J Pest Sci
DOI 10.1007/s10340-015-0663-1

ORIGINAL PAPER

Marking and retention of harlequin bug, *Murgantia histrionica* (Hahn) (Hemiptera: Pentatomidae), on pheromone-baited and unbaited plants

Guillermo Cabrera Walsh[1] · Anthony S. Dimeglio[2] · Ashot Khrimian[2] · Donald C. Weber[2]

Received: 23 September 2014 / Revised: 28 March 2015 / Accepted: 31 March 2015

Abstract Harlequin bug (*Murgantia histrionica*) is an important pest of cole crops in the USA. The adults and nymphs feed on aboveground plant tissues by sucking cell contents and can seriously damage the host. Current insect control measures on cole crops target mainly lepidopteran pests, and the insecticides generally used do not control harlequin bug, so alternative management practices need to be explored. Previous research has established the existence of a male-produced pheromone attractive to both sexes and nymphs of *M. histrionica*. In this work, two systems of marking bugs were tested to verify if the mark affected fitness traits such as survival and host location. In a second phase, marked individuals were placed on trap host plants baited with synthetic pheromone lures to test whether migration rates were related to *M. histrionica* density on the trap plants and the presence of the attractants. Neither marking system affected the survival or orientation of the subjects compared to unmarked individuals. The pheromone lures significantly increased the attractiveness of the trap plants, but did not increase the retention time of the plants compared to unbaited plants. Emigration from the trap plants showed a constant rate and seemed unrelated to bug density on the plants. However, a mean peak density of ca. 36 bugs/plant was calculated. Beyond this number, density tended to decrease. These successful marking methods and retention time models support development of *M. histrionica* management with trap crops, by providing tentative control thresholds and decision rules.

Keywords 10,11-epoxy-1-bisabolen-3-ol · Mark-release-recapture · Trap crops · Migration rates · Stink bug

Key message

- Mark-release experiments were used to test harlequin bug (*Murgantia histrionica*) movement in the field to baited collard plants.
- Synthetic male pheromones and mustard oils were used to bait trap plants.
- Marking did not affect the survival or orientation of the bugs.
- Baits increased the attractiveness of the trap plants, but not the retention time of the plants compared to unbaited plants.
- A mean peak density of 36 bugs/trap plant was calculated.

Introduction

Harlequin bug, *Murgantia histrionica* (Hahn) (Hemiptera: Pentatomidae), is an important pest of cole crops (Brassicaceae) in the southern USA. The adults and nymphs feed on aboveground plant tissues, by lacerating plant cells and flushing the contents (Peiffer and Felton 2014), leaving white blotches on the leaves, affecting crop quality. High densities can seriously damage or kill the host (Ludwig and Kok 2001; Wallingford et al. 2011). The decrease in the use of broad spectrum insecticides on brassicaceous crops

Communicated by M. Traugott.

✉ Guillermo Cabrera Walsh
gcabrera@fuedei.org

[1] FUEDEI (Invasive Species Research Foundation), Bolívar 1559, B1686EFA Hurlingham, Buenos Aires, Argentina

[2] USDA Agricultural Research Service, Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, MD, USA

Published online: 10 April 2015

has caused an important rebound in the pest status of this insect. Pest control methods on these crops tend to an integrated approach that targets mainly lepidopterans and aphids and only poorly control harlequin bug (Walgenbach and Schoof 2005; Wallingford et al. 2012), so alternative management practices need to be explored.

Previous research has established the identity of the male-produced pheromone attractive to both sexes of *M. histrionica* (Zahn et al. 2008, 2012; Khrimian et al. 2014; Weber et al. 2014). There are also distinct preferences for attraction to different host plants (Wallingford et al. 2013). *Murgantia histrionica* sequesters toxic glucosinolates from its host plants, presumably as a defense against natural enemies (Aliabadi et al. 2002). These studies suggest that both the pheromone and host plant compounds such as isothiocyanates (mustard oils) have great potential for management applications.

The utility of a trap crop, or any trap for that matter, depends on several dynamic factors. The range of attraction of a pheromone, plant or plant compound used as a lure, depends among other things on the feeding status and physiological state of the target pest, abiotic conditions, the availability of favored crops and their proximity, and possibly on pest density as well. The retention of the pest on the trap crop, and the proportion of the population of a given area that is effectively captured by a trap or trap crop, also depends heavily on the order of preference for different hosts in the environment, their stage, and density (Hokkanen 1991; Midega et al. 2010). The addition of a pheromone or other semiochemicals to the trap crop can enhance its effect. Regardless, attraction to the pheromone and to the trap crop could both vary seasonally.

In the case of trap crops, baited or not, there is also the additional question of the immigration–emigration balance, associated to host fidelity. Unless the trap plants keep the target insects on them for a significant period of time and/or this balance is significantly biased toward immigration, trap crops may have no efficacy at keeping the pest away from the protected crop or may even attract more pests to it (Yamanka et al. 2011; Holden et al. 2012). Even the term "significant period of time" may mean different things, such as time for the trap crop to be sprayed with insecticide or time to minimize the damage on the protected crop. This immigration–emigration balance can be assessed with insect marking experiments, but conclusions rest on the fitness and data reliability obtained with the marked bugs.

