Electrophysiological and Behavioral Evidence for (E)-2-Hexenal as a Female-Attracting Pheromone Produced by Disturbed *Megacopta cribraria* (Hemiptera: Plataspidae)[1]

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Abstract *Megacopta cribraria* (F.) (Hemiptera: Plataspidae), commonly known as the kudzu bug, is an invasive insect that was first recorded in the United States in Georgia in 2009. Since its introduction it has spread across the Southeast, infesting soybean crops and significantly reducing crop yield. Chemical pesticides pose environmental concerns and are not compatible with biological control. This investigation was conducted to identify *M. cribraria* pheromones that could be utilized in an insect trap for environmentally friendly monitoring and integrated pest management. Open Y-track olfactometer assays revealed that *M. cribraria* females are attracted to volatiles produced by disturbed *M. cribraria*. Gas chromatography–mass spectrometry analysis of volatile organic compounds produced by disturbed and undisturbed *M. cribraria* revealed the presence of tridecane and (E)-2-decenal in secretions from both disturbed and undisturbed *M. cribraria,* whereas (E)-2-hexenal was detected only in secretions from disturbed *M. cribraria*. The behavioral responses of *M. cribraria* to (E)-2-hexenal and tridecane were measured with open Y-track olfactometer assays. Females were significantly attracted to (E)-2-hexenal while males were not. Neither sex was attracted to tridecane. Electroantennographic assays demonstrated an electrophysiological response of female *M. cribraria* antennae to (E)-2-hexenal but not to tridecane or (E)-2-decenal. Male antennae were unresponsive to the chemicals tested. These data support the conclusion that (E)-2-hexenal is a female-attracting pheromone produced by disturbed *M. cribraria* and suggest that (E)-2-hexenal could be used to lure female *M. cribraria* into pheromone-baited traps.

Key Words *Megacopta cribraria*, Plataspidae, female-attracting pheromone, (E)-2-hexenal, kudzu bug

Megacopta cribraria (F.) (Hemiptera: Plataspidae), also known as the kudzu bug, is an invasive insect native to Asia that was inadvertently introduced into Georgia in 2009 (Suiter et al. 2010). *Megacopta cribraria* feeds and develops mainly on kudzu, *Pueraria montana* (Loureiro) Merrill variety *lobata* (Willdenow), but it also thrives on other legumes including soybeans (*Glycine max* (L.) Merrill). It has spread into 12 states, including portions of all of the southeastern United States (University of Georgia Center for Invasive Species and Ecosystem Health 2016), where it is an economic pest of soybean. *Megacopta cribraria* nymphs and adults suck sap from

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legume stems, petioles, and leaves. Such nutrient loss results in decreased pod fill, seed germination, and overall crop yield (Seiter et al. 2013). In addition to damaging soybean crops, *M. cribraria* is a nuisance when it aggregates on homes and other surfaces (Eger et al. 2010).

Although there are chemicals that control infestations of *M. cribraria* in soybean (Seiter et al. 2015b), application of these pesticides should be based on data that reveal the timing and degree of infestation by this pest (Seiter et al. 2015a) as a part of integrated pest management program. Pheromones can be used to lure insects into traps in order to assess population density, stage of development, and pesticide resistance. Pheromone-baited traps have proven to be valuable in monitoring and controlling insect infestations of soybean plants affected by the neotropical brown stink bug, *Euschistus heros* (F.) (Borges et al. 2011 and references therein). Pheromone-baited traps are a safe tool on both a domestic and agricultural level for monitoring population density and disrupting mating.

Stink bugs, when disturbed, produce a pungent odor that consists of a mixture of volatiles secreted from the metathoracic scent gland (Favaro et al. 2011, 2013). Some of these volatiles serve a defensive function by discouraging predators (Krall et al. 1999, Noge et al. 2012) and are thus classified as allomones; some of these volatiles serve to repel conspecific members and are, therefore, classified as alarm pheromones. These two functions are not necessarily mutually exclusive.

Analysis of composition and function of chemicals produced by the metathoracic scent gland has been performed for a number of species in the Pentatomoidea superfamily. Although each species secretes its own specific blend of components when disturbed, closely related species may secrete identical defensive chemicals or pheromones (Borges et al. 1999). (E)-2-hexenal and *n*-tridecane are produced by disturbed *Carpocoris fuscispinus* (Boheman) (shield bug, family Pentatomidae) (Durak and Kalender 2012). (E)-2-hexenal and (E)-4-oxo-hexenal are produced by disturbed *Piezodorus guildinii* (Westwood) (red banded stink bug, family Pentatomidae) and function as alarm pheromones (Zarbin 2000). (E)-2-hexenal and (E)-decanol are produced by *Nezara viridula* (L.) (southern green stink bug, family Pentatomidae) and function as alarm pheromones (Blum 1996). Tridecane is present in secretions from both disturbed and undisturbed *Tessaratoma papillosa* (Drury) (litchi stink bug, family Tessaratomidae), whereas this stink bug secretes (E)-2-hexenal only when disturbed (Zhao et al. 2012).

