Synergy of Aggregation Pheromone With Methyl (*E*,*E*,*Z*)-2,4,6-Decatrienoate in Attraction of *Halyomorpha halys* (Hemiptera: Pentatomidae)

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ABSTRACT The reported male-produced aggregation pheromone of the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), identified as a mixture of (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, offers new opportunities for its management. We found that black pyramid traps deployed along crop borders in Maryland and West Virginia, containing lures with both stereoisomers of this reported aggregation pheromone combined with methyl (*E*,*E*,*Z*)-2,4,6-decatrienoate (MDT) lures, attracted more adult and nymphal *H. halys* than either the aggregation pheromone or MDT alone. In season-long totals, combined lures acted synergistically by catching 1.9–3.2 times more number of adults, and 1.4–2.5 times more number of nymphs, than expected from an additive effect of the lures deployed individually. There were no significant differences in patterns of male and female captures. MDT alone was not significantly attractive to adults during most of the growing season, but became increasingly attractive to adults and especially nymphs in autumn. Mixed-isomer lures containing eight stereoisomers of 10,11-epoxy-1-bisabolen-3-ol, including the two active stereoisomers, were as effective at catching adults and nymphs with or without MDT as were lures loaded only with the two active stereoisomers in the natural ratio ((3*S*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol: (3*R*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol) of 3.5:1. These results identify a combination of semiochemicals that is attractive season-long for detection, monitoring, and potential control of this polyphagous invasive pest of North America and Europe.

KEY WORDS semiochemical, Pentatomidae, pest monitoring, trap capture

Brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is a polyphagous invasive pest in North America and Europe, now detected in >40 states of the United States, and also in Canada, Switzerland, Germany, France, and Liechtenstein (Lee et al. 2013). Lack of tools for detection, monitoring, and capture has hampered development of management tactics and action thresholds, resulting in damage to crops, increased pesticide applications (Leskey et al. 2012a), and nuisance in structures (Inkley 2012, Leskey et al. 2012b).

Recently, Khrimian et al. (2013) reported that the two main components of the aggregation pheromone (sensu Millar 2005) for this species are (1*S*,4*S*)-4-((*R*)-4-((*S*)-3,3-dimethyloxiran-2-yl)butan-2-yl)-1-methylcyclohex-2-enol and (1*R*,4*S*)-4-((*R*)-4-((*S*)-3,3-dimethyloxiran-2-yl)butan-2-yl)-1-methylcyclohex-2-enol, or, using terpene nomenclature (Connoly and Hill 1991), (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, respectively. Each compound (hereinafter SSRS and RSRS) attracted female, male, and nymphal *H. halys* in field trials, with SSRS being more active than RSRS and the mixture at the naturally occurring 3.5:1 ratio more attractive than either compound alone. Interestingly, the presence of other stereoisomers of 10,11-epoxy-1-bisabolen-3-ol apparently did not inhibit the attraction (Khrimian et al. 2013; A. K. et al, unpublished data).

Methyl (*E*,*E*,*Z*)-2,4,6-decatrienoate (hereinafter MDT), the aggregation pheromone of another Asian stink bug, *Plautia stali* Scott, was previously demonstrated to be attractive to *H. halys,* and useful in monitoring field populations in Japan and the United States (Sugie et al. 1996; Tada et al. 2001; Khrimian 2005; Aldrich et al. 2007, 2009; Khrimian et al. 2008). However, MDT was not attractive to overwintered *H. halys* adults (Leskey et al. 2012a), a critical stage in determining population levels potentially threatening to tree fruit and other crops.

Mention of commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture.

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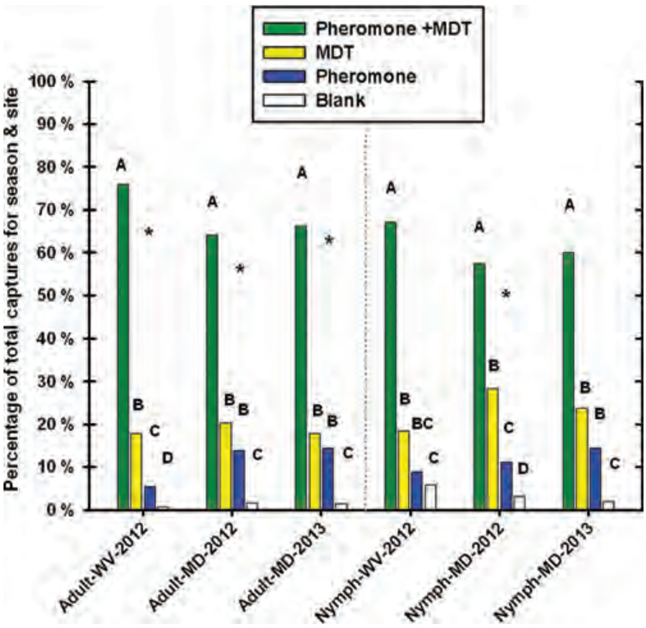