In this work, we first assess three fitness aspects as affected by two different marking techniques, alone and together: comparative life expectancy, host or pheromone detection capabilities, and migration/mobility to host plants. The advantage of using two different marks at the same time resides in the number of combinations, which can greatly increase the number of simultaneous observations defined in a release-recapture experiment without the use of ambiguous or unclear marks derived from similar colors or juxtaposition of multiple marks.

Using marked *M. histrionica* and individual host plants, we then assess migration and retention among plants with and without pheromone lures.

Materials and methods

Marking methods

All the experiments were performed on the North Farm of the Beltsville Agricultural Research Center (BARC), in Beltsville, Maryland, the USA, between June and August 2013, with wild bugs captured in the field 12–48 h prior to the experiments on different brassicaceous crops located at the same research center. The bugs were captured in 2.5-l plastic jugs with plastic kitchen funnels (20 cm wide at the mouth) attached through the jug caps. Plants were inspected visually and the jugs were simply placed beneath bugs, which were prodded or shaken, so that they dropped in the funnel and into the jug. The bugs were kept at room temperature until marking in 30.5 × 30.5 × 61 cm collapsible aluminum cages with two or three collards (*Brassica oleracea* L., acephala group, cv. Champion or Vates) in 3.8-l pots for nutrition. A random subsample of 100 bugs was taken from the cages and sexed. These samples were compared with a simple binomial test to test for significant deviations from a 1:1 sex proportion (VassarStats 2014).

The two marks used were fluorescent pigment powders (Shannon Luminous Materials, Inc., Santa Ana, CA) of the colors blue, golden yellow, and fire red; and oil-based paint markers (Sharpie, Newell Rubbermaid, Inc., Freeport IL) of the colors red, gold, yellow, green, blue, and white. Silver and black paint markers were discarded in preliminary tests because silver chipped off easily and black was difficult to see against the insect's background colors. The bugs were chilled at 3 °C in a refrigerator for 20 + min and held with soft forceps to paint a single streak of about 3 mm across the middle of the pronotum. After allowing the bugs to dry and recover for 1 h, they were dusted with the pigment powders by shaking ca. 15 mg of powder inside 16.9 by 14.9-cm plastic bags and introducing 10–30 bugs inside for 5 min. The marked insects were then placed in ventilated 2-l rectangular boxes with several layers of collard leaves and paper towels for the insects to recover and to cast off excess powder.

Survival

Sixty adult bugs were chilled at 3 °C and then marked with the paint markers as described above, ten with each color.

One hour later, they were combined and then separated at random into three groups of 20, then marked with one of the powder colors, and left to recover overnight on collard plants. Twelve collapsible aluminum cages (30.5 × 30.5 × 61 cm) were placed on the ground three meters apart in a row next to a row of fruiting collards. Cages were numbered 1–12, and a ca. 25-cm-tall potted collard plant was placed in each. The marked bugs (10 for each cage) were selected at random and assigned to 6 randomly selected cages. The remaining cages received 10 unmarked bugs each as controls. Beginning 3 days later, all dead bugs were taken out of the cages every 2–3 days. The dead bugs were sexed and checked for paint and powder marks. The assignment of the insects to different colors and cages at random was meant to control for effects due to location or color combinations.

The lifespan of each bug was recorded together with its sex, paint color, and powder color. A generalized linear model was used to compare lifespan differences related to these same factors. The interactions sex * paint and powder * paint were also assessed in the model.

Pheromone and plant volatiles detection

The attraction of wild, unmarked *M. histrionica* to synthetic pheromone-baited collards was compared to that of collards alone, so as to confirm the increased attractiveness of plants lured with this synthetic pheromone (MIX2, see Table 1) compared to plants alone. The cumulative binomial probability that captures on baited and unbaited plants were equal was calculated using the number of captures of each sex on baited plants as the number of trials, assuming a probability of 0.5.

The capacity of marked bugs to find their way to trap collard plants, alone or with added lures loaded with different mixtures of synthetic male *M. histrionica* pheromone stereoisomers, (3*S*,6*S*,7*R*,10*S*)- and (3*S*,6*S*,7*R*,10*R*)-10,11-epoxy-1-bisabolen-3-ol, referred to, respectively, as SSRS and SSRR (Khrimian et al. 2014), or isothiocyanates (mustard oils), was compared to that of unmarked bugs through a series of release-recapture tests of marked bugs, adults and nymphs, in four different experiments. The types of lures used, delivery method, and number of insects and stage released are specified in Table 1. Individual isomers were prepared according to the synthetic methods of Khrimian et al. (2014). Chemical purities of tested stereoisomers were ≥95 % and stereoisomeric purities were SSRS 95 % *dr* (diastereomeric ratio; percentage of main stereoisomer to sum of minor stereoisomers) and SSRR 95 % *dr*. Mixed-isomer preparations containing both cis- and trans-10,11-epoxy-1-bisabolen-3-ols were prepared from (7R)-4-(6-methylhept-5-en-2-yl)cyclohex-2-enone (Hagiwara et al. 2002) following Zahn et al. (2008). Mixed-isomer lure #2 (MIX2), was a crude mixture of eight stereoisomers of 10,11-epoxy-1-bisabolen-3-ol with 7R configurations. All lures were gray rubber septa (1-F SS 1888 GRY, West Pharmaceutical Services, Lititz, PA) washed in a Soxhlet apparatus with hexane and dried for 12 h before loading with candidate attractants as described in Khrimian et al. (2008).

