Journal of Economic Entomology, 114(4), 2021, 1666–1673
doi: 10.1093/jee/toab088
Advance Access Publication Date 22 May 2021
Research

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Horticultural Entomology

Refining Pheromone Lures for the Invasive *Halyomorpha halys* (Hemiptera: Pentatomidae) Through Collaborative Trials in the United States and Europe

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Subject Editor: Anne Nielsen

Received 2 February 2021; Editorial decision 14 April 2021

Abstract

Brown marmorated stink bug, *Halyomorpha halys*, is native to Asia and has invaded North America and Europe inflicting serious agricultural damage to specialty and row crops. Tools to monitor the spread of *H. halys* include traps baited with the two-component aggregation pheromone (PHER), (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, and pheromone synergist, methyl (2*E*,4*E*,6*Z*)-decatrienoate (MDT). Here, an international team of researchers conducted trials aimed at evaluating prototype commercial lures for *H. halys* to establish relative attractiveness of: 1) low and high loading rates of PHER and MDT for monitoring tools and attract and kill tactics; 2) polyethylene lure delivery substrates; and 3) the inclusion of ethyl (2*E*,4*E*,6*Z*)-decatrieonate (EDT), a compound that enhances captures when combined with PHER in lures. In general, PHER loading rate had a greater impact on overall trap captures compared with loading of MDT, but reductions in PHER loading and accompanying lower trap captures could be offset by increasing loading of MDT. As MDT is less expensive to produce, these findings enable reduced production costs. Traps baited with lures containing PHER and EDT resulted in numerically increased captures when EDT was loaded at a high rate, but captures were not significantly greater than those traps baited with lures containing standard PHER and MDT. Experimental polyethylene vial dispensers did not outperform standard lure dispensers; trap captures were significantly lower in most cases. Ultimately, these results will enable refinement of commercially available lures for *H. halys* to balance attraction and sensitivity with production cost.

Keywords: brown marmorated stink bug, pheromone, monitoring tools, attract and kill

The invasive brown marmorated stink bug, *Halyomorpha halys* (Stål, 1855) (Hemiptera: Pentatomidae), is species native to Asia and was first officially reported in the United States in 2001 (Hoebeke and Carter 2003). Less than a decade later, outbreak populations of *H. halys* inflicted heavy damage to specialty and row crops in the mid-Atlantic. Since that time, this insect has spread throughout the continental United States and to a number of European countries leading to increased agricultural problems (Maistrello et al. 2017, 2018; Leskey and Nielsen 2018). Moreover, this invasive pest is difficult to manage in vulnerable cropping systems due to strong dispersal activity by both adults and nymphs (Lee et al. 2014, Lee and Leskey 2015, Wiman et al. 2015), short residual activity of most foliar-applied products (Leskey et al. 2014), and constant re-invasion from wild host habitat (Leskey and Nielsen 2018). Thus, having sensitive and reliable monitoring tools to detect *H. halys* presence, relative abundance and seasonal activity, are critical for making informed pest management decisions.

Progress toward development of pheromone-based monitoring tools began with discovery that methyl (2*E*,4*E*,6*Z*)-decatrienoate (MDT), the pheromone of *Plautia stali*, another Asian stink bug, was attractive to *H. halys* (Aldrich et al. 2007, Khrimian et al. 2008). While MDT is attractive to nymphs season-long, adults are only attracted to this stimulus during the late-season, often well after damage to the crop has occurred and even after harvest in some cases (Leskey et al. 2012). However, with the identification of the two-component *H. halys* pheromone (PHER), (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 (Khrimian et al. 2014), and the discovery that MDT serves as a synergist (Weber et al. 2014), the pieces were in place to reliably monitor this pest. Indeed, trials conducted with various trap designs baited with these stimuli across the United States (Leskey et al. 2015a, Rice et al. 2018a, Acebes-Doria et al. 2018, 2020), parts of Europe (Morrison et al. 2017a) and the native range in South Korea (Morrison et al. 2017b), demonstrated the attractiveness and sensitivity of lures composed of these olfactory stimuli. Moreover, traps baited with these stimuli have been used as decision support tools to make management decisions in cropping systems (Short et al. 2017, Morrison et al. 2019, Leskey et al. 2020). However, much of the work to date has involved prototype lures that, although effective (Weber et al. 2017), are not optimized in terms of the amount of material used, their release rate and longevity, and the dispenser type relative to cost. Various ratios of the PHER and MDT formulated into lures have been evaluated and found to be attractive to *H. halys* in limited trials (Weber et al. 2020), yet they have not been evaluated widely. Moreover, additional potential olfactory stimuli such as ethyl (2*E*,4*E*,6*Z*)-decatrieonate (EDT) have shown promise (Rice et al. 2018b).

Rapid progress on development of monitoring and management for *H. halys* has been made because of large multi-state or region trials (Acebes-Doria et al. 2020, Ludwick et al. 2020). Here, an international team of researchers conducted trapping trials aimed at evaluating prototype commercial lures for *H. halys* to establish relative attractiveness of: 1) low and high loading rates of PHER and MDT for monitoring and biosurveillance programs; 2) high loading rates of PHER and MDT developed for potential attract and kill usage; 3) use of polyethylene substrate lure dispensers; and 4) inclusion of EDT in lures. We report the results of these studies in the context of refining commercial products to enable widespread adoption by growers and crop consultants.

Materials and Methods

Trial Locations and Lure Components

All trials were conducted in regions of the United States, Italy, and Hungary in areas with established *H. halys* populations in 2018 and 2019. Specific trials, locations, treatments, and dates evaluated can be found in Table 1. All lures were produced by Trécé Inc. (Adair, OK) and shipped directly to collaborators.