Fig. 1. Season-long total captures of *H. halys* in pyramid traps with pheromone and/or MDT lures, and blank, 20 May through 23 October 2012, Arden, WV, and 6 April through 23 October 2012 and 12 April through 24 October 2013, Beltsville, MD. For each site and life stage, bars with different letter differ by Tukey–Kramer test on arcsin-transformed proportion of block total. Asterisk indicates significant interaction between pheromone and MDT lure type (in each case a positive synergism) for the season-long totals.

With the discovery of *H. halys* pheromone, we decided to test the attraction of both the pheromone and MDT, and their combination, to determine if the combination would prove superior to either single component throughout the season.

Materials and Methods

Attractants Used. We tested the following lures in our field trials:

BMSB1—A mixture of eight stereoisomers of 10,11-epoxy-1-bisabolen-3-ol with 7*R* configurations containing SSRS and RSRS and the ratio of *cis* and *trans* stereoisomers 3:1. This mixture was prepared from (7*R*)-1,10-bisaboladien-3-one (Hagiwara et al. 2002) following Zahn et al. (2008). The faster eluting fraction, containing about equal amounts of four *cis* stereoisomers, and the slower eluting fraction, containing equal amounts of four *trans* stereoisomers, were isolated by flash chromatography and mixed at a 3:1 ratio before loading in gray rubber septa (1-F SS 1888 GRY, West Pharmaceutical Services, Lititz, PA) at a 10.66 mg per septum dose that contained 2 mg of SSRS and 0.67 mg of RSRS.

BMSB2—A crude mixture of eight stereoisomers of 10,11-epoxy-1-bisabolen-3-ol with 7*R* configurations prepared analogously to BMSB1 without further purification. The ratio of *cis* and *trans* stereoisomers from the reaction was 1:2. BMSB2 was loaded (AgBio, Westminster, CO) in a proprietary sealed polyethylene sachet at 31 mg of dose to match the 2 mg of SSRS content in BMSB1. Sachets were ≈3 by 3 cm with characteristics adjusted to obtain an overall release of all isomers of ≈0.24 mg/d at 20°C and were rated for >30 d in the field.

SSRS + RSRS—The mixture was prepared as described in Khrimian et al. (2013). The chemical purities of both compounds, assessed by gas chromatography analyses on a conventional (HP-5MS) column, were >95%, and diastereomeric purities, assessed on chiral columns (Chiraldex G-TA# and Hydrodex-β-6TBDM) were >90% *de* (=diastereomeric excess). SSRS and RSRS isomers, in a 3.5:1 ratio, were loaded on the same rubber septa as lure BMSB1 at 4.0 mg (3.11 mg of SSRS and 0.89 mg of RSRS) total dose.

MDT1—MDT contained within proprietary polyethylene sachets, ≈2 by 5 cm, provided by Sterling International, Spokane, WA, at a reported 60 mg loading and rated for >30 d in the field.

MDT2—MDT contained within proprietary sealed polyethylene sachets provided by AgBio, Westminster, CO, at a reported 66 mg loading; sachets were ≈4 by 4 cm with characteristics adjusted to obtain a release rate of ≈0.6 mg/d at 20°C and were rated for >30 d in the field.

Trials Performed. Three season-long trials were performed:

Maryland 2012: from 6 April to 23 October 2012 at Beltsville Agricultural Research Center North Farm (39° 02′ N, 76° 56′ W), MD, we deployed four randomized complete blocks of black pyramid traps described previously (Leskey et al. 2012c) in the border area between woody vegetation and field or vegetable crops, spaced ≈50 m apart, with treatments of BMSB1,

Table 1. Magnitude of effect (ratio of season-long bug captures and 95% binomial CI) from combination of *H. halys* pheromone (BMSB1 or BMSB2) plus MDT (lure MDT1 or MDT2), compared with pheromone and MDT lures deployed alone, and sum of numbers for both lures deployed alone, in Arden, WV, and Beltsville, MD

