JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2008,** *56,* 197–203 **197**

Field Trapping of the Invasive Brown Marmorated Stink Bug, *Halyomorpha halys*, with Geometric Isomers of Methyl 2,4,6-Decatrienoate

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The brown marmorated stink bug, *Halyomorpha halys* (Stål), is a polyphagous pest indigenous to northeastern Asia where it damages various trees, vegetables, and leguminous crops. The bug was recently introduced into the U.S. and could potentially become a pest. In its native range, *H. halys* was reportedly attracted to the aggregation pheromone of the brown-winged green bug, *Plautia stali*, methyl (2*E*,4*E*,6*Z*)-decatrienoate. We also observed that traps baited with this compound are attractive to *H. halys*. We additionally found that methyl (2*E*,4*E*,6*Z*)-decatrienoate (as well as other isomeric methyl 2,4,6-decatrienoates) exposed to daylight in solutions and/or on dispensers used for field trapping can readily isomerize to form complex mixtures of isomers, thus causing a concern about lure stability and longevity. However, our studies demonstrated that preventing isomerization of methyl (2*E*,4*E*,6*Z*)-decatrienoate in dispensers was not essential for field trapping of *H. halys* males, females, and nymphs. We also present evidence that traps baited with methyl (2*Z*,4*E*,6*Z*)-decatrienoate and methyl (2*E*,4*Z*,6*Z*)-decatrienoate (pheromone of *Thyanta* spp. pentatomids), as well as the mixtures of geometric isomers, attract *H. halys*. The *ZEZ* isomer, unknown in nature, as well as the *EEZ* isomer, elicited electrophysiological responses from antennae of *H. halys* males. The field data suggest that the presence of the *EEZ* but not *ZEZ* isomer in the lure is essential for attraction of *H. halys*, and that other isomers are not antagonistic and may even be needed for maximum attraction. Because the pheromone of *H. halys* is unknown at present, lures containing methyl (2*E*,4*E*,6*Z*)-decatrienoate without protection from daylight are suitable for monitoring populations of *H. halys* late in the season.

KEYWORDS: Brown marmorated stink bug; *Halyomorpha halys*; methyl 2,4,6-decatrienoate; geometric isomers; isomerization; field trapping; electroantennogram

INTRODUCTION

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae) is a polyphagous horticultural pest indigenous to northeast Asia (*1*). In Japan, it causes considerable damage to various shade and fruit trees, vegetables, and leguminous crops (*2*). Host plants of economic significance in its native range include citrus, persimmon, soybean, apple, mulberry, paulownia, cherry, peach, pear, and apricot (*1*). *Halyomorpha halys* is also a nuisance pest when the adults aggregate on the outside of buildings and then seek hibernation sites inside (*1*).

The presence of the brown marmorated stink bug was confirmed for the first time in the U.S. in Allentown, Pennsylvania, in 2001, but prior collection records have been found dating back to 1996 (*1*). Since 2001, the bug has been found in several additional Pennsylvania counties and in the states of New Jersey, Delaware, Maryland, Virginia, West Virginia, and Oregon. Kiritani predicted that the mortality of overwintering adults of *H. halys* in Japan will be reduced by as much as 15% with a rise in temperature by 1 °C, and so the range and numbers of this bug may increase if global warming continues (*3*).

Methods for detection and monitoring stink bugs include light trapping, sweep-net sampling, and the use of pheromone traps. Black light traps were used in Japan for monitoring populations

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10.1021/jf072087e CCC: $40.75
Published on Web 12/11/2007

of *H. halys* (*4*). Despite our efforts and that of others, a pheromone for *H. halys* has not yet been identified, but in its native range (*5–7*) and recently in the U.S. (*8*), the bug was attracted in the field to the aggregation pheromone of the brown-winged green bug, *Plautia stali*, methyl (2*E*,4*E*,6*Z*)-decatrienoate (2*E*,4*E*,6*Z*-10:COOMe) (*9*). A possible explanation for this cross-attraction was that *H. halys* exploits the pheromone of *P. stali* to find food and/or hibernation sites (*5, 6, 8, 10*) Pheromone-baited traps attracted more *P. stali* than *H. halys*, but the light traps showed a reverse pattern (*7*). Furthermore, pheromone traps caught *H. halys* in early spring and late fall when light traps did not, whereas adult *H. halys* were caught in light traps mainly in the middle of summer (*5*).

Once *H. halys* was found to be established in the U.S., we became interested in using 2*E*,4*E*,6*Z*-10:COOMe in detection and monitoring of the brown marmorated stink bug. For that purpose, one of us has recently developed the syntheses of 2*E*,4*E*,6*Z*-10:COOMe and all other geometric isomers (*11*). At the onset of our research we envisioned a challenge in handling methyl 2,4,6-decatrienoates given the thermal and, especially, photochemical instability of these esters (*11*). The present paper summarizes the stabilities of 2*E*,4*E*,6*Z*-10:COOMe and its geometric isomers and our three-year collaborative efforts to evaluate these compounds as trap lures for the brown marmorated stink bug in the northeastern U.S.

