Sex Pheromone of the Soybean Aphid, *Aphis glycines* Matsumura, and Its Potential Use in Semiochemical-Based Control

JUNWEI ZHU,[1, 2] AIJUN ZHANG,[4] KYE-CHUNG PARK,[3] TOM BAKER,[1] BRIAN LANG,[5] RUSSELL JURENKA,[3] JOHN J. OBRYCKI,[3, 6] WILLIAM R. GRAVES,[7] J. A. PICKETT,[8] D. SMILEY,[8] KAMLESH R. CHAUHAN,[4] AND JEROME A. KLUN[4]

Environ. Entomol. 35(2): 249–257 (2006)

ABSTRACT The newly invasive soybean aphid, *Aphis glycines* Matsumura, has seriously threatened soybean production in North America, after having spread to >20 states in the United States and several southern provinces of Canada. Control of *A. glycines* has focused on applications of insecticides, which are not a long-term solution to soybean aphid pest management. In autumn, soybean aphids start producing alate females (gynoparae) that search for their overwintering host plants, the common buckthorn, *Rhamnus cathartica*. The gynoparae then produce pheromone-emitting wingless female offspring (oviparae) that attract male aphids. In this study, we report the chemical identification of the soybean aphid sex pheromone using gas chromatography–electroantennogram, gas chromatography–mass spectrometry, and nuclear magnetic resonance spectroscopy. Behavioral activities of males and gynoparous females in the field were also characterized. The potential applications using formulations containing specific soybean aphid pheromone compositions for reducing overwintering populations are discussed.

KEY WORDS sex pheromone, artificial induction of pheromone-emitting females, (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol, (4a*S*,7*S*,7a*R*)-nepetalactone, field trapping

THE SOYBEAN APHID, *Aphis glycines* Matsumura, is a newly invasive insect species that seriously threatens U.S. soybean production. It is the only aphid species that develops large colonies on soybeans, *Glycine max*, in North America (Ragsdale et al. 2004). Since its first appearance in Wisconsin in 2000, it has spread to >20 U.S. states and southern provinces of Canada (soybean aphid watch 2005). Infestations of *A. glycines* reduce plant growth so fewer pods and seeds develop, thereby lowering yields (data from Midwest Soybean Aphid Workshop, 5 February 2004). Soybean aphids also transmit several plant viruses, including alfalfa mosaic, soybean mosaic, soybean dwarf, soybean stunt, and bean yellow mosaic (van-den-Berg et al. 1997, Clark and Perry 2002, Wang and Ghabrial 2002). These viruses distort soybean growth and further reduce yields.

Aphis glycines have a complex life cycle with >15 generations per season that feed and reproduce on its secondary plant host, soybeans (Zhang and Zhong 1982). In the fall, *A. glycines* begin producing winged female aphids (gynoparae) that fly from soybean fields, searching for their overwintering host plant, buckthorns, *Rhamnus* spp. (primary host). In Asia, the most common overwintering hosts are *R. davurica* and *R. japonica* (Zhang and Zhong 1983, Takahashi et al. 1993). In North America, several *Rhamnus* species are used as a primary host (Ragsdale et al. 2004, Voegtlin et al. 2004). Once on overwintering hosts, gynoparae produce pheromone-emitting wingless female offspring (oviparae). Alate males are attracted to oviparae through a specific sex pheromone blend produced from glands on the hind legs of female aphids (Pettersson 1970, 1971). The pheromones have been reported from several other aphid species (Pickett et al. 1992). After mating, oviparae lay eggs that overwinter on the buckthorn. The generation involving males and oviparae occurred in the autumn is the only generation that uses sex pheromones for mate finding during the entire season.

Thus far, pheromones have been identified from <15 aphid species worldwide (Dawson et al. 1990, Pickett et al. 1992, Boo et al. 2000, Goldansaz et al. 2004). All reported aphid pheromone structures are

[1] MSTRS Technologies, Inc., 2501 North Loop Dr., Ames, IA 50010.
[2] Corresponding author. e-mail: jwzhu@iastate.edu.
[3] Department of Entomology, Iowa State University, Ames, IA 50011.
[4] USDA–ARS Plant Sciences Institute, Chemicals Affecting Insect Behavior Laboratory, BARC-West, 10300 Baltimore Ave., Beltsville, MD 20705–2350.
[5] Iowa State University Extension, Decorah, IA 52101.
[6] Department of Entomology, University of Kentucky, Lexington, KY 40546.
[7] Department of Horticulture, Iowa State University, Ames, IA 50011.
[8] Biological Chemistry Division, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK.

