Attraction of the Invasive *Halyomorpha halys* (Hemiptera: Pentatomidae) to Traps Baited with Semiochemical Stimuli Across the United States

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ABSTRACT A recent identification of the two-component aggregation pheromone of the invasive stink bug species, *Halyomorpha halys* (Stål), in association with a synergist, has greatly improved the ability to accurately monitor the seasonal abundance and distribution of this destructive pest. We evaluated the attraction of *H. halys* to black pyramid traps baited with lures containing the pheromone alone, the synergist methyl (2E,4E,6Z)-decatrienoate (MDT) alone, and the two lures in combination. Traps were deployed around areas of agricultural production including fruit orchards, vegetables, ornamentals, or row crops in Delaware, Maryland, North Carolina, New Jersey, New York, Ohio, Oregon, Pennsylvania, Virginia, and West Virginia from mid-April to mid-October, 2012 and 2013. We confirmed that *H. halys* adults and nymphs are attracted to the aggregation pheromone season long, but that attraction is significantly increased with the addition of the synergist MDT. *H. halys* adults were detected in April with peak captures of overwintering adults in mid- to late May. The largest adult captures were late in the summer, typically in early September. Nymphal captures began in late May and continued season long. Total captures declined rapidly in autumn and ceased by mid-October. Captures were greatest at locations in the Eastern Inland region, followed by those in the Eastern Coastal Plain and Pacific Northwest. Importantly, regardless of location in the United States, all mobile life stages of *H. halys* consistently responded to the combination of *H. halys* aggregation pheromone and the synergist throughout the entire season, suggesting that these stimuli will be useful tools to monitor for *H. halys* in managed systems.

KEY WORDS brown marmorated stink bug, pheromone, monitoring, IPM, traps

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Halyomorpha halys (Stål), or brown marmorated stink bug, native to China, Taiwan, Korea, and Japan, is an invasive insect that was accidentally introduced into the United States in the mid- to late 1990s (Hoebeke and Carter 2003). It is a polyphagous pest of many crops in Asia (Panizzi et al. 2000, Lee et al. 2013a) and the United States (Leskey et al. 2012a, Rice et al. 2014). In 2010, outbreak populations in the mid-Atlantic region led to critical levels of damage in many crops, particularly stone and pome fruit (Leskey et al. 2012b).

In response to the threat posed by *H. halys* and in the absence of reliable monitoring and decision-making tools, some growers increased insecticide applications up to fourfold (Leskey et al. 2012c). A number of broad spectrum insecticides are effective against *H. halys* (Nielsen et al. 2008a, Leskey et al. 2012c, Lee et al. 2013b), but their increased uses has led to the severe disruption of integrated pest management programs (Leskey et al. 2012d). Therefore, establishing and validating reliable monitoring tools for *H. halys* is a critical step in the formation of revised integrated pest management programs.

In the northern United States, *H. halys* typically has one generation per year (e.g., in New Jersey: Nielsen and Hamilton 2009a); however, in more southerly locations, the pest undergoes two generations per year. In Maryland and West Virginia, the overwintering adults emerge in May and quickly begin laying eggs, which develop into the first summer generation adults (F1). Once the summer F1 adults mature, usually mid- to late-July, eggs are laid, and then develop into the second (F2) generation adults, which typically peak in early September (Leskey et al. 2012b). While *H. halys* is well-established in the eastern United States, less is known about the species' dynamics in other regions of the country, such as the midwestern and western United States. Effective monitoring tools may be used to support management decisions in the former, while also documenting the spread, establishment, and annual increase in populations in the latter regions.

Typically, native stink bug species have been monitored in agroecosystems using visual counts, sweep nets, beating samples, pheromone-baited traps, and blacklight traps (Krupke et al. 2001, Leskey and Hogmire 2005, Rashid et al. 2006, Kamminga et al. 2009, Borges et al. 2011). For *H. halys*, pyramid traps baited with the aggregation pheromone of the Oriental stink bug, *Plautia stali* Scott, methyl (2E, 4E, 6Z)-decatrienoate (MDT hereafter) have been evaluated (Nielsen et al. 2011, Leskey et al. 2012b, Joseph et al. 2013), and the lure has been found to be cross-attractive to *H. halys* (Aldrich et al. 2007, Khrimian et al. 2008). However, *H. halys* adults are attracted to this stimulus in the United States only in the late season (Leskey et al. 2012b), though there have been reports from Asia of adults responding to the stimulus earlier in the growing season (Funayama 2008). In the United States, this makes it difficult to detect the seasonal abundance and distribution of pest populations throughout much of the growing season using MDT alone.

Recently, Khrimian et al. (2014) identified and synthesized the male-produced aggregation pheromone of *H. halys*. Based on captures in black pyramid traps, this two-component pheromone, (3S,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol and (3R,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol (naturally present in a 3.5:1 ratio), of *H. halys* is attractive to males, females, and nymphs. Furthermore, mixtures of stereoisomers of 10,11-epoxy-1-bisabolen-3-ol were also attractive to *H. halys*, thus indicating that non-pheromonal semiochemicals apparently did not inhibit the attraction (Khrimian et al. 2014, Leskey et al. 2015). Subsequently, Weber et al. (2014) showed that when MDT is deployed in combination with the *H. halys* aggregation pheromone, a synergistic response is observed in baited traps.

Thus, these attractive olfactory stimuli can now be used to document seasonal abundance and distribution of *H. halys*. However, the aforementioned trials were conducted in only a few locations in Maryland and West Virginia, where the population density of *H. halys* was high. Therefore, the goals of this study were to assess season-long field responses of *H. halys* to the aggregation pheromone and synergist alone, in combination, and in different regions of the country with varying climates and population densities.

Materials and Methods

Traps. Pyramid traps used previously for *H. halys* (Leskey et al. 2012d) were used for all trials. Two base panels consisted of multiple plywood sheets affixed together (1 cm in thickness) by the manufacturer (AgBio Inc., Westminster, CO), and painted with flat black latex exterior paint. Leskey et al. (2012b) showed that *H. halys* adults and nymphs responded in greater numbers to this particular visual stimulus compared with other visual stimuli. Each base panel was 1.07 m in height, 52 cm in width at the base, and 8.2 cm in width at the top. Each pyramid base was topped by an inverted plastic collection jar (16 by 10 by 10 cm H:L:W; AgBio, Westminster, CO) with a cone-shaped base, an internal cone opening of 1.6 cm, and vented on all four sides with 3-cm openings covered with vinyl-coated polyester screen (mesh size: 1 by 3 mm^2). Traps were deployed at 31 (2012) and 28 (2013) sites in various habitats (Table 1) in Delaware, Maryland, New Jersey, New York, North Carolina, Ohio, Oregon, Pennsylvania, Virginia, and West Virginia, but generally positioned between agricultural production and unmanaged areas. Traps were deployed from early April until mid-October in 2012 and 2013 and spaced 50 m apart. All *H. halys* adults and nymphs found in collection jars were counted and removed weekly. At that time, the position of each collection jar containing a specific lure treatment within a replicate was rerandomized.