Experimental Trials

Lure Loading

To establish how reducing the amount of PHER by 50%, 75% and 90% in combination with standard and a 2× MDT loading affected trap captures, trials using experimental and standard lures were conducted. Standard lures were similar to those used by Acebes-Doria et al. (2020) and were formulated with 5 mg of the *H. halys* aggregation pheromone and 50 mg of the MDT synergist. Experimental lure formulations relative to loading rates of standard lures included: (PHER:MDT) 0.10:1, 0.25:1, 0.50:1, 0.10:2, 0.25:2, and 0.50:2 (Table 1). Experimental and standard lures were deployed in association with 15.2 × 30.5 cm double-sided clear sticky cards (Trécé Inc., Adair, OK) positioned horizontally and attached atop ~1.5 m wooden stakes with binder clips with tops of cards ~1.25 m above the ground as per Acebes-Doria et al. (2018, 2020). Unbaited traps served as a controls in the trial. Traps were deployed along the transition zone between susceptible crops and unmanaged habitats. Traps were spaced 50 m apart, checked weekly and all *H. halys* adults and nymphs were counted and removed. Lures were replaced at 12-wk intervals and sticky cards were replaced every 2 wk. Lure treatments were re-randomized within a replicate every 2 wk. Each site contained at least three replicates (Table 2).

To establish how increasing the amount of PHER by 2–3× and MDT by 4–12×, experimental lure treatments included: (PHER:MDT) 2:4, 2:6, 3:4, 3:6, and 3:12 (Table 1). Experimental lures were deployed

Table 1. Treatment designations and loading rates of the main *H. halys* pheromone (PHER), and the synergist (MDT), in ratios (PHER:MDT) relative to standard lure loading in experimental trials. Additionally, trials that included EDT are presented relative to their ratio to standard MDT loadings

Low rate loading	High rate loading	Attract and kill ratio	Dispenser substrate	EDT inclusion
PHER:MDT	PHER:MDT	PHER:MDT	PHER:MDT	PHER:MDT:EDT
0.10:1	2:4	0.75:12	1:1	1:0:1
0.25:1	2:6	3:12	1:1	1:1:3
0.50:1	3:4	12:12	0.5:1	1:0:3
0.10:2	3:6	0.75:24		1:0:9
0.25:2	3:12	3:24		
0.50:2		12:24		

Table 2. Country, state or region, number of sites (replicates per site), and dates for each experimental trial. All trials were conducted in 2018, except for EDT inclusion which was conducted in 2019

		No. sites (replicates per site); and dates for each trial				
Country	State or region	Low rate loading	High rate loading	Attract and kill ratio	Dispenser substrate	EDT inclusion
United States	Georgia (GA)				1(5); 9 Aug–24 Oct	
	Maryland (MD)	1(3); 22 May–23 Oct	1(3); 25 Jun–5 Nov	2(3); 24 May–24 Oct, 22 May–23 Oct	3(3); 6 Jul–18 Oct, 2 Jul–22 Oct, 4 Jul–17 Oct	
	New Jersey (NJ)	2(3); 18 Jul–16 Oct; 24 July–18 Oct				
	Oregon (OR)			1(4); 16 Jul–5 Nov		
	Utah (UT)	1(5); 16 Jul–20 Oct				
	Virginia (VA)			1(3); 10 Jul–Sep 24	1(3); 18 Jul–24 Sep	
	West Virginia (WV)	2(3); 21 May–22 Oct	1(3); 25 Jun–5 Nov	1(3); 21 May–22 Oct		3(3); 3 June–28 Oct
Italy	Emilia-Romagna (ER)	2(3); 22 Aug–10 Sep, 25 Aug–10 Sep		3(3); 25 Aug–10 Sep, 22 Aug –10 Sep, 14 Aug–16 Oct	2(3); 22 Aug–10 Sep, 25 Aug–10 Sep	
	Friuli Venezia Giulia (FVG)	1(3); 14 Aug–4 Oct		1(3); 20 Aug–24 Sep		
	Piedmont (PM)			2(3); 14 Aug–12 Oct		

on the exterior of jar tops via steel wire and binder clips atop AgBio Dead-Inn (Westminster, CO) black pyramid traps to enable likelihood of increased *H. halys* captures with increased loading (Acebes-Doria et al. 2018). An unbaited pyramid trap served as a control. Vestergaard netting (D-Terrence/DeadOnContact) was wrapped around the cone of the funnel inside the jar top as a killing agent. All traps were deployed along wooded habitats and spaced 50 m apart. Traps were checked weekly and all *H. halys* adults and nymphs were counted and removed. Lures were replaced at 12-wk intervals and lure treatments within a replicate were re-randomized every 2 wk. Each site contained at least three replicates (Table 2).

Additionally, a preliminary trial was run comparing loadings of PHER:MDT at a standard loading and high (4× higher for each component) from 24 May to 30 October 2018. Two traps for each treatment were deployed in association with clear sticky panels attached to wooden posts at fixed positions in an arboretum in Budapest, Hungary. As with other trials, traps were checked weekly, and sticky traps and lures were changed at 6- and 12-wk intervals, respectively.

Attract and Kill Components Ratio

To develop lures aimed at usage as part of attract and kill programs (Morrison et al. 2019, Leskey et al. 2020), the amounts of PHER and MDT were loaded at 0.75–12× and 12–24×, respectively, relative to standard lures. The following experimental lures were evaluated: (PHER:MDT) 0.75:12, 3:12, 12:12, 0.75:24, 3:24, and 12:24 (Table 1). Experimental lures were deployed on the exterior of commercial jar tops via steel wire and binder clips atop AgBio Dead-Inn (Westminster, CO) black pyramid traps to handle the likelihood of increased captures with increased loading (Acebes-Doria et al. 2018). An unbaited pyramid trap served as a control. Deltamethrin-incorporated Vestergaard netting (D-Terrence/DeadOnContact) was wrapped around the cone of the funnel inside the jar top as a killing agent. All traps were deployed along unmanaged wooded habitats and spaced 50 m apart. Traps were checked weekly and all *H. halys* adults and nymphs were counted and removed. Lures were replaced at 12-wk intervals and lure treatments within a replicate were re-randomized every 2 wk. Each site contained at least three replicates (Table 2).