Lure	Arden, WV, 2012		Beltsville, MD, 2012		Beltsville, MD, 2013	
	Adult season total	Ratio (95% CI)	Adult season total	Ratio (95% CI)	Adult season total	Ratio (95% CI)
Combined	4,231		3783		1821	
MDT	999	4.24 (3.95, 4.54)	1203	3.14 (2.95, 3.36)	510	3.57 (3.24, 3.95)
Pheromone	304	13.93 (12.39, 15.69)	822	4.60 (4.27, 4.97)	383	4.75 (4.26, 5.32)
Synergism*		3.25 (3.05, 3.46)		1.87 (1.77, 1.97)		2.04 (1.88, 2.21)
	Nymph season total	Ratio (95% CI)	Nymph season total	Ratio (95% CI)	Nymph season total	Ratio (95% CI)
Combined	1404		3688		3063	
MDT	386	3.64 (3.25, 4.08)	1823	2.02 (1.91, 2.14)	1212	2.53 (2.36, 2.70)
Pheromone	185	7.59 (6.51, 8.89)	718	5.13 (4.74, 5.57)	743	4.12 (3.80, 4.47)
Synergism*		2.46 (2.23, 2.71)		1.45 (1.38, 1.53)		1.57 (1.48, 1.66)

* Synergism refers to ratio of captures for combined lures, to the sum of captures from single lures, i.e. the more-than-additive effect of the combination of lures.

MDT1, and combined lures (BMSB1 + MDT1) hung together in the trap, and unbaited (control) traps.

West Virginia 2012: from 16 May to 23 October 2012 in Arden, WV (39° 27′ N, 78° 02′ W), we deployed three randomized complete blocks of traps in the border area between woody vegetation and commercial tree fruit spaced ≈50 m apart and with traps and the four treatments identical to the Maryland 2012 trial.

Maryland 2013: from 12 April to 24 October 2013 at the same site in Beltsville, we deployed three randomized complete blocks until 10 May, five blocks from 10 May until 30 August, and four blocks thereafter, using treatments of BMSB2, MDT2, combined

Table 2. Summary of captures by approximate fortnightly collection periods, 16 May through 23 October 2012, Arden, WV

Date	Capture (4 traps/treatment)	Lure treatment: Unbaited control	Pheromone (BMSB1)	MDT (MDT1)	Combined MDT1 + BMSB1
		Adults captured (% of total)	Adults captured (% of total)	Adults captured (% of total)	Adults captured (% of total)
Adult *H. halys*					
16–23 May	7-d total	0 (0)	0 (0)	0 (0)	8 (100)
23 May–4 June	14-d total	0 (0)b	3 (25)b	0 (0)b	9 (75)a*
4–19 June	15-d total	0 (0)	1 (50)	0 (0)	1 (50)
19 June–2 July	13-d total	0 (0)	0 (0)	0 (0)	1 (100)
2–17 July	15-d total	0 (0)	1 (20)	0 (0)	4 (80)
17 July–2 Aug.	16-d total	0 (0)b	2 (11.8)b	0 (0)b	15 (88.2)a*
2–13 Aug.	11-d total	7 (10.4)b	5 (7.5)b	7 (10.4)b	48 (71.6)a*
13–27 Aug.	14-d total	1 (0.8)c	30 (23.3)b	4 (3.1)c	94 (72.9)a*
27 Aug.–10 Sep.	14-d total	4 (2.1)b	63 (33.2)ab	3 (1.6)b	120 (63.2)a
10–24 Sep.	14-d total	1 (0.0)c	68 (2.6)c	333 (12.7)b	2,210 (84.6)a*
24 Sep.–9 Oct.	17-d total	8 (0.4)d	101 (4.8)c	532 (25.2)b	1,473 (69.7)a*
9–23 Oct.	14-d total	17 (4.1)	30 (7.2)	120 (28.9)	248 (59.8)
16 May–23 Oct	Total	38 (0.7)d	304 (5.5)c	999 (17.9)b	4,231 (75.9)a*
		Nymphs captured (% of total)	Nymphs captured (% of total)	Nymphs captured (% of total)	Nymphs captured (% of total)
Nymphal *H. halys*					
16–23 May	7-d total	0	0	0	0
23 May–4 June	14-d total	0	0	0	0
4–19 June	15-d total	0	0	0	0
19 June–2 July	13-d total	1 (14.3)	0 (0)	0 (0)	6 (85.7)
2–17 July	15-d total	2 (1.3)b	11 (6.9)b	8 (5.0)b	139 (86.9)a*
17 July–2 Aug.	16-d total	10 (27.8)	13 (36.1)	8 (22.2)	5 (13.9)
2–13 Aug.	11-d total	15 (3.8)b	15 (3.8)b	38 (9.5)b	331 (83.0)a*
13–27 Aug.	14-d total	53 (10.4)b	73 (14.3)ab	51 (10.0)b	334 (65.4)a
27 Aug.–10 Sep.	14-d total	36 (22.6)	31 (19.5)	68 (42.8)	24 (15.1)
10–24 Sep.	14-d total	2 (0.4)c	12 (2.1)c	135 (23.9)b	416 (73.6)a*
24 Sep.–9 Oct.	17-d total	1 (0.4)c	27 (11.9)bc	58 (25.7)ab	140 (61.9)a
9–23 Oct.	14-d total	1 (3.0)b	3 (9.1)b	20 (60.6)a	9 (27.3)ab
16 May–23 Oct.	Total	121 (5.8)c	185 (8.8)bc	386 (18.4)b	1,404 (67.0)a

