

## **Semiochemically Based Monitoring of the Invasion of the Brown Marmorated Stink Bug and Unexpected Attraction of the Native Green Stink Bug (Heteroptera: Pentatomidae) in Maryland**

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## SEMIOCHEMICALLY BASED MONITORING OF THE INVASION OF THE BROWN MARMORATED STINK BUG AND UNEXPECTED ATTRACTION OF THE NATIVE GREEN STINK BUG (HETEROPTERA: PENTATOMIDAE) IN MARYLAND

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### ABSTRACT

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Pentatomidae), is a newly invasive species in the eastern U. S. that is rapidly expanding its range from the original point of establishment in Allentown, Pennsylvania. Although an attractant pheromone has yet to be identified for *H. halys*, in its native Asian range the insect is cross-attracted to the pheromone of another pentatomid *Plautia stali* Scott whose males produce methyl (*E,E,Z*)-2,4,6-decatrienoate. Previous tests of methyl 2,4,6-decatrienoate isomers in the U. S. verified that *H. halys* is highly attracted to methyl (*E,E,Z*)-2,4,6-decatrienoate, and that the native green stink bug, *Acrosternum hilare* (Say), also is attracted to this compound. Using traps baited with methyl 2,4,6-decatrienoates and the reported pheromone of *A. hilare* (*trans*- and *cis*-(*Z*)- $\alpha$ -bisabolene epoxides), we monitored populations of the brown marmorated and green stink bugs at the Agricultural Research Center, Beltsville, Maryland, for the 2004-2008 growing seasons. Over this time period, the *H. halys* population rose from being undetectable in 2004 to becoming much more abundantly trapped than the native *A. hilare*. Furthermore, *A. hilare* was significantly more attracted to methyl (*E,E,Z*)-2,4,6-decatrienoate than the blend of bisabolene epoxides reported as its pheromone. Supplemental material online at <http://www.fcla.edu/FlaEnt/fe923.htm#InfoLink1>

Key Words *Acrosternum*, *Halyomorpha*, aggregation, kairomone, pheromone, Hemiptera, tachinid

### RESUMEN

La chiche marrón mármol, *Halyomorpha halys* (Stål) (Pentatomidae), es una nueva especie invasora en el este de Estados Unidos, la cual está expandiendo su rango de distribución rápidamente desde su punto original de entrada en Allentown, Pennsylvania. Aunque su feromona aún no se ha identificada, este insecto es atraído en su hábitat nativo (Asia) por la feromona de otro pentatómido, *Plautia stali* Scott, cuyos machos producen (*E,E,Z*)-2,4,6-decatrienoato de metilo. Experimentos previos con distintos isómeros de 2,4,6-decatrienoato de metilo en Estados Unidos demostraron que *H. halys* es fuertemente atraída por (*E,E,Z*)-2,4,6-decatrienoato de metilo, y que la chiche verde nativa *Acrosternum hilare* (Say) es también atraída por este compuesto. Usando trampas cebadas con 2,4,6-decatrienoatos y con la feromona reportada para *A. hilare* (epóxidos de (*E*)- y (*Z*)- $\alpha$ -bisabolenos), realizamos un seguimiento poblacional de ambas especies en la Agricultural Research Center, Beltsville, Maryland, durante las temporadas de crecimiento 2004-2008. En este período, las poblaciones de *H. halys* aumentaron desde niveles no detectables en 2004, a valores de capturas sustancialmente mayores que los correspondientes a la especie nativa. Asimismo, *A. hilare* fue significativamente más atraída al (*E,E,Z*)-2,4,6-decatrienoato de metilo que a la mezcla de epóxidos de  $\alpha$ -bisabolenos, reportados como la feromona de esta especie.

Translation provided by Dr. Andrés González Ritzel.

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae), is an exotic species (Hoebeke & Carter 2003) that is rapidly expanding its range from its original point of establishment in Allentown, Pennsylvania (Hamilton 2009). Collection records for *H. halys* date from 1996, although it was not realized that the insect was foreign until 2001 after the population had exploded. *Halyomorpha halys* is native to northeast Asia and, in Japan, has become a serious fruit pest since post-world war II reforestation of old-growth deciduous forest with Japanese cedar, *Cryptomeria japonica*, and false cypress, *Chamaecyparis obtusa* (Kiritani 2007). According to Kiritani (2007), *H. halys* and 2 other fruit-feeding stink bugs are dependent on cones to complete their life cycle. The bug also has an extraordinary propensity to form aggregations (Toyama et al. 2006), often in houses and other buildings where they are a nuisance similar to boxelder bugs (Tinker 1952). In fact, it has been suggested that an aggregation of *H. halys* entered the U.S. in a cargo container (Hoebeke & Carter 2003) and, subsequently, an aggregation was apparently transported via some type of vehicle to Oregon where *H. halys* also is established (Anonymous 2007). More recently, *H. halys* has been found established in the Zürich region of Switzerland (Wermelinger et al. 2008).