Lure Substrate Dispenser

To establish whether the dispenser used to formulate *H. halys* lures affected captures, captures from traps baited with three experimental polyethylene lure dispensers with varying ratios of PHER:MDT (two dispensers at 1:1 and a third at 0.5:1; Table 1) were compared with captures from traps baited with standard commercial lures (Dual Lure, Trece). Unbaited traps served as a control. Experimental lures were deployed on double-sided clear sticky cards positioned horizontally and attached atop wooden stakes with tops of cards ~1.25 m above the ground along unmanaged, wooded habitats. Lures were affixed to the top of the post via a binder clip to ensure lures did not encounter the glue on the sticky cards and replaced at 12-wk intervals. Sticky cards were replaced every 2 wk. Traps were spaced 50 m apart, checked weekly and all *H. halys* adults and nymphs were counted and removed. Lure treatments were re-randomized every two weeks within a replicate. Each site contained at least three replicates (Table 2).

Inclusion of EDT

Lures were formulated with PHER, MDT, and/or EDT to establish how the presence of MDT and EDT affected *H. halys* captures. Lure treatments included: (PHER:MDT:EDT) 1:0:1, 1:1:3, 1:0:3 and 1:0:9 (Table 1). Experimental lures were deployed on double-sided clear sticky cards positioned horizontally and attached atop wooden stakes with tops of cards ~1.25 m above the ground. An unbaited trap was included as a control. Traps were checked weekly and all *H. halys* adults and nymphs were counted. Traps were deployed along unmanaged habitats. Lures were affixed to the top of the post via a binder clip to ensure lures did not encounter the glue on the sticky cards. Lures were replaced at 12-wk intervals and sticky cards were replaced every 2 wk. Traps were spaced 50 m apart, checked weekly, and all *H. halys* adults and nymphs were counted and removed. Lure treatments were re-randomized every 2 wk within a replicate. Each site contained at least three replicates (Table 2).

Statistical Analyses

Data were analyzed using RStudio (Version 1.1.463; 2009–2018 RStudio , Inc.). Due to consistently large numbers of zero counts across the data sets, all data were analyzed with a zero-inflated Poisson (ZIP) generalized mixed effect models using the "pscl" package (Zeileis et al. 2008, Jackman et al. 2015). Within each experimental trial, data were separated by location, and effect of lure type on trap captures was analyzed. Where there was a significant effect, the "emmeans" package was used to calculate the difference between lure treatments through estimated marginal means and the Tukey method was applied to determine significance levels; these statistical methods have been used for similar trapping studies (Formella et al. 2020, Hou et al. 2021). For preliminary trial conducted in Hungary, only mean ± SE are presented.

Results

Lure Loading

Among low loading treatments, there were significant differences in adult trap captures at nearly all locations (MD-1, $\chi^2 = 72.8$, df = 7, $P < 0.01$; NJ-1, $\chi^2 = 123.4$, df = 7, $P < 0.01$; NJ-2, $\chi^2 = 123.4$, df = 7, $P < 0.01$; WV-1, $\chi^2 = 15.7$, df = 7, $P = 0.03$; WV-2, $\chi^2 = 56.9$, df = 7, $P < 0.01$; ER-1, $\chi^2 = 49.6$, df = 7, $P < 0.01$; ER-2, $\chi^2 = 22.9$, df = 7, $P < 0.01$; and FVG, $\chi^2 = 773.9$, df = 7, $P < 0.01$). In general, lowering loading rates of PHER by 50% or greater reduced trap captures significantly, unless the loading rate of MDT was doubled (Table 2). Nymphal captures were lower than adults, particularly as some trials did not begin until later in the season when nymphal populations had begun to decline. Significant differences in nymphal trap captures were detected at the majority of locations (MD, $\chi^2 = 17.5$, df = 7, $P = 0.01$; NJ-2, $\chi^2 = 108.4$, df = 7, $P < 0.01$; WV-1, $\chi^2 = 79.5$, df = 7, $P < 0.01$; WV-2, $\chi^2 = 293.6$, df = 7, $P < 0.01$; FVG, $\chi^2 = 30.6$, df = 7, $P < 0.01$) with similar patterns of captures as observed with adults (Table 3).

Among high loading treatments, all traps baited with experimental lures captured significantly more adults at all sites (MD-1, $\chi^2 = 817.5$, df = 5, $P < 0.01$; MD-2, $\chi^2 = 814.9$, df = 5, $P < 0.01$; WV, $\chi^2 = 1011.9$, df = 5, $P < 0.01$) and significantly more nymphs at two sites (MD-2, $\chi^2 = 53.9$, df = 5, $P < 0.01$; WV, $\chi^2 = 334.4$, df = 5, $P < 0.01$) compared with unbaited traps (Table 4). A third site, MD-1, yielded very low nymphal captures overall. For adult captures, increasing the loading of PHER and MDT generally increased captures, but even at the highest loading of PHER(3×):MDT(12×), captures often were not significantly different when loading of MDT was reduced to 4× and 6×. However, captures in traps baited with lures with PHER loading at 2× (and MDT at 4× and 6×) did yield significantly lower captures in general (Table 4). Additionally, in a preliminary trial in Hungary, the mean number of adults (±SE) captured in traps with high and standard loading were 23. 1 ± 8.0 and 15. 8 ± 3.2, respectively. Nymphal captures in traps baited with high and standard loading were 75. 9 ± 11.6 and 13. 5 ± 2.4.