Within each row, totals followed by a common letter do not differ by Tukey's HSD test, $P < 0.05$, for that sample period.

Asterisk at end of row indicates significant interaction (positive synergism) between lure types for that sample period, reported as a significant $F_{(1,8)}$ test for interaction term, using arcsin-transformed block proportions (three blocks for all periods).

Table 3. Summary of captures by approximate fortnightly collection periods, 6 April through 23 October 2012, Beltsville, MD

Date	Capture (4 traps/treatment)	Lure treatment			
		Unbaited control	Pheromone (BMSB1)	MDT (MDT1)	Combined MDT1 + BMSB1
		Adults captured (% of total)	Adults captured (% of total)	Adults captured (% of total)	Adults captured (% of total)
Adult *H. halys*					
6–20 April	14-d total	2 (3.4)	9 (15.3)	6 (10.2)	42 (71.2)
20 April–4 May	14-d total	2 (6.3)b	3 (9.4)b	7 (21.9)b	20 (62.5)a*
4–22 May	18-d total	1 (1.0)c	16 (16.5)b	2 (2.1)c	78 (80.4)a*
22 May–5 June	14-d total	0 (0.0)c	27 (26.5)b	3 (2.9)c	72 (70.6)a*
5–19 June	14-d total	1 (0.7)c	18 (12.5)b	6 (4.2)c	119 (82.6)a*
19–29 June	10-d total	0 (0.0)b	2 (9.1)b	0 (0.0)b	20 (90.9)a*
29 June–13 July	14-d total	0 (0.0)c	13 (22.4)b	5 (8.6)bc	40 (69.0)a
13–27 July	14-d total	0 (0.0)b	24 (30.0)ab	0 (0.0)b	56 (70.0)a
27 July–10 Aug.	14-d total	7 (3.1)b	18 (7.9)b	14 (6.1)b	190 (83.0)a*
10–24 Aug.	14-d total	1 (0.7)c	38 (24.8)b	7 (4.6)c	107 (69.9)a
24 Aug.–7 Sept.	14-d total	18 (1.0)c	218 (12.3)b	393 (22.2)b	1,143 (64.5)a*
7–21 Sept.	14-d total	40 (2.6)c	291 (18.9)b	314 (20.3)b	898 (58.2)a
21 Sept.–5 Oct.	14-d total	21 (1.3)d	142 (9.0)c	440 (27.9)b	974 (61.8)a
5–23 Oct.	18-d total	1 (2.9)b	3 (8.8)b	6 (17.6)b	24 (70.6)a
6 April–23 Oct.	Total	94 (1.6)c	822 (13.9)b	1203 (20.4)b	3783 (64.1)a*
		Nymphs captured (% of total)	Nymphs captured (% of total)	Nymphs captured (% of total)	Nymphs captured (% of total)
Nymphal *H. halys*					
6–20 April	14-d total	0	0	0	0
20 April–4 May	14-d total	0	0	0	0
4–22 May	18-d total	0	0	0	0
22 May–5 June	14-d total	0 (0)	4 (22.2)	7 (38.9)	7 (38.9)
5–19 June	14-d total	5 (2.0)c	32 (12.7)bc	63 (25.1)ab	151 (60.2)a
19–29 June	10-d total	3 (0.6)b	38 (8.1)b	6 (1.3)b	425 (90.0)a*
29 June–13 July	14-d total	0 (0)c	27 (10.0)b	40 (14.9)b	202 (75.1)a*
13–27 July	14-d total	2 (3.1)	13 (20.3)	13 (20.3)	36 (56.3)
27 July–10 Aug	14-d total	41 (3.1)c	38 (2.9)c	406 (30.8)b	833 (63.2)a*
10–24 Aug.	14-d total	113 (7.9)c	240 (16.7)b	212 (14.8)b	870 (60.6)a
24 Aug.–7 Sept.	14-d total	36 (1.5)c	311 (12.8)b	1018 (41.8)ab	1,068 (43.9)a
7–21 Sept.	14-d total	5 (4.2)b	10 (8.3)b	36 (30.0)ab	69 (57.5)a
21 Sept.–5 Oct.	14-d total	2 (4.8)b	5 (11.9)ab	17 (40.5)a	18 (42.9)a
5–23 Oct.	18-d total	0 (0)b	0 (0)b	5 (35.7)ab	9 (64.3)a
6 April–23 Oct.	Total	207 (3.2)d	718 (11.2)c	1,823 (28.3)b	3,688 (57.3)a*