Allyl isothiocyanate was also tested as a lure, based on a preliminary field test showing that, among three commercially available common isothiocyanates (allyl, benzyl, and 2-phenylethyl isothiocyanate) derived from the breakdown of natural cruciferous glucosinolates (Fahey et al. 2001) at doses of 10 and 100 µl, allyl isothiocyanate was most attractive to wild *M. histrionica* (unpublished).

Table 1 List of lures and marks used to assess the comparative detection ability of marked and unmarked *M. histrionica*

Test	Lure	Code	Delivery	Mark	No. released	*P* value*
1	MIX2** versus control	MIX2	Rubber septa***	Unmarked	200 adults	N/A
2	Natural blend versus equal quantities of SSRS and SSRR, each with 2 mg SSRS	H4 + H12-2	Rubber septa	Paints + powder	200 marked adults + 200 unmarked adults	0.11
3	Natural blend versus equal quantities of SSRS and SSRR, each with 4 mg SSRS	H4-4 + H12-4	Rubber septa	Red powder	200 marked adults + 200 unmarked adults	0.48
4	Allyl isothiocyanate, 100 µl versus blank	A100	Cotton wicks	Red powder	130 marked nymphs + 130 unmarked nymphs	0.81
5	Allyl isothiocyanate, 10 µl versus blank	A10	Cotton wicks	Red powder	130 marked nymphs + 130 unmarked nymphs	0.56

* *P* value of Fisher's exact test for treatment difference between marked and unmarked bugs

** MIX2 was a mixture of eight stereoisomers of 10,11-epoxy-1-bisabolen-3-ol with 7R configurations synthesized as in Khrimian et al. (2014) without further purification. The ratio of *cis* and *trans* stereoisomers from the reaction was 1:2, and the active pheromone components SSRS and SSRR (which are both *cis* isomers) were in approximate 1:1 ratio

*** All lures were gray rubber septa (1-F SS 1888 GRY, West Pharmaceutical Services, Lititz, PA) washed in a Soxhlet apparatus with hexane and dried for 12 h before loading with candidate attractants as described in Khrimian et al. (2008)

Trap plant plots were prepared with 6–8-week-old collards (cv. Champion or Vates) grown in 3.8-l pots in a greenhouse with potting soil (Gromix BX, Premier Tech Horticulture, Rivière-du-Loup, Québec, Canada), fertilized with slow-release fertilizer (Osmocote® pro, Scotts Miracle-Gro Marysville, OH). Eight healthy, similar sized plants (ca. 30 cm tall) were taken out of the pots and planted in the soil in circles 32 m in diameter. Each plant was 12.6 m from the next. There were 5 such circles, separated 50 meters from each other, in a 3-ha fallow field on BARC North Farm, Beltsville, Maryland (39°01′48″N 76°56′00″W). The top and lure basket of a green Unitrap (Great Lakes IPM, Vestaburg, MI, USA) was positioned immediately over the top of each plant, using a 1-m-tall bendable steel green PVC-coated flower stake. Each basket had a snap-on lid to protect the lure (rubber septa for the pheromones and 1-cm pieces of dental wicks for the mustard oils) from the elements.

This design allowed two trap plants per cardinal direction from the release point (Fig. 1a). Each treatment was assigned at random to one of the plants per directional block, while the other was used as a control. This design allowed testing the different compounds independent of wind direction or any other directional tendency of the subject bugs. Any wild alternative hosts within or close to the circles that emerged after the plowing were removed on sight.

Bugs were released in the center of the circle after dusk because preliminary tests indicated that *M. histrionica* would not readily move about at night. This timing was intended to prevent the bugs from flying or walking away from the arena while distressed by the liberation process and all the previous manipulations. The trap plants were inspected the following day at 0630, 1200 and 1700 h. Plants were inspected at about 1200 h on all ensuing days for 4–6 days. Inspection of all the plants took between 15 and 40 min, depending on the number of bugs collected from them. One plant circle was used per lure combination, except for the allyl isothiocyanate tests which were repeated simultaneously in all the plots. The total catches per treatment (lure type) per inspection were pooled, to avoid working with too many zeroes or the contrasting numbers found on plants of different quadrants (i.e., windward plants are bound to have more bugs than the leeward plants of the same treatments). So each circle was in practice a replicate, not each baited plant/control pair, and each day a repetition. Fisher's exact tests were performed for each test to determine if there were any significant associations between marked or unmarked bugs with either of the treatments of the test, be they different lures, or lures and control plants.

