Predation and parasitism by native and exotic natural enemies of Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) eggs augmented with semiochemicals and differing host stimuli

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GRAPHICAL ABSTRACT

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ABSTRACT

Escape from the natural enemy community by invasive species in their introduced range is a key determinant to their success. Historically in North America, there have been only low levels of predation and parasitism for Halyomorpha halys (Stål), the brown marmorated stink bug. In our study, we sought to determine whether prey-, predator-, or plant-associated stimuli increase mortality of H. halys egg masses, and whether the exotic parasitoid Trissolcus japonicus (Ashmead) is present in West Virginia or New Jersey. We deployed sentinel egg masses over two years in a variety of studies. We found that the H. halys aggregation pheromone was not used as a kairomone by natural enemies, the presence of methyl salicylate and varying host species stimuli did not impact egg mortality, and other predator attractants did not increase predation damage to egg masses. However, we documented Trissolcus japonicus for the first time in Jefferson Co., West Virginia, USA. Ultimately, our study suggests that other related stimuli and potential landscape factors should be investigated for increasing the impact of the natural community on H. halys.

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1. Introduction

Invasive species are a perennial problem for many regions of the world, and are projected to increase (Dukes and Mooney, 1999). These species have a myriad of effects, including disrupting ecosystem regulation (Charles and Dukes, 2007), causing economic damage (Pimentel et al., 2005), and outcompeting native species (Pyšek et al., 2012). One proximate factor implicated in the tendency of an invasive species’ population to increase in the introduced range is through their escape of natural enemies with which they have coevolved, known as the enemy release hypothesis (Elton, 1958; Jeffries and Lawton, 1984; Wolfe, 2002).

This seems to have been the case for *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), or the brown marmorated stink bug. This species was accidentally introduced to the eastern United States in the late 1990s (Hoebeke and Carter, 2003) from the Beijing region in China (Xu et al., 2014). As with many other invasive species that exhibit a lag species was accidentally introduced to the eastern United States in the (Hemiptera: Pentatomidae), or the brown marmorated stink bug. This parasitoid has been undergoing e (Talamas et al., 2015b). This parasitoid has been undergoing e (Wolfe, 2002).

Anastatus bifasciatus (Hymenoptera: Eupelmidae) and *Oecynurus telenomus* (Vassiliev) (Hymenoptera: Encyrtidae), two native parasitoids of *H. halys* in Europe, positively responded to *H. halys*-induced plant volatiles from *Vicia faba* (L.) plants (Rondón et al., 2017). More generally, prior research has shown that plant volatiles and other chemicals are important for host-finding by natural enemies. For example, the common green leaf volatile, methyl salicylate, has been shown to be important for recruiting natural enemies in agriculture (Rodriguez-Saona et al., 2011), and can lead to reductions in pest pressure (Malling et al., 2011). One predator species of *H. halys*, *P. maculiventris* is also attracted to methyl salicylate (Dickens, 1999). The recruitment of *P. maculiventris* may also be altered by deploying its pheromone in the lab and field (SantAna et al., 1997). The parasitoid *T. podisi* has been shown to exploit herbivore-induced plant volatiles to locate its host, *Euschistus heros* (F.) (Hemiptera: Pentatomidae) (Moraes et al., 2008). Similarly, *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae) and *Oecynurus telenomus* (Vassiliev) (Hymenoptera: Encyrtidae), two native parasitoids of *H. halys* in Europe, positively responded to *H. halys*-induced plant volatiles from *Vicia faba* (L.) plants (Rondón et al., 2017). More broadly, this raises the question of the role that predator- and plant-associated stimuli have on the natural enemy community of *H. halys*.

Recently, there has been mounting evidence that natural enemies may use kairomones associated with *H. halys* for host-finding (reviewed in Weber et al., 2017). For example, one study found that tridecan, an *H. halys*-associated compound (Nixon et al., 2018), acted as a kairomone in attracting *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) (Fraga et al., 2016). In addition, that study also found that *H. halys*-damaged bean pods were attractive to *O. insidiosus*. Zhong et al. (2017) found that female *Tr. japonicus* were attracted by the *H. halys*-produced volatile, n-tridecan, but strongly repelled by (E)-2-decenyl in the laboratory. Another study found that *Astatia occidentalis* Cresson, a predator of *H. halys* (Rice et al., 2014), uses methyl (E,E,Z)-2,4,6-deca trienoate (hereafter, MDT) as a host-finding kairomone (Cottrell et al., 2014). MDT is the aggregation pheromone of *Plautia stali* Scott (Hemiptera: Pentatomidae), which is cross-attractive to *H. halys*, and has been used for monitoring *H. halys* across the United States (e.g. Khrimian et al., 2008; Leskey et al., 2015a) and Asia (e.g. Fujisawa, 2001; Morrison et al., 2017b). Furthermore, a separate study found that two native parasitoids, *Trissolcus erugatus* Johnson and *Telenomus podisi*, were attracted to (2E,4Z)-2,4-decadienoate (Tognon et al., 2016), which is the aggregation pheromone of *Euschistus conspersus* Uhler (Hemiptera: Pentatomidae). Traces of that species’ aggregation pheromone were also found on conspecific egg masses (Tognon et al., 2016). This potentially implicates the aggregation pheromone of *H. halys* as being important for the host-finding behavior of its parasitoids. While prior work has shown that the cues on the surfaces of *H. halys* eggs may inhibit egg laying by some native North American parasitoids (Tognon et al., 2017), no one has specifically evaluated the *H. halys* aggregation pheromone as a potential kairomone. Finally, the *H. halys* aggregation pheromone is structurally very similar to the aggregation pheromones of native stink bug species in North America, such as *Mungangia histrionica* (Hahn) (Khrimian et al., 2014a). Both species have 2-
component aggregation pheromones, and share one of the stereoisomers with each other, thus the *H. halys* aggregation pheromone may actively be used by the native natural enemy community in the USA.