Within each row, totals followed by a common letter do not differ by Tukey's HSD test, $P < 0.05$, for that sample period.
Asterisk at end of row indicates significant interaction (positive synergism) between lure types for that sample period, reported as a significant $F_{(1,12)}$ test for interaction term, using arcsin-transformed block proportions (four blocks for all periods).

lure (BMSB2 + MDT2), and unbaited black pyramid traps identical to and spaced the same as 2012 trials.

Also, during 30 August–26 September 2013 at the Beltsville, MD site, we compared lures SSRS + RSRS and BMSB2, both with and without MDT2, for a total of four treatments. All of the traps were the same in form, deployment, and spacing as above, in four randomized blocks.

Hercon Vaportape II (DDVP; Hercon Environmental, Emigsville, PA) was added as a killing agent to prevent escape from traps, and replaced at 4-wk intervals. All septum lures (BMSB1 and SSRS + RSRS) were replaced every 2 wk, and sachets (BMSB2, MDT1, and MDT2) were replaced every 4 wk. Traps were emptied twice per week (Maryland trials) or weekly (West Virginia trials) and all adults and nymphs enumerated, with adults determined as to sex.

Statistical Analysis. Adult and nymphal numbers captured in each treatment within each block during each 2-wk period starting or ending with a lure change, were transformed as the arcsine square root of proportions within each block and subjected to analysis of variance (ANOVA) to test overall treatment effect as well as the effect of the two attractants and their interaction (SAS Institute 1998, Cary, NC). If the ANOVA F-test was significant at $\alpha = 0.05$, then a Tukey's HSD (honestly significant difference) means comparison was used to determine if the treatments differed. The same analysis was performed on the season-long totals for the four treatments for each season and life stage (adults and nymphs). For the pure versus mixed isomer trial, the same statistical approach was used. In addition, for season totals, we constructed binomial confidence intervals for comparison of the combined lure with separate MDT and pheromone lures, and to test the synergistic effect, by comparison of the combined lure treatment with the sum of the two individual treatments (Zar 2009, Pezzullo 2013).

To analyze differences in response over the season, we compared proportion of captures in the four treatments by month (April through October for adults, and June through October for nymphs) for the 2012 Maryland dataset. We used chi-square tests between all combinations of months, Bonferroni-corrected experiment-

Table 4. Summary of captures by approximate fortnightly collection periods, 12 April through 24 October 2013, Beltsville, MD