Lure Comparisons. *2012.* Three treatments were compared in pyramid traps. A gray rubber septum (1-F SS 1888 GRY, West Pharmaceutical Services, Lititz, PA) impregnated with 10.7 mg of the *H. halys* aggregation pheromone (8 mg of cis-10,11-epoxy-1-bisabolen-3-ol and 2.7 mg of trans-10,11-epoxy-1-bisabolen-3-ol stereoisomers prepared from (7R)-citronellal; T.L.C. unpublished data) served as one treatment. This composition contained 2 mg of (3S,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol (SSRS) and 0.7 mg of (3R,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol (RSRS). Before loading the septa with material, as described by Khrimian et al. (2008), the septa were washed in a Soxhlet apparatus with hexane and then dried for 12 h. A second treatment had MDT lures only (Sterling International Inc., Spokane, WA; hereafter referred to as Rescue) containing a reported ∼119 mg of material. Unbaited traps served as the control. Lures were hung inside the collection jar at the top. *H. halys* pheromone lures were replaced every 2 wk, while MDT lures were replaced every 4 wk. All collection jars were also provisioned with a piece of Hercon Vaportape II (Hercon Environmental, Emigsville, PA) that contained dichlorvos as a killing agent to prevent escape of insects from traps (Leskey et al. 2012b). The kill strip was changed every 2 wk or every 4 wk, depending on whether 2.5- or 5-cm-long (×2.5-cm-wide) pieces were used, respectively.

Table 1. Summary of the sites used for sampling in 2012 and 2013 in the United States

State	Town	Crop	Coordinates		# Repetitions	2012		2013		2012 Adults	2013 Adults
			Latitude	Longitude		Start[a]	Stop	Start[1]	Stop	Mean ± SE	Mean ± SE
EI											
MD	Smithsburg	Fruit	39.68	−77.59	3	11 April	5 Nov.,	–	–	3.17 ± 0.70	–
MD	Woodbine	Ornamental	39.32	−77.03	3	1 May	18 Oct.,	–	–	21.99 ± 6.91	–
NC	Asheville	Woods edge[b]	35.56	−82.52	1	16 April	26 Oct.,	–	–	5.87 ± 1.80	–
NC	Mills River	Corn and soybeans	35.43	−82.56	1	16 April	26 Oct.,	–	–	0.10 ± 0.05	–
NC	Edneyville	Apple and peach	35.36	−82.41	1	16 April	26 Oct.,	–	–	0.01 ± 0.01	–
OH	Columbus	Soybean	40.02	−83.04	3	13 July	12 Oct.,	–	–	1.76 ± 0.35	–
VA	Winchester	Apple and peach	39.01	−78.27	3	11 April	24 Oct.,	–	–	2.08 ± 0.58	–
VA	Winchester	Apple	39.10	−78.29	3	11 April	24 Oct.,	–	–	6.94 ± 1.30	–
VA	Winchester	Apple	39.20	−78.20	3	11 April	24 Oct.,	–	–	7.27 ± 1.72	–
WV	Kearneysville	Apple	39.37	−77.87	3	12 April	2 Nov.,	–	–	5.43 ± 1.50	–
WV	Martinsburg	Apple	39.45	−78.03	3	12 April	6 Nov.,	–	–	4.49 ± 1.30	–
MD	Laytonsville	Ornamental	39.24	−77.17	3	1 May	18 Oct.,	16 April	16 Oct.,	9.99 ± 2.34	43.88 ± 7.89
NY	Warwick	Apple and peach	41.23	−74.38	3	20 April	14 Sept.,	1 May	21 Oct.,	1.54 ± 0.44	10.06 ± 2.19
NC	Mills River	Tomato and pepper	35.42	−82.56	2	16 April	26 Oct.,	27 May	14 Oct.,	0.07 0.04	1.67 ± 0.49
NC	Asheville	Woods edge[b]	35.62	−82.57	1	16 April	26 Oct.,	27 May	14 Oct.,	0.96 ± 0.42	42.16 ± 14.17
OH	Columbus	Apple	40.01	−83.04	3	28 June	12 Oct.,	3 May	20 Sept.,	2.07 ± 0.32	2.17 ± 0.40
PA	Lancaster	Mixed fruit[c]	39.98	−76.31	3	19 April	23 Oct.,	1 April	21 Oct.,	2.98 ± 0.97	3.92 ± 0.83
PA	Gardners	Apple	40.00	−77.25	3	17 April	31 Oct.,	1 April	21 Oct.,	9.56 ± 1.40	4.97 ± 1.23
PA	Biglerville	Apple and peach	39.93	−77.26	3	13 April	26 Oct.,	1 April	21 Oct.,	2.00 ± 0.59	2.64 ± 0.50
VA	Glenvar	Sweet corn and pepper	37.27	−80.14	3	18 April	16 Sept.,	19 April	24 Oct.,	2.08 ± 0.71	4.69 ± 1.12
WV	Gerrards-town	Fruit	39.39	−78.08	3	12 April	6 Nov.,	3 April	23 Oct.,	6.41 ± 1.98	1.38 ± 0.24
MD	Thurmont	Fruit	39.65	−77.40	3	–	–	2 April	5 Nov.,	–	18.32 ± 3.62
MD	Woodbine	Fruit	39.32	−77.11	3	–	–	4 April	5 Nov.,	–	16.39 ± 2.80
NY	Campbell Hall	Apple	41.43	−74.24	1	–	–	1 May	21 Oct.,	–	7.25 ± 1.75
NC	Mills River	Peach	81.57	−35.43	1	–	–	27 May	14 Oct.,	–	1.26 ± 0.49
NC	Morganton	Apple, peach	35.66	−81.66	1	–	–	27 May	14 Oct.,	–	9.47 ± 3.09
NC	Vale	Abandoned apple[d]	81.39	−35.55	1	–	–	27 May	14 Oct.,	–	32.47 ± 6.07
PA	North East	Grape	42.06	−79.86	4	–	–	27 May	24 Oct.,	–	0.02 ± 0.01
VA	Winchester	Apple	39.10	−78.29	3	–	–	16 April	15 Oct.,	–	22.24 ± 3.66
VA	Winchester	Apple	39.12	−78.25	3	–	–	16 April	15 Oct.,	–	36.87 ± 5.86
ECP											
MD	Beltsville	Corn and soybean	39.01	−76.82	3	2 May	17 Oct.,	–	–	1.43 ± 0.49	–
MD	Upper Marlboro	Corn and soybean	38.86	−76.78	3	2 May	17 Oct.,	–	–	0.38 ± 0.08	–
MD	Upper Marlboro	Mixed crops[e]	38.86	−76.78	3	2 May	17 Oct.,	–	–	0.83 ± 0.26	–
MD	Elkton	Fruit	39.67	−75.82	3	13 April	8 Nov.,	–	–	7.05 ± 2.10	–
DE	Newark	Mixed vegetables[f]	39.67	−75.75	6	13 April	26 Oct.,	12 April	11 Oct.,	3.55 ± 0.59	5.62 ± 0.89
NJ	Cream Ridge	Peach	40.12	−74.52	5	19 April	25 Oct.,	8 April	7 Oct.,	2.81 ± 0.73	2.48 ± 0.98
NJ	Hammonton	Blueberry	39.63	−74.82	5-3	24 April	24 Oct.,	11 April	25 Oct.,	0.97 ± 0.17	1.70 ± 0.23
NJ	Landisville	Grape	39.54	−74.94	3-5	25 April	31 Oct.,	1 May	10 Oct.,	4.12 ± 0.77	2.47 ± 0.36
NY	Long Island	Peach	40.96	−72.81	2	–	–	15 April	15 Oct.,	–	0.05 ± 0.03
NY	Long Island	Apple	40.97	−72.66	1	–	–	15 April	15 Oct.,	–	0.22 ± 0.00
NY	Long Island	Mixed crops[g]	41.01	−72.48	1	–	–	15 April	15 Oct.,	–	0.07 ± 0.00
PNW											
OR	Sherwood	Ornamental	45.38	−122.82	1	27 April	26 Oct.,	–	–	0.74 ± 0.33	–
OR	Fairview	Blackberry	45.56	−122.43	1	27 April	26 Oct.,	–	–	0.15 ± 0.07	–
OR	Eagle Creek	Blueberry, Blackberry	45.37	−122.34	3	–	–	19 April	23 Sept.,	–	0.13 ± 0.04
OR	Troutdale	Raspberry	45.50	−122.37	3	–	–	19 April	27 Sept.,	–	0.29 ± 0.07