Volatile secretions produced by disturbed species in the family Plataspidae have not been characterized. The purpose of this study was to conduct an initial investigation of the function and composition of the pungent odor produced by disturbed *M. cribraria*. In particular, we investigated whether this defensive secretion functions as a pheromone to attract or repel conspecific members, analyzed the volatile organic compounds (VOCs) in the secretion, and tested the electrophysiological responses of males and females to specific components of the secretion.

By discovering the pheromones produced by *M. cribraria*, an effective, efficient, and safe method to monitor and control this pest can be achieved through pheromone-baited traps. Pheromone research adds to available control options, facilitates integrated pest management, and expands our understanding of the physiology of *M. cribraria*.

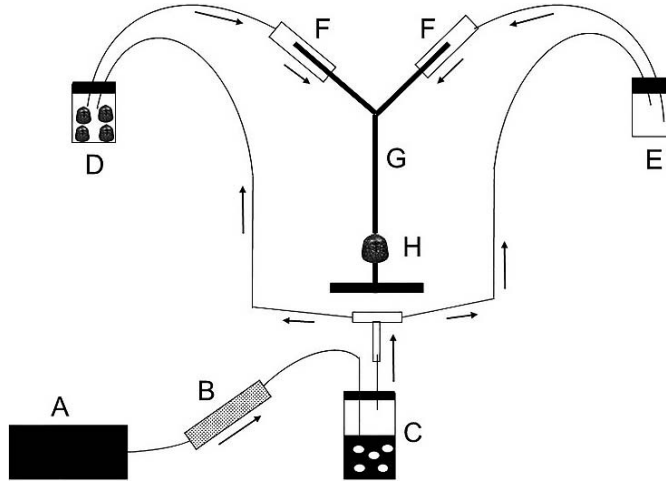

Fig. 1. Open Y-track olfactometer. (A) air pump; (B) charcoal filter; (C) humidifier with distilled water; (D) scent vial; (E) empty vial; (F) open syringe placed over each arm of the olfactometer; (G) copper tubing; (H) placement of *Megacopta cribraria* at beginning of each test. Diagram is not drawn to scale.

Materials and Methods

Collection and maintenance of *M. cribraria*. During the fall of 2012, 2013, and 2014 more than 300 *M. cribraria* were collected from a kudzu patch in Brevard, NC. *Megacopta cribraria* were maintained in a Bugdorm-2120 insect tent on a diet of kudzu and snow peas (*Pisum satium* L.). *Megacopta cribraria* were randomly selected for experimentation during late fall.

Olfactometer construction and assays. Two identical olfactometers (one for each sex) were constructed (Fig. 1). The apparatus consisted of a vertical, copper Y-shaped rod (Y-track) and a system to deliver humidified, scented air to one arm of the Y-track and humidified, unscented (control) air to the other arm. Room air was pumped via plastic tubing through a charcoal filter and a bottle containing distilled water. The filtered, humidified air was directed to either a test vial (containing either *M. cribraria* or a specific chemical) or a control vial and finally to an open glass syringe placed over each arm of the Y-track.

Assays were performed in a fume hood to minimize outside odors. A single *M. cribraria* was placed at the base of a Y-track and observed as it climbed up the vertical rod and chose one arm of the Y-track (either scent or control). After each insect, the Y-track was washed with acetone and distilled water to remove any potential pheromone trails. Specimens that did not respond within 60 s were replaced. After half of the replicates were tested, the Y-track was rotated 180 degrees to compensate for any potential Y-track asymmetry. For each trial, one olfactometer was used for males and the other was used for females. The

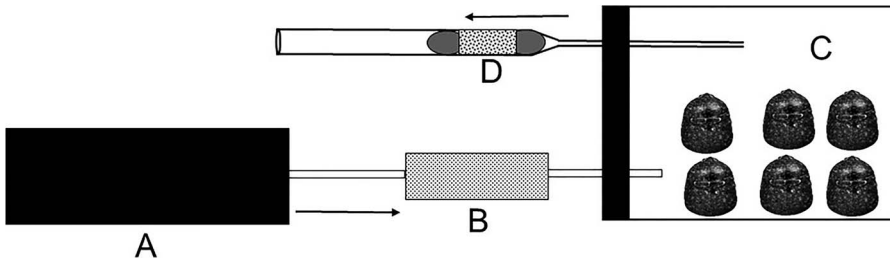

Fig. 2. Apparatus to trap volatile organic compounds emitted by *Megacopta cribraria*. (A) air pump; (B) charcoal filter; (C) volatile chamber; (D) volatile collection trap. Diagram is not drawn to scale.

olfactometer used for a specific sex was alternated for each subsequent trial. All plastic parts were replaced and glass parts rinsed with water between experiments.