Attract and Kill Components Ratio

At all sites, significant differences were detected among captures (MD-1, $\chi^2 = 1509.4$, df = 6, $P < 0.01$; MD-2, $\chi^2 = 1191.2$, df = 6, $P < 0.01$; OR, $\chi^2 = 3483.9$, df = 6, $P < 0.01$; VA, $\chi^2 = 3483.9$, df = 6, $P < 0.01$; WV, $\chi^2 = 1460.5$, df = 6, $P < 0.01$; ER-1, $\chi^2 = 105.1$, df = 6, $P < 0.01$; ER-2, $\chi^2 = 90.5$, df = 6, $P < 0.01$; ER-3, $\chi^2 = 453.7$, df = 6, $P < 0.01$; FVG, $\chi^2 = 959.4$, df = 6, $P < 0.01$; PM-1, $\chi^2 = 384.7$, df = 6, $P < 0.01$; and PM-2, $\chi^2 = 599.8$, df = 6, $P < 0.01$). Increases in captures were observed in traps baited with lures with increasing amounts of PHER, with greatest captures typically at ratios of PHER at 12× and MDT at 24× loading. However, loading at 12× for both PHER and MDT, and 3× for PHER and 24× for MDT also yielded high captures in traps baited with these lures indicating that high PHER loadings were critical to achieving high captures, but reductions in PHER loading could be offset to some degree with higher MDT loadings. Nevertheless, reductions in PHER to 0.75× even with 24× loading of MDT in lures yielded significantly lower captures in traps (Table 5).

Significant differences were also detected in nymphal captures at nearly all locations (MD-1, $\chi^2 = 84.2$, df = 6, $P < 0.01$; MD-2,

Table 3. Mean number of *H. halys* adults and nymphs (±SE) captured in traps baited with lures with varying low loading rates of PHER:MDT relative to a standard loading in the United States in 2018. Means within a row followed by a different letter are significantly different at $P < 0.05$

Site	Life stage	Treatments (PHER:MDT)							
		0.10:1	0.25:1	0.50:1	0.10:2	0.25:2	0.50:2	Standard	Control
MD	Adults	2. 1 ± 0.5c	2. 5 ± 0.6bc	3. 5 ± 0.6abc	2. 7 ± 0.6abc	3. 2 ± 0.6abc	4. 3 ± 0.9ab	4. 4 ± 0.8a	0. 0 ± 0.0d
NJ-1	Adults	2. 7 ± 0.6a	3. 6 ± 0.9a	2. 9 ± 0.6a	4. 1 ± 1.1a	5. 9 ± 2.4a	4. 1 ± 1.1a	3. 3 ± 1.0a	0. 2 ± 0.1b
NJ-2	Adults	3. 9 ± 0.7c	6. 0 ± 1.1abc	5. 5 ± 0.9abc	5. 7 ± 1.2abc	6. 5 ± 1.2ab	6. 3 ± 1.1abc	8. 0 ± 1.7a	0. 0 ± 0.0d
UT	Adults	0. 0 ± 0.0	0. 0 ± 0.0[a]	0. 1 ± 0.0	0. 1 ± 0.0	0. 0 ± 0.0[a]	0. 1 ± 0.0	0. 1 ± 0.0	0. 0 ± 0.0
WV-1	Adults	3. 2 ± 0.7c	4. 2 ± 0.8bc	4. 4 ± 0.7bc	3. 7 ± 0.7bc	4. 8 ± 0.9ab	5. 8 ± 0.9a	5. 5 ± 0.9ab	0. 0 ± 0.0c
WV-2	Adults	5. 3 ± 0.8d	6. 9 ± 1.1bcd	7. 4 ± 0.9bc	6. 0 ± 1.2cd	7. 2 ± 1.1bdc	8. 8 ± 1.3ab	9. 6 ± 1.4a	0. 1 ± 0.1d
ER-1	Adults	10. 5 ± 2.7ab	9. 0 ± 1.9ab	10. 6 ± 1.4ab	12. 6 ± 2.4ab	13. 4 ± 1.8a	14. 8 ± 2.5a	11. 1 ± 1.4ab	1. 1 ± 0.5c
ER-2	Adults	15. 7 ± 3.1bc	15. 7 ± 2.9bc	11. 7 ± 2.4c	19. 0 ± 3.0ab	18. 2 ± 3.1ab	15. 8 ± 2.4bc	22. 3 ± 3.7a	0. 4 ± 0.2d
FVG	Adults	56. 2 ± 7.4e	63. 3 ± 7.d4	74. 3 ± 8.1bc	69. 7 ± 6.7cd	81. 5 ± 10.2b	91. 2 ± 12.9a	90. 5 ± 11.9a	2. 1 ± 0.5f
MD	Nymphs	0. 0 ± 0.0ab[a]	0. 0 ± 0.0ab[a]	0. 2 ± 0.1a	0. 1 ± 0.0ab	0. 2 ± 0.1a	0. 1 ± 0.0ab	0. 1 ± 0.1ab	0. 0 ± 0.0b
NJ-1	Nymphs	0. 5 ± 0.2a	0. 8 ± 0.4	1. 0 ± 0.4	1. 9 ± 0.8	0. 3 ± 0.2	0. 4 ± 0.1	0. 5 ± 0.2	0. 0 ± 0.0
NJ-2	Nymphs	1. 0 ± 0.5bc	1. 7 ± 0.5ab	3. 1 ± 0.9ab	1. 4 ± 0.5ab	3. 3 ± 1.0ab	3. 6 ± 1.a0	3. 9 ± 1.3a	0. 1 ± 0.0c
UT	Nymphs	0. 0 ± 0.0	0. 0 ± 0.0	0. 0 ± 0.0	0. 0 ± 0.0	0. 0 ± 0.0	0. 0 ± 0.0	0. 0 ± 0.0	0. 0 ± 0.0
WV-1	Nymphs	0. 1 ± 0.1c	0. 5 ± 0.2bc	0. 1 ± 0.1bc	0. 2 ± 0.1abc	0. 8 ± 0.4ab	1. 1 ± 0.4a	0. 8 ± 0.5ab	0. 0 ± 0.0d
WV-2	Nymphs	0. 3 ± 0.2c	0. 5 ± 0.2bc	1. 1 ± 0.4ab	0. 6 ± 0.2bc	0. 6 ± 0.3bc	1. 3 ± 0.4ab	1. 8 ± 0.7a	0. 0 ± 0.0d[a]
ER-1	Nymphs	0. 0 ± 0.0	0. 0 ± 0.0	0. 0 ± 0.0	0. 1 ± 0.1	0. 0 ± 0.0	0. 1 ± 0.1	0. 1 ± 0.1	0. 0 ± 0.0
ER-2	Nymphs	0. 0 ± 0.0	0. 3 ± 0.0	0. 0 ± 0.0	0. 2 ± 0.1	0. 6 ± 0.4	0. 2 ± 0.1	0. 3 ± 0.2	0. 0 ± 0.0
FVG	Nymphs	11. 6 ± 4.1a	6. 1 ± 2.0ab	11. 8 ± 3.3a	19. 0 ± 8.a1	10. 1 ± 2.9a	24. 3 ± 9.6a	40. 3 ± 27.1a	0. 0 ± 0.0b