[a] Dates that sampling began and stopped in 2012 and 2013.
[b] Included *Paulownia*, locust, tulip poplar, tree of heaven, multiflora rose, etc.
[c] Apple, cherry, and peach.
[d] Abandoned for 3–4 yr prior to sampling.
[e] Included tomato, pepper, blueberry, and sweet corn.
[f] Included peppers, tomatoes, eggplant, okra, sweet corn, and snap beans.
[g] Included apple, cherry, and vegetables.

2013. Four treatments were compared in pyramid traps. One treatment included a gray rubber septum impregnated with 31 mg of a crude mixture synthesized from (7*R*)-citronellal (Leskey et al. 2015), containing 2 mg of (3*S*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol and 3.2 mg of (3*R*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol. A second treatment included the same *H. halys* pheromone lure in combination with a lure reportedly containing ~119 mg of MDT (Sterling International Inc.; i.e., Rescue). A third treatment included the *H. halys* pheromone lure in combination with a different MDT lure (AgBio Inc.) reportedly containing ~66 mg of material. Again, unbaited traps served as the control. Lures and the kill strip were hung inside the collection jar at the top, and kill strips were changed at intervals previously described. *H. halys* pheromone lures were replaced every 2 wk, while MDT lures were replaced every 4 wk.

Trapping Location Regions. Trapping sites were designated as falling into one of three regions: Eastern Coastal Plain (ECP), Eastern Inland (EI), and Pacific Northwest (PNW; Table 1). Definitions of regions are derived from U.S. Geological Survey (USGS; http://www.usgs.gov/) maps and studies. The ECP region is based on the USGS definition of the area comprising the Northern Atlantic Coastal Plain aquifer (Masterson et al. 2013), while the EI region is circumscribed by the USGS-defined Ohio, Tennessee, and parts of the Great Lakes and Mid-Atlantic watersheds (e.g., Watershed Boundary Dataset; http://nhd.usgs.gov/wbd.html,

last accessed 13 April 2015). The PNW region roughly corresponds to the PNW watershed from the USGS.

Statistics. Two repeated measures analyses of variance (ANOVAs) were used with the same model form, one for the nymph and the other for adult *H. halys*. Because of the heterogeneous nature of the sampling dates in 2012 and 2013, for data analysis, weeks were labeled consecutively during the growing seasons, and any sampling dates falling within a given week were assigned the new consecutive number. These numbers were used as the repeated measures in place of the actual sampling dates to unify the sampling dates in a given week across the study sites.

To describe lure treatment effects on the abundance of nymphs and adults during different parts of the season, the data were classified as occurring in the early (1 April to 15 June), mid- (16 June to 15 August), or late season (16 August to 15 November). Each model explained the abundance of the response life stage by the part of the season in which it occurred (early, mid, or late), the lure treatment (control, aggregation pheromone [PHER] only, or MDT in 2012 and control, PHER only, PHER + AgBio MDT, or PHER + Rescue MDT in 2013), a season–lure treatment interaction, and geographical region (ECP, EI, or PNW). The residuals did not conform to the assumptions of normality, and were, therefore, log-transformed. Upon a significant result, pairwise comparisons were performed with Tukey's honestly significant difference (HSD). Because there were different treatments between years, the procedure described above was repeated for the 2013 data. Finally, an analysis of the differences among regions pooled the samples across all baited traps within a region (unbaited traps were excluded from the analysis). All statistical analyses were performed in JMP v.5.0 (SAS Institute Inc. 2010, Cary, NC), and based on $\alpha = 0.05$. Mapping was performed using R Software (R Statistical Computing Group 2014, Vienna, Austria).