Because *M. histrionica* was abundant in the environment, there was no certain way of making sure which unmarked bugs recovered on the trap plants were from the releases and which were not. However, as the collard circles were isolated within the plowed field, it was assumed that any resurgence in the numbers of bug captures after a few days into the test were from volunteer bugs. Resurgence was defined as a recovery of bug numbers on the trap

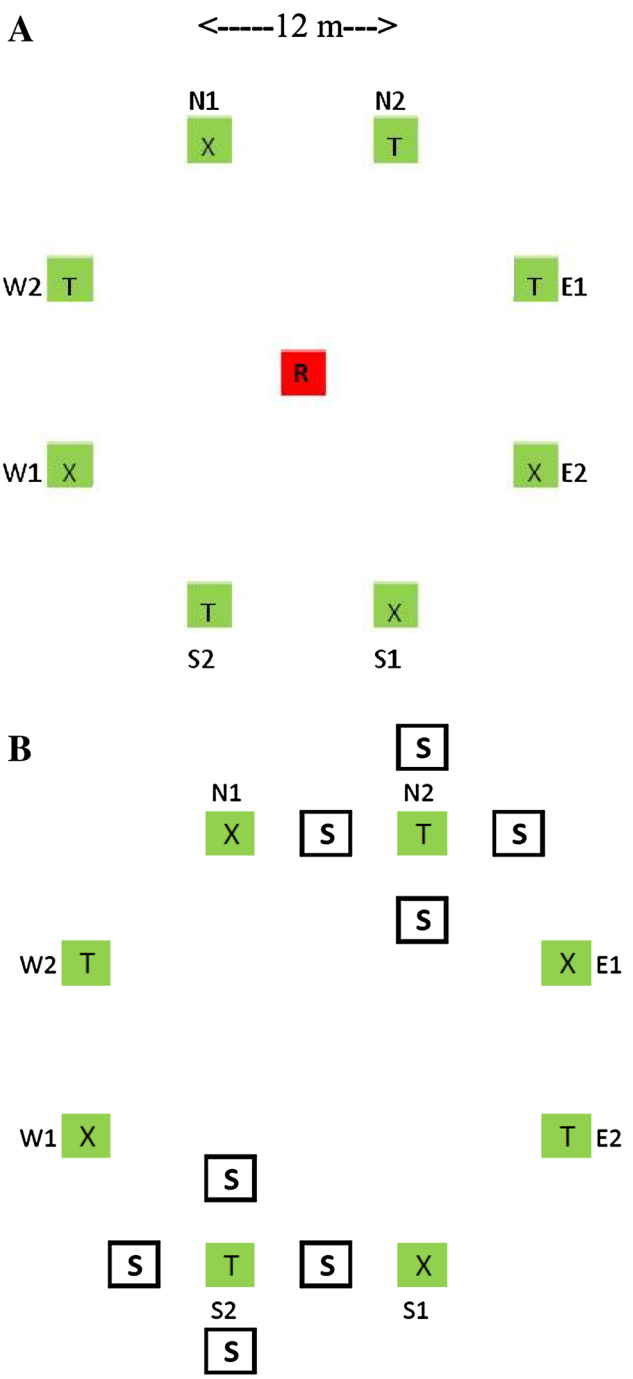

Fig. 1 Experimental plots design: **a** simple release circle: *R*, release point; *N1*, *N2*, *E1*, *E2*, etc., first and second plant, respectively, of each cardinal direction; *X*, control plant; *T*, lured plant; **b** same, with the addition of satellite plants (S) (2 mustards + 2 collards, planted in random order)

plants after at least 1 day of zero or near zero catches. So the total numbers of marked and unmarked bugs captured before the resurgence of wild volunteers were compared with Mann–Whitney *U* tests (Systat 2004), to test for differences in the detection and/or movement capability compared to unmarked *M. histrionica* adults and nymphs.

We preferred not to use marked nymphs because the mark would be lost at molting. So we performed the pheromone tests with adults. However, in view of the appearance of wild adults interfering with the experiments, we decided to use nymphs for the mustard oil tests (which were deployed immediately after the pheromone tests). Because the circles were isolated within a barren field, we assumed that wild nymphs would not get to the trap plants before the ones released within the trap plant circles, as adults could have by flight. Using different stages was deemed immaterial for the objectives of the study because we only meant to compare the number of marked and unmarked bugs of a same population arriving at the plants, not the relative attraction of pheromones to mustard oils.

Retention by trap plants of marked and wild bugs

The retention of *M. histrionica* on the trap collards with added pheromone lure (MIX2) was evaluated in the field by releasing at night 120 oil paint-marked adults on plants of the collard circles. Twelve bugs (planted bugs) were released on 10 collards (home plants), two from each circle, located opposite each other. The same six oil paint colors described in the first section were used, and colors gold, yellow, white, and blue were repeated, so they were marked with a curved instead of a straight streak the second time. Each release plant was surrounded by two additional collards and two mustard greens (*Brassica juncea* (L.) Czernajew cv. 'Southern Giant Curled) (satellite plants), planted at random on one cardinal point 5 meters away from the host collard (Fig. 1b). These plants were meant to attract away the marked bugs or intercept the ones leaving the chosen release plant. Every morning at 0630 h (while it was still cool, before the bugs were prone to take flight), the home plants were checked for marked bugs, trying to disturb them as little as possible. Every other collard plant in the circles and the satellite plants were also checked (60 collards and 20 mustards in total), allowing calculation of a mean daily change in bug numbers on each plant. The number and origin of the marked bugs were recorded, the distance from their original plants, as well as the number of wild bugs establishing on each plant. The mean number of wild *M. histrionica* invading the home plants per day was calculated and compared to the mean number of marked bugs abandoning them. The association between both was evaluated with Pearson's correlation. Neither the marked nor the wild bugs were picked off, and the numbers were allowed to increase naturally to replicate the effect of crowding on bug retention by the trap plants.