MATERIALS AND METHODS

Gas-Chromatographic Analyses. A Shimadzu 17A gas chromatograph (Columbia, MD) equipped with a 30 m x 0.25 mm ID, 0.25 μm film thickness, HP-5 capillary column (Agilent Technologies, Santa Clara, CA), a flame ionization detector, a Shimadzu AOC-20s autosampler, and AOC-20i autoinjector was used. Analyses were done in the splitless mode using hydrogen as a carrier gas at 1.2 mL/min. Column temperature was maintained at 70 °C for 5 min and then raised to 230 at 10 °C/min. Injection temperatures were 180–200 °C for methyl 2,4,6-decatrienoates and 260 °C for the other compounds presented in the Supporting Information.

Electron-Impact (EI) Mass Spectra. EI-MS (70 eV) were obtained with an Agilent Technologies 5973 mass-selective detector interfaced with a 6890N GC equipped with a 30 m x 0.25 mm ID, 0.25 μm film thickness, HP-5MS capillary column. Helium was used as the carrier gas at 1 mL/min. Column temperature was maintained at 70 °C for 5 min and then raised to 230 at 10 °C/min. Injection temperatures were 180–200 °C for methyl 2,4,6-decatrienoates and 260 °C for other compounds.

Coupled Gas Chromatographic-Electroantennographic Detection (GC-EAD). The GC-EAD system used was as previously described (*12, 13*). A Hewlett-Packard (HP) 6890 gas chromatograph equipped with a 60 m x 0.25 mm ID, 0.25 μm film-thickness DB-WAXETR capillary column (J&W Scientific Inc., Folsom, CA) in the splitless mode with hydrogen as carrier (1.4 mL/min) was used for GC-EAD analyses (120 °C for 2 min, then programmed to 250 at 15 °C/min and held for 10 min, inlet temperature of 260 °C, and transfer line temperature of 300 °C). The capillary column effluent and nitrogen makeup gas (10 mL/min) were split (~1:1) by a fixed outlet splitter (SGE, Incorporated, Austin, TX) to a flame ionization detector (FID) and EAD. After both antennae were excised from a bug, they were positioned between two gold wire electrodes, which were immersed in saline-filled (0.9% NaCl) wells (1.25 mm diameter; about 3 mm apart) in a small acrylic plastic holder (8 cm long x 0.8 cm wide x 0.6 cm thick). The output recording electrodes were connected to a high-impedance 1:100 amplifier with automatic baseline drift compensation. The EAD branch of the capillary column was introduced into a stream of humidified air (about 500 mL/min) passed through a condenser mounted on top of the GC and cooled by circulating near 0 °C water through the insulation layer of the condenser from a benchtop refrigeration unit (RTE-100, NESLAB instruments, Inc., Portsmouth, NH). The antennal preparation holder was placed in the distal end of the condenser, and the temperature was maintained at ~5 °C. The flame ionization and electrophysiological output signals were recorded using HP ChemStation software.

Table 1. Methyl 2,4,6-decatrienoate Lures Used for Field Trapping of *H. halys* in 2003–2005

	composition, GC %				
lure	2E,4E, 6Z-10:COOMe	2E,4Z, 6Z-10:COOMe	2Z,4E, 6Z-10:COOMe	2E,4E, 6E-10:COOMe	others[a]
EEZ-A	76.7		5.1	5.0	
EEZ-B	87.3			7.7	2.2[b]
EEZ-C	94.8		0.2	5.0	
EZZ-D		92.7			5.6[c]
EZZ-B	55.9	22.9		13.3	
EZZ-C		97.0		1.2	
ZEZ-C			93.0		7.0[d]

[a] Identified compounds. [b] Methyl (2*E*,4*E*)-decadien-6-ynoate. [c] 2*E*,4*Z*-8:COOMe. [d] 2*Z*,4*E*,6*E*-10:COOMe.

Chemicals and Supplies. Mention of a proprietary company or of commercial products does not constitute an endorsement by USDA. Unless otherwise specified, all chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI). The Tinuvin 328 UV stabilizer was purchased from Ciba Specialty Chemicals (Basel, Switzerland). Gray rubber septa were purchased from The West Company (Kearney, NE) and were extracted with acetone/hexane, 1:1, in a Soxhlet extractor prior to use. Custom made traps were supplied by Sterling International, Inc., Spokane, WA. These clear plastic oval traps (27 cm × 15 cm) had two 6.5 cm diameter funnels with 1.0 cm holes in the small end that allowed entrance into the trap. The trap bottom contained a flat 10 cm plastic plug that was removable for servicing the trap. The photo of the trap is provided in the Supporting Information.

Methyl 2,4,6-decatrienoates used for field trapping of *H. halys* are presented in **Table 1**. Letters following the configuration descriptors indicate the sources of the lures: A = Shin-Etsu Chemical Company (Tokyo, Japan); B = Khrimian (this paper); C = Khrimian (*11*); D = University of California, Riverside (Dr. J. G. Millar). Syntheses of *EEZ*-B and *EZZ*-B lures utilized a semihydrogenation of 2,4-decadien-6-ynols in the key step. However, this synthetic route lacked stereoselectivity and, subsequently, a superior synthetic scheme was developed (*11*). Therefore, the synthetic scheme and details of preparations of *EEZ*-B and *EZZ*-B lures are presented in the Supporting Information. According to our GC analysis, *EZZ*-D consisted of 92.7% 2*E*,4*Z*,6*Z*-10:COOMe and 5.6% of an impurity that, based on the mass spectrum (*m*/*e*: 154 (45%), 123 (26), 111 (100), 95 (32), 81 (42)) and judging from the synthetic method (*14*), apparently was 2*E*,4*Z*-8:COOMe. The *EZZ*-isomer is thermally unstable under GC conditions, and appears as a broad peak as described by Millar (*14*).

