

Development and comparison of trunk traps to monitor movement of *Halyomorpha halys* nymphs on host trees

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Abstract

Halyomorpha halys Stål (Hemiptera: Pentatomidae) has recently become a major orchard pest in the Mid-Atlantic, USA. Large *H. halys* populations can develop on wild tree hosts adjacent to orchards, posing an ongoing threat to fruit. Adults and nymphs feed on tree fruit, causing economic injury. Understanding the seasonal patterns of nymphal host use among trees at the orchard-woodland interface may aid the development of integrated pest management strategies for this pest. In laboratory and field experiments, modified versions of published trap designs – ‘Circle’, ‘Hanula’, ‘M&M’ (after Moeed & Meads) traps – were compared for their effectiveness for capturing *H. halys* nymphs walking up and down tree trunks. In the laboratory, second instars were released at the top and bottom of ailanthus (tree of heaven), *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae), logs and captures were recorded after 24 h. Circle and M&M traps, respectively, were most effective for capturing nymphs walking up and down. In the field, traps were deployed on ailanthus trees next to apple orchards and captures were recorded weekly from 24 July to 11 September 2013. As in the laboratory, Circle and M&M traps captured the greatest number of upward- and downward-walking nymphs. Hanula traps were least effective in both experiments. In the field, 88% of total captures were of nymphs walking up trees. This was at least partially explained by behavioral assays in the laboratory demonstrating that nymphs exhibited negative gravitaxis and positive phototaxis. Stage-specific trends in captures of instars walking up during field sampling were observed. These results suggest that trunk traps can be used to address important ecological questions about seasonal patterns of host use by *H. halys* nymphs.

Introduction

Brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), is invasive from Asia and has recently become a serious agricultural pest in the Mid-Atlantic region of the USA (Leskey et al., 2012a; Rice et al., 2014). It has had an especially pronounced impact on the tree fruit industry, as it commonly feeds on deciduous tree species (Lee et al., 2013). *Halyomorpha halys* is highly polyphagous and reportedly uses >100 plant species as feeding or reproductive hosts, including wild trees in woodlands that often abut orchards in the

Mid-Atlantic region (Rice et al., 2014). Its season-long movement between woodlands and orchards has put continuous pressure on commercial orchards, resulting in increased insecticide use (Leskey et al., 2012b). The current necessity for aggressive insecticide-based management of *H. halys* is deemed ecologically and economically unsustainable; hence, the development of effective and sustainable management strategies based on an improved understanding of its behavior and ecology is paramount.

Studying the movement of insect pests is critical to monitoring and predicting their presence and potential impact throughout the season. The flight activity of alate insect pests has been studied more extensively (Muirhead-Thompson, 1991; Mazzi & Dorn, 2012) than has the walking dispersal of flightless agricultural pests. Among hemipteran pests, the distance walked by the

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bean bug *Riptortus pedestris* (Fabricius) (Tabuchi et al., 2007) in a restricted space and in a given amount of time was measured in laboratory studies. Lee et al. (2014) demonstrated that the dispersal capacity of *H. halys* nymphs by walking is considerable. In the laboratory, nymphs walked up to 41 m in 1 h and up to 8 m in 15 min on smooth horizontal and vertical surfaces, respectively. When attractive olfactory stimuli were deployed in a mowed grassy field near a woodlot, Lee et al. (2014) reported that responding nymphs walked over a distance of 20 m within 4–5 h. These findings suggest that *H. halys* nymphs can move readily among host plants growing near one another. Moreover, *H. halys* nymphs exhibited higher survivorship and faster development when reared on mixed diets of wild and cultivated tree hosts than on single diets of most of the same hosts (AL Acebes-Doria, unpubl.), concurring with Funayama (2006), and further suggesting the likelihood that nymphs move among hosts during the season.

In field studies, pitfall traps have been used to measure the abundance and movement of litter- and soil-dwelling arthropods (Duelli et al., 1990; Lalonde et al., 2012), but less commonly to monitor the movement of agricultural pests. The only well-studied agricultural pest in this regard is the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), as adults walk into agricultural fields from surrounding habitats (Boiteau et al., 2003). Traps to monitor the abundance and movement of arthropods walking on standing trees in forest ecosystems were used by Bar-Ness et al. (2012), Hanula & New (1996), Moeed & Meads (1983), and Pinzón & Spence (2008), and in an agricultural system by Mulder et al. (1999).

Given that *H. halys* nymphs are commonly found on wild and cultivated tree hosts growing near one another, show optimal survivorship and development on a diet of mixed hosts, and can walk over considerable distances in the vertical and horizontal dimensions, we explored the utility of traps for capturing *H. halys* nymphs walking on the trunk of host trees. The traps were based on modifications of published designs used for other arthropod species in agricultural (Mulder et al., 1999) and forest systems (Moeed & Meads, 1983; Hanula & Franzreb, 1998). Here, the results of laboratory and field experiments that evaluated and compared the effectiveness of three traps designed to monitor *H. halys* nymphs moving up and down trees are reported and discussed in relation to the potential for using them to study seasonal movement patterns of *H. halys* nymphs on host trees at the orchard-woodland interface.