The retention periods in days of marked bugs that moved to another, unbaited, plant were compared to those of the planted bugs with a Mann–Whitney test (Systat 2004). In other words, the time on the lured plants was compared to the time on the unbaited plants on which the marked bugs settled voluntarily. This could indicate if the lure plays any part in determining the retention on a trap plant, in addition to attracting it to the plant.

The relationship between crowding and retention was evaluated by comparing the number of marked bugs, as they diminished on each home plant, to the increase in wild bugs with a Spearman's correlation matrix. Every plant was followed until all the marked bugs either died or left the experimental plots.

The probability density function $f(x)$ of emigration from home plants was obtained by fitting the number of days (N) that planted bugs stayed on their plants to a geometric distribution:

$$f(x) = p(1-p)^{x-1}$$

$x = 1, 2, \ldots$, with probability $= p$, mean $= 1/p$, and variance $= (1 - p)/p^2$ (Everitt 2002). Significance of the fit was evaluated with a Kolmogorov–Smirnov goodness-of-fit test (Systat 2004). A good fit would indicate that the rate at which the bugs leave the home plant could be considered constant.

The fluctuations in the number of wild bugs on the home plants at daily intervals were recorded. The peak densities per plant were tested for normality with a Shapiro–Wilks test, and the mean was taken as the inflection point at which bug density could only be expected to stay fixed or decrease, in other words, the point at which the net migration rate $= 0$ (Carey 1993). One underlying assumption is that under these experimental conditions only migration influenced population fluctuations, while the other typical components of population growth, such as reproduction, predation, and mortality could be disregarded (Schowalter 2011).

Results

Survival of marked adults

The sex ratio in the original field samples, or the marked bugs released, was never significantly different from 1:1 throughout the duration of the experiments ($U = 20$, $P = 0.75$, $n = 12$). No marks were ever lost in the survival cages, and both the paint and the powders were visible to the naked eye to the end (a maximum of 30 days). Neither the powder nor the oil paints affected *M. histrionica*

survival. Survival of bugs in the field cages (mean = 11.5 days, SE = 0.77) showed no statistical difference for sex, powders, or paint ($F = 0.1$, d.f. = 1, $P = 0.75$; $F = 0.14$, d.f. = 2, $P = 0.87$; $F = 1.48$, d.f. = 6, $P = 0.19$, respectively). Sex * paint and paint * powder interactions also gave non-significant results ($F = 0.57$, d.f. = 25, $P = 0.94$).

Movement of marked and unmarked bugs to trap plants

The MIX2 pheromone versus blank test (Table 1, test 1, N/A means not applicable) resulted in 52 adult bugs, out of a total of 200 adults released, 26 of each sex, on the MIX2-baited plants, and 0 on the unbaited plants, after 4 days (Table 1). This treatment effect is significantly different from 1:1 at a binomial probability of $P = 1.5 \times 10^{-8}$. The attraction of *M. histrionica* for MIX2 is strong, with no sexual difference in response observed.

Figure 2 illustrates the total number of marked and unmarked *M. histrionica* captured per inspection. The graphs show that bug arrival to the trap plants occurred almost entirely after the first morning inspection; supporting the observation that *M. histrionica* does not forage at night. Recapture peaks of adults were 15–18 h after lure deployment. However, this period was ca. 60 h in the mustard oil tests in which marked nymphs were released instead of adults. About 15–33 % of all the marked adult bugs and 27 % of the marked nymphs released were recovered, depending on the lures used. In general terms, marked bugs were recaptured within 3 days or not at all.

Fisher's exact tests were non-significant for tests 2–5 (Table 1), indicating no evidence that the marking process caused a bias in lure or host plant detection. Given this result, the number of bugs on all the plants was pooled to compare the number of marked and unmarked (Fig. 2).

The number of paint + powder-marked and unmarked bugs trapped was statistically not different until wild bug resurgence ($U = 7.5$, $P = 0.29$, $n = 10$) (Test 2, Table 1; Fig. 2a). The resurgence pattern of unmarked bugs also gives graphical evidence that wild bugs would tend to reach the trap plants 36–40 h after the lures were in place. Tests with powder-marked bugs showed no significant differences in the captures of marked and unmarked bugs either ($U = 2$, $P = 0.27$, $n = 6$; $U = 4$, $P = 0.25$, $n = 8$; $U = 7.5$, $P = 0.88$, $n = 8$ for tests 3, 4, and 5, respectively) (Fig. 2b–d).