Preparation of Rubber Septum Dispensers. Ten rubber septa were placed in a 100 mL round-bottom one-neck Morton flask (Ace Glass, Inc., Vineland, NJ), and were covered with 7 mL of a hexane solution containing 25.0 mg of test compound, plus light stabilizer Tinuvin 328 (1.5–50%) in some treatments. The flask was rotated on a closed rotary evaporator (without applying a vacuum) for 1.5–2 h or until the liquid was completely absorbed into the septa. The septa were placed in a fume hood for 1 h to evaporate most of the remaining hexane and were subsequently used for field trapping, stability studies, or release rate measurements.

Volatiles Collection/Stability Studies. Esters *EEZ*-C, *ZEZ*-C, and *EZZ*-C were used in these tests. One freshly prepared dispenser of each treatment (see **Table 2**) was placed individually in glass chambers (6.35 cm × 2.54 cm (I.D.), sealed with two O-ring joints (Kontes, Vineland, NJ). The tubes were placed in a light-protected compartment, and a constant air flow of 60 mL/min carried headspace volatiles onto 60 mm x 6 mm (I.D.) Gerstel Tenax TA tubes (Supelco, Bellefonte, PA) at 22 °C. After an ~8 h collection period, tubes were eluted with 1.5 (3 × 0.5) mL of acetone and were analyzed by GC to determine the initial composition of volatiles. The same dispensers were placed directly in traps or were pinned through a narrow hole in standard PVC plumbing pipes (65 mm x 17 mm, I.D.), to protect them from direct sunlight, and then placed in traps. The traps were hung on trees

Table 2. Stabilities of *EEZ*-C, *ZEZ*-C, and *EZZ*-C Ester in Solutions and on Rubber Septa

						isomeric composion, % GC			
entry	ester	medium	tinuvin added, %	exposure conditions	exposure duration	*EEZ*	*ZEZ*	*EEE*	others
1	*EEZ*-C	hexane		freshly made		94.8	0.2	5.0	
2	*EEZ*-C	hexane		−20 °C	45 days	92.8	0.2	7.0	
3	*EEZ*-C	hexane		25 °C, dark	10 days	89.0	0.2	10.3	0.4
4	*EEZ*-C	CH_2Cl_2		25 °C, laboratory	2 days	37.1	30.4	11.8	20.7
5	*EEZ*-C	septum		25 °C[a]	10 h	89.7	1.8	6.3	2.2
6	*EEZ*-C	septum		field, U[b]	7 days	52.1	21.3	16.4	10.1
7	*EEZ*-C	septum		field, U[b]	14 days	44.7	19.0	12.7	23.6
8	*EEZ*-C	septum		field, P[c]	7 days	80.6	4.9	9.3	5.2
9	*EEZ*-C	septum	1.5	field, U[b]	7 days	50.3	18.4	13.3	18.0
10	*EEZ*-C	septum	15	field, U[b]	10 days	55.9	14.6	12.2	16.1
11	*EEZ*-C	septum	50	field, U[b]	10 days	55.6	15.1	12.3	16.3
12	*ZEZ*-C	hexane		freshly made			93.0		7.0[d]
13	*ZEZ*-C	hexane		−20 °C	90 days	1.2	91.1		7.7[d]
14	*ZEZ*-C	hexane		25 °C, dark	10 days	1.5	86.9		11.6[d]
15	*ZEZ*-C	septum		25 °C[a]	10 h	2.0	87.3	1.1	8.4
16	*ZEZ*-C	septum		field, U[b]	7 days	18.5	52.2	9.0	20.3
17	*ZEZ*-C	septum		field, U[b]	14 days	18.2	46.1	9.0	25.0
18	*ZEZ*-C	septum		field, P[c]	7 days	4.9	81.1	2.6	11.4
19	*ZEZ*-C	septum	15	field, U[b]	10 days	15.3	55.4	8.0	20.2
20	*ZEZ*-C	septum	50	field, U[b]	10 days	15.5	57.9	7.5	19.2
21	*EZZ*-C	hexane		freshly made				1.2	97.0[e]
22	*EZZ*-C	hexane		25 °C, laboratory	2 days	11.8	5.1	9.1	72.2[f]

[a] Dispenser preparation followed by volatiles collection in dark (see Materials and Methods section). [b] Unprotected from daylight in a trap. [c] Protected in a PVC pipe in a trap. [d] Main impurity 2*Z*,4*E*,6*E*-10:COOMe. [e] 2*E*,4*Z*,6*Z*-10:COOMe. [f] Complex mixture of 2*E*,4*Z*,6*Z*-10:COOMe and other isomers.

bordering agricultural fields at the Beltsville Agricultural Research Center (BARC) in May 2004. After the time periods specified in **Table 2**, the dispensers were retrieved, and volatiles were collected and analyzed as described above.