Moreover, there have been several advances in describing the semiochemicals associated with *H. halys* specifically, as well as their behavioral effects. For example, Khrimian et al. (2014b) discovered the *H. halys* aggregation pheromone to be two closely related stereoisomers of murgantiol, including (3S,6S,7R,10S)-10,11-epoxy-1-bisabolene-3-ol and (3R,6S,7R,10S)-10,11-epoxy-1-bisabolene-3-ol (hereafter, aggregation pheromone) in a 3.5:1 ratio. Subsequently, the attraction of the aggregation pheromone was found to be synergized by the addition of MDT (Weber et al., 2014), and enhanced by ethyl (2E,4E,6Z)-deca-2,4,6-trienoate (Rice et al., 2018). Finally, field tests of mixtures of the 16 stereoisomers from the parent compound of the aggregation pheromone did not reveal inhibitory effects on attraction in the field (Leskey et al., 2015b). Because *H. halys* often form large, mixed-sex groups when releasing their aggregation pheromone, it is likely that egg laying takes place in the presence of these semiochemical cues (W.R.M, pers. obs.), and may thus be a potential kairomone for egg predators and parasitoids in the area.

In other systems, plant volatiles have been used to increase the capture of parasitic wasps in vineyards (James and Grasswitz, 2005), increase the mortality of root weevils in citrus orchards and blueberry fields by attracting nematodes (Ali et al., 2012), increase parasitism of beetle larvae in forest systems (Wei et al., 2008), and reduce aphid populations in soybean (Mallingier et al., 2011). These illustrate the importance that semiochemicals could have for increasing the biological control of *H. halys* in unmanaged areas. The aims of the current study were to 1) document the distribution of *Tr. japonicus* in Maryland, New Jersey, and West Virginia, USA, and 2) assess whether semiochemicals (prey-, predator-, and plant-associated stimuli) can enhance biological control of *H. halys* eggs in the field.

2. Materials and methods

2.1. Study sites & source of egg masses

There were a total of 15 sites established in this study to monitor for *Tr. japonicus*, nine in both 2015 and 2016, though most sites varied between years (Table 1). Most sites were located in unmanaged woody habitats in or near parks, or adjacent to commercial production of corn, soybean, or apple. All the sites except one were located within the Shenandoah Valley of West Virginia and Maryland, USA. The other site was located in New Jersey.

There were two main sources of fresh egg masses used for the surveys in this study. The first source was from lab-reared and semi-field cage reared *H. halys* at the Appalachian Fruit Research Station ( Kearneysville, WV) or the Rutgers Agricultural Research and Extension Center (Bridgeport, NJ). For WV lab-reared egg masses, overwintering adult aggregations were initially used as the source, and collected using wooden overwintering shelters that contained layers of cardboard (as described in Bergh et al., 2017). They were fed a diet of organic carrots, surred tomatoes, raw sunflower seeds, and water ad libitum under 16:8 L:D, >50% RH, and 23 °C. Secondarily, adults were also maintained in a semi-field cage (1.8 × 1.8 × 1.8 m L:W:D; 1 × 1 mm mesh) under ambient light and temperature conditions (season mean: 22.7 ± 0.02 °C, 70.7 ± 1.0% RH) outside. The adults were provided with potted *Helianthus* sp., *Solanum lycopersicum* L., *Ailanthus altissima* Swingle, *Phaseolus vulgaris* L., *Amaranthus* sp., *Capsicum annuum* L., *Abelmoschus esculentus* Moench, and *Glycine* max (L.) Merr. as well as water ad libitum.

In addition to these sources, fresh egg masses in 2016 trials were supplemented with weekly shipments of SEMs from the New Jersey Department of Agriculture’s Phillip Alampi Beneficial Insect Rearing Laboratory (Trenton, NJ). Egg masses were shipped overnight express and were between 12 and 30-hr old, and immediately placed out in surveys the same day as described in Section 2.5. In the 2015 and 2016 NJ studies, all egg masses were obtained from the NJ Beneficial Insect Rearing Laboratory and were ≤24 h old for all studies when deployed.

2.2. 2015 Influence of kairomone presence on egg mass predation and parasitism

The sentinel egg mass (SEM) survey performed in 2015 was intended to test whether there was greater predation on prey stimuli-baited compared with unbaited sentinel egg masses, and was explicitly extensive in order to evaluate the background mortality by native natural enemies, as well as detect *Tr. japonicus* in western Maryland and West Virginia. Fresh sentinel egg masses (<24 h old) were collected from laboratory and semi-field colonies by gently applying a thin film of water along the edges of egg masses using a fine-pointed artist’s brush, and then gently lifting the egg mass from underneath with a spatula.

