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Short Communication

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Enhanced Response of *Halyomorpha halys* (Hemiptera: Pentatomidae) to Its Aggregation Pheromone with Ethyl Decatrienoate

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Abstract

The invasive stink bug species, *Halyomorpha halys* (Stål) (Hemiptera; Pentatomidae), severely damages multiple agricultural commodities, resulting in the disruption of established IPM programs. Several semiochemicals have been identified to attract *H. halys* to traps and monitor their presence, abundance, and seasonal activity. In particular, the two-component aggregation pheromone of *H. halys*, (3*S*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol and (3*R*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol (PHER), in combination with the pheromone synergist, methyl (2*E*,4*E*,6*Z*)-decatrienoate (MDT), were found to be attractive. Here, we report that an analogous trienoate, ethyl (2*E*,4*E*,6*Z*)-decatrienoate (EDT), enhances *H. halys* captures when combined with PHER. In trials conducted in Eastern and Western regions of the United States, we observed that when traps were baited with the *H. halys* PHER + EDT, captures were significantly greater than when traps were baited with PHER alone. Traps baited with EDT alone were not attractive. Thus, the addition of EDT to lures for attracting *H. halys* to traps may further improve monitoring efficiency and management strategies for this invasive species.

Key words: brown marmorated stink bug, BMSB, pheromone, attractants, trapping

Introduction

Halyomorpha halys (Stål) (Hemiptera; Pentatomidae) is an invasive stink bug that causes severe economic damage to fruits, vegetables, field crops, nuts, and ornamental nursery plants (Rice et al. 2014). Originating in Asia, established populations of *H. halys* were first detected in Pennsylvania in 2001 (Hoebeke and Carter 2003), and have since been reported throughout the United States, four Canadian provinces, many European countries (www.stopbmsb.org, Rice et al. 2014, Kriticos et al. 2017), and most recently in South America (Faúndez and Rider 2017). This invasive insect severely disrupts established IPM programs as growers now rely on calendar-based insecticide applications to reduce economic damage (Leskey et al. 2012a,b), often leading to secondary pest outbreaks (Leskey et al. 2012c), thus emphasizing the need for effective monitoring techniques.

Prior to its introduction to North America, researchers in Asia reported *H. halys* captures in traps baited with the aggregation pheromone of the oriental stink bug *Plautia stali* Scott (Hemiptera; Pentatomidae), methyl (2*E*,4*E*,6*Z*)-decatrienoate (MDT) (Sugie et al. 1996), suggesting this compound could be used to monitor and detect *H. halys* populations in invaded regions. In the United States, MDT combined with visually attractive black pyramid traps successfully captured *H. halys* adults and nymphs in the latter part of the growing season, but early season monitoring remained difficult because *H. halys* adults did not respond to MDT at that time (Leskey et al. 2012d). In 2014, the *H. halys* aggregation pheromone (PHER) (3*S*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol (SSRS) and (3*R*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol (RSRS) (in approximate 3.5:1 ratio of SSRS:RSRS) was identified and synthesized

(Khrimian et al. 2014) and traps baited with PHER + MDT captured *H. halys* nymphs and adults throughout the entire season.

When combined in a single trap, PHER + MDT act synergistically, attracting more *H. halys* than the additive effect of individual PHER or MDT lures to traps (Weber et al. 2014). The combined lures successfully captured *H. halys* in traps across the United States, Europe, and in Asia, suggesting these compounds can monitor and detect *H. halys* throughout the world (Leskey et al. 2015a; Morrison et al. 2016a, 2017). In apple orchards, traps baited with PHER + MDT have been successfully used to develop decision support tools (Short et al. 2017) and PHER + MDT lures have been used as the basis for attract-and-kill strategies (Morrison et al. 2016b). Because *H. halys* is cross-attracted to the aggregation pheromone of other stink bug species (Sugie et al. 1996; Aldrich et al. 2007, 2009; Khrimian et al. 2008), is also attracted to nonpheromonal stereoisomers of murgantiol (Leskey et al. 2015b), and exhibits a synergistic response when PHER is combined with MDT (Weber et al. 2014, Leskey et al. 2015a), we examined if other compounds with similar chemical structure were attractive to *H. halys* or if they enhanced the response to PHER. These included ethyl (2*E*,4*E*,6*Z*)-decatrienoate (EDT) and the pear ester compound, ethyl (*E*,*Z*)-2,4-decadienoate (EDD), which was found to be attractive to female codling moth (Knight et al. 2001). Identification of additional *H. halys* attractants may provide more sensitive monitoring and detection tools, and aid in management techniques such as trap-based economic thresholds and attract-and-kill strategies.