Volatile collection system. All equipment was cleaned with acetone. The volatile collection trap consisted of 10 to 15 mg of HayeSep Q 80/100 (Sigma-Aldrich, St. Louis, MO) that was suspended in acetone and pipetted into a Pasteur pipette plugged with glass wool. The HayeSep Q was held in place by an additional glass wool plug. The volatile collection traps were wrapped in aluminum foil and refrigerated at 4°C until used. The volatile chamber consisted of a 10-ml glass vial fitted with a two-hole rubber stopper wrapped in Teflon tape. Room air was pumped via plastic tubing through a charcoal filter and then through a glass tube inserted through one hole of the rubber stopper in the volatile chamber. Emissions from the volatile chamber passed through the volatile collection trap that was inserted in the other hole of the rubber stopper (Fig. 2).

To collect volatiles emitted by *M. cribraria*, 12 *M. cribraria* of each sex (a total of 24 insects) were placed in a volatile chamber. For undisturbed samples, the insects were gently inserted into the chamber with a paintbrush. For the disturbed samples, the insects were individually pressed between two fingers before being inserted into the chamber. The negative control volatile chamber was empty. Volatiles were collected for 4 h in a fume hood. Collection traps were removed and stored in sealed bags on dry ice for 2 h.

Coupled gas chromatography–mass spectrometry (GC-MS) analysis of volatiles. Volatiles adsorbed by HayeSep Q were eluted with hexane and analyzed using a gas chromatograph (Agilent 6850 series) fitted with a ZB-5MSi column (30 m $\times$ 0.25 mm inner diameter $\times$ 0.25 μm film, 5% phenyl/95% dimethylpolysiloxane) (Phenomenex Inc.) and interfaced to an Agilent 5973 mass selective detector (70 eV; BioNetwork Asheville, Asheville-Buncombe Technical Community College, Candler, NC). The GC temperature program was 50°C/2 min, ramped 5°C/min to 250°C. One microliter of solution was introduced into a splitless injection inlet at 250°C, and transfer line temperature was 280°C. Helium was used as the carrier gas. Mass spectra were compared to the NIST 05 database. Identifications of (E)-2-hexenal and tridecane based on mass spectrometry were verified by comparison and coinjection with standards.

Electroantennographic assays. A diagram of the apparatus is shown in Fig. 3. The reference electrode consisted of a sanded silver wire inserted into a severed

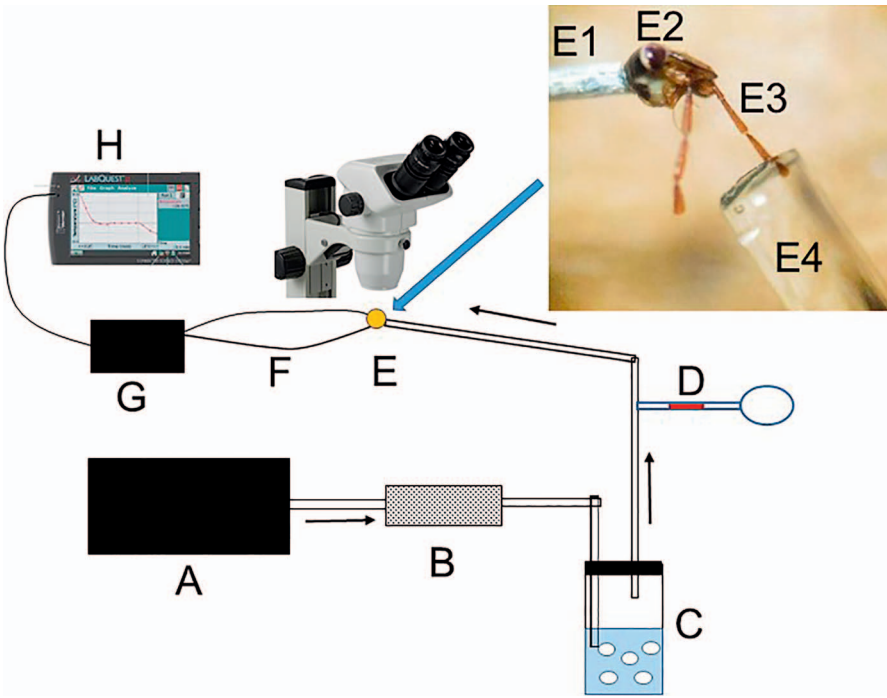

Fig. 3. Diagram of the apparatus for the electroantennographic assays. (A) air pump; (B) charcoal filter; (C) humidifier with distilled water; (D) puffer with filter paper containing volatile chemical; (E1) electrode of sanded silver wire inserted into (E2) the insect's head; (E3) clipped antenna inserted into (E4) electrode containing electrically conductive gel; (F) wires from electrodes to (G)Vernier EKG sensor; (H) Vernier labquest data recorder. Diagram is not drawn to scale.

head from *M. cribraria*. The tip of one antenna (with the distal half of the third segment removed) attached to the severed head was inserted into the recording electrode, which consisted of silver wire inserted into a glass capillary filled with a conducting gel. A stereomicroscope at 30× magnification was used to observe the insertion of the electrodes. A Vernier EKG Sensor (Vernier Software and Technology, Portland, OR) was used to measure the potential difference in millivolts. The signal was recorded on a Vernier LabQuest data logger.