Materials and methods

Insects

Adult *H. halys* were collected from overwintering sites in northern Virginia (USA) (38°44'24.71"N, 78°04'55.49"W) in February 2013 and held in a dark chamber at 4 °C inside a black garbage bag with crumpled newspaper. In late April 2013, the adults were removed from cold storage and held in groups of ca. 30 males and ca. 30 females in 30 × 30 × 30 cm plastic screened BugDorm cages (BioQuip Products, Rancho Dominguez, CA, USA) in a room at Virginia Tech's Alson H. Smith Jr. Agricultural Research and Extension Center (AHS-AREC), Winchester (VA, USA). Rearing room conditions were ca. 25 °C, 70% r.h., and L16:D8 photoperiod, and the insects were provided a mixture of popcorn kernels, barley, buckwheat, soybeans, dried figs, unsalted dry-roasted peanuts, and sundried tomatoes in a Petri dish, and 2–5 peach or apple fruits. Water was provided in a plastic container with a cotton wick inserted through the lid. Folded paper towels on the cage floor and excised ailanthus (tree of heaven), *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae), foliage in a plastic vase with water served as oviposition substrates and egg-laying began by the 3rd week of May. *Halyomorpha halys* typically deposit eggs in masses of 28 (Nielsen et al., 2008); newly deposited egg masses were removed daily in situ and held in groups of five in covered polystyrene Petri dishes (10 × 1.5 cm; Thermo Fisher Scientific, Pittsburgh, PA, USA) with water-soaked cotton. First instars of *H. halys* aggregate around and on the empty egg mass until molting to the second instar (Taylor et al., 2014), then disperse and begin feeding on plant tissue. Egg masses were monitored regularly for eclosion of first instars and their molt to the second instar. Recently molted second instars from each dish were transferred to a separate polyester, screened popup rearing cage (30.5 × 30.5 × 30.5 cm; BioQuip Products) containing fresh peach and apple fruit, the dry diet described previously, and water. Second instars at 2–4 days following their molt were used in laboratory studies conducted between 28 June and 10 July 2013.

Traps

Three trunk trap designs were modified from versions used in other studies. The 'Circle' trap (Figure 1A) was invented by and named after a pecan grower who used it to monitor pecan weevil (Mulder et al., 1999), whereas the 'Hanula' trap (Figure 1B) was developed to capture arthropods walking or crawling on tree boles (Hanula & New, 1996). Both of these traps were originally designed to capture insects walking up tree trunks, although for this

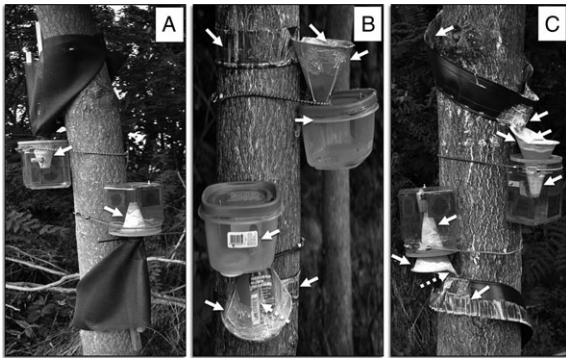


Figure 1 (A) Circle, (B) Hanula, and (C) M&M (Moeed & Meads) traps used to capture *Halyomorpha halys* nymphs walking on *Ailanthus altissima* logs and trees in laboratory and field studies, respectively, at Winchester (VA, USA). Solid arrows indicate surfaces coated with fluon and the broken arrow indicates the roughened area in the interior of the funnel.

study, complementary traps to capture downward-moving insects also were manufactured by inverting the funnelling or guiding device and collection container (Figures 1A,B). The third design was named the 'M&M' trap (Figure 1C) after its inventors (Moeed & Meads, 1983), and was based on their design for sampling invertebrate fauna moving up and down forest trees in New Zealand. Hereafter, 'up' traps of each design refer to those intended to capture *H. halys* nymphs walking up tree trunks and 'down' traps refer to the inverted version of each, devised to intercept nymphs walking downward.

Although the materials and designs of the three traps differed, the common underlying principle was that insects walking on the trunk would be guided into a collection container via a material that tightly encircled the trunk. Selected surfaces on or in each trap were coated strategically with a fluoropolymer resin, PTFE-30 or fluon (Bio-Quip Products), a slippery material that served to prevent *H. halys* escape from the collection container or from walking over the funnelling or guiding feature of the traps (Figure 1). All down traps had four holes (6 mm diameter) on the bottom of the collection container that were covered with plastic screen (1.0 × 0.7 mm mesh size; Saint-Gobain Adfors America, Grand Island, NY, USA), allowing drainage of water. The collection container for each trap was held in place against the tree trunk by two 25.4-cm-long flexible cords with a hook at each end. After each trap was installed on tree trunks, any remaining gaps or spaces that could have affected their effectiveness were sealed using transparent High Tech Sealant (Ace Hardware, Oak Brook, IL, USA).