0046-225X/06/0249–0257$04.00/0

comprised of either a mixture of the monoterpenoids (4a*S*,7*S*,7a*R*)-nepetalactone and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol or one of these two compounds alone, except the damson-hop aphid, *Phorodon humili*, which uses only (1*S* and *R*,4a*R*,7*S*,7a*S*)-nepetalactol as its pheromone (Birkett and Pickett 2003). The pheromone of two *Aphis* species, closely related to the soybean aphid, is comprised of different blend ratios of (4a*S*,7*S*,7a*R*)-nepetalactone and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (Campbell et al. 1990, Dawson et al. 1990, Pickett et al. 1992). The ratios of these two compounds in aphid sex pheromone communication seems extremely important, because specificity in male response relies on the species-specific ratio that occurs in several sympatric species (Hardie et al. 1992, Boo et al. 2000).

This study reports on the chemical identification of the sex pheromone of the soybean aphid and behavioral responses of male and gynoparous soybean aphids to synthetic pheromones. We also discuss their potential uses in pheromone mass trapping and mating disruption for suppressing the overwintering population, therefore reducing the damage on soybeans in the next growing season.

Materials and Methods

Insects. Soybean aphids collected from soybean fields (Ames, IA) were raised on potted soybean plants. They were initially kept in a growth chamber maintained at 25 ± 2°C and under L:D, 16:8 h photoperiod as a stock colony producing only asexual, parthenogenetic females. To induce sexual forms of soybean aphids in the laboratory, 10 boxes of 20 winged or wingless asexual females were transferred and reared on individual soybean plants under a short-day photoperiod (L:D, 10:14 h) and temperature program (L:D, 20:12°C) that mimics late autumn conditions in the upper midwest of the United States. The progeny from these aphids was checked every week and segregated by age throughout the next 4–5 wk until winged aphids emerged. After the alate females (gynoparae) were observed, buckthorn leaves (*Rhamnus carthartica*) were provided, and the alate females gave birth to the sexually active oviparae that start producing sex pheromone. The later emerging male aphids (winged) that were also produced under these conditions were transferred into individual boxes and kept for electroantennogram and gas chromatography-electroantennogram (EAG and GC-EAD) analyses.

Pheromone Collection and Purification. Air entrainment was used for collecting putative sex pheromone components from oviparae. Thirty mature oviparae, maintained on buckthorn leaves with the ends of cut branches held in water-filled test tubes, were placed in a ventilated, 960-ml wide-mouth glass bottle. Activated charcoal–filtered air was drawn from the container at a rate of 0.5 liters/min. Volatiles were entrained onto an adsorbent collector system comprised of a glass tube containing 250 mg of Super Q, 80/100 mesh (Alltech, Deerfield, IL). The collection lasted for 6 h, and volatiles were desorbed by eluting with 3 ml of distilled hexane. The hexane eluent was concentrated to a volume of ≈50 μl. Pheromones from >500 laboratory-induced oviparous soybean aphids were collected for further analytical and electrophysiological analyses.

To accumulate pure forms of the two soybean aphid pheromone compounds, (4a*S*,7*S*,7a*R*)-nepetalactone and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol, we used a micropreparative GC fractionation system previously described in Zhang et al. (2004). A Hewlett-Packard 6890 gas chromatograph combined with a Gerstel preparative fraction collector (Gerstel, Baltimore, MD) was equipped with a 60 m by 0.53-mm ID, 0.50-μm film thickness DB-1 capillary column (J&W Scientific, Folsom, CA). Injector temperature was 32°C and increased to 230°C at 60°C/min to transfer solute onto the column; column temperature was held at 50°C for 2 min and then programmed to 250°C at 30°C/min and held for 10 min. Split ratio of column effluent to flame ion detection (FID) and fraction collector was set at 4:96. The collector was cooled to −20°C by circulating MeOH from a benchtop refrigeration unit (Julabo F25-MP; Julabo USA, Mertztown, PA). The collection efficiency was ≈70%.