A constant stream of filtered, humidified room air was directed onto the antenna. Baseline measurements were taken for 3 s to measure the "noise" potential before exposure of the antenna to a chemical stimulus using a puffer. The puffer consisted of a Pasteur pipette with a bulb. The tip of the puffer was inserted into the air-stream tubing. A piece of filter paper (8 × 60 mm) loaded with 25 μl of paraffin oil (catalog no. 18512, Sigma-Aldrich) or 25 μl of a 10:1 solution of paraffin oil and test chemical was inserted into the puffer. For a trial with each head, an antenna was first exposed to a puff of room air to establish the amplitude of the noise potential, then

to a puff of paraffin oil, and then to 3 or 4 puffs of paraffin oil plus test chemical. The number and sex of heads used for each test chemical are indicated in the results.

To determine the amplitude of the noise potential, the lowest noise potential was subtracted from the highest noise potential. To determine the amplitude of the induced-response potential, the lowest response potential was subtracted from the highest response potential. To calculate the net amplitude of the induced-response potential (reported here as the EAG response), the amplitude of the noise potential was subtracted from the amplitude of the induced-response potential. The EAG responses (in millivolts) were averaged for each trial.

Reagents. Trans-2-hexenal 99% (no. 158130250, ACROS Organics) and *n*-tridecane (99+%, no. 139510250, ACROS Organics) were purchased from Thermo-Fisher Scientific (Waltham, MA). Paraffin oil (no. 18512) was purchased from Sigma-Aldrich.

Statistical analyses. Data from the olfactometer assays were analyzed using a one-proportion *Z* test. Data from the electroantennographic assays were analyzed using a *t* test for two independent means. The conventional standard of statistical significance at a *P* value less than 0.05 was used.

Results

Sex-specific behavioral response of *M. cribraria* to scents from disturbed *M. cribraria*. Based on the knowledge that *M. cribraria* releases a pungent odor when disturbed, we tested the hypothesis that this odor contains communicative volatiles. Open Y-track olfactometer assays were conducted to determine female and male *M. cribraria* responses to scents from disturbed *M. cribraria*. Eight females and eight males were each pressed between two fingers to create the disturbed scent and then placed in the scent container. A container with no insects was used as the negative control. The responses of 60 *M. cribraria* of each sex to volatiles from the scented container and the control container were tested in a series of four trials with 15 insects of each sex per trial (Fig. 4). Approximately 78% of the female *M. cribraria* chose the arm that led to the scent from disturbed *M. cribraria*. This statistically significant result ($P = 0.008$) indicates that females are attracted to volatiles in the scent from disturbed *M. cribraria*. In contrast, only 57% of the males chose the arm that led to scent from disturbed insects. This choice was not statistically significant ($P = 0.189$) and indicates that males are neither attracted to nor repelled by volatiles in the scent from disturbed *M. cribraria*.

Analysis of VOCs. VOCs were collected from undisturbed and disturbed *M. cribraria* and analyzed by coupled GC-MS. Of the numerous VOCs detected by the gas chromatograph with retention times of 3 to 41 min, seven were identified based on matches in the NIST 05 database (Table 1). Surprisingly, the total abundance of VOCs in the disturbed and undisturbed samples was similar (data not shown). By far, tridecane was the most abundant VOC in both samples; E-2-decenal was the second most abundant VOC in both samples (Table 1). These two compounds represented approximately 80% of the VOCs in each sample; no other compound represented more than 3% of the VOCs in either sample. Octacosane (2.89%), decamethyl cyclopentasiloxane (0.93%), benzothiazole (0.76%), and 2-ethylhex-