Date	Capture (4 traps/treatment)	Lure treatment: Unbaited control	Pheromone (BMSB2)	MDT (MDT2)	Combined MDT2 + BMSB2
		Adults captured (% of total)	Adults captured (% of total)	Adults captured (% of total)	Adults captured (% of total)
Adult *H. halys*					
12–25 April	13-d total	0 (0.0)	2 (14.3)	0 (0.0)	12 (85.7)
25 April–10 May	15-d total	0 (0.0)b	3 (33.3)	0 (0.0)b	6 (66.7)a*
10–23 May	13-d total	0 (0.0)c	12 (17.9)b	7 (10.4)c	48 (71.6)a
23 May–7 June	15-d total	0 (0.0)d	27 (21.4)b	9 (7.1)c	90 (71.4)a*
7–21 June	14-d total	0 (0.0)b	26 (45.6)a	3 (5.3)b	28 (49.1)a
21 June–5 July	14-d total	0 (0.0)c	18 (12.5)b	6 (4.2)bc	120 (83.3)a*
5–19 July	14-d total	0 (0.0)b	13 (40.6)b	0 (0.0)b	19 (59.4)a*
19 July–2 Aug.	14-d total	2 (0.9)c	36 (16.3)b	6 (2.7)c	177 (80.1)a*
2–16 Aug.	14-d total	2 (4.0)b	8 (16.0)b	1 (2.0)b	39 (78.0)a*
16–30 Aug.	14-d total	3 (0.9)b	42 (12.9)b	37 (11.4)b	243 (74.8)a*
30 Aug.–13 Sept.	14-d total	6 (0.9)c	90 (13.7)b	153 (23.3)b	408 (62.1)a
13–26 Sept.	13-d total	23 (0.0)c	73 (10.4)bc	206 (29.2)b	403 (57.2)a
26 Sept.–8 Oct.	12-d total	4 (1.2)c	28 (8.4)b	79 (23.6)b	224 (66.9)a*
8–24 Oct.	16-d total	1 (7.7)	5 (38.5)	3 (23.1)	4 (30.8)
12 April−24 Oct.	Total	41 (1.5)c	383 (13.9)b	510 (18.5)b	1821 (66.1)a*
		Nymphs captured (% of total)	Nymphs captured (% of total)	Nymphs captured (% of total)	Nymphs captured (% of total)
Nymphal *H. halys*					
12–25 April	13-d total	0	0	0	0
25 April–10 May	15-d total	0	0	0	0
10–23 May	13-d total	0	0	0	0
23 May–7 June	15-d total	0	0	0	0
7–21 June	14-d total	1 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)
21 June–5 July	14-d total	5 (0.3)c	97 (5.5)bc	531 (29.9)b	1,143 (64.4)a
5–19 July	14-d total	1 (0.4)c	113 (45.0)ab	28 (11.2)bc	109 (43.4)a
19 July–2 Aug.	14-d total	21 (3.0)b	177 (25.4)ab	92 (13.2)ab	408 (58.5)a
2–16 Aug.	14-d total	39 (8.6)b	114 (25.1)a	135 (29.7)ab	166 (36.6)a
16–30 Aug.	14-d total	28 (1.9)c	156 (10.3)bc	296 (19.6)b	1030 (68.2)a*
30 Aug.–13 Sept.	14-d total	4 (1.1)c	72 (19.7)b	111 (30.4)b	178 (48.8)a
13–26 Sept.	13-d total	0 (0.0)b	3 (14.4)ab	12 (28.6)ab	27 (64.3)a
26 Sept.–8 Oct.	12-d total	1 (5.3)	10 (52.6)	6 (31.6)	2 (10.5)
8–24 Oct.	16-d total	0	0	0	0
12 April−24 Oct.	Total	100 (2.0)c	743 (14.5)b	1,212 (23.7)b	3,063 (59.8)a

Within each row, totals followed by a common letter do not differ by Tukey's HSD test, $P < 0.05$, for that sample period.
Asterisk at end of row indicates significant interaction (positive synergism) between lure types for that sample period, reported as a significant $F_{(1,4(b\text{-}1))}$ test for interaction term (where b = block no.), using arcsin-transformed block proportions (three blocks until 10 May; five blocks from 10 May through August 30, and four blocks after 30 August).

wise $\alpha = 0.05$ (corresponding to comparison-wise $\alpha = 0.0024$ for adults [21 paired tests], and $\alpha = 0.005$ for nymphs [10 paired tests]) to determine the patterns of similarity in response by month over the season. Post hoc chi-square cell contributions were used to determine which treatments contributed most to the observed differences, if the overall chi-square test was significant, and the chi-square test was repeated with this treatment omitted to determine if the resulting proportions still differed by season. Male and female captures in the four treatments for all 2-wk periods and the season-long totals for Maryland in 2013 were tested as to gender differences using 2-way chi-square tests (treatments were excluded if the expected cell size was <5; Zar 2009).

Results and Discussion

The combined pheromone (BMSB1 or BMSB2) plus MDT lure proved significantly superior for both nymphal and adult captures than either the pheromone or the MDT lure alone, at both locations (Fig. 1). For all three season-long totals (MD and WV locations), there was a significant synergy (i.e., a greater-than-additive effect) for adults, and there was also a significant synergy for the 2012 nymphal captures in Maryland. Adult captures with the combined lure exceeded MDT captures by 3.1–4.2 times, and pheromone captures by 4.6–13.9-fold. Similarly, combined lures caught 2.0–3.6 times as many nymphs as MDT alone, and 4.1–7.6 times as many as the pheromone blends alone (Table 1). In season-long totals, combined lures caught from 1.9 to 3.2 times the number of adults, and 1.4 to 2.5 times the number of nymphs than expected, compared with an additive effect of the lures deployed individually ("synergism" in Table 1).