Table 1

<table>
<thead>
<tr>
<th>Field ID</th>
<th>Town</th>
<th>County</th>
<th>State</th>
<th>GPS coordinates</th>
<th>2015</th>
<th>2016</th>
<th>Exp</th>
<th>Total # SEMs deployed</th>
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<tr>
<td>1</td>
<td>Sharpsburg</td>
<td>MD</td>
<td>39°25'59.38&quot;N</td>
<td>77°47'35.53&quot;W</td>
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<td>b</td>
<td>14</td>
<td></td>
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<td>Beaver Creek</td>
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<td>39°32'8.65&quot;N</td>
<td>77°37'4.18&quot;W</td>
<td>X</td>
<td>b</td>
<td>8</td>
<td></td>
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<td>3</td>
<td>Hagerstown</td>
<td>MD</td>
<td>39°36'59.98&quot;N</td>
<td>77°44'4.15&quot;W</td>
<td>X</td>
<td>b</td>
<td>12</td>
<td></td>
</tr>
<tr>
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<td>MD</td>
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<td>b</td>
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<td>77°49’20.79”W</td>
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<td>b</td>
<td>12</td>
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<tr>
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<td>77°44’34.87”W</td>
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<td>b</td>
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<td>77°46’3.72”W</td>
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<td>b</td>
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</tr>
<tr>
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<td>X</td>
<td>b</td>
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<tr>
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<td>b</td>
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<td>X</td>
<td>b</td>
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<tr>
<td>15</td>
<td>Sharpsburg</td>
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<td>77°53’14.76”W</td>
<td>X</td>
<td>b</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

The experiments that were performed at each site, with experiments indicated by letters below.

Exp 2.2 & 2.3 Influence of kairomone presence on egg mass predation and parasitism.

Exp 2.4 Wild host plant species sentinel egg mass survey.

Exp 2.5 Cultivated and uncultivated wild host sentinel egg mass comparison.

Exp 2.6 Influence of prey-, predator-, and plant-based semiochemicals on egg mass predation and parasitism.
While this may have removed some of the natural cues present on the egg mass (Bin et al. 1993, Jones et al., 2014), the same process was used for each treatment and experiment, thereby controlling for any contribution of this to observed relative differences in results among treatments. The egg mass was directly transferred to a piece of card stock (3.8 × 6.4 cm) with a small piece of double-sided tape (Scotch, 3 M, St. Paul, MN, USA) in the center of the card. Sterilized playground sand (Pavestone, Hagerstown, MD, USA) was sprinkled onto exposed areas of tape after attachment of the egg mass in order to avoid exposing sticky surfaces, which could potentially impede parasitism or predation. Excess sand was removed by gently tapping the card. The card was split into 4 quadrants, and subsequently photographed before and after deployment under a microscope (SMZ1500, Nikon Inc., Melville, NY, USA) with a camera attached (Paxcam ARC camera, Villa Park, IL, USA). Six photos were taken of the sentinel egg mass: an overall picture (2x), a picture in each of the four quadrants (5x), and a picture in the center of the egg mass (5x) (for details see Morrison et al., 2016b). The total number of eggs in an egg mass was associated with the respective egg mass (overall mean: 25.3 ± 0.4; range: 8–40) and was used to calculate percent parasitism, predation, and other variables. Sets of two egg masses were placed at a given site on the same day that the eggs were collected from the colony to control for the age of the egg mass. One egg mass remained unbaited, while the other egg mass was paired with a high dose \( H. \) halys aggregation pheromone lure placed < 1 m away on the same branch. The \( H. \) halys lure contained approximately 34.1 mg of \( (3 \) \( S \))-6,7-dimethoxy-5-oxabicyclo[4.3.0]non-9-en-2-ol and 7.9 mg of \( (3 \) \( R \))-6,7-dimethoxy-5-oxabicyclo[4.3.0]non-9-en-2-ol (Chem-Tica, Heredia, Costa Rica; Morrison et al., 2016a; Weber et al., 2017). The egg masses were separated by at least 50 m at a site. Sentinel egg mass cards were placed on the abaxial surface of a leaf about 2 m from the ground on \( A. \) altissima and affixed in place by threading two insect pins (#2 size) through the card and the leaf. Egg masses were exposed for 72 h before being retrieved, put in a cooler, and brought back to the lab. A total of 98 sentinel masses consisting of 2406 eggs were deployed from 15 Jun to 21 Aug 2016, corresponding to the period of peak egg-laying by \( H. \) halys (Sargent et al., 2011). The following was noted on retrieved egg masses: the total number of eggs with \( H. \) halys hatched (evident by egg breakers on the chorion), eggs with chewing predation and piercing/sucking predation, live parasitoid emergence, and those that did not emerge (undeveloped). Parasitoids were allowed to emerge for at least 6 weeks after retrieval from the field. Laboratory-reared colony and the semi-field colony were the sole sources of fresh eggs in 2015. After parasitoids were allowed to emerge, the remaining eggs were dissected with a dissecting needle (#2 insect pin) under a stereomicroscope. Prior work has shown that this is important for fully documenting the full effect of native parasitoids (Cornelius et al., 2016b). In particular, the following were noted inside eggs: fully developed parasitoids (complete, intact adult wasp), undeveloped parasitoids (either pupa, wasp larva, or the presence of a whitish, opaque yolk inside the egg), developed nymphs (intact but died prior to hatching with legs and antennae), and undeveloped nymphs (green, yellow, or brown mass in coloration).