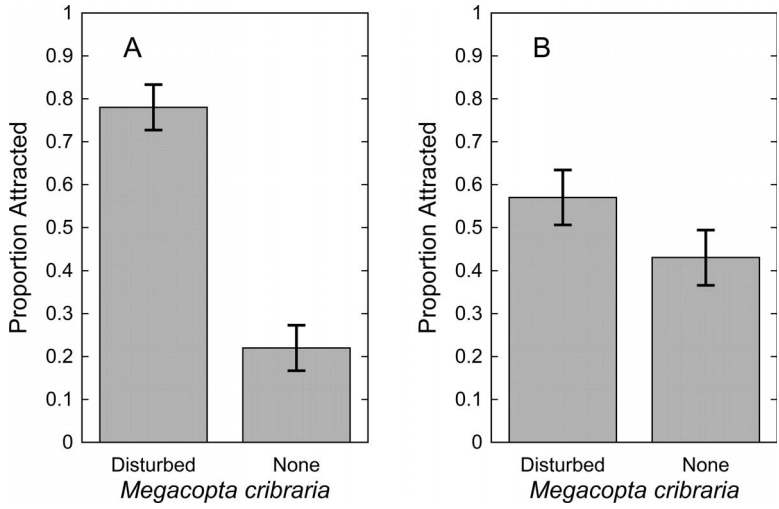

Fig. 4. Responses of female (panel A) and male (panel B) *Megacopta cribraria* to volatiles produced by disturbed *M. cribraria*. Assays were conducted with a Y-arm olfactometer. One arm of the olfactometer led to volatiles from a container with 16 disturbed *M. cribraria* (eight males and eight females). The other arm led to emissions from a container with no insects. Error bars represent a 95% confidence interval based on $\pm$ 1.96 $\times$ SE (standard error).

anol (0.15%) were present in the sample from undisturbed *M. cribraria* but notably absent from disturbed *M. cribraria*.

The most abundant compound (0.24% of total) that was unique to the VOCs from disturbed *M. cribraria* eluted with a retention time of 4.782 min (Fig. 5, peak A). Analysis of this peak by mass spectrometry revealed a 91% match with (E)-2-hexenal in the NIST 05 database. A pure sample of (E)-2-hexenal eluted with a retention time of 4.685 min (data not shown) and showed a 96% match with (E)-2-hexenal in the NIST 05 database. A compound in both the disturbed sample (Fig. 5, peak B) and the undisturbed sample (Fig. 5, peak C) eluted with a retention time of 4.802 min. There was no match in the NIST 05 database for the mass spectrum of peak C.

Sex-specific behavioral response of *M. cribraria* to (E)-2-hexenal. Based on our observation that (E)-2-hexenal is the predominant VOC that is unique to emissions from disturbed *M. cribraria*, we decided to test the hypothesis that (E)-2-hexenal attracts female *M. cribraria*. To test this hypothesis, open Y-track olfactometer assays were conducted to determine the response of *M. cribraria* to (E)-2-hexenal (10 μl) placed in the scent container. An empty container was used as the negative control. The responses of 64 *M. cribraria* of each sex to (E)-2-hexenal were tested in a series of four trials (Fig. 6). Approximately 70% of the female *M. cribraria* chose the arm that led to the scent from (E)-2-hexenal. This statistically significant result ($P = 0.001$) indicates that females are attracted to (E)-2-hexenal. In contrast, only 45% of the males chose the arm with (E)-2-hexenal.

Table 1. Volatile organic compounds emitted by *Megacopta cribraria* and identified by mass spectroscopy.

Retention Time (min) Disturbed/ Undisturbed	*Megacopta cribraria*			
	Disturbed (% of total)	Undisturbed (% of total)	Database Match (%)	Compound
4.782/———	0.24	<0.05	91	(E)-2-hexenal
———/9.384	<0.05	0.15	83	2-Ethylhexanol
———/13.013	<0.05	0.93	91	Decamethyl cyclopentasiloxane
———/14.885	<0.05	0.76	91	Benzothiazole
15.902/15.909	14.25	16.89	87	E-2-decenal
16.952/16.959	66.33	61.08	95	Tridecane
———/38.658	<0.05	2.89	91	Octacosane

This choice was not statistically significant ($P = 0.45$) and indicates that males are neither attracted to nor repelled by (E)-2-hexenal.

Because tridecane was the major component in the emissions from both disturbed and undisturbed *M. cribraria*, we predicted neither sex would exhibit any preference for this compound. When tested in a series of olfactometer assays, approximately 44% of the female *M. cribraria* and 45% of male *M. cribraria* chose the arm that led to tridecane volatiles. These statistically insignificant results (data not shown; $P = 0.32$) indicate that females and males are indifferent to tridecane.

Response of *M. cribraria* antennae to (E)-2-hexenal. Because pheromone receptors are largely located in insect antennae, electroantennography provides evidence of communicative chemicals. We constructed an apparatus (Fig. 3) to measure the response (defined as a change in voltage) induced by brief exposure of male and female *M. cribraria* antennae to a VOC. That response was compared to the response induced by paraffin oil (control), which was used as a carrier for each VOC. The net amplitude of the induced-response potential (referred to here as the EAG response) was calculated as previously described.

The average of the responses of 10 female antennae and 6 male antennae to (E)-2-hexenal and paraffin oil are shown in Fig. 7. The females exhibited a statistically significant ($t = 1.72$; df = 9; $P = 0.047$) response to (E)-2-hexenal whereas the males did not ($t = 0.116$; df = 5; $P = 0.454$). When the responses of eight females and six male antennae to (E)-2-decenal were measured, no significant response was observed for females ($t = 0.453$; df = 7; $P = 0.326$) or for males ($t = 0.070$, df = 5, $P = 0.472$). Similarly, no significant response was observed when the responses of four female ($t = 0.819$; df = 3; $P = 0.211$) and five male antennae ($t = 0.138$, df = 4, $P = 0.446$) to tridecane were measured.