Release Rate Measurements. The experiment was conducted with *EEZ*-C and *ZEZ*-C esters loaded on rubber septa (2.5 mg/lure, 5 replicates). After the initial volatile collections (Week 0, **Figure 1**), the dispensers were pinned inside PVC plumbing pipes as described above and were placed in traps hung on tree branches at BARC in August 2004. The emissions from dispensers were determined weekly for a 4-week period as described above. The acetone extracts were quantified using external standards of *EEZ*-C and *ZEZ*-C esters (**Figure 1**).

Field Bioassay. On September 11, 2003, traps were set in a randomized block design with each treatment replicated at three sites in the area of Allentown, PA. One location consisted of various fruit trees growing in a residential backyard. Each of the remaining two sites were mature *Paulownia tomentosa* groves established at the Rodale Working Tree Center Institute. The following treatments were used: *EEZ*-A, *EEZ*-B, *EZZ*-D, and *EZZ*-B (2.5 mg/rubber septum) plus a control septum impregnated with hexane. Traps were placed approximately 3 m above the ground so that they contacted trunks, limbs, or foliage, and captured bugs were removed and counted weekly. The distance between traps was approximately 5–10 m. The experiment was continued until December 5.

In 2004, the initial test was conducted from August 19 through September 15 with the following treatments: *EEZ*-C, *ZEZ*-C, and *EEZ*-C/*ZEZ*-C, 3:1, plus a control. All treatments (2.5 mg ester/septum) were placed in PVC plumbing pipes as described above to protect lures from direct sunlight. The lures were changed approximately every two weeks. On September 15, two more treatments were added: *EZZ*-C protected from sunlight and *EZZ*-C unprotected from sunlight. The traps were placed about 10 m apart in two *P. tomentosum* groves and were monitored until October 11, 2004.

In 2005, traps with four treatments: *EEZ*-C, *ZEZ*-C, and 3:1 *EEZ*-C/*ZEZ*-C, all protected as described above, and *EEZ*-C unprotected, were set on September 14, and the test was conducted through October 22. All treatments contained 2.5 mg of material per rubber septum. The experiment was replicated at six sites by placing traps on host plants, such as paulownia trees, grapes (*Vitis lambrusca*), and Russian olive (*Elaeagnus angustifolia*). The lures were changed approximately every two weeks, and trap counts were conducted every 3–4 days.

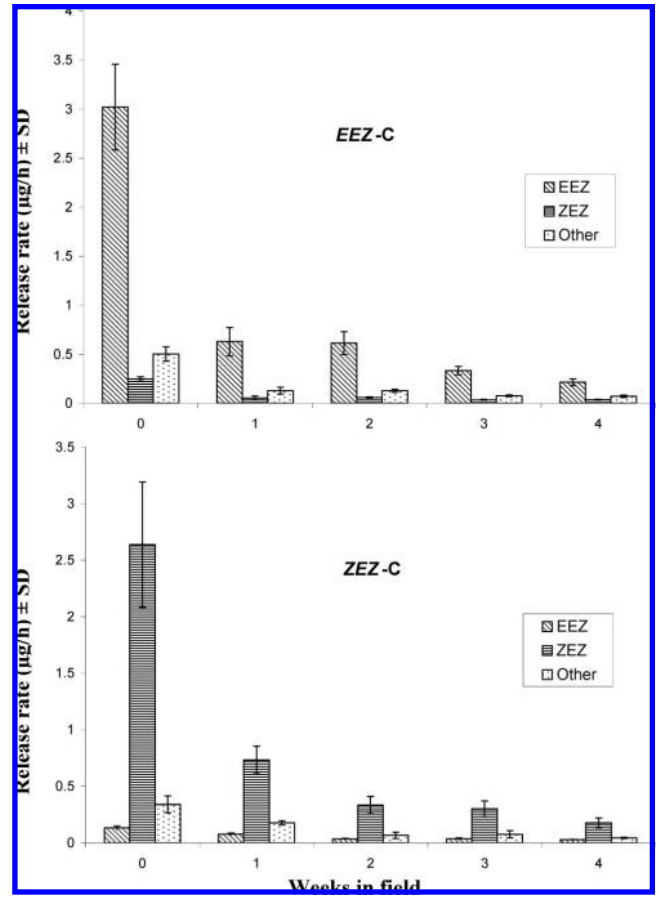

Figure 1. Release rates of *EEZ*-C and *ZEZ*-C esters from rubber septa (2.5 mg/lure; 5 replicates) protected from direct sunlight in standard PVC plumbing pipes (65 mm x 17 mm, I.D.). Each cluster of bars represents the composition of volatiles.