Retention of marked bugs on baited and unbaited plants

The mean retention time for marked bugs on baited and unbaited plants (3.15 and 3 days, respectively) did not show a statistical difference ($U = 1600$, $P = 0.44$, $n = 306$). The mean (±SE) number of marked bugs leaving the home plants per day was 1.78 (±0.20). The mean (±SE) increase of wild bugs per plant per day was 3.22 (±0.88).

Mean distance (±SE) of marked bugs that were found away from their home plants was 30.2 (± 7.74) m (range 5–80 m) suggesting that migration was not necessarily toward the closest available hosts.

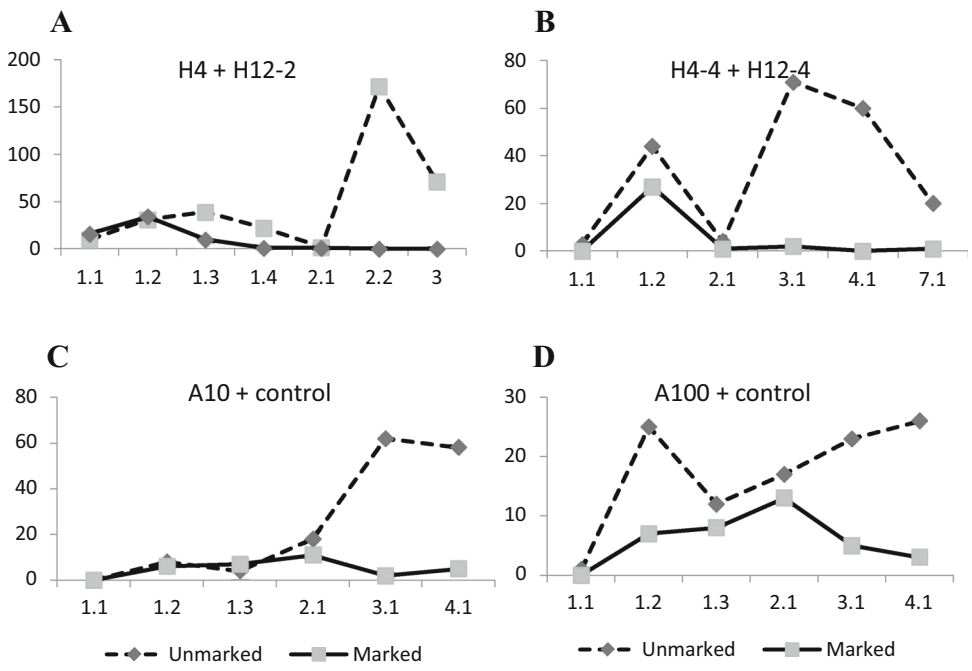

Fig. 2 Pooled number of marked and unmarked *Murgantia histrionica* bugs captured in different experiments. Treatment description in Table 1. *Y*-axis: No. of bugs captured *X*-axis: inspection time, 1.1 = 1 day, first inspection; 1.2 = 1 day, second inspection, etc

Density at the end of the experiment had an overall mean of 27.4 (±6.74 SE) bugs per plant. The correlation between the number of marked bugs and wild bugs on each plant was not significant ($r_s = -0.27$, $P \leq 0.232$, $n = 140$). The number of marked bugs per home plant per day (Fig. 3) conformed significantly to a geometric distribution (K-S = 0.03, P (2-tail) = 0.92, $n = 333$), indicating a constant emigration rate.

The peak bug densities across the experimental plants conformed to a normal distribution (SW = 0.877, $P \geq 0.081$), with a mean (±SE) peak of 36.5 bugs (±5.85) (range 11–69). The mean net bug influx to the trap plants with marked bugs, calculated as the mean of the difference between the number of bugs on each plant at time t minus the number at $t-1$, increased rapidly to a high peak on the 1 day and later oscillated from positive to negative values (Fig. 4).

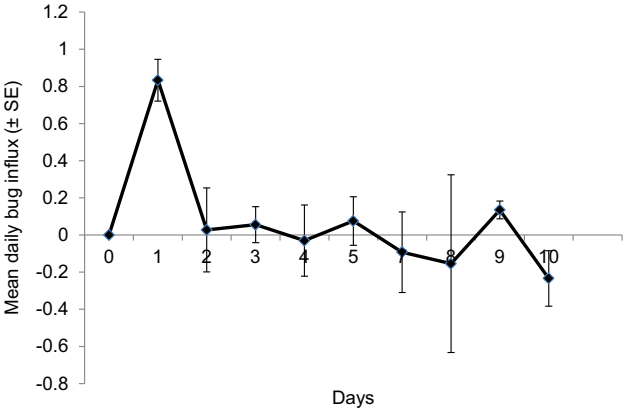

Fig. 4 Mean net bug influx (±SE) to the trap plants with marked bugs (retention by trap plants of marked and wild bugs experiment: 10 collards with 12 marked bugs each). Points represent the mean of the net difference between the *number of bugs* on each plant at time t minus the number at $t-1$

Discussion

Our results allow us to infer that neither of the marking systems assessed affects the survival of adult *M. histrionica.* Also, in spite of the impossibility to distinguish released unmarked bugs from wild bugs, the arrival at trap plants of marked and unmarked bugs on the trap plants was not significantly different up to the point of bug resurgence. We assume that this resurgence is in fact of wild bugs because it normally exceeded the total number of bugs released, it typically happened at a similar time post-release, and because there was always a depression before a second, more sustained captures peak.