Circle traps were based on a funnel (Figure 1A) intended to guide walking insects to the apex, where a

second funnel led them into the collection container. The main funnel was constructed from a 63.5 × 45.7-cm piece of charcoal-colored, New York Wire Sunguard 85 plastic screen (Saint-Gobain Adfors America) with a mesh size of 1.0 × 0.70 mm. The long edge of the screen was folded to form the funnel and stapled to a wood lath (25.4 × 7.6 × 5.1 cm). A second wood lath (33 cm long) was stapled to the screen directly opposite the shorter one and the two laths were aligned parallel to the trunk and separated by 5.1-cm-long screws attached at both ends of each, creating a space between the two sides of the screen funnel that enabled entry by insects. The bigger aperture of the screen funnel was wrapped tightly around and attached to the trunk by staples along its edge. A piece of PVC pipe (7.6 cm long, 2.54 cm i.d.) was fastened to the smaller aperture of the screen funnel using a cable tie (20.3 cm). The interior surface of the pipe had been roughened using sandpaper to provide traction for insects walking on it and the pipe was glued to the entrance of a plastic funnel from a Dead-Inn trap container (AgBio, Westminster, CO, USA) that led to the inside of the collection container. The outer surface of the plastic funnel was coated with fluon (Figure 1A), to prevent escape by insects that had entered the container.

The guiding portion of Hanula traps (Figure 1B) consisted of a black plastic strip (4.45 cm wide, 0.75 mm thick; Master Mark Plastics, Albany, MN, USA) wrapped tightly around and stapled to the trunk along both edges, so that its guiding edge was perpendicular to the long axis of the trunk. The length of these strips was tailored to the circumference of individual trees to which they were attached. Most of the outer surface of the strip was coated with fluon, leaving a small section uncoated so that insects could walk either up or down on only that section. Insects encountering the edge were anticipated to follow it around the circumference of the trunk until reaching the uncoated section, beneath or above which was a ca. 400-ml plastic funnel (13.5 cm diameter at top opening) coated on strategic portions of its inner and outer surfaces with fluon (Figure 1B). For the up traps, an area on the inner surface of the plastic funnels directly above the uncoated section of the strip was roughened for traction (Figure 1B). A semi-circular notch cut from the upper lip of each funnel enabled the funnel to be fitted tightly and flush against the plastic strip. The bottom of the funnel inserted into a 2.4-cm-diameter hole in the lid of a plastic Rubbermaid collection container (17.8 × 17.8 × 8.6 cm, Easy Find Lids; Rubbermaid Incorporated, Atlanta, GA, USA). For the up traps of the Hanula design, a 5 × 5-cm section was removed from the lid of each collection container directly above the funnel entrance and replaced by a piece of transparent plastic glued to the lid, enabling light

transmission from above, as occurred in up traps of the other designs.

The guiding portion of M&M traps (Figure 1C) was constructed of a strip of 8.9 cm wide and 0.75 mm thick black plastic (Master Mark Plastics) wrapped diagonally around the trunk. Like the Hanula traps, the lengths of these strips also were individually tailored to fit the circumference of tree trunks. One edge was stapled to the trunk and the other edge was ca. 3.8 cm away from the trunk, forming a diagonal collar around the tree. In principle, these traps were designed to guide walking insects along the edge attached to the tree trunk to a funnel at the lowest or uppermost point, according to the trap orientation on the tree. An opening in the stapled edge led to a ca. 240-ml plastic funnel (8.6 cm diameter at top opening) (Hopkins Manufacturing, Emporia, KS, USA) that in turn led into the funnel of a Dead-Inn collection container that emptied inside the container. For the up traps, an area on the inner surface of the plastic funnel starting from the surface directly above the opening in the strip was roughened (Figure 1C) to allow traction for nymphs walking on it. The inner surface of the plastic collar for down traps and the outer surface of the plastic collar for up traps, and portions of the interior and exterior surfaces of the trap funnels were coated with fluon (Figure 1C) to prevent bugs from crossing to the opposite side.

Evaluation of trap efficiency

Capturing and retaining released nymphs in the laboratory. Separate experiments – evaluating and comparing trap efficiency for capturing *H. halys* nymphs, and assessing nymphal retention in the collection containers – were conducted in a walk-in growth chamber (4.4 × 2.7 × 2.1 m) maintained at 27.5 °C, ca. 65% r.h., and L16:D8 photoperiod, provided by overhead fluorescent lighting. *Ailanthus* logs (mean ± SE = 13.0 ± 0.5 cm diameter, 1.65 ± 0.03 m long) were cut from trees felled at the edge of woodlands at the AHS-AREC. The logs were oriented vertically in the center of 15.1-l plastic nursery pots filled with soil to within ca. 5.4 cm of the rim. The top 5 cm of the inside of each bucket was coated with fluon to prevent released nymphs from escaping.

For the capture efficiency study, an up and a down trap of the same design was installed on individual logs. Traps of each design were installed on four logs; down traps were ca. 46 cm from the top of the log and up traps ca. 46 cm above the soil surface in the pot.