Statistical Analysis. All field experiments were conducted using a randomized block design with treatments (pheromone) and location (host plants) as main effects (blocks). All data were transformed {log(*X*

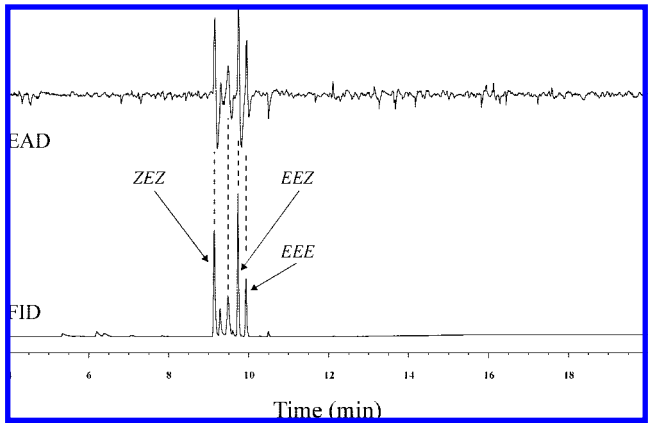

Figure 2. Simultaneous responses of FID and EAD of adult male *H. halys* antennae to the mixture of methyl 2,4,6-decatrienoates in CH_2Cl_2 (**Table 1**., Entry 4) on a DB-WAXETR capillary column.

+ 1)} before analysis with ANOVA, and means were separated using Tukey's Studentized Range (HSD) Test (*15*).

RESULTS

Chemical Stabilities. With the exception of one isomer, 2*E*,4*Z*,6*Z*-10:COOMe (*14*), methyl 2,4,6-decatrienoates survive GC conditions (injection temperatures 180–200 °C), as judged from the integrity of their peaks in the GC-MS analyses and NMR spectra (*11*). Hence, we used a GC method to study stabilities of the selected isomers starting from preparation and storage, through formulation on rubber septa and, finally, exposure to field conditions. As illustrated in **Table 2**, a freshly prepared hexane solution of 2*E*,4*E*,6*Z*-10:COOMe (batch *EEZ*-C, entry 1) could be stored in the freezer without much change (entry 2) but isomerized noticeably to form 2*E*,4*E*,6*E*-10:COOMe if the solution was stored at ambient temperature in the dark (entry 3). The course of isomerization changed dramatically when a solution of *EEZ*-C was exposed to ambient conditions unprotected from daylight (entry 4). As a result of an apparent photochemical reaction, a complex mixture of isomers was formed, with 2*Z*,4*E*,6*Z*-10:COOMe being the most noticeable among the newly formed compounds. Analysis of volatiles emitted from a rubber septum impregnated with *EEZ*-C showed a loss of geometric purity from 94.8 to 89.7% (entry 5), but far greater isomerization occurred when the dispenser was placed in a trap and exposed to field conditions unprotected from sunlight (entries 6 and 7). A similar trend was observed with the *ZEZ*-C ester (entry 12), which was fairly stable in a freezer (entry 13) but thermally isomerized to the *ZEE* isomer (entry 14) and was prone to rapid and random photochemical isomerization when exposed to light (entries 15–17). Attempts to stabilize lures by adding as much as 50% Tinuvin 328 UV light absorber failed (entries 9–11, 19, and 20). However, we found that placing rubber septum lures in a standard PVC pipe, thus shielding from direct sunlight, provided reasonable protection for both *EEZ*-C, and *ZEZ*-C samples, preserving as much as ~80% of geometric purity after one week of exposure (**Table 2**, entries 8 and 18; **Figure 1**). 2*E*,4*Z*,6*Z*-10:COOMe (batch *EZZ*-C) was also found to be unstable in daylight, with three identifiable byproduct being *EEZ*, *ZEZ*, and *EEE* isomers (**Table 2**, entries 21 and 22).

GC-EAD Study. We conducted simultaneous GC-FID and EAD recordings with the mixture shown in **Table 2**, entry 4, and found that male *H. halys* antennae responded to *EEZ* and *ZEZ* isomers (**Figure 2**), as well as other unidentified isomers present in the mixture.

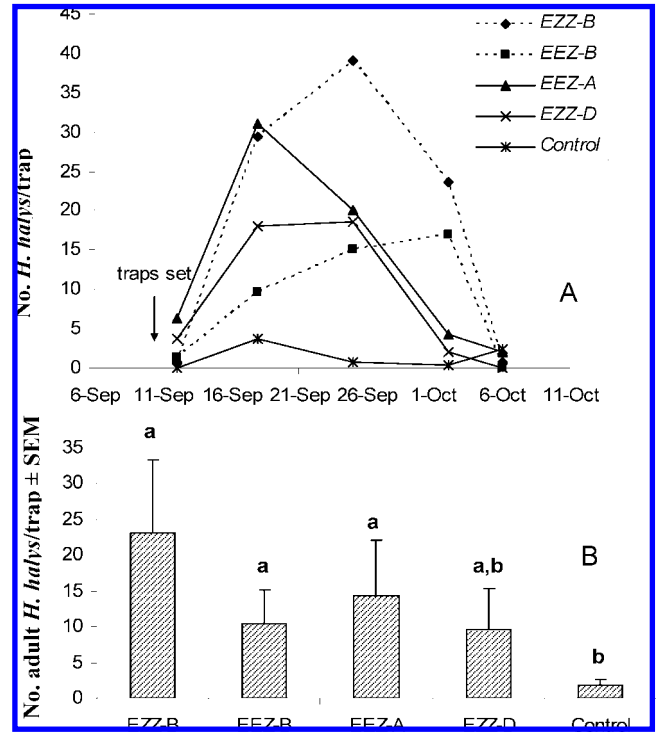

Figure 3. (**A**) Weekly mean captures of adult *H. halys* in attractant-baited traps from September 11 to October 6, 2003, at three sites in Allentown, PA. (**B**) Average number of adult *H. halys* captured. Bars with the same letter were not significantly different ($P < 0.05$). ♀/♂ *EZZ*-B, 1.1; *EEZ*-B, 0.8; *EEZ*-A, 1.2; *EZZ*-D, 0.9, control, 0.5.