Pheromone-baited traps offer a convenient and potentially powerful means to monitor insect populations, and some stink bug pheromones are known (Millar 2005), but no pheromone is known for *H. halys*. However, in Japan *H. halys* is cross-attracted (Tada et al. 2001a, 2001b; Funayama 2008) to the pheromone of another pentatomid, *Plautia stali* Scott, whose males produce methyl (*E,E,Z*)-2,4,6-decatrienoate (Sugie et al. 1996). In tests begun in 2003 at Allentown (Khrimian 2005; Khrimian et al. 2008), we verified that *H. halys* adults and nymphs are, indeed, attracted to methyl (*E,E,Z*)-2,4,6-decatrienoate and related geometric isomers. Surprisingly, methyl 2,4,6-decatrienoates also attracted significant numbers of the native green stink bug, *Acrosternum hilare* (Say) (Aldrich et al. 2007), even though methyl 2,4,6-decatrienoates have not been found from *A. hilare* and the reported pheromone components of this bug (*trans*-/*cis*-(*Z*)- $\alpha$ -bisabolene epoxides) (Aldrich et al. 1989; Aldrich et al. 1993; McBrien et al. 2001) are chemically unlike methyl 2,4,6-decatrienoates.

Here we report the results of our efforts to monitor *H. halys* and *A. hilare* at the Beltsville Agricultural Research Center from the 2004 through 2008 growing seasons using traps baited with methyl 2,4,6-decatrienoates and *trans*-/*cis*-(*Z*)- $\alpha$ -bisabolene epoxides, the reported pheromones of *Plautia stali* (Sugie et al. 1996) and *A. hilare* (Aldrich et al. 1989; McBrien et al. 2001), respectively. Over this time period, the population

of *H. halys* in Maryland increased from being undetectable in 2004 to becoming much more abundantly trapped than *A. hilare* in 2007 and 2008. Previously reported data for 2004 and 2005 (included herein, Aldrich et al. 2007) showed that *A. hilare* is particularly attracted to methyl (*E,E,Z*)-2,4,6-decatrienoate.

## MATERIALS AND METHODS

### Chemical Standards and Treatments

Methyl (*E,Z,Z*)-, (*Z,E,Z*)- and (*E,E,Z*)-2,4,6-decatrienoates were synthesized as previously described (Khrimian 2005), as were (4*S*)-*cis*- and (4*S*)-*trans*-(*Z*)- $\alpha$ -bisabolene epoxides (*Z*)-(1*R*,2*S*,4*S*)-4-(1',5'-dimethyl 1',4'-hexadienyl)-1,2-epoxy-1-methylcyclohexane and (*Z*)-(1*S*,2*R*,4*S*)-4-(1',5'-dimethyl 1',4'-hexadienyl)-1,2-epoxy-1-methylcyclohexane, respectively, (Chen et al. 2000).

Lures for methyl 2,4,6-decatrienoate treatments were prepared by impregnating gray rubber septa (West Pharmaceutical Services, Kearney, NE) with single synthetic isomers (Khrimian 2005) as previously described (Aldrich et al. 2007; Khrimian et al. 2008). For treatments with the reported pheromone of *A. hilare*, septa were impregnated similarly with a 5:95 ratio of *trans*-/*cis*-(*Z*)- $\alpha$ -bisabolene epoxides. Lures containing methyl 2,4,6-decatrienoates were loaded with 4.0 or 2.5 mg of each active ingredient per septum, and bisabolene epoxide lures were loaded with a total of 2.5 mg of the epoxide mixture per septum. In tests combining the bisabolene epoxides with methyl (*E,E,Z*)-2,4,6-decatrienoate, 2 septa were placed together in traps, 1 with the methyl ester and a second with the epoxides. Lures were rebaited weekly or biweekly as specified in figure legends.

### Field Trapping

Field experiments were carried out at BARC, Prince George's County, Maryland, from 2004 through 2008 beginning in late spring or early summer through mid-Oct. A summary of the field experiments is presented in Table 1.

From 2004 through 2007, four replicates per treatment were tested with traps hung in the same location each year about 1.8 m above ground from tree branches ( $\geq 20$  m apart) in patches of mixed deciduous forest bordering a field consisting of alternating strips of corn and soybean (see supplemental material). Replicates were grouped into sets of traps, with ( $\geq 100$  m between sets), and traps were rotated one position within a set every 2-3 weeks. In 2004 and 2005, container traps (30 cm tall transparent plastic, 10 cm removable bottom, and two 9.5 inwardly projecting mesh funnels see supplemental material) were fabricated by Sterling International, Inc. (Spokane, WA). In 2006, a new kind of baffle trap (see supplemental

TABLE 1. FIELD EXPERIMENTS CONDUCTED AT BETLSVILLE, MD, 2004-2008.