Parasitoids that emerged from eggs were preserved in 75% v/v ethanol and placed individually in 5/8 dram glass vials with polyethylene-lined screw tops. Labels containing locality and collection information were added to each vial. Vials were sent to C. Dieckhoff (Beneficial Insects Introduction Research, USDA-ARS, Newark, DE), and E.T. (Florida State Collection of Arthropods, formerly of Systematic Entomology Laboratory, USDA-ARS, Washington, D.C.) for identification using tools developed specifically for egg parasitoids of \( H. \) halys (Talamas et al. 2015a).

2.3. 2016 Influence of kairomone presence on egg mass predation and parasitism

The goal of the sentinel egg mass survey in 2016 was to more intensively sample Jefferson Co., WV to establish if \( Tr. \) japonicus was present in addition to quantifying prey-based stimuli effects on egg mortality. The protocol between years was identical, except that smaller notecards (3.8 × 3.2 cm) were used in 2016. In addition, egg masses were affixed to leaves with small 2.5-cm plastic clothespins (#8360WH, Factory Direct Craft, Springboro, OH) instead of insect pins, which may be less likely to induce volatile emissions in the deployment plants. In total, 136 egg masses consisting of 3503 eggs were deployed from 27 May to 29 Aug 2016 on \( A. \) altissima (Table 1), in sets of two eggs, one baited and the other unbaited as described in 2.2.

2.4. Wild host plant species sentinel egg mass survey

In order to evaluate whether the host species on which \( H. \) halys egg masses are deployed affects predation and parasitism, a separate series of sentinel egg masses were deployed as described in 2.2 except for the alterations noted in 2.3 and in the following. Sentinel egg masses were placed on either \( A. \) altissima (tree of heaven, exotic from Asia), \( Juglans \) nigra L. (black walnut, native to North America), or \( Robinia \) pseudacacia L. (black locust, native to North America), all of which are considered to be preferred hosts of \( H. \) halys (Bergmann et al., 2018). Eggs were deployed at two sites, Jefferson Orchards (see Field ID# 15 in Table 1) and at the Appalachian Fruit Research Station (see Field ID# 9 in Table 1). At a given field site, nine sentinel egg masses were deployed, with three per species of tree. A separate tree was used for each egg mass, each separated by at least 10 m from any other egg mass. Sets of eggs were deployed on a weekly basis from 15 Jul to 29 Aug 2016, for a total of 126 egg masses consisting of 3417 eggs.

2.5. Cultivated and uncultivated wild host sentinel egg mass comparison

In a further examination of the role that host stimuli on the parasitism and predation of \( H. \) halys, we deployed sentinel egg masses on a cultivated host (peach, \( Prunus \) persica (L.)) and adjacent uncultivated, wild hosts along a wooded border (including the following species: \( Acer \) spp., \( Quercus \) spp., \( P. \) serotina Ehrhart, \( J. \) nigra). Field sites consisted of one peach block (0.85 ha; Table 1), and adjacent wooded borders in NJ. Between 8 and 16 sentinel egg masses were deployed five times between 17 Jun – 3 Aug 2015 and 14–20 egg masses were deployed seven times between 1 Jun – 7 Aug 2016. In each deployment, half of the eggs were placed on randomly selected trees along the edge of a peach orchard (and the other half were placed in the uncultivated wooded border adjacent to the field (ca. 23 m from the peach block). In total, 204 egg masses were deployed in the two years. Egg masses were checked under a stereo-dissecting microscope for the number of eggs and presence of stylet sheaths (an indicator of predation) prior to deployment. Egg masses were maintained on their ovipositional substrate, transferred to cardstock, and secured with non-toxic glue (Elmer’s non-toxic School Glue, Elmer’s Products, Inc., High Point, NC, USA). Subsequently, egg masses were deployed, then collected 48 h after and returned to the laboratory for assessment of predation. Afterwards, egg masses were held at room temperature or 25 °C 16:8 L:D for emergence of \( H. \) halys or parasitoids.

2.6. Influence of prey-, predator-, and plant-based semiochemicals on egg mass predation and parasitism

In order to evaluate the effect of a plant volatile (methyl salicylate), predator-derived compounds, and \( H. \) halys-associated compounds, a sentinel egg mass survey was performed in two recently fallow production fields that had been colonized by various grasses in WV at the Appalachian Fruit Research Station and in NJ at the Rutgers
A total of 24, 1 × 1 m plots were tilled, weeded, and sprayed with a post-emergent herbicide (tradename: Fusilade DX; active: butyl (R)-2-[4-[[5-trifluoromethyl]-2-pyridyloxy]phenoxy]propanoate) at standard application rate for each site in early April 2015. Buckwheat (Fagopyrum esculentum Moench), sweet alfalfa (Lobularia maritima (L.)), coriander (Coriandrum sativum L.), faba bean (Vicia faba), and sunflower (Helianthus spp.) were seeded at each site at rates of 0.5, 0.04, 0.2, 7.6, and 0.5 g per m², respectively. The plots were arranged in blocks, each with 6 plots, with at least 50 m between plots. In each block, there were six treatments that were randomly assigned to the plots. Plots contained H. halys-associated odors only, or H. halys associated odors plus one of four natural enemy attractants: P. maculiventris (predator) pheromone, Cocinella septempunctata (L.) (Coleoptera: Coccinellidae) (predator) pheromone, methyl salicylate (herbivore-induced plant volatile, general natural enemy attractant: Rodriguez-Saona et al., 2011) and all the semiochemicals together (combo). A control containing no semiochemicals was also present in each block.