For each repetition, up and down traps of the three designs were evaluated simultaneously. Twenty second instars were released at the base (for up traps) or at the top (for down traps) of each log. Only one trap orientation was targeted for evaluation based on the release position of

nymphs at each repetition (i.e., if nymphs were released at the base, only the up traps were being evaluated, and vice versa) ($n = 2$ logs per trap design). On each log, the number of nymphs that were captured by the trap being evaluated (i.e., up or down), by the opposite trap, and those in the release area, elsewhere on the log, or trap surface exterior, or/and missing were recorded at 24 h after release. The experiment was repeated 4× between 28 June and 3 July 2013, using different insects for each repetition. The position of logs with different trap designs was re-randomized within the chamber prior to each repetition.

For the retention study, 20 second instars were placed inside the collection container of up and down trap designs. For a given repetition, only the up or down trap of each trap design ($n = 2$ logs per design) was evaluated and each test was repeated on four separate days. The live and dead nymphs in each collection container were counted after 24 h.

Capturing nymphs in the field. A pair of up and down traps of each design was installed on each of four mature, female *A. altissima* trees growing next to orchards at the AHS-AREC. Mean (± SE) diameter at breast height of the trees used was 17.4 ± 1.3 cm. Down traps were installed at 1–1.25 m above the tree base, whereas up traps were 0.8–1 m above the base. For these studies, a ca. 5.1-cm-long strip of Hercon Vaportape II (Hercon Environmental, Emigsville, PA, USA) containing dimethyl-2, 2-dichlorovinyl phosphate was suspended inside each collection container using a paper clip attached to a 5.1-cm-long corner brace that was screwed to the side or roof of the container. Use of these ‘kill strips’ in *H. halys* monitoring traps and their replacement at 2-week intervals, as was done in this study, is standard protocol (Joseph et al., 2013; Weber et al., 2014) and ensured the retention of captured nymphs. Captures of *H. halys* nymphs and adults in each trap were recorded weekly from 24 July to 11 September 2013, and the developmental stage of nymphs was determined following the morphological descriptions of Hoebeke & Carter (2003).

In response to lower captures of *H. halys* in down traps than in up traps during the first field evaluation (see Results) and following the observations of Lee et al. (2014), a follow-up study examined whether captures in down traps could be increased by presenting an attractive olfactory stimulus near the tree base and, by extension, whether the lower captures in the first experiment were due to fewer nymphs walking down than up or to some other factor. To examine this, we exploited the fact that both *H. halys* adults and nymphs are attracted to the vicinity of lures containing its aggregation pheromone, a mixture of (3S,6S,7R,10S)-10,11-epoxy-1-bisabolene-3-ol

and (3*R*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolene-3-ol (Khrimian et al., 2014) and the pheromone synergist methyl (*E,E,Z*)-2,4,6-decatrienoate (MDT) (Weber et al., 2014), and that nymphs are known to respond quite quickly and over a distance of many meters to these stimuli (Lee et al., 2014). A 10-mg pheromone lure and a 66-mg MDT lure (AgBio) were attached to the top of a black pyramid trap (122 cm high, 51 cm wide at the base) (AgBio), the standard pheromone trap for *H. halys* (Leskey et al., 2012c; Joseph et al., 2013; Weber et al., 2014), positioned 3 m away from the base of trees on which a down trap was installed; each down trap design was installed on four trees. The lures were deployed for 2 days then removed for 2 days and this alternating deployment pattern was conducted over 24 consecutive days from 13 September until 7 October 2013, for a total of 12 days with and without lures. The *H. halys* nymphs captured in each down trap were counted after each 2-day sample interval.

Examination of nymphal phototaxis and gravitaxis

Behavioral experiments to examine possible cues affecting the direction of nymphal movement were conducted in the laboratory using second instars within 2–3 days following their molt. To examine their response to light, a wooden dowel (122 cm long, 1 cm diameter) was leveled horizontally between retort stands at 23 cm from each end. The dowel was 51 cm above a table surface and had a mark at the middle that served as the release point and marks at 30.5 cm on either side of the midpoint that served as response points. Light was provided by a 27-W Daylight 24 quad tube compact fluorescent bulb (Trademark Global, Lorain, OH, USA) atop a 1.4-m tall reading lamp with a flexible neck that enabled the light to be directed along the length of the dowel from either end. Using a small paintbrush, a single nymph was released at the midpoint and observed until it had walked to the left or right response point. The light source was alternated between the right and left ends of the dowel between consecutive observations, each of which used a different nymph. As a control, the same observations were conducted using a battery-operated LED headlamp with red filter at 150 lumens (Top Gun LED Headlamp; Primos Hunting, St. Flora, MS, USA) worn by the observer.

To examine their gravitactic response, the dowel was leveled vertically and held by a retort stand at 23 cm from the top. All observations were conducted using the red light headlamp. Single nymphs were released at the midpoint and observed until they had walked to the response point above or below. All experiments evaluating phototactic and gravitactic responses were conducted at 23 °C on a single day, with 30 replicates per treatment. Between

uses for individual nymphs, the dowel was cleaned with wet tissue, air-dried, and re-leveled either horizontally or vertically.