Year <sup>1</sup>	Duration	Site Description <sup>2</sup>	Treatment <sup>3</sup>	Trap <sup>4</sup>	N
2004	1 Jul-26 Oct	Deciduous/soybean-corn	EZZ	C	4
			ZEZ	C	4
2005	11 Aug-19 Oct	Deciduous/soybean-corn	EZZ	C	4
			ZEZ	C	4
2006	6 Jun-26 Oct	Deciduous/soybean-corn	EEZ	C	4
			EEZ	B	4
	15 Aug-26 Oct	Deciduous/soybean-corn	BX	C	4
			BX	B	4
			EEZ+BX	C	4
			EEZ+BX	B	4
2007	11 May-18 Oct	Deciduous/soybean-corn	EEZ	B	4
			BX	B	4
			5 x BX	B	4
	9 Aug-18 Oct	Deciduous/soybean-corn	live male <i>HH</i>	C-L	4
	18 Sep-18 Oct	Deciduous/soybean-corn	dead male <i>HH</i>	C-L	4
2008	18 Apr-31 Oct	Deciduous/soybean-corn	EEZ	B	2
	18 Apr-31 Oct	Coniferous/corn	EEZ	B	2

<sup>1</sup>Data for 2004 and 2005 from Aldrich et al. (2007).

<sup>2</sup>Aerial view of field sites in supplemental online material.

<sup>3</sup>EEZ = methyl (*E,Z,Z*)-2,4,6-decatrionoate; ZEZ = methyl (*Z,E,Z*)-2,4,6-decatrionoate; EEZ = methyl (*E,E,Z*)-2,4,6-decatrionoate; BX = 5:95 ratio of *trans-cis*-(*Z*)- $\alpha$ -bisabolene epoxides. Note: EZZ readily rearranges in sunlight to EEZ and other isomers (Khrimian et al. 2008).

<sup>4</sup>Photographs of traps shown in online supplemental material; C = container-type trap; B = baffle-type trap; C-L = container trap with bottom insert for laboratory-reared *H. halys* (*HH*) males.

material) was tested side-by-side with the container traps deployed for the first 2 years (4 replicates/treatment/trap type) (Table 1). Baffle traps were constructed from 0.25 inch corrugated plastic (Coroplast®, Dallas, TX) after the design of Mizell (1996) (61 cm tall and 30.5 cm at the base), except that the collector consisted of the top of a reusable yellowjacket trap (Sterling International, Inc.) held in place with wire pins. In 2007 and 2008, the baffle trap was used exclusively except when traps were baited with *H. halys* males as described below (Table 1).

From 9 Aug through 18 Oct 2007, 4 traps were added in which 2-3 live, laboratory-reared *H. halys* males were used to bait traps. *Halyomorpha halys* were reared as previously for other stink bugs (Aldrich et al. 1991) but with organically grown green beans (*Phaseolus vulgaris*) (My Organic Market, College Park, MD) and buckwheat (*Fagopyrum esculentum*) seeds in addition to sunflower seeds (*Helianthus annuus*). Adult males at least 2 weeks old were placed with a bean in transparent plastic half pint cups having several 3-4-mm diameter holes punched in the bottom halves of the cups, then the cups were inverted and inserted into the bottom of the container traps instead of the normal trap bottom such that volatiles from the male bugs would be released inside the trap. Another set of traps was added on 18 Sep prepared as described above except that

instead of live *H. halys* males, specimens of methylene chloride-extracted males were pinned inside the plastic cups to test for possible visual response to chemically neutral *H. halys* males.

From 18 Apr through 31 Oct 2008, four baffle traps baited with methyl (*E,E,Z*)-2,4,6-decatrionoate and 4 unbaited control traps were deployed as described above except that 2 sets of traps were hung from deciduous trees around the perimeter of the agricultural field in the same location as in previous years, and 2 sets of traps were deployed in 2 patches of mature coniferous trees located approximately 500 m northeast of the deciduous/soybean-corn site (see supplemental material). One conifer patch consisted exclusively of loblolly pine (Pinaceae: *Pinus taeda* L.); the other patch consisted mainly of eastern white pine (*Pinus strobus* L.), plus some Austrian (*Pinus nigra* Arnold) and Virginia pines (*Pinus virginiana*).

Among phytophagous stink bug species in the subfamily Pentatominae (Heteroptera: Pentatomidae), sexual communication is a bimodal process whereby long-distance attraction is mediated by sex pheromones, and shorter-range mate location is mediated by substrate-borne vibrational songs (Bagwell et al. 2008). Thus, pentatomines attracted chemically from long-range may fail to enter traps in the absence of vibrational signals; therefore, bugs in and within 10 cm of traps were counted as being attracted.

## Feeding Choice Test

Seeds of 2 Asian conifer species that are fed upon by *H. halys* in Japan (Kiritani 2007) were obtained from the U.S. National Arboretum, Washington, D.C., for feeding choice experiments: Japanese cedar, *Cryptomeria japonica*, and false cypress, *Chamecyparis obtusa*. 2 treatments (sunflower seeds versus *Cr. japonica*, and sunflower seeds versus *Ch. obtusa*), each with 8 replicates, were tested. A Petri dish with a filter paper in the bottom served as an arena for each replicate. One shelled sunflower seed, and either clumps of 8 *Cr. japonica* or 6 *Ch. obtusa* seeds were glued to the filter paper with wallpaper paste (Metylan® standard, Roman Adhesives, Inc., Calumet City, IL). The numbers of conifer seeds were chosen to approximate the weight of a sunflower seed. Additionally, a cotton-plugged, glass shell vial of water was provided. Each Petri dish received 5 second instar *H. halys*, and treatments were placed on the same shelf in an environmental chamber at 25°C, 72% relative humidity, and 16:8 h (L:D) photoperiod. The number of nymphs found on each seed treatment was recorded at 1-2-h intervals each day for 5 consecutive days, and dead nymphs were removed and replaced as they were found.