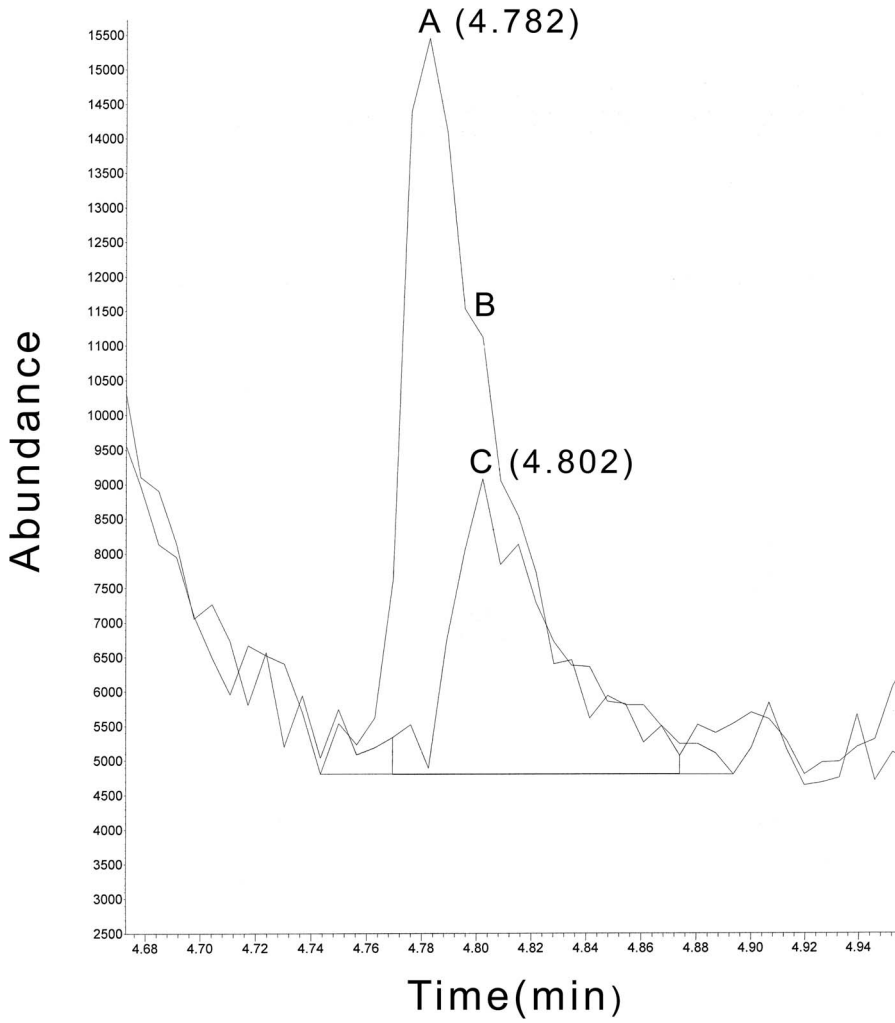

Fig 5. Gas chromatographic analysis of volatile organic compounds from disturbed (peaks A and B) and undisturbed (peak C) *Megacopta cribraria*. Profile of compounds with retention times of 4.68 to 4.96 min.

Discussion

The results of the olfactometer assays (Fig. 4) with volatiles from disturbed *M. cribraria* demonstrated that female *M. cribraria* are attracted to volatiles emitted by disturbed *M. cribraria*, whereas males are neither attracted nor repelled by this mixture of volatiles. The GC-MS data revealed that (E)-2-hexenal is a component of volatile emissions from disturbed *M. cribraria* but is not present at a detectable level in volatile emissions from undisturbed *M. cribraria*. The GC-MS data also revealed that tridecane and (E)-2-decenal are the major components of volatiles emitted by

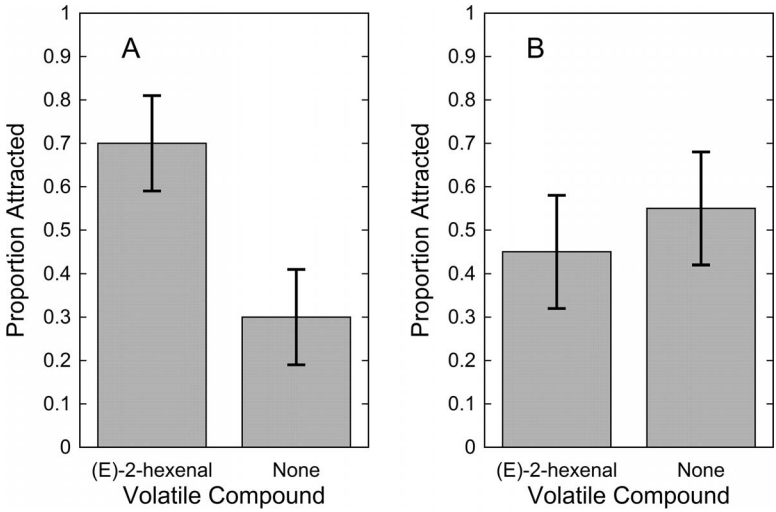

Fig. 6. Responses of female (panel A) and male (panel B) *Megacopta cribraria* to (E)-2-hexenal. Assays were conducted with a Y-arm olfactometer. One arm of the olfactometer led to vial containing (E)-2-hexenal. The other arm led to an empty vial. Error bars represent a 95% confidence interval based on $\pm$ 1.96 $\times$ SE (standard error).