Over the course of the season, the combined pheromone plus MDT treatment was often superior, and never inferior, to other treatments (Tables 2–4). For 29 of 40 2-wk periods tested for adults, and 10 of 29 2-wk periods tested for nymphs, the combined lure provided captures significantly higher than all other treatments; in no 2-wk period were captures of the

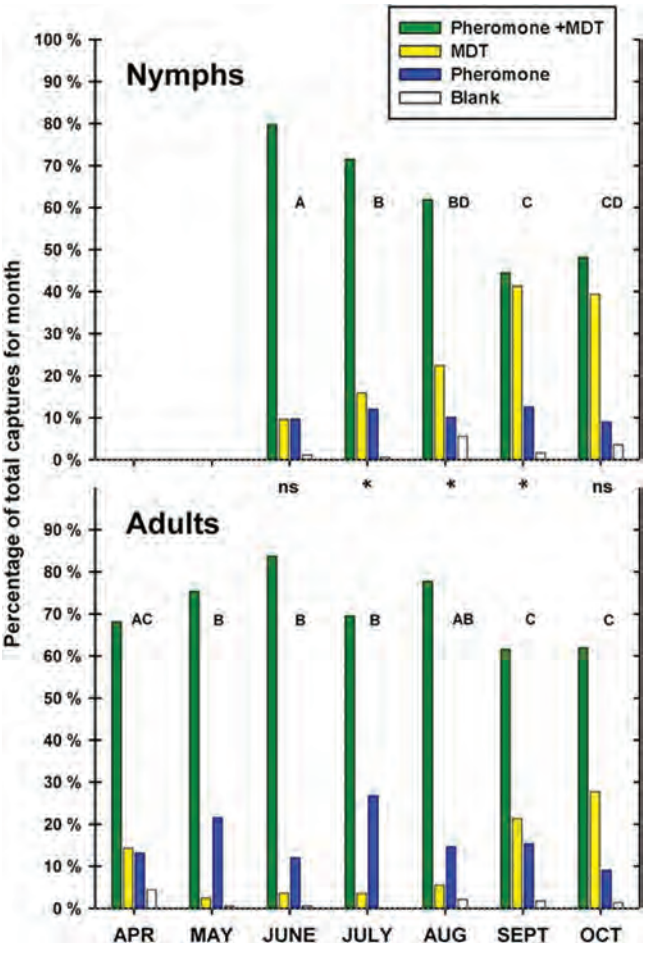

Fig. 2. Seasonal response of *H. halys* nymphs and adults to pheromone, MDT, and combination. Letters indicate a difference in proportion of captures according to Bonferroni-corrected chi-square multiple-comparison tests (experiment-wise $\alpha = 0.05$; comparison-wise $\alpha = 0.0024$ for adults [21 paired tests], and $\alpha = 0.005$ for nymphs [10 paired tests]). Asterisks (or ns = no significant difference) indicate difference in proportion of captures between adults and nymphs within the same month (Bonferroni-corrected chi-square multiple-comparison tests with experiment-wise $\alpha = 0.05$; comparison-wise $\alpha = 0.01$ [five tests]). Monthly totals for 6 April through 23 October 2012, Beltsville, MD; nymphs were not captured before early June.

combined lures significantly lower than any other treatments for adults or for nymphs (Tables 2–4).

Figure 2 illustrates some significant seasonal trends: adults responded more strongly to pheromone alone than to MDT alone for the months of May through August, whereas in September and October this response was reversed (Fig. 2). Similarly, ≈14% of newly active overwintered adults were captured in the MDT-only traps in April, in a pattern similar to that seen in reproductively diapausing fall adults. These differences are not unexpected because adults are generally not responsive to MDT alone until mid-August (Leskey et al. 2012a), but researchers in Asia have in some seasons reported captures of overwintered adults early in the season (Funayama 2008). Nymphs showed stronger seasonal treatment differences in captures (Fig. 2). The increased response to MDT at the end of the season is well documented in Asia and North America (Leskey et al. 2012a).