Release Rates. Figure 1 shows emission rates of *EEZ*-C and *ZEZ*-C esters from rubber septa dispensers placed in PVC pipes inside traps under conditions similar to field trapping. Initial release rates of the esters from freshly prepared dispensers were 2.5–3.0 μg/h, but they dropped rapidly to 0.5–1.0 μg/hr after one week of aging in the field. The compositions of volatiles were reasonably close to numbers presented in **Table 2**, entries 8 and 18. At two weeks of field exposure and further into the study, both emission rates and composition of volatiles did not change as dramatically as in the first week.

Field Bioassays. In 2003, *H. halys* captures were observed from September 11 to December 5, but catches were low after early October and, therefore, are not presented. **Figure 3** shows weekly captures of adult *H. halys* (panel **A**) and cumulative average numbers of bugs caught (panel **B**). The average number of adult *H. halys* captured in traps baited with *EZZ*-D from September 11 to October 6 was not different from the numbers captured in any of the other treatment or the control traps. However, treatments *EZZ*-B, *EEZ*-B, and *EEZ*-A captured adult *H. halys* at levels that were greater than the control ($F_{4,8}$; $P = 0.014$). In **Figure 3**, panel **B**, each treatment bar is represented by nearly equal numbers of males and females. In addition to adults, we caught some immature *H. halys* in most of the treatments (a total of 8 third-instars, 5 fourth-instars, and 26 fifth-instars). There were no differences in the number of immature *H. halys* captured in any of the treatment or untreated control traps ($F_{4,8}$; $P = 0.272$).

We started the 2004 field trial on August 19 with the *EEZ* and *ZEZ* isomers because both compounds gave clear EAG responses, and the *ZEZ* isomer was the main photoisomerization product of the *EEZ* ester in laboratory and field conditions (**Table 2**, entries 4 and 6). Weekly total catches of *H. halys* tallied across blocks within treatments in this experiment were low (**Figure 4**). Adding the *EZZ*-C isomer (protected and

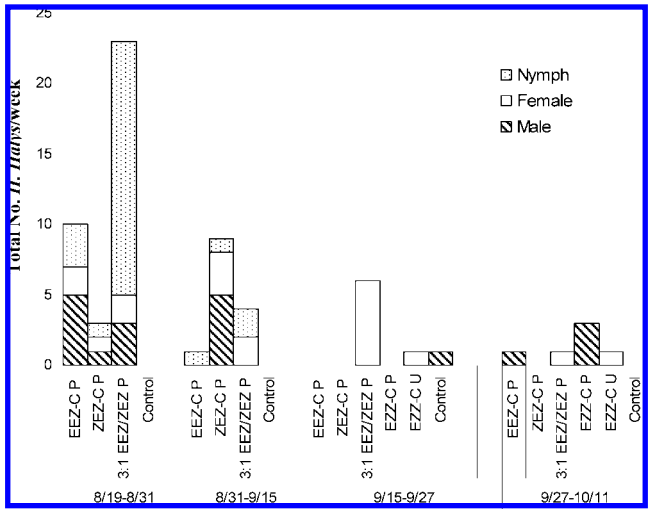

Figure 4. Weekly total catches of *H. halys* at six sites in Allentown, PA, in 2004, with *EEZ*-C, *ZEZ*-C, and 3:1 *EEZ*-C/*ZEZ*-C treatments. Treatments with the suffix "P" were protected from direct sunlight, and those with the suffix "U" were unprotected from direct sunlight. There were no differences among treatments in the total number of males ($F_{3,15} = 1.49$; $P = 0.26$), females ($F_{3,15} = 1.34$; $P = 0.30$), or total adults ($F_{3,15} = 2.65$; $P = 0.09$).

unprotected) to the experiment on September 15 did not improve catches, further indicating that the population levels were depressed and variable between sites. As a result, there were no differences between treatments in the average number of males ($F_{3,15} = 1.49$; $P = 0.256$), females ($F_{3,15} = 1.34$; $P = 0.299$), or total adults ($F_{3,15} = 2.65$; $P = 0.087$) captured. However, treatment differences were observed regarding the number of immature *H. halys* captured ($F_{3,15} = 4.54$; $P = 0.019$) (**Figure 4**). Traps baited with 3:1 *EEZ*-C/*ZEZ*-C captured more immature *H. halys* than the untreated control traps, but not statistically more than traps baited with *EEZ*-C or *ZEZ*-C alone. The numbers of immature *H. halys* captured in traps baited with either *EEZ*-C or *ZEZ*-C were not different from those for the untreated control.