Results

Lure Comparisons. *2012 Studies.* Across all 31 sites (Fig. 1; Table 1), 34,898 nymphs and 21,439 adults were captured in baited traps, while 1,568 nymphs and 586 adults were captured in unbaited controls. Lure treatment significantly affected the number of *H. halys* adults ($F = 597.8$; df = 2, 305; $P < 0.0001$) captured in baited traps, although captures were also affected by time of the season ($F = 115.4$; df = 2, 279.5; $P < 0.0001$; Fig. 2) as well as the interaction between season and lure type ($F = 714.2$; df = 4, 279.5; $P < 0.0001$). Adult captures in traps baited with the *H. halys* aggregation pheromone were significantly greater than those baited with MDT in the early season (Fig. 2a) and with the unbaited control season long (Figs. 2a-c). *H. halys* adults were attracted to traps baited with MDT beginning in mid-season (late July), when captures were statistically similar to those in traps baited with the aggregation pheromone (Fig. 2b). However, late in the season, traps baited with MDT lures alone captured significantly more adults than those with the aggregation pheromone alone (Fig. 2c).
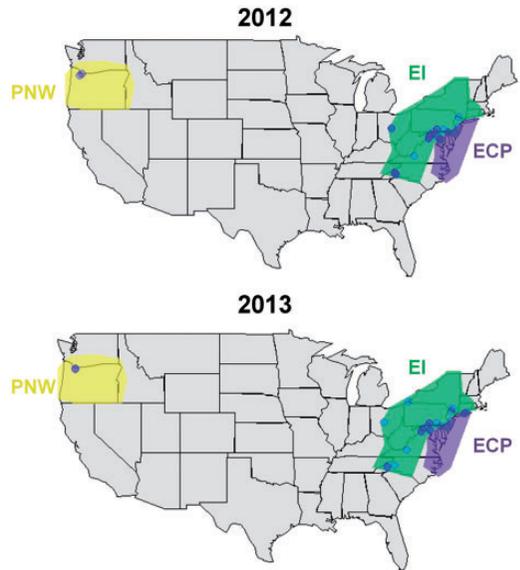

Fig. 1. Map of the sites in the United States used in the study in 2012 and 2013. Region abbreviations correspond to those found in Table 1, and are defined as: EI, Eastern Inland (green shading); ECP, Eastern Coastal Plain (purple shading); PNW, Pacific Northwest (yellow shading). Definitions for the regions are derived from the watershed designations of the USGS. Areas of darker blue shading on map indicate overlapping dots denoting field sites. For an interpretation of the colors, please consult the online PDF.

For nymphs, significant differences in trap captures were affected by treatment ($F = 1457.8$; df = 2, 305; $P < 0.0001$) and time of the season ($F = 99.0$; df = 2, 390.9; $P < 0.0001$). The interaction between lure type and season was also significant ($F = 383.9$; df = 4, 390.9; $P < 0.0001$). In the early season, almost no nymphs were present in the field ($\sim$ mean 0.01 nymphs week^{-1} trap^{-1}) and there were no significant differences among treatments (Fig. 2d). In the mid- and late season, however, traps baited with the aggregation pheromone or with MDT captured significantly more nymphs than unbaited traps (Fig. 2e and f). In addition, traps baited with MDT captured significantly more nymphs than those traps baited with the aggregation pheromone (Fig. 2e and f).

2013 Studies. Across the 28 sites, 54,673 nymphs and 47,850 adults were captured in baited traps (Fig. 1), while the unbaited controls captured 1,780 nymphs and 687 adults. As in 2012, the lure treatments significantly affected adult captures ($F = 262.3$; df = 3, 305; $P < 0.0001$) as did the time of season ($F = 262.3$; df = 2, 133.5; $P < 0.0001$), with a significant interaction between lure and period in the season ($F = 567.0$; df = 6, 133.5; $P < 0.0001$). Traps baited with a combination of the aggregation pheromone and MDT lures from AgBio or Rescue captured significantly more adults than traps baited with the pheromone alone and unbaited traps season long (Fig. 3a–c). In the late season, however, traps baited with the combined
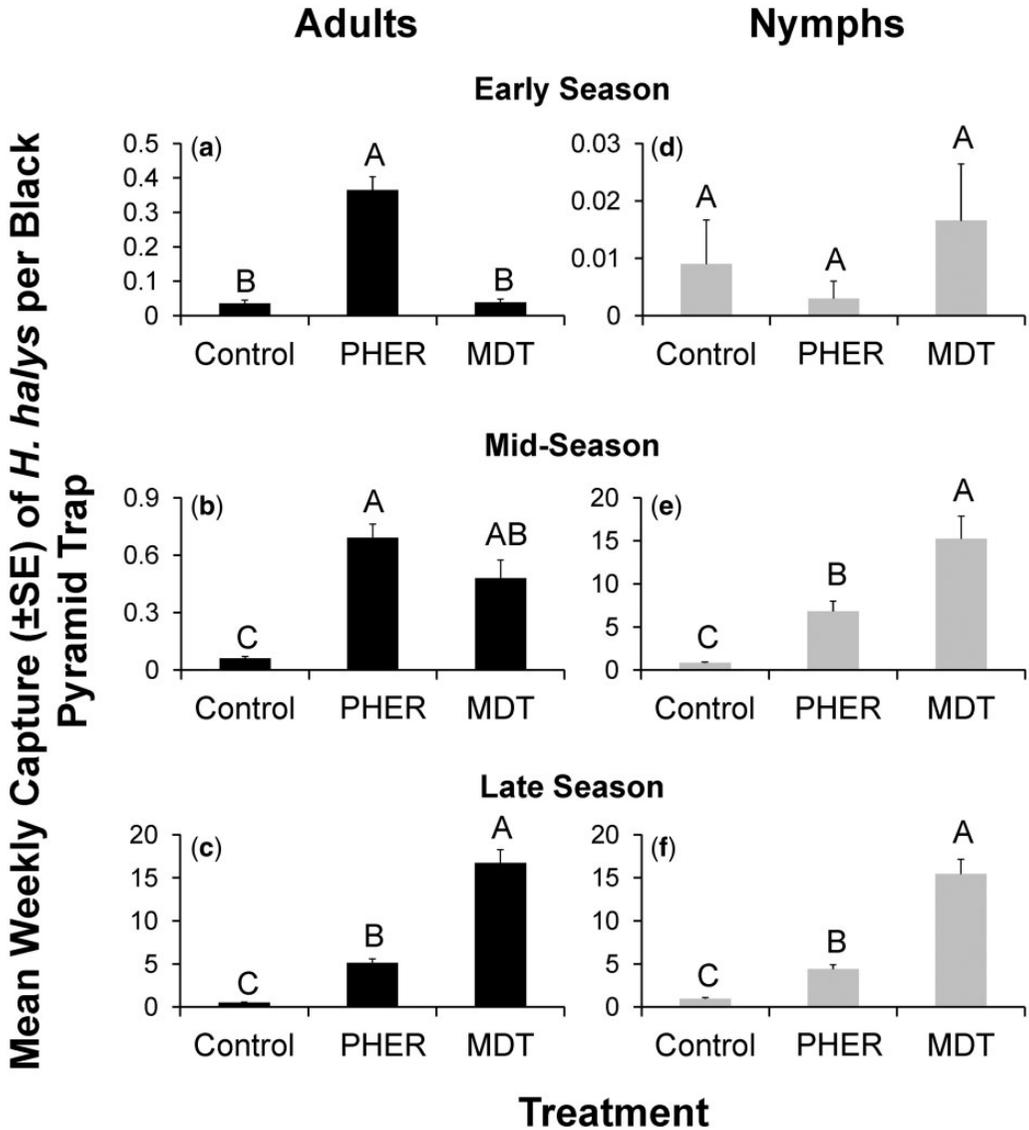
Fig. 2. Captures of *H. halys* (±SE) adults (black bars) or nymphs (gray bars) on a weekly basis with black pyramid traps in 2012 throughout the United States in the early season (a and d), mid-season (b and e), and late season (c and f). The control remained unbaited, while MDT refers to lures containing the synergist methyl (2E,4E,6Z)-decatrienoate, and PHER indicates lures containing the *H. halys* aggregation pheromone. Bars with shared letters are not significantly different from one another (Tukey's HSD, α = 0.05).