Data analysis

All analyses were conducted using JMP Pro v. 11 (SAS Institute, Cary, NC, USA). Percentage data from laboratory experiments that did not satisfy the assumptions of parametric tests were transformed using arcsine \sqrt{x} , whereas nymphal captures in the field were subjected to a log(*n*+1) transformation. One-way ANOVA was used to analyze the capture data within up and down orientations among trap designs from the laboratory studies. The difference between the numbers of nymphs captured walking up and down was compared for each trap design using Student's *t*-test. The percentage of nymphs not captured by the trap being evaluated during the trap efficiency experiment was compared among trap designs and within trap orientation using two-way ANOVA. The numbers of live and dead nymphs retained after 24 h were compared using Student's *t*-test. For the initial field experiment, two-way ANOVA was used to compare captures among trap designs and between trap orientations, and Student's *t*-test was used to compare captures of nymphs and adults in the field. For the follow-up field study, comparison of captures among down trap designs within the periods with and without lures used one-way ANOVA, whereas a Matched Pairs *t*-test (which accounted for the repeated measurement) was used to compare captures between the periods with and without lures for each trap design. Multiple mean comparisons among the trap designs used Tukey's post-hoc test. Data from the experiments examining nymphal phototaxis and gravitaxis were analyzed using a χ^2 two-tailed test, followed by a χ^2 one-tailed test when the null hypothesis of the two-tailed test was rejected.

Results

Evaluation of trap efficiency for capturing and retaining released nymphs in the laboratory

The trap efficiency experiment revealed a significant effect of trap design on the number of second instars captured moving up ($F_{2,21} = 26.53$, $P < 0.0001$) and down ($F_{2,21} = 23.58$, $P < 0.0001$) *ailanthus* logs (Figure 2). Among the up traps, significantly more nymphs were captured in Circle and M&M traps than in Hanula traps and there was a non-significant difference between Circle and M&M traps (Figure 2). Significantly more nymphs walking down were intercepted by M&M and Hanula traps than by the Circle trap, and captures in M&M and Hanula traps did not differ (Figure 2). More upward than downward-walking nymphs were captured in Circle traps

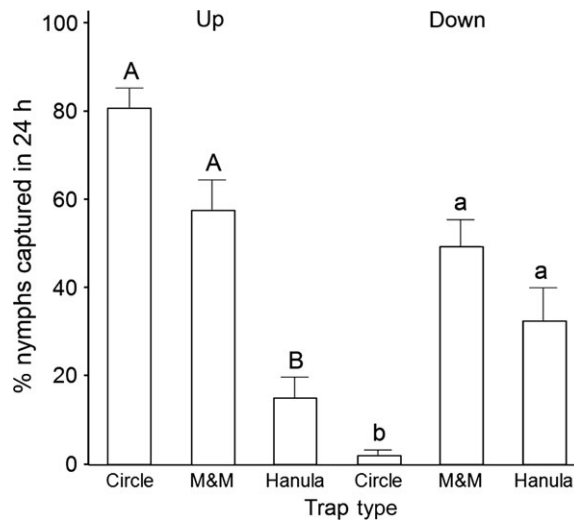


Figure 2 Mean (\pm SE) percentage of second instar *Halyomorpha halys* captured in three types of trunk trap (see Figure 1) and two orientations (up, capturing upward-walking nymphs; down, capturing downward-walking nymphs) at 24 h after their release at the bottom or top of *Ailanthus altissima* logs in a growth chamber ($n = 4$ per replicate per trap design and orientation). The experiment was repeated $4\times$. Bars within an orientation capped with the same letters are not significantly different (Tukey's HSD test: $P > 0.05$).

($t = -15.93$, d.f. = 14, $P < 0.0001$), whereas there was no difference between captures in up and down traps of the Hanula ($t = -0.88$, d.f. = 14, $P = 0.40$) or M&M ($t = 1.97$, d.f. = 14, $P = 0.07$) trap designs.

Significantly more nymphs were captured in the opposing trap when down traps were evaluated ($11.3 \pm 2.3\%$) than when up traps were assessed ($3.1 \pm 1.0\%$); there were no significant differences in captures in opposing traps among the trap designs (Table 1). A few nymphs were found on the release area and elsewhere on the log or trap exterior surface, but there were no differences among up or down trap trials or among trap designs (Table 1). Significantly more nymphs were missing 24 h after release during the assessment of down traps ($56.3 \pm 4.7\%$) than of up traps ($40.0 \pm 6.2\%$) (Table 1). Additionally, there was a significant interaction between trap design and the orientation of assessed traps (i.e., release location of nymphs) in the percentage of missing nymphs (Table 1). Significantly more nymphs were missing 24 h after release when Hanula up traps were evaluated (nymphs released at the base of logs) and for down traps of the Circle and Hanula designs (nymphs released at the top) compared with the other designs and orientations (Table 1).