[a]Captures ranged between 0.01 and 0.04 individuals per trap.

Table 4. Mean number of *H. halys* adults and nymphs (±SE) captured in traps baited with lures with varying high loading rates of PHER:MDT relative to standard pheromone lures in the United States in 2019. Means within a row followed by a different letter are significantly different at $P < 0.05$.

		Treatments (PHER:MDT)					
Site	Lifestage	2:4	2:6	3:4	3:6	3:12	Control
MD-1	Adults	20.9 ± 6.4b	10.7 ± 2.8c	35.2 ± 9.6a	24.5 ± 7.0ab	29.4 ± 8.8ab	0.0 ± 0.0d
MD-2	Adults	55.4 ± 13.6a	36.8 ± 11.3b	23.8 ± 6.0c	38.0 ± 8.0b	58.1 ± 12.9a	0.2 ± 0.1d
WV	Adults	96.2 ± 20.6a	111.4 ± 23.7a	108.7 ± 21.8a	105.2 ± 21.3a	120.0 ± 24.3a	2.0 ± 0.1b
MD-1	Nymphs	0.9 ± 0.3	1.6 ± 0.4	1.7 ± 0.5	1.6 ± 0.5	1.8 ± 0.6	0.0 ± 0.0
MD-2	Nymphs	7.6 ± 1.6a	5.9 ± 1.3a	5.0 ± 1.4a	7.1 ± 2.1a	7.6 ± 1.9a	0.2 ± 0.1b
WV	Nymphs	12.3 ± 2.7b	12.2 ± 3.1b	19.9 ± 5.6a	19.7 ± 4.8a	19.5 ± 5.3a	0.1 ± 0.1c

Table 5. Mean number of *H. halys* adults and nymphs (±SE) captured in traps baited with lures with varying ratios of PHER:MDT relative to standard pheromone lures in the United States and Italy. Means within a row followed by a different letter are significantly different at $P < 0.05$.