Nymphs, relative to adults, were captured more in traps with MDT lures only, and also more in the unbaited traps (MD 2012 season totals $\chi^2 = 154.86$; $P < 0.0001$; df = 3). Monthly proportions differ between nymphs and adults in the months when both are abundant, July through September, reflecting the higher proportion of nymphs in MDT-only traps, and for July, a lower response to pheromone-only lures (differences by Bonferroni-corrected chi-square tests with experiment-wise $\alpha = 0.05$ [comparison-wise $\alpha = 0.01$] indicated by asterisks on Fig. 2; [excluding blank lure treatment; df = 2 for all]; June: $\chi^2 = 6.63$, $P = 0.036$; July: $\chi^2 = 25.03$, $P < 0.0001$; August: $\chi^2 = 64.88$, $P < 0.0001$; September: $\chi^2 = 276.51$, $P < 0.0001$; and October: $\chi^2 = 4.32$, $P = 0.115$). The somewhat lower adult captures in MDT-only traps is probably explained by their interest in mating, given that MDT does not indicate presence of potential mates. Adults could also be more selective because of their ability to fly to

Table 5. Test of BMSB2 lure (nonpurified mixture of eight isomers of 10,11-epoxy-1-bisabolen-3-ol with 7*R* configuration) versus SSRS + RSRS, a combined "pure lure" of SSRS and RSRS in the natural ratio of 3.5:1, with and without MDT2 lure, Beltsville, MD, 30 August through 26 September 2013, in four randomized blocks, total captures for period

Lure	Adult	Nymph
Combined BMSB2 + MDT2	811a	205a
SSRS + RSRS plus MDT2	729a	194a
SSRS + RSRS	226b	25b
BMSB2	163b	75ab

Within each life-stage (column), total trap captures followed by a common letter do not differ by Tukey's HSD test, $P < 0.05$, using arcsin-transformed block proportions for the 27-d trapping period.

preferred locations including traps; they could leave the vicinity of traps where, owing to our twice-weekly rerandomization, the plume of attractive volatiles had disappeared. Conversely, nymphal captures were probably enhanced in unbaited traps because of prior attraction to baited traps in the previous sampling period.

Male and female bugs did not differ in response to lure types. For season-long totals in Maryland 2013, 1,210 females and 1,168 males were captured (with 29 not determined because of escape or dismemberment by predators). Season-long response did not differ ($\chi^2 = 3.54$; $P = 0.316$; df = 3), nor did response differ by sex for any of the 2-wk trapping periods (χ^2 tests $P < 0.05$; treatments with total capture of five or less were excluded).

When we tested lures containing SSRS and RSRS in the natural ratio of 3.5:1 versus the nonpurified mixed-isomer pheromone lure, the purity of pheromone lure did not affect captures with or without MDT over the 27-d trapping period (Table 5). There was no interaction between MDT and lure type; both adult and nymphal captures were enhanced approximately fourfold by addition of MDT. Results demonstrate the effectiveness of the nonpurified mixed-isomer lure, a much less expensive alternative to pure pheromone components. However, because our field experiments did not compare different ratios of pheromone and MDT attractants, there is further need to optimize these joint loadings and release rates for trap captures.

The reason for the attraction of *H. halys* adults and nymphs to MDT is not known. The North American native *Chinavia hilaris* (Say) is also attracted to MDT, as are several tachinid species (Aldrich et al. 2007). There are several other pentatomids attracted to pheromones of other species (Endo et al. 2010, Tillman et al. 2010, Laumann et al. 2011). Proposed explanations include location of host plants (Tada et al. 2001, Endo et al. 2006), dispersal to overwintering sites (Khrimian et al. 2008), and density-based protection from natural enemies (Aldrich et al. 2007). All of these functions are plausible and not mutually exclusive. The increase in attraction by MDT of nonreproductive adults in the fall supports prediapause feeding as well as overwintering site location. Throughout the growing season, even young nymphs are attracted to MDT, presumably as a feeding signal; in the fall, the increasing attractiveness of MDT to nymphs, which are unable to overwinter without additional feeding and development, strongly suggests a food-related cause for this response.

As Millar (2005) pointed out, the chemistry of the Pentatomidae and other true bugs, while far from complete, has greatly surpassed our knowledge of the biological functions of this group's semiochemicals. Several factors complicate interpretation of pheromone response and trap capture: often weak response to pheromone, depending on mating and physiological state; requirement in courtship for shorter-range substrate-borne vibrational communication; and the polyphagous and highly mobile habits of many species. Add to this the use by several species of other species' semiochemicals, and the situation is complicated, making trap captures difficult to relate to behavior and abundance in the field.

Nevertheless, for *H. halys*, with the combination of economical mixed-isomer aggregation pheromone and MDT, we now have a practical season-long attractant combination for all mobile life stages, a key management tool for this polyphagous invasive pest of North America and Europe.

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