## Statistical Analysis

Fisher's exact chi-square, exact binomial and exact multinomial tests were performed for the analyses by StatXact (Mehta & Patel 2005). When applicable the numbers of insects were analyzed as an  $R \times C$  table with Fisher's chi-square test to determine if the distribution of the insects differed for the various factors. If the overall test was significant, then Fisher's Chi-square tests for  $2 \times 2$  tables, binomial or multinomial tests were performed to determine where the distributions differed. In some instances there was no table, and only binomial or multinomial tests could be used to test for differences.

## RESULTS

*Halyomorpha halys* populations increased from being undetectable in 2004 to being abundantly trapped in 2007 and 2008 (Fig. 1). Adults of both sexes and nymphs (second through fifth instars) of each species were caught in roughly equal numbers (data for *A. hilare* not shown). Captures of *A. hilare* adults remained constant from 2006 through 2008, while captures of *H. halys* adults went from being less than those of *A. hilare* in 2006 to being about 4 times greater than *A. hilare* for 2007 and 2008 (Fig. 2). In tests conducted in 2008 deploying traps from deciduous or coniferous trees bordering agricultural fields (see online supplemental material), all *A. hilare* indi-

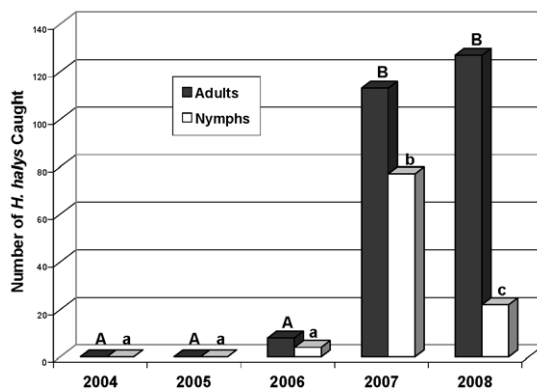


Fig. 1. The total number of *Halyomorpha halys* adults and nymphs caught inside or within 10 cm of 2 traps baited with isomers of methyl 2,4,6-decatrionoate and deployed at the same location in the field at Beltsville, Maryland, from 2004 through 2008 (2004: treatment = methyl (*E,Z,Z*)-2,4,6-decatrionoate\*, 4 mg active ingredient/rubber septum in container trap, rebaited biweekly; 2005: methyl (*E,Z,Z*)-2,4,6-decatrionoate, 4 mg a.i./rubber septum in container trap, rebaited biweekly; 2006-2008: methyl (*E,E,Z*)-2,4,6-decatrionoate, 2.5 mg a.i./rubber septum in baffle trap, rebaited weekly). [\*Note: Methyl (*E,Z,Z*)-2,4,6-decatrionoate readily isomerizes to methyl (*E,E,Z*)-2,4,6-decatrionoate and other isomers when exposed to light (Khrimian et al. 2008).]

viduals and all but 1 adult male *H. halys* were captured at the deciduous/soybean-corn site ( $P < 0.0001$ ). Significant numbers of bugs were never caught in control traps (usually none were caught); therefore, data for control traps are omitted from Figs. 1-5.

The 2006 data for the 2 different types of traps showed that baffle traps worked as well as or bet-

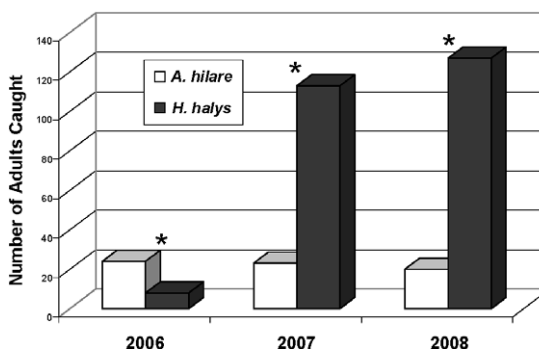


Fig. 2. The total number of *Halyomorpha halys* and *Acrosternum hilare* adults caught inside or within 10 cm of 2 traps baited with methyl (*E,E,Z*)-2,4,6-decatrionoate and deployed at the same location in the field at Beltsville, Maryland, from 2006 through 2008 (2.5 mg active ingredient/rubber septum in baffle trap, rebaited weekly). Asterisks over bars indicate significant differences between captures for each species within a year ( $P < 0.0001$ ).