both disturbed and undisturbed *M. cribraria*. The results of the olfactometer assays with (E)-2-hexenal demonstrated that female *M. cribraria* are attracted to (E)-2-hexenal, whereas males are not. Olfactometer assays demonstrated that neither sex was attracted to tridecane. EAG results revealed a female response to (E)-2-hexenal and no response by either sex to (E)-2-decenal or tridecane. Together, these data provide evidence that (E)-2-hexenal is a female-attracting pheromone (Kikuyama et al. 1995) secreted by disturbed *M. cribraria*. The female-specific attraction to volatiles from disturbed *M. cribraria* is of particular interest because, to the best of our knowledge, sex-specific attraction to volatiles produced by other disturbed members of the Pentatomoidea superfamily has not been reported.

(E)-2-decenal was investigated due to its presence in the disturbed *M. cribraria* volatiles, its relatively large proportion of the volatiles (approximately 15%), and its structural similarity to (E)-2-hexenal. However, (E)-2-decenal did not induce a significant voltage response in female antennae. Tridecane was investigated due to its prevalence in secretions from members of the family Pentatomidae and because it was the most abundant VOC produced by *M. cribraria* (Table 1). Our assays detected no response of *M. cribraria* to tridecane. Gunawardena and Herath (1991) reported that tridecane enhances the activity of (E)-2-hexenal as a fumigant and repellent, perhaps by regulating the evaporation of this volatile compound.

Further pheromone research is warranted due to the rapid territorial expansion of *M. cribraria* and its threat to soybean production in the United States. Future research on pheromones produced by *M. cribraria* should include electroantennographic assays to determine the response of each sex of *M. cribraria* to each of the

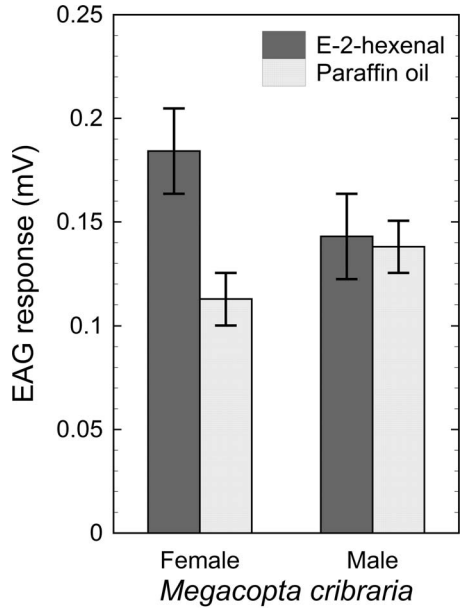

Fig. 7. Electroantennographic analysis of responses of female and male *Megacopta cribraria* antennae to (E)-2-hexenal. The net amplitude of the induced-response potential response (mV) is shown. Error bars represent standard error of the mean.

defensive secretion compounds. In addition, the chemical concentration effect on behavioral response to the defensive secretion should be determined. GC-MS should be used to analyze and compare the female and male volatiles to discern which sex, if not both, is producing the communicative compounds. The electrical and behavioral response should also be examined during each season as communication among *M. cribraria* may vary with season, as observed for the brown marmorated stink bug *Halyomorpha halys* (Stal) (Funayama 2008, Leskey et al. 2012). The effectiveness of a pheromone-baited trap for monitoring *M. cribraria* in soybean fields needs to be tested using (E)-2-hexenal as the bait.

This study identified (E)-2-hexenal as a female-attracting *M. cribraria* pheromone that could be used in an insect trap for environmentally friendly monitoring and integrated pest management. Monitoring the movement of this pest from kudzu to soybean fields can assist in determining the timing for application of pesticides and other control measures. The development of a pheromone-baited trap is an important step in effectively managing this invasive species and soybean pest.

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References Cited

Blum, M. 1996. Semiochemical parsimony in the Arthropoda. Annu. Rev. Entomol. 41: 353–374.

Borges, M., M.C.B. Moraes, M.F. Peixoto, C.S.S. Pires, E.R. Sujii and R.A. Laumann. 2011. Monitoring the neotropical brown stink bug *Euschistus heros* (F.) (Hemiptera: Pentatomidae) with pheromone-baited traps in soybean fields. J. Appl. Entomol. 135: 68–80.