Methods

Laminate configuration lures produced by Hercon Environmental (Emigsville, PA) containing PHER, EDT, and/or EDD were evaluated in the field in 2015 and/or 2016. Both EDD and EDT were a minimum of 90% isomeric purity (*E*,*Z* for EDD and *E*,*E*,*Z* for EDT). Lures that contained PHER were synthesized by Bedoukian Research (Danbury, CT) and contained ~12.5% of the active SSRS and RSRS isomers. In studies conducted in 2015 and 2016, the sum of active isomers was ~10 and ~20%, respectively. For both years, the ratio of SSRS:RSRS was ~40:60. In 2015, specific treatments evaluated included the following: 1) 10-mg PHER lure; 2) 10-mg PHER lure + 125-mg MDT; 3) 10-mg PHER + 250-mg EDT; 4) 10-mg PHER + 250-mg EDT + 500-mg of EDD; 5) 250-mg EDT alone; and 6) 500-mg of EDD. All lures were deployed in black pyramid traps (AgBio, Inc., Westminster, CO) as described in Leskey et al. (2015b), and an unbaited trap served as a control. Traps were deployed between agricultural production and unmanaged habitat and spaced 50 m apart. Lures were deployed inside collection jars and all jars were also provisioned with a 2.5-cm piece of Hercon Vaportape II (Hercon Environmental, Emigsville, PA) that contained dichlorvos as a killing agent to prevent escape of trapped insects. Traps were checked weekly and all adult and nymphal *H. halys* were counted and removed. Lures and kill strips were changed every 2 wk. In MD and WV, traps were deployed between 10 June and 1 July. In OR, traps were deployed between 26 June and 17 July. Three replicates of each treatment were deployed at each location (Table 1). In 2015, trap *H. halys* capture rates were low. Therefore, for analysis, adult and nymphal captures were combined to evaluate the effect of the semiochemical treatments on trap captures, and a generalized linear model was created based on a quasi-Poisson distribution to account for overdispersion in the dataset. The total number of *H. halys* individuals (adults and nymphs) was used as an aggregate response, with semiochemical treatment (10-mg PHER + 125-mg MDT, 10-mg PHER + 250-mg EDT, 10-mg PHER + 250-mg EDT + 500-mg EDD, 250-mg EDT, 500-mg EDD, 10-mg PHER, or an unbaited control) as a fixed, explanatory variable. Field location was used as a random blocking variable, while sampling date was used as a repeated measure to account for temporal autocorrelation between samples. Wald tests for significance were based on a χ^2-distribution. Post hoc pairwise comparisons between the treatments were performed using Tukey's HSD. All tests were performed in the R statistical environment with $\alpha = 0.05$ (R Core Development Team 2015).

In 2016, the response of *H. halys* to EDT and/or PHER was evaluated in field experiments in NY, NJ, MD, and OR (Table 1) again using black pyramid traps spaced 50 m apart. Each site contained three replicates. Each trap was assigned one of the following treatments: 1) USDA Standard; 2) 50-mg PHER +125-mg EDT; 3) 50-mg PHER + 250-mg EDT; 4) 10-mg PHER + 250-mg EDT; 5) 10-mg PHER + 125-mg EDT; 6) 50-mg PHER; 7) 10-mg PHER; and an 8) unbaited control. The USDA Standard lure was

Table 1. Location and GPS coordinates of fields sites that compared *Halyomorpha halys* captures in black pyramid traps using different lures

Site	Latitude	Longitude
Pen Mar, MD	39° 43′5.39″ N	77° 31′21.08″ W
Ringgold, MD	39° 42′41.53″ N	77° 31′49.88″ W
Laytonsville, MD	39° 15′16.93″ N	77° 09′35.43″ W
Bardane, WV	39° 22′42.30″ N	77° 50′41.30″ W
Cream Ridge, NJ	40° 7′2.34″ N	74° 31′25.54″ W
Charbonneau, OR	45° 16′48.87″ N	122° 45′14.50″ W
Highland, NY	41° 44′46.23″ N	73° 57′56.92″ W

All sites were used in 2015 and 2016 with the exception of Cream Ridge, NJ, and Laytonsville, MD, locations which were used only in 2015.

Table 2. Mean No. *H. halys* adult and nymphs ± SE captured in pyramid traps baited with semiochemical stimuli in 2015

Treatment	Mean ± SE
PHER (10 mg) + MDT (125 mg)	5.00 ± 1.80 A
PHER (10 mg) + EDT (250 mg)	2.50 ± 0.78 B
PHER (10 mg) + EDT (250 mg) + EDD (500 mg)	2.30 ± 0.91 B
EDT (250 mg)	1.30 ± 0.58 C
EDD (500 mg)	0.57 ± 0.29 C
Unbaited Control	0.56 ± 0.21 C
PHER (10 mg)	0.31 ± 0.14 C

Rows with different letters indicate significant differences (Tukey's HSD, $\alpha = 0.05$).