Electrophysiological and Chemical Analyses. For each collected pheromone sample, 2–3 μl was analyzed on both a DB-5 and DB-225 column (30 m by 0.25 mm ID; J & W Scientific) in a Hewlett-Packard 5890 Series II gas chromatograph interfaced to a Hewlett-Packard 5972 Mass Selective Detector (GC-MS). The injector temperature was set at 200°C, and the split valve was opened 1 min after injection. The column temperature started at 40°C for 1 min after the injection and then linearly increased to 250°C at a rate of 5°C/min. Mass spectra were recorded from 30 to 450 a.m.u. after electronic impact ionization at 70 eV. The chemical structures of the putative components were tentatively identified by comparison of retention times and mass spectra with those of authenticated chemical standards and reference spectra in a mass spectral library (Wiley 138K; John Wiley and Sons, New York, NY).

To ensure that possible additional components were not missed, we also performed combined GC/EAGs on the airborne-collected extract to determine if other compounds might be present to which male antennal neurons can detect. For both GC-EAG and EAG experiments, we adopted an improved aphid EAG technique (Park and Hardie 1998) that includes the whole intact body of the soybean aphid. This technique resulted in a highly improved signal-to-noise ratio and greater sensitivity. The GC-EAG hardware consisted of a Hewlett-Packard 5890 Series II gas chromatograph equipped with the same columns described above. An effluent split allowed simultaneous flame ionization (FID) and EAG of pheromone components. Helium was used as the carrier gas with a flow rate of ≈30 ml/min, and the effluent split ratio was maintained at 1:1. Aphid pheromone extracts were injected in splitless mode. The GC temperature program was the same as the GC-MS analysis described above. The outlet for the EAG was continuously sup-

plied with a purified, humidified air stream flowing over the antennal preparation at a speed of 0.5 m/s. A restrained soybean aphid was mounted on a plastic base using thin copper wire restraints. A capillary recording Ag-AgCl electrode filled with saline (0.1 M KCl solution) was inserted into one of the aphid's compound eyes and used as the reference (ground) electrode, while the other electrode filled with the same solution was inserted into the intersegmental membrane between the third and the fourth antennal segments. The EAG setup consisted of a high-impedance DC amplifier with automatic baseline drift compensation, and a GC-EAG program (GC/EAG version 2.4; Syntech, Hilversum, The Netherlands) was used to record and analyze the amplified EAG and FID signals on a PC computer.

In the EAG dose–response tests, stimulus cartridges consisted of Pasteur pipettes containing a piece of filter paper (8 by 15 mm) on which a stimulus had been applied. Serial dilutions (0.001–10 mg in decadic steps) of the two aphid pheromone compounds dissolved in 10 μl of hexane were applied. A control puff from a cartridge with just hexane was applied after each puff of a tested stimulus. The sequence of exposure to the stimulus compound on each antenna proceeded from the lowest to the highest concentration.

Chemicals. The two aphid pheromone compounds, (4a*S*,7*S*,7a*R*)-nepetalactone and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol, were synthesized at Rothamsted Research Laboratory, UK, and USDA-ARS Beltsville Laboratory. The purity of these two compounds was ≈99 and 93% as analyzed by GC-MS, respectively.

Nuclear Magnetic Resonance Spectroscopy. The extracts of several airborne collections were combined, and the two pheromone candidate compounds were further purified using a micro-preparative GC fractionation system for stereochemistry determination before nuclear magnetic resonance (NMR) analyses. NMR spectra were recorded in a C_6D_6 solution on a Bruker QE Plus spectrometer at 300 MHz for ^1H and 75 MHz for ^{13}C. The chemical shifts are expressed in ppm (δ scale) relative to the residual solvent for ^1H (C_6H_6 at δ 7.20) or to the central peak of solvent ^{13}C signal (C_6D_6 at 128.5). (4a*S*,7*S*,7a*R*)-Nepetalactone: ^1H NMR (C_6D_6): δ 0.69–0.80 (2H, m), 1.03 (^3H, d, J = 6.44 Hz, $CHCH_3$), 1.14 (^3H, br s, CH_3), 1.51–1.40 (2H, m), 2.01–2.05 (2H, m), 2.08–2.21 (1H, m), 5.82 (1H, br m, = CH); ^{13}C NMR (C_6D_6): δ 169.45, 134.12, 114.68, 49.39, 40.76, 39.70, 33.05, 30.95, 20.33, 15.30. (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol: ^1H NMR (C_6D_6): δ 0.75–0.92 (1H, m), 0.95 (^3H, d, J = 6.44 Hz, $CHCH_3$), 1.05 (1H, m), 1.41 (^3H, br s, CH_3), 1.46–1.55 (1H, m), 1.60–1.75 (2H, m), 1.80–1.90 (1H, m), 2.09 (1H, OH, d, J = 5.91 Hz), 2.23 (1H, q, J = 8.05 Hz), 4.61 (1H, t, J = 5.91 Hz, O-CH-O), 6.07 (1H, br s, = CH); ^{13}C NMR (C_6D_6): δ 135.18, 113.21, 94.94, 50.73, 39.48, 36.15, 33.63, 31.24, 20.83, 16.42.