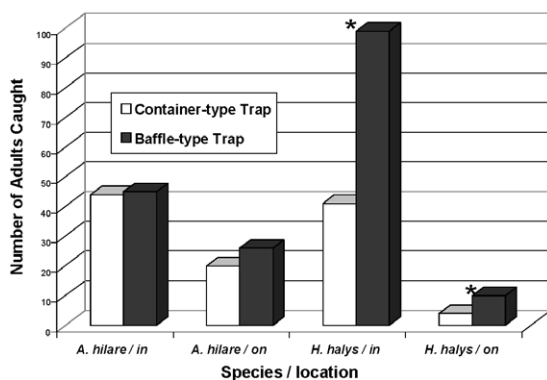


Fig. 3. Comparison of the efficiency of container-type and baffle-type traps for the total number of adults of *Halyomorpha halys* and *Acrosternum hilare* during 2006 ("in" = inside the trap; "on" = standing on the trap or within 10 cm of the trap; treatment = methyl (*E,E,Z*)-2,4,6-decatrionoate, 4 baffle traps, 2.5 mg active ingredient/rubber septum, rebaited weekly). Asterisks over bars indicate significant differences between captures for the trap types ( $P < 0.0001$ ).

ter than container traps in capturing *H. halys* and *A. hilare* adults (Fig. 3, and supplemental online material). The distribution of insects and location (i.e., inside versus on traps) were significantly different for container- and baffle-type traps ( $\chi^2 = 10.5$ ,  $P < 0.0147$ ,  $df = 3$ ). *Halyomorpha halys* adults and nymphs were more apt to enter baffle traps than container traps ( $P < 0.0001$ ); therefore, in 2007 baffle traps were used for all treatments except when container traps were baited with *H. halys* males.

The combined captures (bugs inside and within approximately 10 cm of traps) of *H. halys* and *A. hilare* for 2006 and 2007 by date are shown in Figs. 4 and 5, respectively. For *H. halys*,

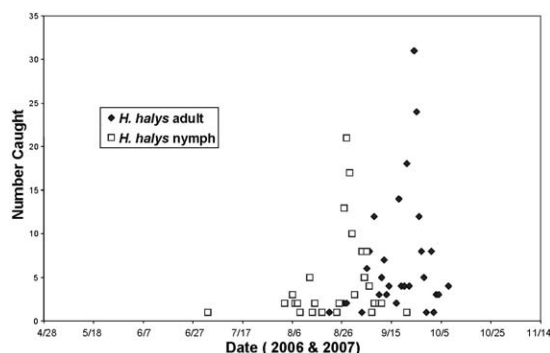


Fig. 4. The total number *Halyomorpha halys* adults and nymphs caught inside or within 10 cm of traps baited with methyl (*E,E,Z*)-2,4,6-decatrionoate by date during 2006 and 2007 (4 baffle traps, 2.5 mg active ingredient/rubber septum, rebaited weekly).

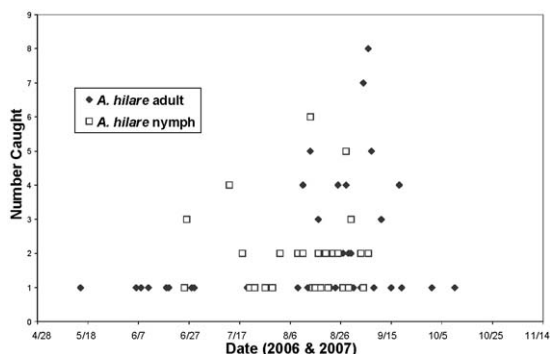


Fig. 5. The total number *Acrosternum hilare* adults and nymphs caught inside or within 10 cm of traps baited with methyl (*E,E,Z*)-2,4,6-decatrionoate by date during 2006 and 2007 (4 baffle traps, 2.5 mg active ingredient/rubber septum, rebaited weekly).

nymphs were the first individuals to be caught in mid-summer, followed by the appearance of adults from late Aug to early Oct. For *A. hilare*, a few adults were caught in May and Jun, and then nymphs appeared in mid-summer, followed by adults from Aug to early Oct. Some of the adults for both *H. halys* and *A. hilare* that were caught in the latter part of the season had soft, incompletely sclerotized cuticle, indicating that they were newly molted adults.

The attraction *H. halys* and *A. hilare* adults in 2006 to traps baited with methyl (*E,E,Z*)-2,4,6-decatrionoate (EEZ), a 5:95 ratio of *trans*-/*cis*-(*Z*)- $\alpha$ -bisabolene epoxides (BX), and the combination EEZ+BX, versus unbaited control traps is shown in Fig. 6. The distribution of *H. halys* and *A. hilare* was significantly different for these treatments