		Treatment (PHER:MDT)						
Site	Lifestage	0.75:12	3:12	12:12	0.75:24	3:24	12:24	Control
MD-1	Adults	14.01 ± 4.1b	17.6 ± 5.5b	23.23 ± 6.5b	15.3 ± 4.1b	18.6 ± 6.1b	46.7 ± 11.5a	0.3 ± 0.2c
MD-2	Adults	37.5 ± 10.1c	46.2 ± 8.5cc	76.5 ± 15.5a	44.8 ± 12.2bc	58.3 ± 11.4b	82.4 ± 16.4a	0.2 ± 0.1d
OR	Adults	15.0 ± 3.6d	16.2 ± 4.2cd	19.9 ± 4.1b	23.1 ± 5.1ab	19.5 ± 5.0bc	24.4 ± 4.9a	1.1 ± 0.3e
VA	Adults	41.2 ± 8.0e	91.5 ± 15.5c	122.7 ± 22.2b	62.8 ± 12.4d	89.6 ± 15.3c	173.7 ± 22.6a	0.9 ± 0.3f
WV	Adults	41.2 ± 10.3c	40.1 ± 9.0c	72.9 ± 14.5b	40.6 ± 9.2c	60.5 ± 14.3b	88.9 ± 16.0a	0.5 ± 0.2d
ER-1	Adults	18.8 ± 4.4bc	19.0 ± 3.6bc	21.0 ± 3.9ab	17.5 ± 4.0bc	15.5 ± 2.9c	26.0 ± 4.6a	1.8 ± 0.6d
ER-2	Adults	20.5 ± 3.9ab	15.1 ± 2.5b	21.0 ± 4.4a	20.9 ± 2.8a	17.6 ± 2.8a	17.0 ± 2.4ab	1.3 ± 0.5c
ER-3	Adults	22.0 ± 3.3e	31.5 ± 3.9c	35.1 ± 3.9b	26.1 ± 5.0d	31.8 ± 4.4bc	41.8 ± 5.7a	0.3 ± 0.1f
FVG	Adults	138.7 ± 9.6c	155.1 ± 1.3b	146.1 ± 10.5bc	141.0 ± 11.4c	177.6 ± 15.4a	177.2 ± 12.6a	3.7 ± 0.9d
PM-1	Adults	28.0 ± 6.6d	43.8 ± 6.8c	60.5 ± 8.1a	39.7 ± 7.9c	46.1 ± 6.0c	51.4 ± 6.3b	0.3 ± 0.1e
PM-2	Adults	51.9 ± 8.8d	70.4 ± 11.2c	82.9 ± 13.3ab	48.8 ± 8.6d	73.6 ± 13.0bc	90.0 ± 14.8a	0.56 ± 0.2e
MD-1	Nymphs	0.8 ± 0.3b	1.2 ± 0.4ab	2.2 ± 1.1ab	2.3 ± 0.9a	1.4 ± 0.7ab	2.2 ± 0.6a	0.1 ± 0.0c
MD-2	Nymphs	4.0 ± 1.3c	7.3 ± 2.0c	8.8 ± 2.2a	4.2 ± 2.0bc	9.3 ± 3.7b	8.6 ± 2.1a	0.1 ± 0.1d
OR	Nymphs	13.5 ± 3.0b	17.2 ± 3.0ab	15.2 ± 2.9b	21.8 ± 4.2a	17.0 ± 3.5ab	16.2 ± 3.5b	2.3 ± 0.8c
VA	Nymphs	3.7 ± 0.9c	8.6 ± 2.9b	10.6 ± 2.2ab	7.8 ± 2.0b	7.3 ± 1.7b	14.4 ± 3.0a	0.1 ± 0.1d
WV	Nymphs	2.9 ± 0.7d	3.6 ± 0.7cd	7.7 ± 2.2ab	4.9 ± 0.8bcd	5.6 ± 1.7bc	9.8 ± 2.8a	0.1 ± 0.0e
ER-1	Nymphs	0.3 ± 0.1	0.3 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.5 ± 0.4	0.1 ± 0.1
ER-2	Nymphs	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ER-3	Nymphs	7.6 ± 1.5c	15.6 ± 4.3b	15.4 ± 3.7b	14.3 ± 3.0b	14.6 ± 2.4b	24.7 ± 7.7a	0.4 ± 0.2d
FVG	Nymphs	14.6 ± 3.9cd	12.0 ± 2.7d	21.8 ± 7.3bc	21.6 ± 3.4b	32.7 ± 8.4a	37.1 ± 9.5a	0.6 ± 0.3e
PM-1	Nymphs	19.1 ± 6.9bc	15.6 ± 3.6c	44.3 ± 14.4a	29.9 ± 11.0ab	25.0 ± 6.0b	24.6 ± 8.0a	0.3 ± 0.2d
PM-2	Nymphs	8.4 ± 2.6b	12.0 ± 3.3bc	18.4 ± 4.4a	17.4 ± 5.8a	15.1 ± 5.8a	12.7 ± 7.4bc	0.0 ± 0.0c

$\chi^2 = 70.5$, df = 6, $P < 0.01$; OR, $\chi^2 = 310.7$, df = 6, $P < 0.01$; VA, $\chi^2 = 136.2$, df = 6, $P < 0.01$; WV, $\chi^2 = 333.1$, df = 6, $P < 0.01$; FVG, $\chi^2 = 374.3$, df = 6, $P < 0.01$; ER-3, $\chi^2 = 475.2$, df = 6, $P < 0.01$; PM-1, $\chi^2 = 207.4$, df = 6, $P < 0.01$; and PM-2, $\chi^2 = 575.7$, df = 6, $P < 0.01$). Similar patterns of capture were also observed with greatest captures typically in traps baited with lures formulated with the greatest amounts of PHER, at PHER:MDT ratios of 12:12 and 12:24 (Table 5).

Lure Substrate Dispensers

Among traps baited with lures using different polyethylene substrates and PHER:MDT ratios relative to standard lure dispensers and unbaited traps, significant differences in adult captures were detected at all locations (GA, $\chi^2 = 86.34$, df = 4, $P < 0.01$; MD-1, $\chi^2 = 36.1$, df = 4, $P < 0.01$; MD-2, $\chi^2 = 279.3$, df = 4, $P < 0.01$; MD-3, $\chi^2 = 58.1$, df = 4, $P < 0.01$; VA, $\chi^2 = 121.0$, df = 4, $P < 0.01$; ER-1, $\chi^2 = 277.6$, df = 4, $P < 0.01$; and ER-2, $\chi^2 = 377.8$, df = 4, $P < 0.01$). For nymphs, significant differences in trap captures were detected in only a few locations (MD-2, $\chi^2 = 75.1$, df = 4, $P < 0.01$; MD-3, $\chi^2 = 79.8$, df = 4, $P < 0.01$; and VA, $\chi^2 = 76.6$, df = 4, $P < 0.01$). In general, none of the traps baited with experimental polyethylene dispensers performed as well as those baited with standard lure dispensers (Table 6).

Inclusion of EDT

Captures in baited traps were significantly different for adults (WV-1, $\chi^2 = 298.9$, df = 5, $P < 0.01$; WV-2, $\chi^2 = 326.6$, df = 5, $P < 0.01$; and WV-3, $\chi^2 = 341.6$, df = 5, $P < 0.01$) and nymphs (WV-1, $\chi^2 = 19.3$, df = 5, $P = 0.02$; WV-2, $\chi^2 = 65.614$, df = 5, $P < 0.01$; and WV-3, $\chi^2 = 14.993$, df = 5, $P = 0.01$) at all sites. The inclusion of EDT (even up to a 9× loading) in lures did not improve trap captures relative to the standard PHER:MDT (1:1) lure. However, when MDT was not present, but EDT was included in increasing amounts (1×, 3× and 9×), captures generally increased significantly for adults, indicating that increasing amounts of EDT can enhance *H. halys* captures when combined with PHER (Table 7).