H. halys was more abundant in the 2005 field test. **Figure 5** shows the levels of adult bugs captured from September 19 through October 19, 2005 (panel **A**) and also summarizes, in the panel **B**, the cumulative average number of adult *H. halys* caught with *EEZ*-C (protected and unprotected), *ZEZ*-C, and a 3:1 mixture of *EEZ*-C and *ZEZ*-C (both protected from direct sunlight). Traps baited with unprotected *EEZ*-C captured more *H. halys* than did the control traps ($F_{4,20} = 8.22$; $P = 0.0004$) and so did protected *EEZ*-C and 3:1 *EEZ*-C/*ZEZ*-C. Importantly, traps containing unprotected *EEZ*-C and protected 3:1 *EEZ*-C/*ZEZ*-C captured more *H. halys* than traps containing the protected *ZEZ*-C isomer. The experimental design did account for some variation as the block effect for different trap host plants was significant ($F_{5,20} = 3.36$; $P = 0.023$).

DISCUSSION

All geometric isomers of methyl 2,4,6-decatrienoate absorb UV light at 290–300 nm (*11, 16–18*) and thus should readily isomerize on exposure to sunlight, a natural source of near-UV irradiation (200–400 nm). To the best of our knowledge, only two isomeric methyl 2,4,6-decatrienoates have been reported from nature: 2*E*,4*E*,6*Z*-10:COOMe, the aggregation pheromone of the brown-winged green bug, *Plautia stali*, and 2*E*,4*Z*,6*Z*-10:COOMe, a component of the sex pheromones of *Thyanta* spp. pentatomids (*14, 19, 20*). Both pheromones are produced

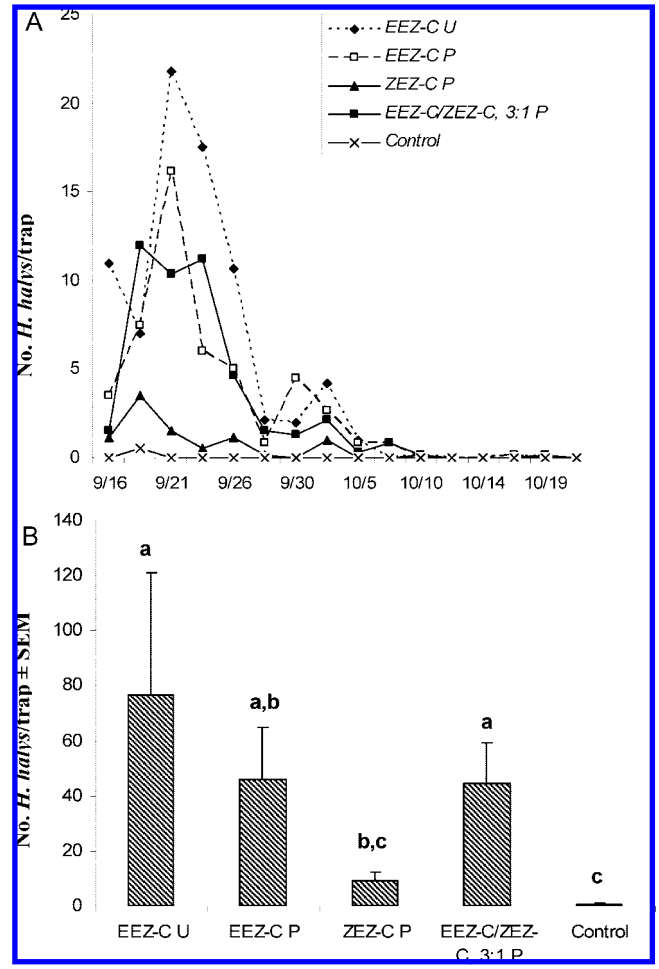

Figure 5. (**A**) Captures of *H. halys* adults from September 16 to October 19, 2005, in traps baited with different treatments unprotected (U) or protected (P) from direct sunlight in PVC tubes. Each treatment (2.5 mg/septum) was replicated at six sites. (**B**) Average number of adult *H. halys* captured. Bars with the same letter were not significantly different ($P < 0.05$).

by insects as single geometric isomers; however, before our studies no information was available about their stabilities on the exposure to daylight (e.g., under field trapping conditions, which may be of importance in evaluation of the potential of these lures for sampling stink bug populations). The only known plant sources of compounds related to methyl 2,4,6-decatrienoates are certain *Euphorbia* species (not *H. halys* hosts), which produce 2,4,6-decatrienoic acid (as well as analogous 2,4,6-dodecatrienoic acid) as mixtures of geometric isomers esterified with diterpenols and triterpenols (*16–18*). The biochemical origin of these esters has not been researched, and it is possible that complex mixtures of geometric isomers arise from photochemical isomerizations. Interestingly, *EEZ* esters are the most abundant in the *Euphorbia* plant extracts followed by *ZEZ* isomers, reminiscent of the volatile composition after exposure of rubber septa impregnated with 2*E*,4*E*,6*Z*-10:COOMe to light (**Table 2**, entries 6 and 7). Our stability studies showed that up to 80% of geometric purity of the lures could be achieved by shielding them from direct sunlight, but we did not pursue this matter further because such isomerization did not reduce attraction of *H. halys* in the field.