The semiochemical treatments were constructed as follows. The H. halys-associated odors were composed of a high dose lure containing 42 mg of the aggregation pheromone of H. halys, 10,11-epoxy-1-bisabol-3-ol (ChemTica International, Heredia, Costa Rica: Morrison et al., 2016a), as well as 66 mg of the cross-attractive methyl (2E,6E)-2,4,6-decatrien-1-ol (AgBio, Inc., Westminster, CO, USA) (H. halys stimuli, hereafter). The H. halys stimuli were changed every 4 weeks during the sampling period. The P. maculiventris (predator) pheromone was composed of a neat solution of (E)-2-hexenal (727.5 μl: Sigma-Aldrich, St. Louis, MO, USA) and α-terpineol (1383 μl: Sigma-Aldrich) added to a 4-ml semi-permeable container with a screw cap in the same relative proportions as found in headspace emitted from the predator (Aldrich et al., 1984). Prior work has shown that the P. maculiventris pheromone can increase biological control of other species, such as the Colorado potato beetle, Leptinotarsa decemlineata (Say) (Coleoptera: Chrysomelidae) (Aldrich and Cantelo, 1999). For the C. septempunctata (predator) pheromone, 75 μl of 2-isopropyl-3-methoxy-1-benzylpyrazine, which has been shown to be involved in the attraction of lady beetles (Al Abassi et al., 1998), was dissolved in 1500 μl of mineral oil (Sigma Aldrich) in a 4-ml semi-permeable container. Both P. maculiventris and C. septempunctata (predator) lures were changed every 2 weeks during the sampling period. Finally, the methyl salicylate treatment employed Praelure (AgBio, Inc., Westminster, CO), with a single lure deployed in a plot, and changed once every 4 weeks.

Sentinel egg masses from both the WV colony and the NJ Department of Agriculture (NJDA) were deployed 3–4 times during the season in WV, while only NJDA egg masses were used in NJ. During each deployment a single egg mass was placed in every plot from 10 Jul to 4 Sep 2015. The season was split up into three periods: early (10 Jul to 30 Jul), mid (1 Aug to 17 Aug), and late (1 Sep to 4 Sep 2015). A total of 168 egg masses were deployed following the methods in Section 2.2, with the exception of the egg dissections. The total number of eggs in each egg mass was tracked before deployment, and the number of eggs in each egg mass with predator damage and parasitoid emergence was recorded after retrieval.

2.7. Statistical analysis

To analyze the presence of kairomone on sentinel egg mass mortality, two multivariate ANOVAs (MANOVAs) were used, one for each sampling year (2015 & 2016) as a result of methodological differences between the datasets. In each model, the percentage of eggs that did not emerge (undeveloped), eggs with predator damage (chewing or piercing/sucking), eggs with live parasitoid emergence, eggs with parasitoids that did not emerge but were fully developed, eggs with undeveloped parasitoids, eggs with developed nymphs, and eggs with undeveloped nymphs were treated as an aggregate response variable. The presence of H. halys aggregation pheromone (yes or no) was used as a fixed explanatory variable, with deployment site as a blocking factor. After inspection of residuals, the data were arcsine(x)1/2-transformed to meet the assumptions of normality. Upon a significant result from the MANOVA, sequential ANOVAs were performed to assess the variables in which significant differences were found. For this and all other tests, R software was used (R Core Team, 2016), with α = 0.05. Likewise, a MANOVA was used to analyze the results from the wild host species sentinel egg mass survey in 2016 and the cultivated vs. wild host egg mass comparison in 2015–2016. For the wild host survey, the same arcsine-transformed aggregate response variable as above was used, but host species (black locust, black walnut, or tree of heaven) was used a fixed explanatory variable. Post-hoc paired t-tests were conducted between the number of eggs with predation and the number with live emergence of parasitoids to see whether predation or parasitism was a more important egg mortality factor compared to the null hypothesis of equal damage from both. For the cultivated and uncultivated host comparison, an aggregate variable with percent of eggs parasitized, number of eggs with predation, and number of undeveloped eggs was coded as an arcsine-transformed aggregate response variable. MANOVA was performed using Wilk’s approximate F-statistics, and sequential ANOVAs were performed upon significant result from the overall test.

Community composition of emerged and guarding parasitoid taxa was examined descriptively using simple summary calculations of frequency. For the purposes of examining the community composition, parasitoids that emerged or were guarding egg masses were combined from the kairomone egg mass survey and wild host plant survey, but were separated by sampling year.