aggregation pheromone and Rescue MDT lures captured more than with the combination including the AgBio lure, probably because of nearly twice the amount of reported active ingredient in the Rescue compared to the AgBio lures (Fig. 3c).

For captures of nymphs, there was a significant effect of lure treatment ($F = 964.4$; df = 3, 305; $P < 0.0001$) and time of season ($F = 113.9$; df = 2, 112.1; $P < 0.0001$), including the interaction between the two ($F = 280.2$; df = 6, 112.1; $P < 0.0001$). In the early season, very few nymphs were present and there was no significant difference among treatments (Fig. 3d). In the mid- and late season, traps baited with

the aggregation pheromone in combination with the Rescue MDT lure captured significantly more nymphs than all other treatments (Fig. 3d and e). This was followed by the aggregation pheromone plus the AgBio MDT lure, which had significantly greater captures than the pheromone alone and the unbaited control (Figs. 3d, e). Traps baited with the aggregation pheromone alone captured significantly more nymphs than the unbaited controls (Fig. 3d and e).

Season-long Captures of *H. halys* in the United States. In 2012, adults that had dispersed from overwintering sites were captured early in the season (mid-April onward) in traps baited with the aggregation
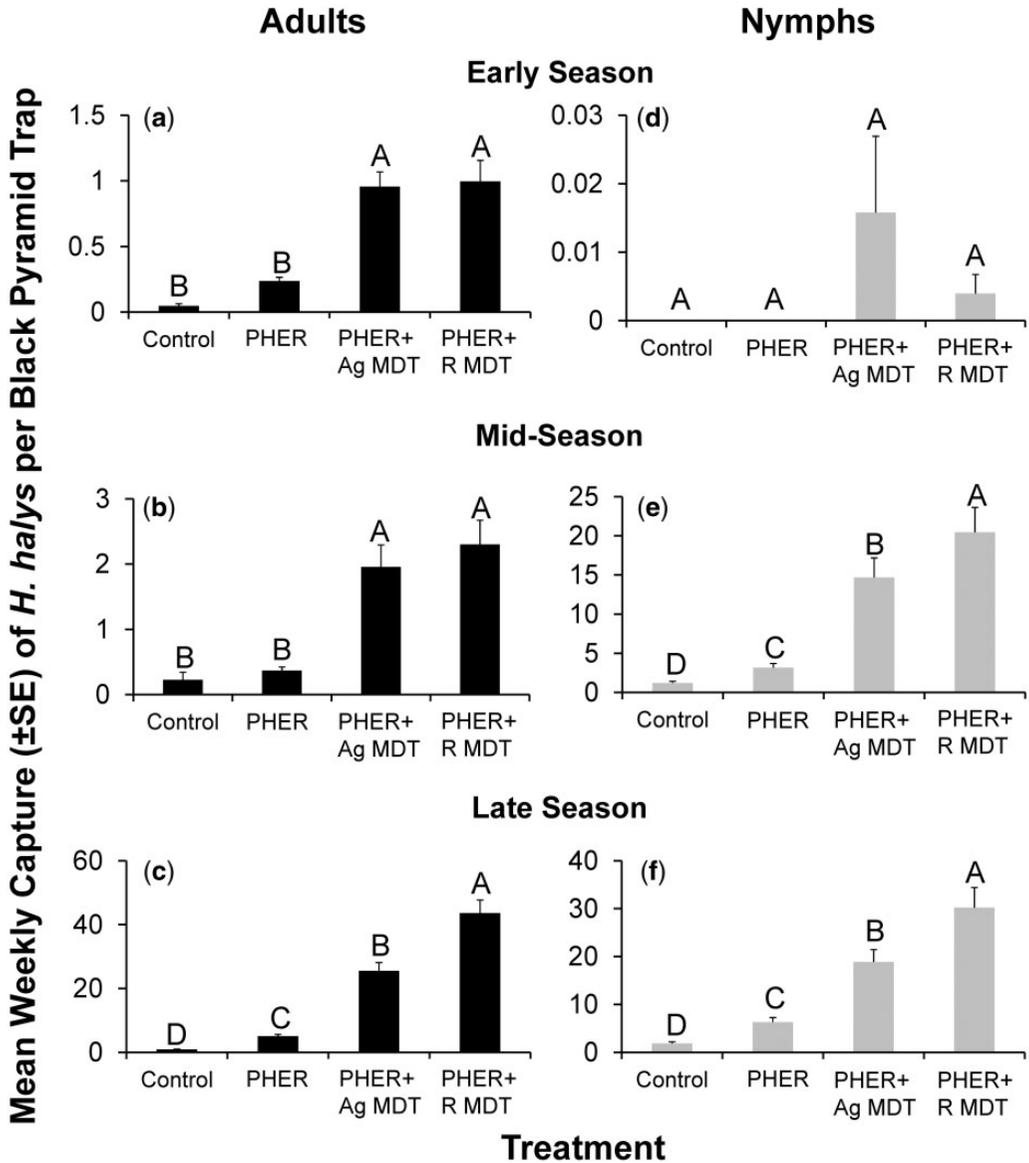
Fig. 3. Captures of *H. halys* (±SE) adults (black bars) or nymphs (gray bars) on a weekly basis with black pyramid traps in 2013 throughout the United States in the early season (a and d), mid-season (b and e), and late season (c and f). The control remained unbaited, while MDT refers to lures containing the synergist methyl (2*E*,4*E*,6*Z*)-decatrienoate, and PHER indicates lures containing the *H. halys* aggregation pheromone. Two sources of MDT were used in 2013, one from AgBio Inc. (Ag), and one from Sterling International Inc. (Rescue, R). Bars with shared letters are not significantly different from one another (Tukey's HSD, α = 0.05).