Table 3. Seasonal long total traps captures (Mean ± SE) of *H. halys* in pyramid traps with EDT and/or PHER during 2016

Treatment	Adults	Nymphs
USDA Standard	5.50 ± 0.96 A	2.10 ± 0.38 a
PHER (50 mg) + EDT (250 mg)	3.50 ± 0.61 A	1.40 ± 0.31 a
PHER (50 mg) + EDT (125 mg)	2.90 ± 0.53 A	0.97 ± 0.15 b
PHER (10 mg) + EDT (250 mg)	1.80 ± 0.37 B	0.64 ± 0.12 b
PHER (10 mg) + EDT (125 mg)	2.10 ± 0.47 A	0.64 ± 0.11 b
PHER (50 mg)	0.90 ± 0.19 C	0.31 ± 0.07 c
PHER (10 mg)	0.47 ± 0.12 C	0.31 ± 0.06 c
Unbaited Control	0.07 ± 0.02 C	0.13 ± 0.04 c

Rows that do not share letters are significantly different from each other within life stage (Tukey's HSD, $\alpha = 0.05$).

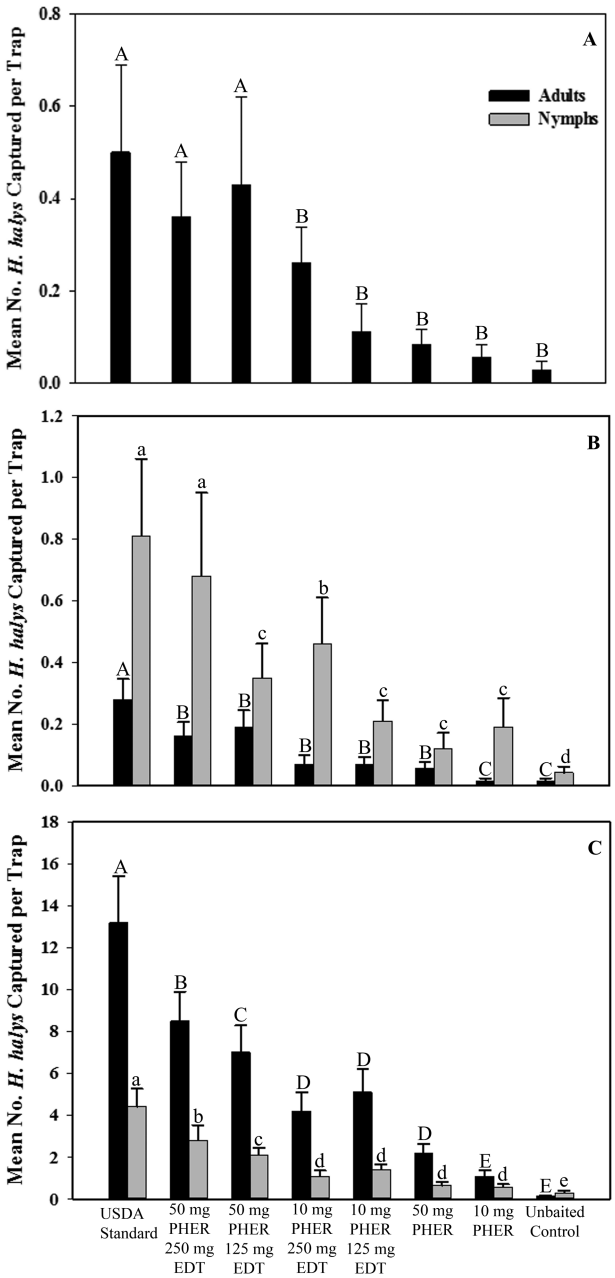

Fig. 1. Trap captures of *H. halys* in early (A), mid (B), and late-season (C), with EDT and/or PHER treatments: 1) USDA Standard; 2) 50-mg PHER + 250-mg EDT; 3) 50-mg PHER + 125-mg EDT; 4) 10-mg PHER + 250-mg EDT; 5) 10-mg PHER + 125-mg EDT; 6) 50-mg PHER; 7) 10-mg PHER; and an 8) unbaited control.

produced by the USDA and consisted of a single rubber septum (1-F SS 1888 GRY, West Pharmaceutical Services, Lititz, PA), loaded with 10.66 mg of 10, 11-epoxy-1-bisabolen-3-ol mixture containing 2 mg of the SSRS and 0.67 mg of the RSRS components (for formulation details, see Weber et al. 2014) in combination with a 66 mg MDT lure (ChemTica Int., S.A., Santo Domingo, Costa Rica, AgBio, Inc.). This lure combination has been widely evaluated in other trap-based trials (see Leskey et al. 2015a, Morrison et al. 2015, Short et al. 2017, Weber et al. 2017). Treatments 2–7 were produced by Hercon (Hercon Environmental, Emigsville, PA).