Finally, MANOVA was used to analyze the semiochemical assay similarly to above, with the percentage of eggs with predation, and eggs with parasitism coded as the aggregate response variable. Data were arcsine-transformed to conform to the assumptions of normality. Semiochemical treatment (control, H. halys stimuli, lady beetle attractant, spined soldier bug attractant, methyl salicylate, or all stimuli), deployment period (early, mid, or late), and their interaction were coded as fixed explanatory variables. Field site was used as a blocking variable (New Jersey or West Virginia). Finally, post-hoc comparisons between the number of eggs with predation and those with live parasitoid emergence was conducted with a paired t-test in order to ascertain whether predation or parasitism was a more important source of egg mortality.

3. Results

3.1. Influence of kairomone presence on egg mass predation and parasitism

In 2015, 98 sentinel egg masses were deployed at 8 sites. The presence of the H. halys aggregation pheromone did not significantly influence the frequency of predation, parasitism, or number of remaining undeveloped eggs from sentinel egg masses (MANOVA: Wilk’s Approx. F1,97 = 0.364, P = 0.872; Fig. 1, left column). Likewise, the presence of the aggregation pheromone had no significant influence on the number of developed parasitoids, undeveloped parasitoids, developed nymphs, or undeveloped nymphs (Table 2). There was about four times more predation damage than eggs with live emergence of parasitoids in 2015 (t = 3.69, df = 98, P < 0.001; Fig. 1).

In 2016, 136 sentinel egg masses were deployed at 7 sites. However, as in 2015, the presence of the H. halys aggregation pheromone did not affect the occurrence of predation, parasitism, or number of undeveloped eggs from sentinel egg masses (MANOVA: Wilk’s Approx. F1,135 = 1.19, P = 0.314; Fig. 1, right column). On average, the amount of predation of H. halys eggs was similar in 2016 and 2015. The presence of the H. halys aggregation pheromone did not significantly affect the number of developed parasitoids, undeveloped parasitoids, developed nymphs, or undeveloped nymphs (Table 2). There was about 3–4 times more predation damage on eggs than eggs with parasitism (t = 11.1, df = 135, P < 0.001; Fig. 1).
and in adjacent wooded borders. There were no instances of parasitism on average, 3.3. Cultivated and uncultivated wild host sentinel egg mass comparison

In 2015, the two least common were a species of Pteromalidae (potential parasitoid, *Anastatus reduvii* (Howard), which was twice as abundant as the next most common parasitoid, *Anastatus reduvii* (Howard) (Fig. 4, left panel). The least abundant parasitoid was *Telemomus podisi*. In 2016, 19 parasitoids were found guarding egg masses, and 167 parasitoids emerged from egg masses (Fig. 5). Notably, *Trissolcus japonicus* was found both guarding and emerging from six sentinel egg masses placed in the field in Jefferson Co., West Virginia from two sites with a mean (± SE) parasitism rate of 32.8 ± 12% of eggs in a mass (Fig. 5). While *Tr. japonicus* was the most common parasitoid found in 2016, the two least common were a species of Pteromalidae (potential

### Table 2

Summary of egg dissections from kairomone-baited sentinel egg mass survey deployed in 2015 and 2016 in western Maryland and West Virginia, USA.

<table>
<thead>
<tr>
<th>Response</th>
<th>2015 survey</th>
<th>2016 survey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pheromone absent</td>
<td>Pheromone present</td>
</tr>
<tr>
<td></td>
<td>Mean Eggs per Egg Mass ± SE</td>
<td>Mean Eggs per Egg Mass ± SE</td>
</tr>
<tr>
<td>Developed Parasitoids</td>
<td>0.04 ± 0.04a</td>
<td>0.06 ± 0.06a</td>
</tr>
<tr>
<td>Undeveloped Parasitoid</td>
<td>0.27 ± 0.16a</td>
<td>0.26 ± 0.15a</td>
</tr>
<tr>
<td>Developed Nymphs</td>
<td>0.21 ± 0.13a</td>
<td>0.48 ± 0.29a</td>
</tr>
<tr>
<td>Undeveloped Nymphs</td>
<td>1.27 ± 0.75a</td>
<td>1.00 ± 0.61a</td>
</tr>
<tr>
<td>Developed Parasitoids</td>
<td>0.91 ± 0.36A</td>
<td>0.17 ± 0.12A</td>
</tr>
<tr>
<td>Undeveloped Parasitoid</td>
<td>2.15 ± 0.76A</td>
<td>1.32 ± 0.49A</td>
</tr>
<tr>
<td>Developed Nymphs</td>
<td>2.15 ± 0.74A</td>
<td>2.32 ± 0.71A</td>
</tr>
<tr>
<td>Undeveloped Nymphs</td>
<td>5.90 ± 1.22A</td>
<td>6.28 ± 1.18A</td>
</tr>
</tbody>
</table>

1 N = 98 total sentinel egg masses dissected in 2015 (N = 48 without pheromone, and N = 50 with pheromone).
2 *H. halys* aggregation pheromone formulated in a high dose release lure from AgBio, Inc. with 42 mg of 10,11-epoxy-1-biasabolen-3-ol.
3 N = 144 total sentinel egg masses dissected in 2016 (N = 68 without pheromone, and N = 76 with pheromone).
4 See definitions in text of Materials and Methods.
5 Lower case letters represent comparisons between egg masses with and without pheromone in 2015, while upper case letters are for comparisons in 2016 (Tukey's HSD, α = 0.05).
hyperparasitoid) followed by Trissolcus euschisti (Ashmead).