Field Trapping Test. In September and October of the 2001 and 2002 seasons, field trapping tests were conducted in three soybean fields (Iowa State University Farms, Ames, IA). The ability to trap males using either single or different ratio blends of aphid pheromone compounds was measured in soybean fields of 6,000–12,000 m^2. There were three to five replicates in each field, with a total number of 10 replicates. Water traps were constructed using yellow plastic bowls (250 ml; Solo Cup Co., Wheeling, IL) for collecting aphids. Each trap contained ≈100 ml of water and two drops of odorless detergent. Traps were set 1 m above the ground. The two soybean aphid pheromone components were protected from UV degradation by using brown borosilicate vials (Chromacol, Trumbull, CT). Pheromones were emitted through a 1-mm-diameter hole drilled in the plastic cap of the vial. The lures were suspended centrally 3–4 cm above the water level. Traps were deployed on the field edges and inside soybean fields. Trap positions were spaced 10 m apart. Traps were checked three times a week, and within each replicate, trap position was randomized to minimize the effects of habitat heterogeneity. The captured aphids from each trap were transferred to petri dishes and brought back to the laboratory for counting and species identification.

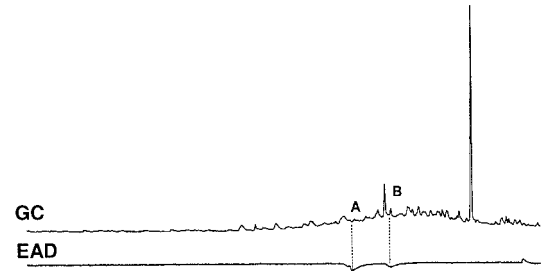

Fig. 1. GC-EAD analyses using a DB-5 column of extracts of soybean aphid sex pheromone entrainment on antennae of conspecific males.

Soybean aphids caught in the pheromone traps were identified based on characters (caudal setae and sensoria on the antennae) of specimens (preserved in 90% alcohol) under a Olympus dissecting microscope (Olympus, Melville, NY), using keys published in Zhang and Zhong (1983), Takahashi et al. (1993), and Voegtlin et al. (2004). Although other aphid species were caught in the pheromone traps, their identities were not confirmed because of the complexity of aphid species identification.

Statistical Analysis. Unless otherwise mentioned, aphid captures (means) were compared by one-way analysis of variance (ANOVA) followed by Fisher's PLSD test (SPSS 10.0 for Windows) for significance at $\alpha = 0.05$.

Results

Soybean Aphid Pheromone Identification and Electrophysiology. GC/EAG analyses of extracts from the airborne collection of calling oviparae on male antennae of soybean aphids revealed two EAG active peaks (Fig. 1). The GC-MS analyses of the same extract determined the mass spectra of peak A: 168 (81), 135

Table 1. Comparisons of pheromone titers and blend ratios between oviparae of laboratory-induced and field-collected soybean aphids (SBA)

SBA sources	Pheromone titers (ng/h/ovipara ± SD)		Pheromone ratios (% ± SD)	
	Nepetalactone	Nepetalactone	Nepetalactol	Nepetalactone
Laboratory-induced	0.38 ± 0.13	0.82 ± 0.27	32 ± 0.7	68 ± 0.6
Field-collected	0.59 ± 0.03	1.14 ± 0.06	34 ± 0.2	66 ± 0.2

Data based on analyses of extracts of pheromone-producing oviparae from laboratory-induced colony or collected from their winter host plants, buckthorn (*Rhamnus cathartica*), in Ames, IA. For laboratory-induced colony, pheromones from a total of 106 calling females in three batches were analyzed. Approximately 60 field-collected calling females in two batches were extracted. Student *t*-test, $N = 2–3$, pheromone titers: nepetalactone, $t = 0.93$, $P > 0.05$; nepetalactol, $t = 1.27$, $P > 0.05$, pheromone ratios: nepetalactone and nepetalactol, $t = 1.99$, $P > 0.05$.

