to understand how these results correspond to crop damage and stink bug persistence within the plots over a longer period of time. Ultimately, trap cropping shows incredible promise and, if optimized properly, may serve as a viable alternative tactic for growers managing *H. halys*.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Protein assessment.

Data S2. Reliability of harmonic radar in vegetable production.

Data S3. Results of the reliability of harmonic radar in vegetable production.