The marking experiments described support population and management studies on *M. histrionica.* Attraction range and sampling range (Wall and Perry 1987; Schlyter 1992) of traps and trap crops can be evaluated on the assumption of no fitness reductions on behalf of the marked bugs.

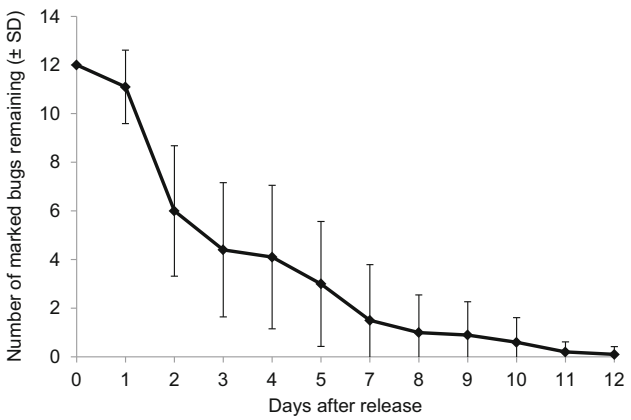

Fig. 3 Retention of marked *Murgantia histrionica* on MIX2-baited collards (10 collards with 12 marked bugs each): mean (±SD) *number of marked bugs* remaining over time

The significance of the retention of an insect on its host plant is very difficult to evaluate because of the multitude of variables involved. Plant quality, architecture and size of the plant, relative attractiveness to other host plants in the environment, competition and crowding, proximity of alternative hosts, and previous feeding history may all have a part in the insect's decision to leave or stay on the host (Holden et al. 2012). In these experiments, some of these variables were controlled, plant quality was ensured, the preferred hosts were used, plant size was standardized, and the effect of crowding was evaluated statistically. Wild alternative hosts within and close to the circles that emerged after the plowing were removed on sight, and preferred hosts were available in every direction at 5–32 meters, or up to 350 meters in a W-E direction.

Murgantia histrionica departure rate was constant, and the peak density was normally distributed. Results suggest that neither crowding nor rates of density increase played a part in the migration of the marked bugs, at least at the densities observed in our field. The non-significant correlation between marked bugs and crowding, combined with the unpredictable distance at which marked bugs would establish, suggests a stochastic element both in the search and establishment behaviors for *M. histrionica*. Alternatively, both processes could be related to the number of calling males (males emitting aggregation pheromones), a factor we could not assess in this experiment. Further experimentation may be able to establish this with a greater degree of certainty.

Pheromone MIX2-baited collards attracted *M. histrionica* significantly more than unbaited collards. Yet planted and volunteer bugs stayed a similar length of time

on the plants, indicating that, although plants baited with MIX2 attracted more bugs, they did not retain them any longer than unbaited plants. These results are not necessarily unexpected; they may indicate that crowded plants are not attractive to newcomers because of plant or insect volatiles (Pettersson et al. 1998) or that the neural pathways modulating nutrition-dependent dispersal are separate from responses to pheromones, which usually use labeled-line neural channels (Smith and Getz 1994; Galizia 2014). This implies that whereas peak densities may be achieved faster on baited trap plants than on the crop, peak density would not vary in one or the other. Consequently, *M. histrionica* would have to be eliminated from the trap plants at, or below, the peak density, before "spillover" of bugs to the crop.

Nevertheless, the maximum density on the trap plants is a dynamic variable that will depend mainly on the surrounding insect density and plant size, type, and quality, and many other possible factors (Holden et al. 2012). At very high field densities, this maximum density may be expected to increase. Yet this study provides a rudimentary figure to help develop decision rules for different management situations involving trap crops. Simple sampling protocols could be designed to determine when to spray a trap crop (e.g., when mean density reaches 36 bugs/plant), to relate these thresholds to economic thresholds or to calculate when trap plants stop being effective (i.e., sending into the environment as many bugs as they are attracting).

Regardless of the research still needed, this study provides some initial tools for the management of this growing pest.

Author contribution statement

GCW and DCW conceived and designed the experiments. GCW, DCW, and ASD performed the experiments and collected and analyzed the data. AK synthesized the pheromones and prepared the lures. All authors participated in writing the manuscript.

Acknowledgments The authors wish to acknowledge the help of the farm crew at the Beltsville Agricultural Research Center (BARC) in preparing the crops and plowed land used in these experiments, Mike Athanas for supplying the marking material and laboratory help, and Megan Herlihy for assistance in the laboratory.