Table 6. Mean number of *H. halys* adults and nymphs (±SE) captured in traps baited with lures using different polyethylene substrates in 2018. Loading for each treatment of PHER:MDT relative to standard lures. Means within a row followed by a different letter are significantly different at $P < 0.05$

		Treatment (PHER:MDT)				
Site	Lifestage	1:1	1:1	0.5:1	Standard	Control
GA	Adults	4. 0 ± 0.9b	3. 7 ± 0.8b	6. 3 ± 1.1a	7. 2 ± 1.4a	0. 1 ± 0.0c
MD-1	Adults	2. 4 ± 0.2b	1. 8 ± 0.2b	1. 9 ± 0.2b	5. 6 ± 0.2a	0. 0 ± 0.0c
MD-2	Adults	3. 4 ± 0.9b	3. 6 ± 0.8b	2. 4 ± 0.6b	13. 5 ± 2.0a	0. 1 ± 0.0c
MD-3	Adults	2. 7 ± 0.6b	2. 9 ± 0.6b	2. 8 ± 0.6b	6. 7 ± 0.9a	0. 0 ± 0.0c
VA	Adults	3. 7 ± 0.6b	3. 2 ± 0.5b	2. 9 ± 0.5b	8. 7 ± 1.0a	0. 1 ± 0.0c
ER-1	Adults	12. 3 ± 1.6ab	17. 3 ± 2.7a	12. 6 ± 1.8ab	19. 8 ± 2.6a	1. 0 ± 0.3b
ER-2	Adults	13. 8 ± 2.7b	14. 0 ± 2.5b	13. 5 ± 2.2b	24. 8 ± 4.1a	0. 3 ± 0.1c
GA	Nymphs	1. 4 ± 0.4	1. 6 ± 0.5	2. 0 ± 0.5	3. 4 ± 0.6	0. 0 ± 0.0
MD-1	Nymphs	0. 2 ± 0.1	0. 2 ± 0.1	0. 2 ± 0.1	0. 6 ± 0.2	0. 0 ± 0.0
MD-2	Nymphs	0. 3 ± 0.2b	0. 2 ± 0.1b	0. 2 ± 0.1b	3. 6 ± 1.2a	0. 0 ± 0.0b
MD-3	Nymphs	0. 3 ± 0.1bc	0. 8 ± 0.4abc	0. 5 ± 0.2b	1. 9 ± 0.6a	0. 0 ± 0.0c
VA	Nymphs	0. 7 ± 0.2b	3. 2 ± 0.7a	0. 6 ± 0.2b	5. 1 ± 1.4a	0. 0 ± 0.0c
ER-1	Nymphs	0. 0 ± 0.0	0. 1 ± 0.1	0. 0 ± 0.0	1. 0 ± 0.8	0. 0 ± 0.0
ER-2	Nymphs	0. 0 ± 0.0	0. 3 ± 0.2	0. 0 ± 0.0	1. 1 ± 0.6	0. 0 ± 0.0

Table 7. Mean number of *H. halys* adults and nymphs (± SE) captured in traps baited with lures loading with the *H. halys* pheromone and varying ratios of MDT and/or EDT relative to standard lures in 2019. Means within a row followed by a different letter are significantly different at $P < 0.05$

		Treatment (PHER:MDT:EDT)					
Site	Life stage	1:0:1	1:1:3	1:0:3	1:0:9	Standard	Control
WV-1	Adults	2. 2 ± 0.6c	10. 8 ± 2.4a	3. 3 ± 0.9bc	5.3±.1.3b	11. 0 ± 2.7a	0. 0 ± 0.0d
WV-2	Adults	3. 6 ± 0.7d	13. 7 ± 3.1a	6. 0 ± 1.1c	9.4±.1.9b	11. 6 ± 2.5ab	0. 0 ± 0.0e
WV-3	Adults	9. 6 ± 2.6c	25. 2 ± 5.0a	11. 1 ± 2.6bc	15.8±.3.4b	24. 7 ± 5.2a	0. 1 ± 0.0d
WV-1	Nymphs	0. 2 ± 0.1bc	0. 9 ± 0.3a	0. 1 ± 0.1c	0. 4 ± 0.2abc	0. 7 ± 0.2ab	0. 0 ± 0.0c
WV-2	Nymphs	0. 9 ± 0.3b	2. 4 ± 0.6a	1. 2 ± 0.3b	1. 5 ± 0.4ab	1. 6 ± 0.4ab	0. 0 ± 0.0c
WV-3	Nymphs	5. 7 ± 1.9a	10. 4 ± 2.7a	5. 8 ± 1.8a	8. 2 ± 2.6a	16. 8 ± 6.8a	0. 0 ± 0.0b

Discussion

In some insects, specific ratios of pheromonal stimuli are necessary to achieve optimal sensitivity and attractiveness, while the presence of known antagonists can reduce captures (Zhang et al. 2005, Leskey et al. 2006). In the case of *H. halys,* traps baited with lures comprising natural and synthetic ratios of the two aggregation pheromone components, (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, generally did not yield significantly different captures (Weber et al. 2020). Moreover, the presence of nonpheromonal 10,11-epoxy-1-bisabolen-3-ol stereoisomers in lures did not reduce trap captures, indicating they were not antagonistic (Leskey et al. 2015b). These results enable further flexibility in formulating *H. halys* lures, though the presence of the pheromonal synergist, MDT, must be considered as this compound increases trap captures significantly compared with traps baited with PHER alone (Weber et al. 2014, Leskey et al. 2015a). In previous trials, experimental lures were coupled with commercially available MDT lures; increasing PHER and/or MDT loading in lures generally increased trap captures (Weber et al. 2020). Here, we evaluated the effect of reducing PHER by 50–90%, while either providing standard or doubled MDT loading. Reductions in PHER loading had a significant impact on trap captures when coupled with lures with standard MDT loading; when MDT loading was doubled, captures improved. Thus, although PHER loading can be reduced in commercial lures, it must be offset by increases in MDT to maintain sensitivity for monitoring programs.