(94), 97 (82), 84 (79), 81 (56), 71 (88), 67 (52), 58 (59), 55 (66), 43 (77), 41 (100). The GC-MS analyses of the same extract determined the mass spectra of peak B: 166 (78), 151 (8), 138 (17), 123 (83), 109 (51), 95 (81), 81 (100), 69 (95), 67 (66), 55 (39), 41 (72), 39 (65). Compound B had the same retention time and identical mass spectrum as one of the monoterpenes, (4a*S*,7*S*,7a*R*)-nepetalactone, identified from the catnip plant, *Nepata cataria* (Peterson et al. 2002). The mass spectrum of compound A with M_r 168 was similar to that of (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol, which has been reported as a pheromone component used by several aphid species (Dawson et al. 1990).

The ^{1}H NMR data for peak B were in good agreement with those of the natural (4a*S*,7*S*,7a*R*)-nepetalactone isolated from catnip oil. The structure of (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol was also confirmed by ^{1}H NMR (C_6D_6) analysis: characteristic peaks at 4.61 ppm (1H, t, J = 5.91 Hz, O-CH-O) and 2.09 (1H, OH, d, J = 5.91 Hz). The 5.91-Hz coupling between H_1 and OH in the ^{1}H NMR spectrum is characteristic of (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol based on an organic synthetic product obtained from (2*S*,9*S*,)-5,9-dimethyl-2(*N*-methylphenylamino)-3-oxabicyclo [4,3,0]-4-none (Dawson et al. 1996). Based on the results from GC-MS and NMR analyses, we concluded that the chemical structures of the soybean aphid pheromone components are (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol and (4a*S*,7*S*,7a*R*)-nepetalactone.

We also compared pheromone titers and ratios emitted between oviparae induced in the laboratory artificially and those collected from buckthorn shrubs in the field during the fall. The results showed that there were no differences in the amounts of the two pheromone compounds produced by field-collected individuals compared with those from our laboratory colony; nor were there any differences in the blend ratios emitted (Table 1).

The EAG analyses of both males and gynoparae revealed that olfactory receptors on their antennae were responding to the identified pheromone compounds (Fig. 2). Male antennae (Fig. 2, top) were highly sensitive to the two pheromone compounds at relatively low doses, with the response decreasing significantly when the dose exceeded 0.1 mg. In contrast, gynoparae (Fig. 2, bottom) showed a higher EAG response when pheromone exceeded a 0.1-mg dose, particularly in response to (4a*S*,7*S*,7a*R*)-nepetalactone.

Behavioral Responses of Soybean Aphids to Synthetic Pheromone Compounds. Field tests using traps baited with 10 mg of the two synthetic aphid pheromone compounds showed that a blend containing (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol and (4a*S*,7*S*,7a*R*)-nepetalactone at a ratio of 35:65 caught the highest numbers of males and gynoparous females compared with single compounds (Fig. 3). Traps with either (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol or (4a*S*,7*S*,7a*R*)-nepetalactone alone captured more males and gynoparaous females than those of the control. The order of attractiveness for the three treatments for both males and gynoparae was as follows: blend > (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol > (4a*S*,7*S*,7a*R*)-nepetalactone.

Dose–response tests conducted in the same field in 2002 showed that traps with a dose of 30 mg soybean aphid pheromone blend caught the highest numbers of both males and gynoparae (Fig. 4). We also tested effects of different amounts of (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol added to the nepetalactone on their attractiveness to males and gynoparae. At least 10% (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol in the two-component blend was essential to attract males (Fig. 5). Gynoparae responded equally well to traps baited with pheromone lures containing no (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol as they did to those with no (4a*S*,7*S*,7a*R*)-nepetalactone.

EAG Responses of Male Soybean Aphids Pre-Exposed to Pheromone. EAG responses were measured from antennae of male soybean aphids after they were pre-exposed to a dispenser containing 5 g pheromone for 10 min. Significantly lower EAG responses were elicited from antennae of the pre-exposed soybean aphids than to those naive males (Fig. 6).