3.5. Influence of semiochemicals on egg mass predation and parasitism

A total of 168 sentinel egg masses were deployed in New Jersey and West Virginia at floral patches augmented with prey, natural enemy, or plant stimuli. However, the presence of supplemental semiochemicals did not significantly affect the predation and parasitism of egg masses (MANOVA: Wilk’s Approx. $F_{3,163} = 0.980$, $P = 0.461$; Fig. 6). Nonetheless, there was a significant effect of deployment period on these biological control indicators (Wilk’s Approx. $F_{2,163} = 3.34$, $P < 0.01$).

Table 3

<table>
<thead>
<tr>
<th>Response</th>
<th>Black locust$^1$</th>
<th>Black walnut</th>
<th>Tree of heaven</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Eggs per Egg Mass ± SE</td>
<td>Mean Eggs per Egg Mass ± SE</td>
<td>Mean Eggs per Egg Mass ± SE</td>
</tr>
<tr>
<td>Developed Parasitoids$^2$</td>
<td>0.00 ± 0.00a$^3$</td>
<td>0.23 ± 0.22a</td>
<td>0.12 ± 0.09a</td>
</tr>
<tr>
<td>Undeveloped Parasitoid</td>
<td>1.88 ± 0.97a</td>
<td>1.22 ± 0.48a</td>
<td>1.45 ± 0.68a</td>
</tr>
<tr>
<td>Developed Nymphs</td>
<td>2.38 ± 0.99a</td>
<td>1.98 ± 0.83a</td>
<td>1.05 ± 0.69a</td>
</tr>
<tr>
<td>Undeveloped Nymphs</td>
<td>4.93 ± 1.33a</td>
<td>5.17 ± 1.38a</td>
<td>4.73 ± 1.37a</td>
</tr>
</tbody>
</table>

$^1$ N = 40 total sentinel egg masses dissected per host species in 2016.
$^2$ See definitions in text of Materials and Methods.
$^3$ Letters represent comparisons among wild host species within a row (variable) (Tukey’s HSD, $\alpha = 0.05$).
the level of predation (ANOVA: $F_{2,163} = 6.66, P < 0.001$; Fig. 7), but not on parasitism ($F_{2,163} = 0.247, P < 0.782$). For instance, there was almost twice as much predation in the late deployment period compared to the early period (Tukey’s HSD, Fig. 7). The interaction between period and the semiochemical treatment was not significant (MANOVA: Wilk’s Approx. $F_{5,163} = 0.629, P = 0.890$). Finally, there was 166 times more eggs with predator damage than with parasitism ($t = 11.1$, $df = 163, P < 0.0001$).

4. Discussion

This is the first study, to our knowledge, that has directly tested whether prey-, predator-, and plant-associated stimuli have the ability to improve the biological control of H. halys egg masses in the field. In particular, we evaluated whether the H. halys aggregation pheromone may be used by parasitoids or predators in the eastern United States as a kairomone for prey presence. Our results suggest that the H. halys pheromone neither attracted nor repelled natural enemies from conspecific egg masses. However, while the aggregation pheromone may not play a major role in host-finding by natural enemies, other studies indicate that there may be alternative H. halys-associated semiochemicals that natural enemies may be able to use. For example, Jones et al. (2014) demonstrated that naturally-laid H. halys eggs are more heavily parasitized than sentinel egg masses, hinting at other possible kairomones associated with the egg mass that are lost when they are manipulated by researchers after being laid. Indeed, H. halys secretes other compounds, such as tridecane, (E)-4-oxo-2-hexenal, and (E)-2-decenal (Nixon et al., 2018). To some extent, prior work has demonstrated that these volatiles affected the behavior of the parasitoids Tr. japonicus, A. bifasciatus, O. telenomica, and the predator Orius insidiosus (Fraga et al., 2016; Rondoni et al., 2017; Zhong et al., 2017). For other stink bugs in the western US, a recent study found that Trissolcus erugatus and Telenomus podisi were attracted to egg extracts from the native Euschistus conspersus (Tognon et al., 2016). Those researchers also found that recreated mixtures of H. halys egg chemical cues inhibited parasitism by the same two parasitoids (Tognon et al. 2016, 2017). The host breadth of natural enemies as well as the intensity and length of geographic coevolution with H. halys are likely to affect the response of any given species to the volatile emissions from eggs and adults by H. halys. More work is needed in examining other prey-associated cues in the field to understand their potential for increasing biological control of H. halys.