References

Aliabadi A, Renwick JA, Whitman DW (2002) Sequestration of glucosinolates by harlequin bug *Murgantia histrionica*. J Chem Ecol 28:1749–1762

Carey JR (1993) Applied demography for biologists with special emphasis on insects. Oxford University Press, New York, pp 10–11

Everitt BS (2002) The Cambridge dictionary of statistics, 2nd edn. Cambridge University Press, Cambridge, p 163

Fahey JW, Zalcmann AT, Talalay P (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56:5–51

Galizia CG (2014) Olfactory coding in the insect brain: data and conjectures. Eur J Neurosci 39:1784–1795

Hagiwara H, Okabe T, Ono H, Kamat VP, Hoshi T, Suzuki T, Ando MJ (2002) Total synthesis of bisabolane sesquiterpenoids, α-bisabol-1-one, curcumene, curcuphenol and elvirol: utility of catalytic enamine reaction in cyclohexenone synthesis. Chem Soc Perkin Trans 1:895–900

Hokkanen HMT (1991) Trap cropping in pest management. Annu Rev Entomol 36:119–138

Holden MH, Ellner SP, Lee DH, Nyrop JP, Sanderson JP (2012) Designing an effective trap cropping strategy: the effects of attraction, retention and plant spatial distribution. J Appl Ecol 49:715–722

Khrimian A, Shearer PW, Zhang A, Hamilton GC, Aldrich JR (2008) Field trapping of the invasive brown marmorated stink bug, *Halyomorpha halys*, with geometric isomers of methyl 2,4,6-decatrienoate. J Agr Food Chem 56:197–203

Khrimian A, Shirali S, Vermillion KE, Siegler MA, Guzman F, Chauhan K, Aldrich JR, Weber DC (2014) Stereochemical determination of the aggregation pheromone of harlequin bug, *Murgantia histrionica* (Hemiptera: Pentatomidae). J Chem Ecol 40:1260–1268

Ludwig SW, Kok LT (2001) Harlequin bug, *Murgantia histrionica* (Hahn) (Heteroptera: Pentatomidae) development on three crucifers and feeding damage on broccoli. Crop Prot 20:247–251

Midega CAO, Khan ZR, Pickett JA, Nylin S (2010) Host plant selection behaviour of *Chilo partellus* and its implication for effectiveness of a trap crop. Entomol Exp Appl 138:40–47

Peiffer M, Felton GW (2014) Insights into the saliva of the brown marmorated stink bug *Halyomorpha halys* (Hemiptera: Pentatomidae). PLoS ONE 9(2):e88483. doi:10.1371/journal.pone.0088483

Pettersson J, Karunaratne S, Ahmed E, Kumar V (1998) The cowpea aphid, *Aphis craccivora*, host plant odours and pheromones. Entomol Exp Appl 88:177–184

Schlyter F (1992) Sampling range, attraction range, and effective attraction radius: Estimates of trap efficiency and communication distance in coleopteran pheromone and host attractant systems. J Appl Entomol 114:439–454

Schowalter TD (2011) Insect ecology: an ecosystem approach. Academic Press, London, pp 175–178

Smith BH, Getz WM (1994) Nonpheromonal olfactory processing in insects. Annu Rev Entomol 39:351–375

SYSTAT Software, Inc. (2004) SYSTAT 11. Richmond, CA

VassarStats: Website for statistical computation (2014) http://vassarstats.net. Accessed 29 July 2014

Walgenbach JF, Schoof SC (2005) Insect control on cabbage, 2004. Arthropod Manag Tests 30:E14

Wall C, Perry JN (1987) Range of attraction of moth sex-attractant sources. Entomol Exp Appl 44:5–14

Wallingford AK, Kuhar TP, Schultz PB, Freeman JH (2011) Harlequin bug biology and pest management in brassicaceous crops. J Integr Pest Manag 2:H1–H4

Wallingford AK, Kuhar TP, Schultz PB (2012) Toxicity and field efficacy of four neonicotinoids on harlequin bug (Hemiptera: Pentatomidae). Fla Entomol 95:1123–1126

Wallingford AK, Kuhar TP, Pfeiffer DG, Tholl DB, Freeman JH, Doughty HB, Schultz PB (2013) Host plant preference of harlequin bug (Hemiptera: Pentatomidae), and evaluation of a trap cropping strategy for its control in collard. J Econ Entomol 106:283–288

Weber DC, Cabrera Walsh G, DiMeglio AS, Athanas MM, Leskey TC, Khrimian A (2014) Attractiveness of harlequin bug, *Murgantia histrionica* (Hemiptera: Pentatomidae), aggregation pheromone: Field response to isomers, ratios and dose. J Chem Ecol 40:1251–1259

Yamanka T, Teshiba M, Tuda M, Tsutsumi T (2011) Possible use of synthetic aggregation pheromones to control *Plautia stali* in kaki persimmon orchards. Agric For Entomol 13:321–331

Zahn DK, Moreira JA, Millar JG (2008) Identification, synthesis, and bioassay of a male-specific aggregation pheromone from the harlequin bug, *Murgantia histrionica*. J Chem Ecol 34:238–251

Zahn DK, Moreira JA, Millar JG (2012) Erratum to: Identification, synthesis, and bioassay of a male-specific aggregation pheromone from the harlequin bug, *Murgantia histrionica*. J Chem Ecol 38:126