In some cases, higher loading rates are desirable to increase sensitivity of trap captures. For example, traps baited with lures containing standard monitoring loading of PHER and MDT, respectively, resulted in significantly lower captures than those baited with lures with a 4× greater so-called biosurveillance loading in most cases. However, captures in traps baited with the so-called standard and biosurveillance loadings were significantly correlated indicating they were reflecting the same relative densities of insects throughout the season (Acebes-Doria et al. 2018). Here, we were interested in determining if a ratio of PHER that was two- or three-fold higher (than standard lures) in combination with increased loading of MDT resulted in increasing captures. Similar to Weber et al. (2020), we observed increased captures in traps baited with lures containing higher loadings of PHER and MDT. With standard PHER:MDT lures, the area over which a single baited trap reliably captures *H. halys* has been estimated to be ~1.67 ha and ~5.0 ha in areas with and without host plants present, respectively (Kirkpatrick et al. 2019). A logical next step would be to repeat this work with lures at higher loading rates to determine whether trapping area increases, thereby explaining increased captures with lures containing higher loading rates.

High loading rates also have been used for lures deployed in attract and kill studies for *H. halys* management in apple orchards (Morrison et al. 2019, Leskey et al. 2020). These tactics work by combining PHER+MDT lures in association with a host plant (apple tree) to increase attraction and retention of *H. halys* and a killing agent to remove them from the population (Morrison et al. 2016). In previous studies, lures either had 10-fold greater amounts of MDT (Leskey et al. 2020) or 6-fold greater amounts of PHER (Morrison et al. 2019). Both approaches proved successful, but components were not optimized relative to attractiveness versus cost. Here, we found that increasing the loading of PHER and MDT increased overall captures as has been observed in previous studies. However, increasing the loading rate of PHER 0.75× to 12× had a greater impact on captures than did increasing the amount of pheromone synergist MDT. As pointed out by Weber et al. (2017), there never has been an upper limit found in terms of loading rate and overall captures, but there may be a point where there could be diminishing returns for monitoring programs, as traps can become saturated with captured individuals in high density locations. On the other hand, attract and kill programs can potentially benefit from increased numbers of individuals being attracted and removed from the population. Grower interest in adoption of pheromone-based management tools such as attract and kill and pheromone-based monitoring tools remains high (Ludwick et al. 2020), especially if labor for attract and kill can be reduced through killing agents such as insecticide-treated nets (Kuhar et al. 2017, Ibrahim et al. 2020).

Other factors such as lure dispenser can affect overall lure efficacy. For example, traps baited with polyethylene and rubber septum dispensers formulated with the sex pheromone of *Synanthedon scitula* (Harris) performed differently under field conditions. Captures in traps baited with the polyethylene dispenser captured significantly more *S. scitula* initially, but then captures dropped off due to antagonistic breakdown contaminant whereas those baited with rubber septa performed consistently over a six month period (Zhang et al. 2013). Here, we evaluated three types of polyethylene dispensers and found that none performed as well as current standard dispensers. We did not analyze the polyethylene dispensers for contaminants, but in other studies, temperature also had an impact on their efficacy, as polyethylene dispensers used as lure dispensers for *Conotrachelus nenuphar* (Herbst) attractants were ineffective as lures in traps at temperatures below ~15°C (Leskey and Zhang 2007). Perhaps, a similar problem occurred here. We also evaluated the effect of including EDT as an additional lure component for *H. halys*. Similar to Rice et al. (2018a), we observed an enhancement of trap captures when EDT was included in lures containing PHER only. However, this enhancement was not as great when compared with adding MDT alone or in combination with EDT to lures.

Ultimately, lure efficacy for a particular pest species can be established quickly through large-scale collaborative trials (Ludwick et al. 2020). Indeed, for *H. halys*, initial trials evaluating traps baited with experimental PHER and MDT lures were evaluated in the invaded range across the United States (Leskey et al. 2015a) and the native range in South Korea (Morrison et al. 2017b). Commercial lures also were evaluated extensively across the United States (Acebes-Doria et al. 2020) and Europe (Morrison et al 2017a). Here, we use the same model to enable refinement of lures for both monitoring and biosurveillance tools as well as pheromone-based management tactics such as attract and kill. Our results indicate that flexibility exists in ratios of PHER and MDT used to formulate lures for either purpose in terms of attractiveness, sensitivity and cost and that current dispensers appear to be far superior to others tested to date.

Acknowledgements

We thank Chris Hott, Lee Carper, Anthony Rugh, Nate Brandt, Carson Wise John Cullum, and Erica Rudolph for excellent technical assistance. This research was supported by USDA-NIFA-SCRI 2016-51181-25409. This research was also supported by the Hungarian Ministry for Innovation and Technology within the framework of the Higher Education Institutional Excellence Program (NKFIH-1159-6/2019) in the scope of plant breeding and plant protection research of Szent István University. Mention of commercial trade names is for the purposes of providing scientific information only and does not imply endorsement by the U.S. Department of Agriculture. The U.S. Department of Agriculture is an equal opportunity employer.

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