In this study, we also examined the role that plant stimuli have in the foraging of natural enemies on H. halys egg masses. For example, we evaluated the potential of methyl salicylate to increase biological control of H. halys in the field. While methyl salicylate is a key induced plant volatile implicated in plant immunity and defense across many plant taxa (Heil et al., 2016), our results suggest that methyl salicylate is not an important factor in recruiting the natural enemies most likely to attack H. halys. Recent work has found that the parasitoid Trissolcus basalis (Wollaston) is not attracted to volatiles induced in Vicia faba by feeding and oviposition of H. halys, and that the presence of multiple herbivores, including H. halys, actually disrupted established semiochemical webs between T. basalis and Nezara viridula (L.) (Martorana et al., 2017). Indeed, plant volatiles may also only be of minor importance to H. halys itself, as a recent study has shown that the aggregation pheromone had a stronger effect on attraction and retention of the species than fruit volatile blends (Morrison et al., 2018). In looking at predation and parasitism of H. halys eggs on various hosts, there was no evidence for differential attraction of natural enemies to eggs deployed on black locust, black walnut, or tree of heaven. There was also no difference between biological control of egg masses deployed on cultivated hosts (peaches) compared with uncultivated hosts (wooded borders). This is in accordance with prior literature, which has found that the plant genus in plots with native or exotic vegetation did not affect the parasitism or predation rates of sentinel egg masses (Cornelius et al., 2016a). Another study found that parasitism was similar among three vegetable crops (bell pepper, squash, and tomato), but that predation was greater on sentinel egg masses deployed on bell pepper than the other two crops (Cornelius et al., 2016b). Herlihy et al. (2016) generally found greater parasitism of H. halys sentinel egg masses on wild hosts than on apple or soybean, and while our results show that there may be a trend towards increased predation on wild hosts compared to cultivated hosts, this was not statistically significant. Thus, while parasitism and predation may be greater in certain habitat types, the exact effect of host species appears variable and inconsistent on the level of biological control of H. halys.

Specific natural enemy attractants, including the spined soldier bug pheromone and C. septempunctata pheromone, also had no effect on the predation or parasitism of H. halys egg masses. This could be due to a few reasons. For example, it could be that the concentration of the natural enemy attractant was not sufficient for attracting adequate numbers of predators in the field. After the semiochemical assay was run in the current study, Morrison et al. (2016b) tested seven species of lady beetles and demonstrated that lady beetles are not significant sources of mortality for H. halys egg masses. Further, Pote and Nielsen (2017) found that only one out of four tested lady beetle species contributed to the mortality of H. halys eggs or nymphs, and even then, egg hatch was only lowered by 10% relative to the control in the laboratory. This explains why the lady beetle pheromone may not have been effective in increasing predation of sentinel egg masses. Morrison et al. (2016b) also found that < 10% of the tested spined soldier bugs fed on H. halys egg masses, and when they did feed, they consumed < 5% of the eggs (but see Pote and Nielsen 2017), which may have affected the efficacy of the spined soldier bug treatment. Broadly, native species are thought to provide less biological control for invasive species than the predators in their native ranges. Regardless of the mechanism, the native predators that were recruited in this study did not appear to have an impact on H. halys egg masses.

Generally, predation in this study was much higher than parasitism. For example, the percentage of eggs with predation was several to many times more than those with parasitism across the sentinel egg mass surveys performed in 2015 and 2016. This aligns with other studies, which found for example that 80% of the biological control activity on H. halys egg masses was from predator activity (Ogburn et al., 2016). In addition, as the season progressed, especially for sentinel egg masses placed in floral patches, there was an almost doubling of the amount of overall predation. Orthoptera has particularly been implicated in feeding on H. halys egg masses, including Gryllidae and Tettigoniidae (Morrison et al., 2016b; Poley, 2017; Pote and Nielsen, 2017), and may be responsible for a good portion of missing egg masses in the field.

**Fig. 7.** The percentage of eggs in an egg mass (out of ~28 eggs) with predator damage during the early (10 Jul to 30 Jul), mid (1 Aug to 17 Aug), and late (1 Sep to 4 Sep 2015) deployment periods for the sentinel egg masses augmented with prey-, predator-, and plant-based semiochemicals in New Jersey and West Virginia, USA in floral patches.
the season progressed during the current study, tettigoniids captured in the vicinity during the early period were mostly small immatures, but increased significantly in size until most were adults by the end of the sampling period (Morrison, pers. obs.). Morrison et al. (2016b) demonstrated that adult predators were more efficient than immatures in consuming H. halys eggs, suggesting a possible link in the increased rate of death late in the season to the development of hemimetabolous predators.

Importantly, we have documented for the first time the presence of Tr. japonicus from egg masses in West Virginia, adding a new state to the host distribution of the adventive population in the USA. This brings the total number of states within the USA with published and unpublished findings of the species to 10, which includes Oregon, Washington, Maryland, Pennsylvania, and the District of Columbia (Herlihy et al., 2016; Milnes et al., 2016; Talamas et al., 2015b; G. Krawczyk, pers. comm.), though more soon-to-be-published detections are on the horizon. Originally discovered in the USA in 2014, there now seems to be an established and expanding population of Tr. japonicus in the country, with separate introductions on the east and west coasts. Our study has documented its presence two years after its initial discovery, suggesting it is readily able to overwinter in the mid-Atlantic US. In fact, a recent study found that Tr. japonicus is more cold tolerant than its primary host, H. halys (Santacruz et al., 2017). While H. halys may increase its cold tolerance through exposure to shortened photo-period and decreased temperature, the same study found these same techniques for the purposes of providing scientific information only, and does not constitute endorsement by the United States Department of Agriculture. The USDA is an equal opportunity employer.

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