Our three-year field study verified previous reports (*5–7*) that methyl (2*E*,4*E*,6*Z*)-decatrienoate is attractive to *H. halys* males, females, and nymphs, but we also found that traps baited with other geometric isomers and mixtures thereof were attractive

to this bug. Among these isomers were 2*Z*,4*E*,6*Z*-10:COOMe and 2*E*,4*Z*,6*Z*-10:COOMe (**Figures 3** and **5**). On the basis of our stability studies, we hypothesized that lures containing 2*Z*,4*E*,6*Z*-10:COOMe and 2*E*,4*Z*,6*Z*-10:COOMe attract *H. halys* by a virtue of photoisomerization that produces 2*E*,4*E*,6*Z*-10:COOMe. The fact that the *ZEZ*-C P treatment (**Figure 5**), protected from direct sunlight and apparently containing only a small amount of the *EEZ* isomer (e.g., see **Table 1**, entry 18), caught significantly fewer *H. halys* than other treatments containing larger proportions of the *EEZ* isomer supports this hypothesis. However, a high content of the *EEZ* isomer in the lure may not be necessary for attraction of *H. halys*, and other geometric isomers of methyl decatrienoate did not seem to be antagonistic and may even actually be needed to achieve maximum attraction. For instance, the *EEZ*-C U lure unprotected from direct sunlight and, hence, producing a complex mixture of isomers (45–52% *EEZ*; see **Table 1**, entries 4, 6, and 7), was not significantly different from the daylight-protected *EEZ*-C P lure (**Figure 5**), which may have retained up to 80% of the geometric purity during the field test (**Table 1**, entry 8). Also, traps baited with the *EZZ*-B lure containing 56% of 2*E*,4*E*,6*Z*-10:COOMe and 23% of 2*E*,4*Z*,6*Z*-10:COOMe was not significantly different from *EEZ*-B or *EEZ*-A lures having higher percentages of 2*E*,4*E*,6*Z*-10:COOMe (**Figure 3**). Although electroantennographic recordings indicated responsiveness of male *H. halys* to the *ZEZ* isomer, this compound does not seem to be an important component in field attraction (**Figure 5**).

The fact that protection of lures containing 2*E*,4*E*,6*Z*-10:COOMe from direct sunlight was unnecessary for field attraction is advantageous from a practical standpoint. Given that a straightforward synthesis of 2*E*,4*E*,6*Z*-10:COOMe has been described (*11*), commercial development of a lure for the brown marmorated stink bug may be feasible. In both 2003 and 2005, the peak captures of *H. halys* in chemically baited traps took place in mid-September (**Figures 3** and **5**), which is similar to the observations of Tada et al. (*5, 6*) However, we have been unable to trap *H. halys* with 2*E*,4*E*,6*Z*-10:COOMe early in the season, as one study reported (*5*). A possible explanation for the late attraction of *H. halys* to the aggregation pheromone of *P. stali*, is that this period coincides with the dispersal of both bugs to overwintering sites (*5, 6*). Interestingly, our recent studies conducted at Beltsville, Maryland, showed that traps baited with 2*E*,4*E*,6*Z*-10:COOMe also significantly attracted the native North American species, *Acrosternum hilare* (Say), plus various tachinid fly parasitoids of native stink bugs, even though *A. hilare* apparently does not produce 2*E*,4*Z*,6*Z*-10:COOMe (*8*). A similar cross-attraction of a pentatomid bug *Piezodorus hybneri*, to the aggregation pheromone of a competitor bug, *Riptorus clavatus*, was reported by Endo et al. while studying these pests of soybean in Japan (*10*). The authors hypothesized that *P. hybneri* utilizes the pheromone of *R. clavatus* as a kairomone to find food plants.

Our ongoing research concerns trying to understand why the various stink bugs are attracted to 2*E*,4*E*,6*Z*-10:COOMe and devising protocols to use this chemical to monitor and possibly control the brown marmorated stink bugs, as well as other economically important stink bugs.

ACKNOWLEDGMENT

We thank Dr. J. G. Millar, University of California, Riverside, CA, for supplying a sample of 2*E*,4*Z*,6*Z*-10:COOMe, and Shin-Etsu Chemical Co., for a sample of 2*E*,4*E*,6*Z*-10:COOMe. The assistance of N. H. Dees and F. Guzman, USDA-ARS, CAIBL in syntheses and analyses is much appreciated as are the efforts of K. Bernhard, Cooperative Extension of Lehigh County (PA), A. Rucker and A. Neilsen, Rutgers University, NJ, for assisting with the field components of this work. We also thank Rodale Working Tree Center Institute, Allentown, PA, for allowing us to conduct much of our trapping efforts on their farm.

Supporting Information Available: Detailed preparations of *EEZ*-B and *EZZ*-B lures; syntheses of *EEZ*-B and *EZZ*-B lures (Scheme 1); trap photo. This material is available free of charge via Internet at http://pubs.acs.org.

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Received for review July 12, 2007. Revised manuscript received October 12, 2007. Accepted October 22, 2007.

JF072087E