pheromone alone (Fig. 4a). Mid-season adult captures were very low until late July, when captures in traps baited with the aggregation pheromone or MDT likely reflected the presence of new F1 adults (Fig. 4b). Nymphs were captured in the early season beginning in late May in traps baited with the aggregation pheromone or with MDT, and nymphal captures increased in the mid-season (Figs. 4d, e). The largest populations of *H. halys* adults and nymphs were present in the field during the late season, as captures of adults in late

August were over 25 times greater than during the early season (Figs. 4c, f).

In 2013, traps baited with the aggregation pheromone plus MDT (either AgBio or Rescue) combination and those baited with the aggregation pheromone alone detected adults early in the season (mid-April onward), although captures were much higher in traps baited with the lure combinations (Fig. 5a). Nymphs also were detected in the early season beginning in late May in traps baited with the aggregation pheromone
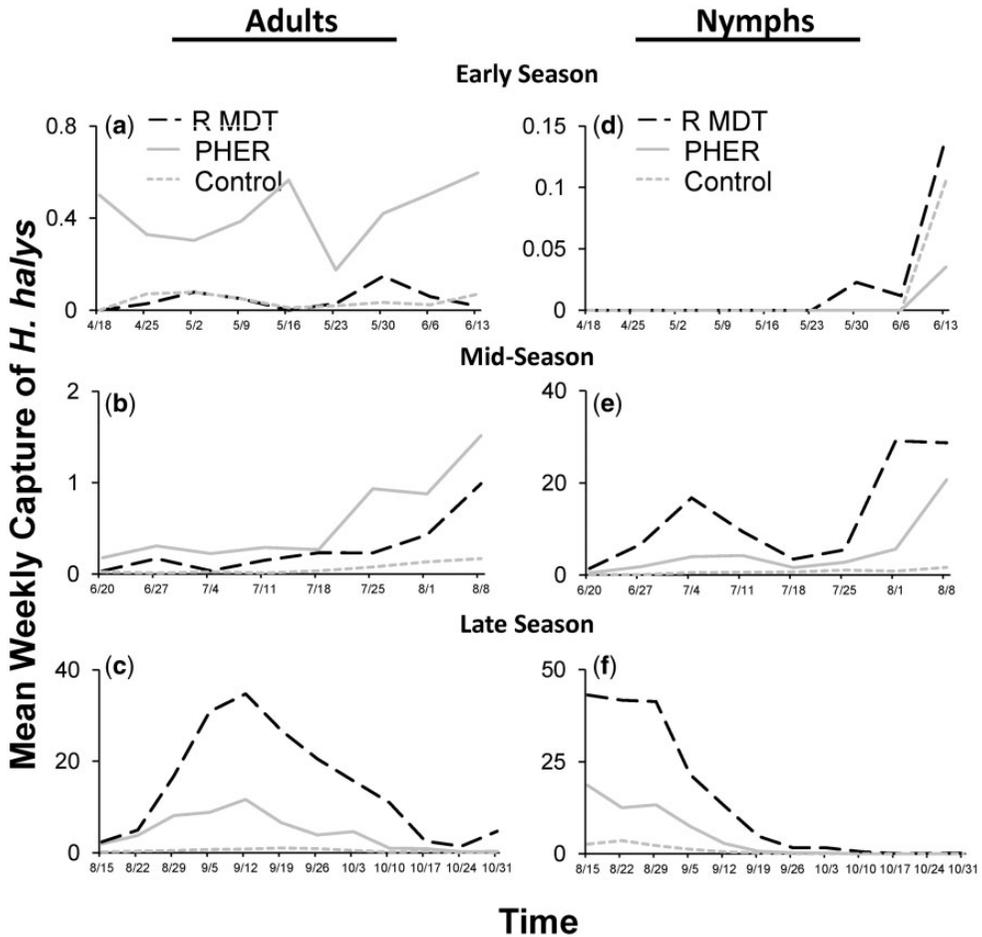
Fig. 4. Season-long mean weekly captures of *H. halys* adults (right column) and nymphs (left column) in traps during 2012 in the early (a and d), mid- (b and e), and late season (c and f), throughout the United States. The control remained unbaited, while MDT refers to lures containing the synergist methyl (2E,4E,6Z)-decatrienoate, and PHER indicates lures containing the *H. halys* aggregation pheromone. Error bars were left off of the lines for the sake of clarity.

and MDT (Fig. 5c). In the mid-season, numbers of adults and nymphs continued to increase as new F1s were being detected in traps baited with the combination stimuli in particular (Figs 5b, d). The largest populations of *H. halys* adults and nymphs were present in the field in the late season, as captures of adults in late August were over 25 times greater than during the early season (Figs 5c, f).

Regional Differences in Captures of *H. halys*. In 2012, there was a significant effect of region (Fig. 1, and defined in Methods under Trapping Location Regions) on the abundance of *H. halys* nymphs ($F = 78.1$; df = 2, 106.9; $P < 0.0001$) and adults ($F = 65.4$; df = 2, 106.9; $P < 0.0001$; Fig. 6a) captured in baited traps. Traps deployed in the EI region captured significantly more adults than the ECP or PNW regions. For nymphs, captures were significantly lower in the PNW compared with either of the eastern regions. This trend was similar in 2013 for nymphs ($F = 312.8$; df = 2, 113; $P < 0.0001$) and adults ($F = 253.0$; df = 2, 113; $P < 0.0001$; Fig. 6b).