Because of sampling for a longer period during 2016, the season was split into an early (23 May to 15 June), mid (16 June to 14 August), and late (15 August to 11 October) period, as has been done in previously published literature (Leskey et al. 2015a, Morrison et al. 2017). Two separate generalized linear models were run for each *H. halys* life stage (nymphs or adults). The overall models contained the semiochemical treatment (USDA Standard, 50-mg PHER + 250-mg EDT, 50-mg PHER + 125-mg EDT, 10-mg PHER + 250-mg EDT, 10-mg PHER + 125-mg EDT, 50-mg PHER, 10-mg PHER, and an unbaited control), period (early, mid, or late), and the interaction of the two as fixed explanatory variables. Upon a significant result for the period and interaction term, separate repeated measures models were run for each life stage in each sampling period to better account for the seasonal variation in behavioral response to semiochemical-baited traps.

Results and Discussion

In 2015, in total, 394 nymphs and 57 adults were captured. Among treatments, traps baited with 10-mg PHER+ 125-mg MDT yielded the greatest captures (χ^2 = 77.4, df = 6, 251, P = < 0.0001), as MDT serves as a synergist for PHER (Weber et al. 2014, Leskey et al. 2015). When combined, PHER + EDT enhanced *H. halys* captures in baited traps (Table 2). Among treatments, captures in traps baited with only EDT, PHER, and EDD did not differ from the unbaited control. The fruit aroma EDD is found in pear and apple (Jennings et al. 1964, Berger et al. 1984) and has been used as a synergist in codling moth, *Cydia pomonella* (L.), pheromone traps (Trona et al. 2010, 2013) but did not increase trap captures of *H. halys* when combined with PHER + EDT. In recent studies, inclusion of host plant volatiles did not lead to increased captures in traps baited with PHER + MDT (Morrison et al. 2017), similar to what was observed here.

In 2016, we compared a number of PHER + EDT lure combinations in traps, varying the amount of each; these were compared to traps baited with PHER alone and a widely tested USDA Standard lure (10-mg PHER + 66-mg MDT). In total, we captured 2,334 nymphs and 6,213 adults. In season-long capture comparisons, we again observed significant differences among treatments for *H. halys* adults (χ^2 = 49.1, df = 7, 2880, P < 0.0001) and nymphs (χ^2 = 227.5, df = 7, 2873, P < 0.0001) (Table 3). Traps baited with PHER + EDT yielded significantly greater adult and nymphal captures than traps baited with PHER alone, while there were statistically equivalent captures of adults when baited with 50-mg PHER + 250-mg EDT compared with traps baited with the USDA Standard lure (PHER + MDT), indicating, again that EDT enhanced the response to PHER.

During the early season when overall populations were low, traps baited with the USDA Standard lure, 50-mg PHER + 125-mg EDT, or 50-mg PHER + 250-mg EDT, captured significantly greater numbers of adult *H. halys* compared to all other baited and unbaited traps (χ^2 = 274.7, df = 7, 576, P < 0.0001; Fig. 1A). During mid-season, traps baited with the USDA Standard lure captured significantly more adults than all other treatments (χ^2 = 327.3, df = 7, 1152, P < 0.0001; Fig. 1B). For nymphal captures during the mid-season, traps baited with USDA Standard or 50-mg PHER + 250-mg EDT captured significantly greater *H. halys* than all other treatments (χ^2, df = 7, 1152, P < 0.0001; Fig. 1B). During the late season, the USDA Standard lure captured significantly more *H. halys* adults (χ^2 = 317.3, df = 7, 5957, P < 0.0001), and nymphs (χ^2 = 625, df = 7, 1923, P < 0.0001) compared with all other treatments, and traps baited with 50-mg PHER + 250-mg EDT captured greater numbers of adults and nymphs compared with other treatments, except the USDA Standard lure (Fig. 1C) . Overall, EDT enhanced the response of *H. halys* adults and nymphs to its aggregation pheromone season-long, as was found for MDT (Weber et al. 2014, Leskey et al. 2015a).

Both EDT and MDT provoke an electroantennogram response in *H. halys* (Bedoukian and Grant, unpublished data), perhaps due to similar structure, or another stink bug species produces EDT as a pheromone and *H. halys* is cross attractive.

Novel aggregation compounds may further improve *H. halys* detection and management decisions. To date, treatment thresholds for chemical control have been developed using traps baited with PHER + MDT in apple orchards (Short et al. 2017). In addition, PHER + MDT lures have been used as components of an attract-and-kill system in apple (Morrison et al. 2016b). The logical next step is to combine EDT with MDT and PHER evaluate whether attraction, sensitivity, and/or the power of these semiochemicals can be increased further. Increased attraction to olfactory stimuli could lead to further improvement in IPM tactics for this invasive species (Morrison et al. 2016b, Rice et al. 2017, Short et al. 2017,) in additional cropping systems.

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