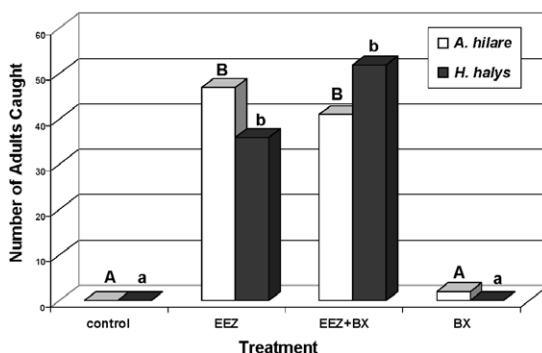


Fig. 6. The total number *Halyomorpha halys* and *Acrosternum hilare* adults caught inside or within 10 cm of traps baited in 2006 with methyl (*E,E,Z*)-2,4,6-decatrionoate (EEZ), a 5:95 ratio of *trans*-/*cis*-(*Z*)- $\alpha$ -bisabolene epoxides (BX), or EEZ+BX, versus unbaited control traps (4 baffle traps/treatment, 2.5 mg/lure, rebaited weekly). Bars followed by different letters are significantly different ( $P < 0.0001$ ).

for each species ( $\chi^2 = 93.82$  and  $83.07$ , respectively,  $P < 0.0001$ ,  $df = 3$ ). *H. halys* and *A. hilare* adults of both sexes were attracted to traps baited with methyl (*E,E,Z*)-2,4,6-decatrionoate, but neither species was attracted to traps baited with the bisabolene epoxide blend. Combining methyl (*E,E,Z*)-2,4,6-decatrionoate with the bisabolene epoxide blend did not significantly increase captures of either bug ( $P = 0.1548$  and  $P = 0.063$  for *A. hilare* and *H. halys*, respectively). The 2007 test results involving EEZ and BX treatments corroborated the 2006 results showing attraction of both species to EEZ-baited but not to BX-baited traps, and baiting traps with a 5 times greater dose of the bisabolene epoxide blend did not result in increased attraction of either species (Fig. 7). In addition, the following tachinid fly parasitoids were caught in 2006 and 2007 in or near traps baited with methyl (*E,E,Z*)-2,4,6-decatrionoate: *Gymnosoma par* (Walker) (11 females), *Euthera tentatrix* Loew (4 females), *Euclytia flava* (Townsend) (6 females and 3 males); or traps baited with the blend of bisabolene epoxides: *Trichopoda pennipes* (F.) (9 females and 3 males), *E. flava* (3 females and 2 males).

In 2007, baiting traps with live, laboratory reared *H. halys* males significantly attracted conspecific adults of both sexes and nymphs (4 females, 11 males and 6 nymphs; 29 Aug to 1 Oct, 2007,  $P < 0.0001$ ). Traps containing solvent extracted, dead adult *H. halys* pinned males did not attract any conspecific individuals from 18 Sep through 16 Oct, during which time traps baited with live males caught 5 male and 2 female *H. halys* adults.

Finally, in feeding choice experiments between sunflower seeds and seeds of Japanese cedar (*Cryptomeria japonica*) or false cypress (*Chame-*

*cyparis obtusa*), second instar *H. halys* showed an overwhelming preference for sunflower seeds. For the sunflower/Japanese cedar test, 78 nymphs were observed aggregated on sunflower seeds, and none were observed on Japanese cedar seeds. For the sunflower/ false cypress test, 78 nymphs were observed aggregated on sunflower seeds, and 2 nymphs were observed on false cypress seeds.

## DISCUSSION

Previous trapping experiments at Allentown, Pennsylvania, showed that methyl (*E,E,Z*)-2,4,6-decatrionoate is apparently essential for attraction of *H. halys*, but methyl (*E,Z,Z*)- and (*Z,E,Z*)-2,4,6-decatrionoates can be used to attract *H. halys* because these isomers substantially isomerize to methyl (*E,E,Z*)-2,4,6-decatrionoate when exposed to sunlight (Khrimian et al. 2008). Therefore, although our 2004 and 2005 tests used methyl (*E,Z,Z*)- and (*Z,E,Z*)-2,4,6-decatrionoates, we are confident that the trap counts recorded at Beltsville, Maryland, from 2004 through 2008 are a true reflection of the range expansion of *H. halys* into central Maryland. From a practical standpoint it is fortunate that methyl (*E,E,Z*)-2,4,6-decatrionoate is most active because this is the most convenient of the methyl 2,4,6-decatrionoate isomers to synthesize (Khrimian 2005), and isomerization of methyl (*E,E,Z*)-2,4,6-decatrionoate in sunlight may even increase attractiveness to *H. halys* (Khrimian et al. 2008).

Our data confirm that *H. halys* is truly an invasive species (Richardson et al. 2000; Colautti & MacIsaac 2004) because in central Maryland the population has risen from being undetectable in 2004 to being more abundantly trapped than the indigenous *A. hilare*. *Halyomorpha halys* expanded its range some 280 km to reach Beltsville, Maryland, where it was first detected in traps in 2005 (1 adult and 3 nymphs) (Aldrich et al. 2007). Thus, the lag time (Mooney & Cleland 2001) from establishment to population explosion is relatively short for *H. halys*. In North America, the brown marmorated stink bug now also has been collected in Ohio, Virginia and Massachusetts, and populations in Pennsylvania and New Jersey have reached damaging levels in some apple orchards (Hamilton 2009). The extent to which *H. halys* becomes an agricultural pest, in addition to a nuisance when overwintering in dwellings, remains to be seen. However, the insect is continuing to spread in North America, and its northern range may be gradually extended as a result of global warming (Kiritani 2006, 2007). The discovery of *H. halys* in Switzerland (Wermelinger et al. 2008) portends a similar situation in Europe.