Discussion

Khrimian et al. (2014) reported that *H. halys* was attracted to traps baited with lures composed of highly purified *H. halys* pheromone in their naturally occurring ratio [3.5:1 of (3S,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol:(3R,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol] in significantly greater numbers than either isomer alone. Leskey et al. (2015) also demonstrated that *H. halys* were attracted to traps baited with lures composed of pheromonal and nonpheromonal 10,11-epoxy-1-bisabolen-3-ol isomers, i.e., lures that had not been highly purified and contained extraneous isomers. Here, we deployed similar lures that were not highly purified (i.e., contained the SSRS and RSRS pheromonal isomers in 3:1 and 1:1.6 ratios in 2012 and 2013, respectively) and demonstrated season-long attraction to these stimuli in traps across the United States. In addition, we observed a synergistic response from *H. halys* when traps contained pheromone and MDT lures, as previously reported by Weber et al. (2014).
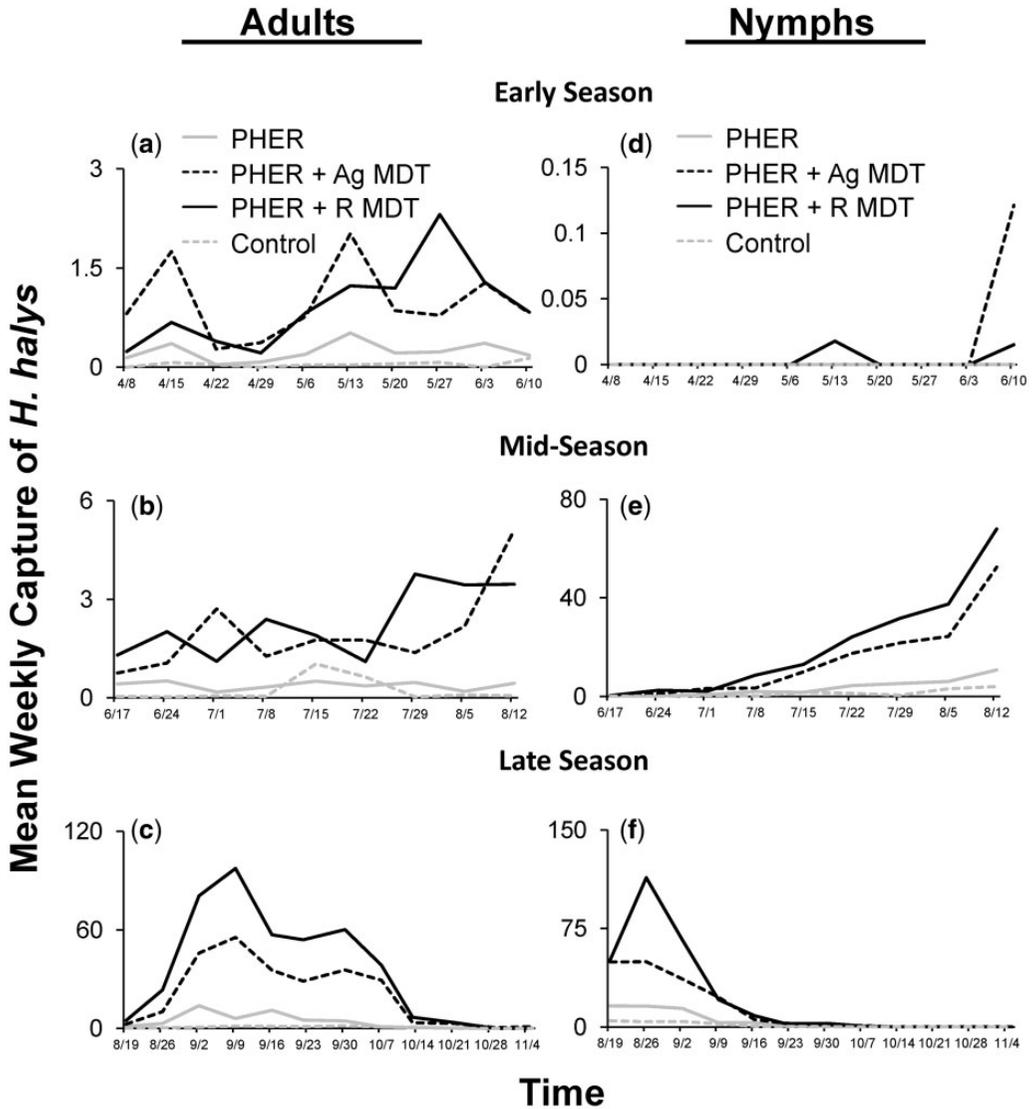
Fig. 5. Season-long mean weekly captures of *H. halys* adults (right column) and nymphs (left column) by traps during 2013 in the early (a and d), mid- (b and e), and late season (c and f), throughout the United States. The control remained unbaited, while MDT refers to lures containing the synergist methyl (2E,4E,6Z)-decatrienoate, and PHER indicates lures containing the *H. halys* aggregation pheromone. Two sources of MDT were used in 2013, one from AgBio Inc. (Ag), and one from Sterling International Inc. (Rescue, R). Error bars were left off of the lines for the sake of clarity.

As found previously and in trials conducted here, traps baited with solely MDT documented adult activity only during the late season (Leskey et al. 2012d). However, with the identification of the *H. halys* pheromone, we were able to monitor activity throughout the active growing season. Indeed, as these trials were conducted season long, we were able to document when adults had dispersed from overwintering sites in the spring to forage in the field (early season captures), seasonal changes in the relative abundance of adults and nymphs, and when adults began to leave the field in the late summer and early fall to seek overwintering sites based on sharply declining captures in early October. These results correspond roughly with other