The nymphal retention study indicated that the percentage of nymphs remaining in the collection containers after

24 h did not differ among the three trap designs ($F_{2,33} = 0.82$, $P = 0.45$) or between trap orientations ($F_{1,34} = 2.46$, $P = 0.13$). The mean (\pm SE) percentage of nymphs remaining in the containers after 24 h was $92.2 \pm 1.5\%$ and significantly more live ($57.1 \pm 3.6\%$) than dead ($35.1 \pm 3.6\%$) nymphs were recovered ($t = 3.05$, d.f. = 35, $P = 0.0043$).

Evaluation of traps for capturing nymphs in the field

Across all trap designs and orientations, significantly more nymphs (mean \pm SE = 7.2 ± 1.3) than adults (0.28 ± 0.06) per trap were captured ($t = 10.68$, d.f. = 382, $P < 0.0001$). As these traps were developed specifically for monitoring nymphs, adult captures were excluded from subsequent analyses. In total, 1 378 nymphs were captured, 88% of which were in up traps, which was significantly more than in down traps (12%) ($F_{1,190} = 53.82$, $P < 0.0001$). There was a significant interaction between trap orientation and trap design in the number of nymphs captured ($F_{1,186} = 70$, $P < 0.0001$). Among the up traps, significantly more nymphs were captured in Circle than in M&M and Hanula traps, and significantly more in M&M than in Hanula traps (Figure 3). Among the down traps, captures were significantly higher in M&M traps than in Hanula or Circle traps, captures in the latter two trap designs did not differ significantly (Figure 3).

In the follow-up study that addressed whether captures in down traps were affected by presenting olfactory stimuli at the tree base, the presence or absence of lures did not significantly increase the number of nymphs captured in any of the trap designs (Circle, Hanula: $t = -1$, d.f. = 3, $P = 0.39$; M&M: $t = 1.22$, d.f. = 3, $P = 0.31$; Figure 4), although there was a numerical effect on captures in M&M traps when lures were present. As in the previous laboratory and field experiments, there was a significant effect of down trap design on nymphal captures when lures were present ($F_{2,69} = 11.48$, $P < 0.0001$) or absent ($F_{2,69} = 4.61$, $P = 0.013$); M&M traps captured more downward-walking nymphs than the others in both scenarios (Figure 4).

Examination of the distribution of instars trapped during each weekly sampling interval was based on data from the up version of Circle traps. Of the 859 nymphs captured in this trap between 24 July and 11 September, 0.7, 26.9, 10.9, 19.8, and 41.7% were in the first through fifth instars, respectively. Captures of second instars predominated between 24 July and 14 August; thereafter, increasing proportions of nymphs in later instars were captured (Figure 5). There was not a similar trend in the distribution of instars moving down trees, possibly due to much lower captures.

Table 1 Location of second instars of *Halyomorpha halys* (mean % \pm SE) relative to each of three types and two orientations of trunk trap (n = 4) (up, capturing upward-walking nymphs; down, capturing downward-walking nymphs) 24 h after their release at the base or top of *Ailanthus altissima* logs

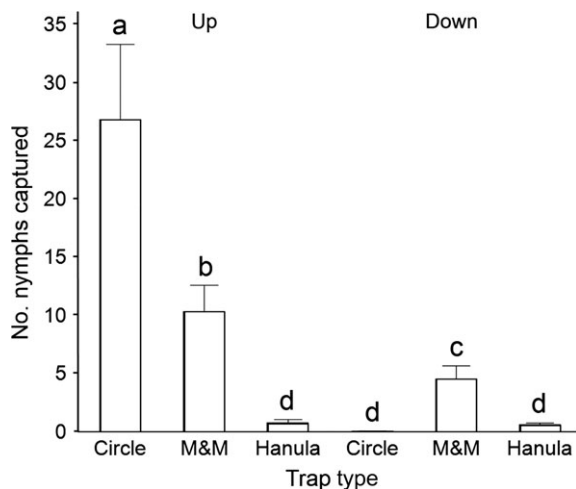
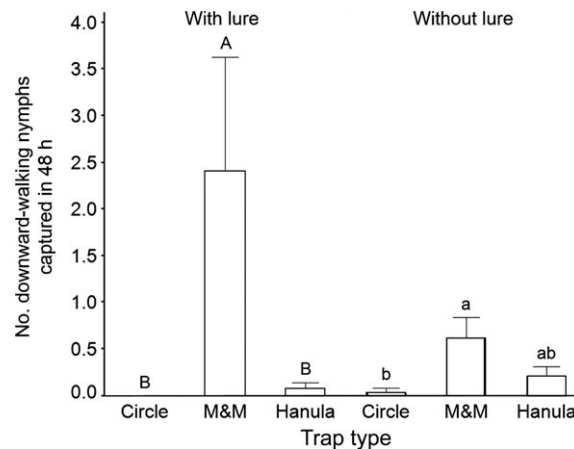
Trap		Opposing non-target trap (Down trap)	Remained at the base of the log	Elsewhere ¹ on the log	Missing ²
Orientation	Design				
Up	Circle	1.25 \pm 1.25	0.63 \pm 0.63	1.25 \pm 0.82	16.25 \pm 5.07c
	M&M	4.375 \pm 1.99	3.75 \pm 3.75	0	34.38 \pm 7.10bc
	Hanula	3.75 \pm 2.06	5.00 \pm 3.66	6.88 \pm 6.88	69.38 \pm 9.84a
Down	Circle	18.13 \pm 4.11	1.25 \pm 0.82	7.50 \pm 2.67	71.25 \pm 6.53a
	M&M	10.00 \pm 4.53	1.25 \pm 1.25	1.25 \pm 1.25	38.13 \pm 5.90bc
	Hanula	5.63 \pm 1.48	0	2.50 \pm 1.34	59.38 \pm 7.41ab