Our finding that traps baited with live, laboratory-reared *H. halys* males attracted conspecific females, males and nymphs suggests that *H. halys*

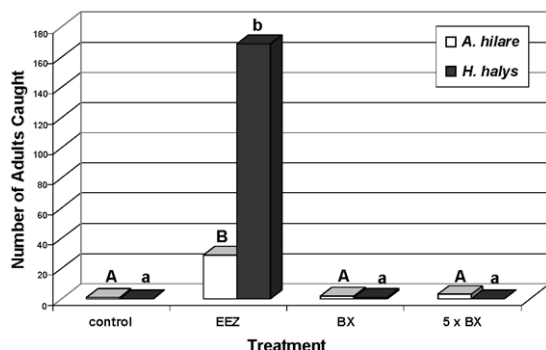


Fig. 7. The total number of *Halyomorpha halys* and *Acrosternum hilare* adults caught inside or within 10 cm of traps baited in 2007 with methyl (*E,E,Z*)-2,4,6-decatrionoate (EEZ), a 5:95 ratio of *trans*-/*cis*-(*Z*)- $\alpha$ -bisabolene epoxides (BX), or 5 septa loaded with BX, versus unbaited control traps (4 baited traps/treatment, 2.5 mg/lure, rebaited weekly). Bars followed by different letters are significantly different ( $P < 0.0001$ ).

males do produce a pheromone despite our inability thus far to isolate any male-specific compounds from them. Alternatively, the captured conspecifics could have been attracted by substrate-borne vibrations from caged males. If attraction was due to the presence of a pheromone, the apparent low level of pheromone production by laboratory-reared *H. halys* males may be caused by an inadequate diet (see Morishima et al. 2005). In this regard it could be significant that we must feed the insects organically grown green beans for them to survive yet the colony never flourishes (J. R. A., personal observation). Nevertheless, in our feeding tests of Japanese cedar and false cypress seeds versus sunflower seeds, second instars showed no propensity to feed on the conifer seeds notwithstanding Kiritani's (2007) claim that *H. halys* must feed on cones of these conifers to complete their life cycle. Funayama (2005) presented evidence that the mature seeds of Japanese cedar are suitable for the development of *H. halys* nymphs, but that indehiscent cones are not. He concluded that most *H. halys* in his region of study (Akita Prefecture, Japan) cannot develop into adults or lay eggs by feeding on Japanese cedar cones because cones do not dehisce until Oct. On the other hand, *H. halys* adults laid eggs on Japanese bird cherry trees, *Prunus grayana* (Rosaceae), in early Jun, and nymphs from these eggs completed a generation by mid-Sep feeding on bird cherry fruit (Funayama 2007). Funayama believes that *H. halys* females in his region develop their ovaries while feeding on early maturing wild cherries (*P. apatala*, *P. verecunda*, and *P. sargentii*), and then immigrate into the later maturing bird cherry (*P. grayana*) to lay eggs (personal communication; Fruit-tree Experiment Station, Akita Prefectural Agriculture, Forestry and Fisheries Research Center, Daigo, Hiraka, Yokote, Akita, 013-0102, Japan; funayamak@pref.akita.lg.jp). The observation of Funayama (2007) that *Prunus* spp. are excellent hosts for *H. halys* in Japan, raises the possibility that North American *Prunus* spp., such as black cherry, *Prunus serotina*, are early season hosts of *H. halys* in the U.S.

The near total absence of *H. halys* in the coniferous forest site at Beltsville is further evidence that the establishment of *H. halys* in North America is not associated with coniferous forests or ornamentals. Our results suggest that *H. halys* was primarily associated with soybean at our study site, and sweep-net sampling of the soybean strips at the Beltsville deciduous/soybean-corn site confirmed the presence of *H. halys* nymphs and adults (J. R. A., unpublished data). Indeed, in Oct 2008 we observed a spectacular invasion of a home near Hagerstown, Maryland, by thousands of *H. halys* adults that were apparently originating from a soybean field about 50 m downhill from the residence (J. R. A. and A. K., personal observation). The abundance of overwintering adults in dwellings where the *H. halys* population has exploded

suggests that the failure to catch adults early in the season in traps baited with methyl (*E,E,Z*)-2,4,6-decatrienoate reflects a seasonal difference in the responsiveness of *H. halys* to this compound.