Borges, M., P.H.G. Zarbin, J.T.B. Ferreira and M.L.M. da Costa. 1999. Pheromone sharing: blends based on the same compounds for *Euschistus heros* and *Piezodorus guildinii*. J. Chem. Ecol. 25: 629–634.

Durak, D. and Y. Kalender. 2012. Structure and chemical analysis of the metathoracic scent glands of *Carpocoris fuscispinus* (Boheman, 1851) (Heteroptera: Pentatomidae) from Turkey. Turk. J. Zool. 36: 526–533.

Eger, J.E., Jr., L.M. Ames, D.R. Suiter, T.M. Jenkins, D.A. Rider and S.E. Halbert. 2010. Occurrence of the Old World bug *Megacopta cribraria* (Fabricius) (Heteroptera: Plataspidae) in Georgia: A serious home invader and potential legume pest. Insecta Mundi 121: 1–11.

Favaro, C.F., A.C. Mauro, M. Rodrigues, J.R. Aldrich and P.H.G. Zarbin. 2011. Identification of semiochemicals in adults and nymphs of the stink bug *Pallantia macunaima* Grazia (Hemiptera: Pentatomidae). J. Braz. Chem. Soc. 22: 58–64.

Favaro, C.F. and P.H.G. Zarbin. 2013. Identification of (Z)-4- and 1-tridecene in the metathoracic gland secretions of stink bugs employing the GC/FT-IR technique. J. Chem. Ecol. 39: 1182–1185.

Funayama, K. 2008. Seasonal fluctuations and physiological status of *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae) adults captured in traps baited with synthetic aggregation pheromone of *Plautia crossota stali* Scott (Heteroptera: Pentatomidae). Jpn. J. Appl. Entomol. Zool. 52: 69–75.

Gunawardena, N.E. and H.M. Herath. 1991. Significance of medium chain n-alkanes as accompanying compounds in hemipteran defensive secretions: An investigation based on the defensive secretion of *Coridius janus*. J. Chem. Ecol. 17: 2449–2258.

Kikuyama, S., F. Toyoda, Y. Ohmiya, K. Matsuda, S. Tanaka and H. Hayashi. 1995. Sodefrin: A female-attracting peptide pheromone in newt cloacal glands. Science 267: 1643–1645.

Krall, B.S., R.J. Bartelt, C.J. Lewis and D.W. Whitman. 1999. Chemical defense in the stink bug *Cosmopepla bimaculata*. J. Chem. Ecol. 25: 2477–2494.

Leskey, T.C., S.E. Wright, B.D. Short and A. Khrimian. 2012. Development of behaviorally-based monitoring tools for the brown marmorated stink bug (Heteroptera: Pentatomidae) in commercial tree fruit orchards. J. Entomol. Sci. 47: 76–85.

Noge, K., K.L. Prudic, and J.X. Becerra. 2012. Defensive roles of (E)-2-alkenals and related compounds in Heteroptera. J. Chem. Ecol. 38: 1050–1056.

Seiter, N.J., J.K. Greene and F.P.F. Reay-Jones. 2013. Reduction of soybean yield components by *Megacopta cribraria* (Hemiptera: Plataspidae). J. Econ. Entomol. 106: 1676–1683.

Seiter, N.J., A.I. del Pozo-Valdivia, J.K. Greene, F.P.F. Reay-Jones, P.M. Roberts and D.R. Reisig. 2015a. Action thresholds for managing *Megacopta cribraria*. J. Econ. Entomol. 108: 1818–1829.

Seiter, N., J.K. Greene, F.P.F. Reay-Jones, P.M. Roberts and J.N. All. 2015b. Insecticidal control of *Megacopta cribraria* (Hemiptera: Plataspidae) in soybean. J. Entomol. Sci. 50: 263–283.

Suiter, D.R., J.E. Eger, Jr,. W.A. Gardner, R.C. Kemerait, J.N. All, P.M. Roberts, J.K. Greene, L.M. Ames, G.D. Buntin, T.M. Jenkins and G.K. Douce. 2010. Discovery and distribution of *Megacopta cribraria* (Hemiptera: Heteroptera: Plataspidae) in northeast Georgia. J. Integ. Pest Mngmt. 1: 1–4.

University of Georgia Center for Invasive Species and Ecosystem Health. 2016. Distribution map. Kudzu bug. 10 August 2016. (www.kudzubug.org/distribution-map).

Zarbin, P.H.G., M. Borges, A.A. dos Santos, A.R.M. de Oliveira, F. Simonelli and F. de A. Marques. 2000. Alarm pheromone system of stink bug *Piezodorus guildinii* (Heteroptera: Pentatomidae). J. Braz. Chem. Soc. 11: 424–428.

Zhao, D., J. Gao, Y. Wang, J. Jiang and R. Li. 2012. Morphology and volatile compounds of metathoracic scent gland in *Tessaratoma papillosa* (Drury) (Hemiptera: Tessaratomidae). Neotrop. Entomol. 41: 278–282.