Factor		d.f.	F	P	F	P	F	P	F	P
Two-way ANOVA	Trap design	2,42	0.44	0.65	0.10	0.90	2.04	0.14	8.34	0.001
	Trap orientation	1,42	11.47	0.002	1.22	0.28	1.78	0.20	7.79	0.008
	Design*orientation	2,42	3.16	0.05	1.30	0.28	1.08	0.35	11.54	<0.0001

Means within the same orientation followed by the same letter are not significantly different (Tukey's HSD test: $P > 0.05$).

¹In or on the guiding device, outside the collection container, and in between the two traps.

²Nymphs that were not captured by the traps under evaluation and not found elsewhere on the log.

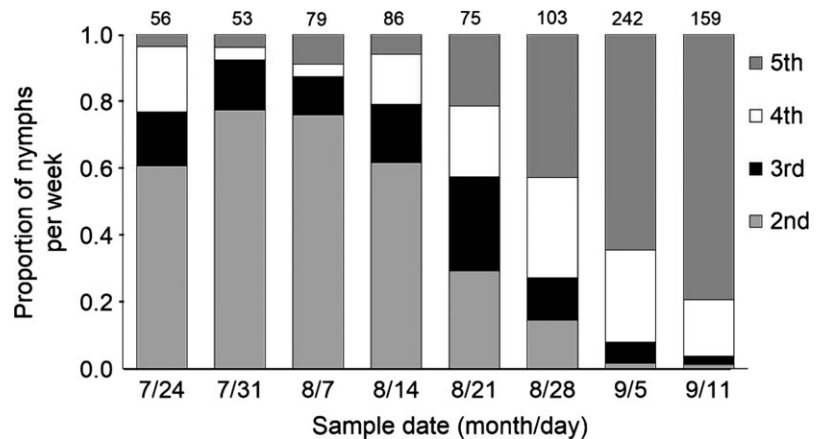
**Figure 3** Mean (+ SE) weekly captures of *Halyomorpha halys* nymphs in three types of trunk trap (n = 4) and two orientations (up, capturing upward-walking nymphs; down, capturing downward-walking nymphs) on *Ailanthus altissima* trees near Winchester (VA, USA) between 24 July and 11 September 2013. Bars within an orientation capped with the same letters are not significantly different (Tukey's HSD test: $P > 0.05$).**Figure 4** Mean (+ SE) captures of downward-walking *Halyomorpha halys* nymphs in three types of trunk traps (n = 4) on *Ailanthus altissima* trees with and without aggregation pheromone lures placed 3 m away from the tree base. There were six alternating 48-h periods with and without lures between 13 September and 7 October 2013. Bars within treatment (with vs. without lure) capped with the same letters are not significantly different (Tukey's HSD test: $P > 0.05$).

Examination of nymphal phototaxis and gravitaxis

Nymphs responded rapidly to the cues presented by walking to one of the response points on horizontally- or vertically oriented dowels in (mean \pm SE =) 20.4 ± 0.75 s. All nymphs exhibited positive phototaxis and moved in

the direction of the light source regardless of whether it was positioned at the left or right end of the horizontal dowel. In the absence of a light cue, there was an equal probability of nymphs walking to the left or right ($\chi^2 = 53.3$, $P = 0.72$). All nymphs released at the

Figure 5 Weekly distribution of *Halyomorpha halys* instars captured in Circle traps ($n = 4$) while walking up the trunk of *Ailanthus altissima* trees near Winchester (VA, USA), between 24 July and 11 September 2013. Numbers capping the bars indicate total captures per week, excluding the six first instars captured.



midpoint of a vertical dowel in the dark exhibited negative gravitaxis by walking upward.

Discussion

As has been shown in previous studies with other arthropods (Moeed & Meads, 1983; Hanula & New, 1996; Mulder et al., 1999), our experiments have demonstrated that *H. halys* nymphs walking up and down the trunk of a known tree host can be intercepted using an appropriate trap. The strong concurrence between the results from laboratory and field studies in the relative effectiveness of the various traps for capturing upward- and downward-walking nymphs provides guidance for future studies addressing the movement of *H. halys* nymphs among its tree hosts.

The collection container types used among the trap designs did not influence their effectiveness in the laboratory study; most nymphs released in each container were recovered from the containers after 24 h and the majority of them were alive. In the field, the use of a killing agent in the container and its replacement at 2-week intervals undoubtedly enhanced the retention of captured nymphs (Joseph et al., 2013). Thus, differences in the relative effectiveness of the traps appear to have been influenced by features other than the collection container.