reports of populations exiting and entering overwintering sites in Asia, the native range of *H. halys* (Lee et al. 2013a), and in the United States (Nielsen and Hamilton 2009b). However, we currently lack information regarding the physiological state of adults that actively orient to pheromonal stimuli in the spring and that cease to be responsive in the fall. Bergh and Leskey (unpublished data) found in a mark–release–recapture study that marked adults exiting fabricated overwintering sites deployed near traps baited with the *H. halys* aggregation pheromone and MDT did not immediately respond to pheromonal stimuli, although they did capture unmarked wild adults during the same time period. In addition, adults overwintering in human-
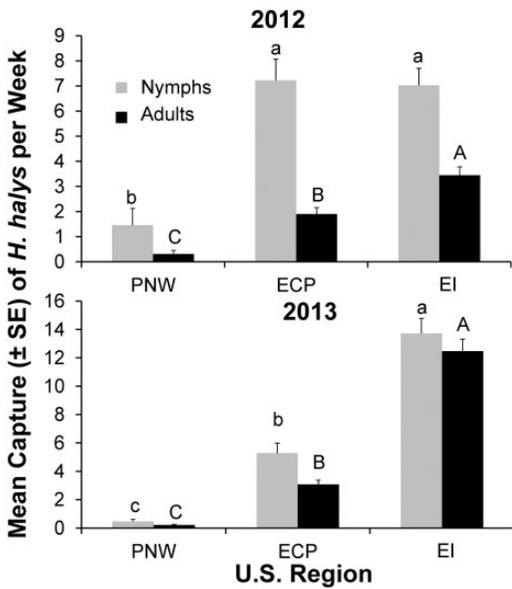
Fig. 6. Mean abundance (±SE) of adult (black bars) or nymphal (gray bars) *H. halys* caught in baited black pyramid traps in different regions of the United States on a weekly basis during (a) 2012 and (b) 2013. Abbreviations: EI, Eastern Inland; ECP, North Atlantic Coastal Plain; PNW, Pacific Northwest. Capitalized letters represent comparisons within adults among regions, while lower case letters signify comparisons within nymphs. Bars with shared letters are not significantly different from one another (Tukey's HSD, $\alpha = 0.05$). The definitions for the regions are derived from watershed designations of the USGS.

made structures during the winter months do not respond to pheromonal stimuli based on indoor trapping studies (T.C.L. unpublished data). Thus, it is likely that the diapausing state and reproductive status of individuals alters *H. halys* response to pheromonal stimuli through some unknown mechanism.

Monitoring tools routinely measure the seasonal abundance and distribution of the target species. Based on our results, it appears that combining the two-component aggregation pheromone of *H. halys* with the MDT synergist provides a reliable means to monitor these parameters for *H. halys*. However, traps do not provide clear estimates as to the number of generations per season and peaks in their activity. *H. halys* is known to be univoltine in central New Jersey (Nielsen and Hamilton 2009a) and bivoltine further south in West Virginia and Maryland (Leskey et al. 2012b) in the eastern United States. In Oregon, *H. halys* is also capable of completing two generations per year despite spanning similar latitudes as New Jersey. However, captures in traps do not necessarily reflect specific peaks in F1 and potentially F2 generations. This is likely owing to the protracted period of adult emergence from overwintering sites (Lee et al. 2014, J.C.B. and T.C.L. unpublished data) that extends from mid-April to late June in Maryland, West Virginia, and Virginia, which may lead to overlapping generations from the mid-season onward. To date, there have been two degree–day (DD) models developed for *H. halys*, one for the United States (Nielsen et al. 2008b) and one for Europe (Haye et al. 2014). From egg to adult, Nielsen et al. (2008b) found that about 537 DD were required to complete development, while Haye et al. (2014) calculated that 588 DD were required. The question as to whether captures in traps could be linked to outputs obtained from these models was beyond the scope of this study, but could provide additional understanding of *H. halys* population dynamics in the future. Ultimately, however, an overall peak of *H. halys* abundance and captures was recorded in early September across all locations, indicating a sustained threat in the late season shortly before or during the harvest of many crops.

Our trapping studies have provided novel information about the relative abundance of *H. halys* across the season and in different parts of the country. Interestingly, adult captures in the EI region were significantly greater than those in the ECP region, despite the fact that *H. halys* has been present in these areas for an equal number of years. It is possible that *H. halys* has not established as well in the ECP region because of specific biotic, abiotic, or both factors. On the other hand, captures in the PNW were significantly lower than in either of the eastern regions, despite the fact that *H. halys* has been present in this region since at least 2004. Other factors, either abiotic or biotic, may again contribute to lower overall populations in the PNW region compared with the eastern regions, although *H. halys* population densities there are continuing to rise and its distribution is continuing to spread (Shearer and Wiman 2014, Wiman et al. 2015) with increasing trap captures in 2014 reflecting this trend.

Effective monitoring traps should be sensitive to insect populations at low or high densities. Although we were able to detect *H. halys* at lower population densities, such as trapping sites in the PNW, increasing the dose or release rate of the stimuli used or optimizing the ratio between MDT and the aggregation pheromone for *H. halys* may lead to an improvement in the sensitivity of lures. In the present study, we used lures formulated with up to 5.2 mg of the active ingredients of the pheromone. However, Leskey et al. (2015) reported that captures increased significantly with increasing dose or loading rate of the pheromone. In addition, the captures reported here were generally greater when the amount of MDT was greater (66 vs. 119 mg) and combined with 10 mg of the aggregation pheromone. Previously, Leskey et al. (2012b) demonstrated that increasing the dose or loading rate of MDT also resulted in increased captures. Thus, if sensitivity of trapping is desired, one method through which it can be achieved is by increasing the amount of the aggregation pheromone and/or MDT loaded into lures, though likely at a significantly greater manufacturing cost. However, there may be an optimal ratio of MDT to *H. halys* aggregation pheromone, which may produce elevated sensitivity through increased attractiveness. Therefore, optimizing the ratio of MDT to

aggregation pheromone to produce the maximally synergistic response to the blend could result in a lure with increased sensitivity that may not necessarily be more costly to produce.

These results have conclusively demonstrated that *H. halys* populations can be reliably trapped season long throughout the United States using pheromonal and cross-attractive synergist stimuli and will undoubtedly benefit growers and pest management specialists who are in need of a reliable monitoring tool to inform on-farm pest management decisions for *H. halys*. The use of monitoring traps may enable thresholds to be developed that will allow growers to more efficiently use insecticides and to move away from calendar-based sprays and toward sustainable pest management. The *H. halys* aggregation pheromone and the synergist may also be incorporated into other traps (e.g., small pyramid traps), which could be easier to deploy and less expensive than the trap used in these studies. Commercial availability of the *H. halys* aggregation pheromone and synergist will provide a tool enabling growers to better combat this highly destructive, invasive species.

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