Perhaps the main reason why the population of *H. halys* is rapidly increasing is that native scionid wasp (Arakawa et al. 2004) and tachinid fly parasitoids (Aldrich et al. 2006) have yet to fully exploit *H. halys* as a new host. The only tachinid to emerge from *H. halys* adults collected in Allentown (2 flies from 834 *H. halys* adults) was *Trichopoda pennipes* (F.) (Aldrich et al. 2006), which is the sole known tachinid parasitoid of *A. hilare* in North America (Arnaud, Jr. 1978). Tachinid flies use pheromones of heteropterans to home-in on potential hosts (Aldrich 1995), so parasitism of *H. halys* by *T. pennipes* may be a clue that the pheromone of *H. halys* is chemically similar to (*Z*)- $\alpha$ -bisabolene epoxide. Furthermore, we found that *T. pennipes* is attracted to the reported "pheromone" of *A. hilare* (Aldrich et al. 1989; Aldrich et al. 1993; McBrien et al. 2001) even though *A. hilare* itself is not attracted to the 5:95 blend of *cis*- and *trans*-(*Z*)- $\alpha$ -bisabolene epoxides. Combining (*Z*)- $\alpha$ -bisabolene epoxides with methyl (*E,E,Z*)-2,4,6-decatrienoate did not increase attraction of *A. hilare*, nor did increasing the dosage of (*Z*)- $\alpha$ -bisabolene epoxides affect *A. hilare* attraction. *Thyanta* spp. are the only New World bugs known to use a methyl 2,4,6-decatrienoate (the (*E,Z,Z*)-isomer) as a pheromone component (Millar 1997; McBrien et al. 2002; Moraes et al. 2005); therefore, *A. hilare* may be eavesdropping on pheromone calling *Thyanta* males. Interestingly, females of the solitary wasp, *Astata occidentalis* Cresson (Sphecidae) use methyl (*E,Z,Z*)-2,4,6-decatrienoate as a kairomone to find *Thyanta* adults with which to provision their nests (Millar et al. 2001; Aldrich et al. 2007). Evans (1957) also lists *Hymenarcys nervosa* (Say) and *Banasa calva* (Say) (Pentatomidae) as preferred prey for *A. occidentalis*, suggesting that these stink bugs may employ methyl 2,4,6-decatrienoates in their pheromones.

Recently, Funayama (2008) has provided the most direct evidence that *H. halys* in Japan is attracted to the pheromone of *P. stali* to find food. By calculating a nutritional level index [= live weight (mg)/pronotum width (mm)<sup>2</sup>] for adults collected in traps baited with methyl (*E,E,Z*)-2,4,6-decatrienoate versus adults hand-collected on food plants, he found that the semiochemically collected insects were nutritionally inferior to those hand-collected from host plants. Our current and past data (Aldrich et al. 2007) indicate that the cross-attraction propensity of *H. halys* for the *P. stali* pheromone has been maintained in the population of *H. halys* invading North America. The fact that pentatomid nymphs, even young nymphs, are often attracted to synthetic pheromones of conspecific males (even when no adult



conspecifics are present in traps to potentially produce substrate vibrations) (Aldrich 1988, 1995) also supports the idea that pheromones may be associated with food because nymphs are obviously not seeking a mate. Funayama (personal communication) suspects that *H. halys* adults and nymphs may need to feed on a series of host plants for optimal development. Similarly, *A. hilare* has a distinct preference for certain native hosts, mainly wild bushes and trees, and a succession of hosts appears to be necessary to sustain a population (Schoene 1933).

Under field conditions in Georgia, Tillman et al. (2009) verified our finding that traps baited with a 5:95 *trans*- to *cis*-(*Z*)- $\alpha$ -bisabolene epoxide blend failed to attract *A. hilare*, whereas traps baited with methyl (*E,E,Z*)-2,4,6-dectrienate significantly attracted *A. hilare*. Additionally, however, it was demonstrated for the first time in the field that the southern green stink bug, *Nezara viridula* (L.), can be trapped using its reported pheromone, a 3:1 *trans*- to *cis*-(*Z*)- $\alpha$ -bisabolene epoxide blend (Aldrich et al. 1987; Baker et al. 1987), but that *N. viridula* is not attracted to methyl (*E,E,Z*)-2,4,6-dectrienate. Males of *Acrosternum* and *Nezara* spp. produce distinctive ratios of the same *trans/cis*-(*Z*)- $\alpha$ -bisabolene epoxides, but *A. hilare* produced only about 0.3  $\mu$ g/male/day versus 9  $\mu$ g/male/day for *N. viridula* and about 20  $\mu$ g/male/day for *A. pennsylvanicum* (Aldrich et al. 1989). Although we now have some indication that *H. halys* males do produce an attractant pheromone, our difficulty in isolating the pheromone suggests that the amount of pheromone produced is naturally low, as is the case as well for *A. hilare*.

In conclusion, *H. halys* seems to have suppressed pheromonal communication while relying more on pheromone cross-attraction to find the array of host plants required for optimal development. The semiochemical situation for *A. hilare* may be similar since production of male-specific compounds is low, and the insect apparently requires a series of host plants to complete development as for *H. halys*. The semiochemical parallels between *H. halys* and *A. hilare* would be validated if and when native North American species of bugs are found that produce methyl (*E,E,Z*)-2,4,6-dectrienate as a part of their aggregation pheromones.

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