Among the three traps, the Circle up trap and the M&M down trap were most effective for capturing nymphs walking up and down trees, respectively, and it appears that the guiding surfaces on those traps were superior for steering nymphs toward and into the collection container compared to the surfaces on the Hanula trap. The conical shape of the screen funnel on the Circle trap and the diagonal collar on the M&M trap may have interfered less with the overall direction of nymphal movement than the abrupt and perpendicular barrier to upward or downward

movement created by the guiding feature of the Hanula trap, which proved to be an inappropriate design for capturing *H. halys* nymphs.

Although the up and down forms of each trap were essentially inverted versions, the effectiveness of the two best designs (Circle and M&M) differed to varying degrees according to their orientation. In the laboratory and field, the up version of the Circle trap captured more upward-walking nymphs than the others, but its down version was consistently least effective. Conversely, the down form of the M&M trap was consistently most effective for capturing downward-moving nymphs, whereas its up version was only moderately effective.

Consistent differences in the relative effectiveness of the various trap designs and orientations in laboratory and field experiments may have been related to effects of one or more design features on nymphal behavior and also to the basic behavior of nymphs. With respect to trap design, nymphs moving down the logs in laboratory experiments often walked over the mesh funnel of Circle traps to the outer surface, whereas upward-walking nymphs were rarely seen to behave in this manner. If *H. halys* nymphs are positively phototactic, differences in directional light cues coming from the apex of up and down versions of the M&M and Circle traps may have influenced their propensity to follow the guiding feature of traps to the apex, resulting in higher captures in up than down traps of these designs. Higher captures in the down version of the M&M trap than in the corresponding version of the Circle trap also may have been affected by the slippery coating on the inside of the M&M plastic guide preventing nymphs from crossing it.

More upward- than downward-walking *H. halys* nymphs were captured in the laboratory and field. Despite observations and reports (Bakken et al., 2015) that *H. halys* egg masses and high nymphal populations are

common on *A. altissima* foliage and female reproductive structures, down traps installed on ailanthus trees in the field captured only 12% of all nymphs recovered. Furthermore, presentation of pheromone plus pheromone synergist lures near the base of trees did not appreciably increase captures in down traps. In the only other field study that used both up and down traps on the trunk of host trees, Moeed & Meads (1983) reported a similar result from New Zealand; only 20% of 22 696 invertebrates captured over 19 months on four tree species were recovered from down traps.

In combination, the effects of positive phototaxis on the direction of nymphal *H. halys* movement and their strongly negative gravitactic behavior may explain much of the difference in captures between up and down traps. In addition, as nymphs are presumed to move onto new tree hosts only via walking up the trunk, lower captures in down traps suggests that they use other behaviors to disperse from trees. Some smaller hemipterans can disperse via phoresy (Magsig-Castillo et al., 2010), on air currents (da Silva-Torres et al., 2013), or by adhering to falling leaves (Daane et al., 2006). It is possible that some *H. halys* nymphs may simply drop from host trees. Indeed, Moeed & Meads (1983) speculated that the disparate captures they recorded between up and down traps may have been due to invertebrates dropping from the canopy rather than walking down the trunk. Dropping may benefit walking insects by requiring less time and energy and by reducing the risk of predation. This behavioral explanation matches our observation that many nymphs released at the top of ailanthus logs fell down and were captured walking up on the same log and that nymphs were commonly missing from those logs.

Dispersal of pentatomid pests from host plants is known to be affected by host quality and the presence of more suitable hosts nearby, as was documented for the southern green stink bug, *Nezara viridula* (L.), and the brown stink bug, *Euchistus servus* (Say), which fed initially on peanuts and then dispersed to cotton when bolls were available (Tillman et al., 2009). In woody ornamentals, the host-utilization pattern of *H. halys* appeared to be influenced by seasonal changes in resource availability; nymphal counts were highest when fruit and seeds were present (Martinson et al., 2015). Moreover, survivorship of *H. halys* nymphs feeding on a single diet of less suitable hosts was highest during the second instar but decreased in later instars (AL Acebes-Doria, unpubl.). Overall survivorship improved significantly when nymphs were reared on a diet of two or more hosts, including pairings of hosts that were rela-

tively unsuitable as single diets. Our field data from Circle up traps suggested that the traps were sensitive to temporal changes in the distribution of instars. Only a few first instars were captured, conforming with observations that they do not disperse from the egg mass until after acquiring symbionts from it and molting to the second instar (Taylor et al., 2014). The preponderance of second instars captured through mid-August may reflect nymphal movement from other hosts onto tree of heaven in search of fulfilling nutritional requirements. Later in the season, increasing proportions of later instars captured suggested that the progeny of the summer generation of adults were entering more advanced stages of development.

In summary, we have demonstrated that Circle and M&M traps can be used complementarily to monitor the upward and downward movements, respectively, of *H. halys* nymphs walking on host trees. Deploying these traps on fruit trees and nearby wild hosts to monitor captures season-long would enable comparisons of nymphal *H. halys* host selection and temporal changes in its host-use patterns at the orchard-woodland interface, thereby aiding the development of ecologically based and temporally precise management strategies for this destructive, invasive pest.

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