Discussion

Oviparae of soybean aphids produce both (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol and (4a*S*,7*S*,7a*R*)-nepetalactone, which are the two most common aphid pheromone compounds identified from a number of other aphid species (Dawson et al. 1990, Pickett et al. 1992, Boo et al. 2000, Goldansaz et al. 2004). The ratio of these two pheromone compounds from *A. glycines* differs from those previously reported, which supports the hypothesis of species specificity in aphid pheromone systems (Pickett et al. 1992, Guldemond et al. 1993). The absolute stereochemistry of (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol has been elucidated, but only with the use

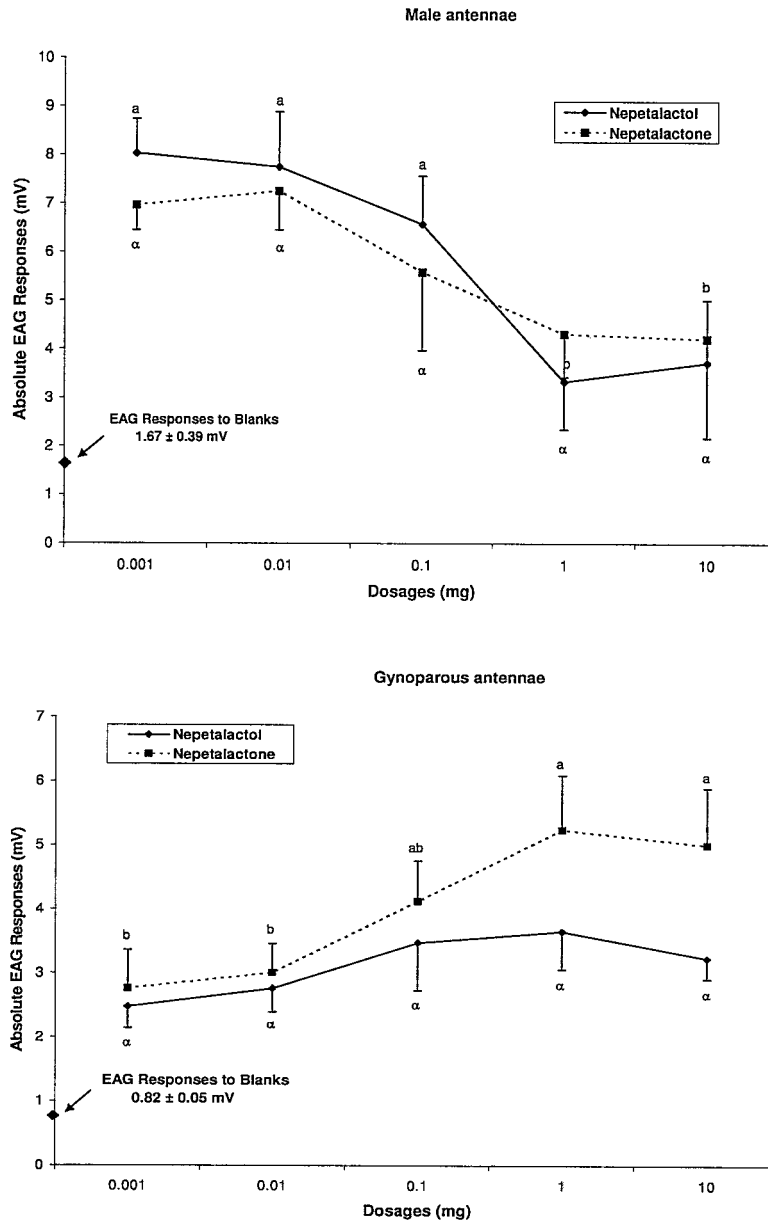

Fig. 2. EAG dose–response curves of the two identified soybean aphid pheromone compounds from both males and gynoparous females. Means with the same letter for a given compound are not significantly different (N = 3–5; gynoparae to nepetalactol: $F = 0.95$; df = 4,20; $P > 0.05$; gynoparae to nepetalactone: $F = 6.42$; df = 5,24; $P < 0.001$; males to nepetalactol: $F = 8.99$; df = 5,12; $P < 0.001$; males to nepetalactone: $F = 2.19$; df = 5,12; $P > 0.05$).

of derivatives from synthetic preparations. This study reports the first attempt to determine its stereochemistry through NMR analyses on naturally collected pheromone compounds. Although the stereochemistry of (4a*S*,7*S*,7a*R*)-nepetalactol at C_1 position could not be established based on the configuration of the natural pheromone, we propose that it is *R*, based on the positive field-trapping results.

One of the biggest obstacles for research on aphid pheromones is the difficulties in locating pheromone-producing oviparae. The alternative to field collection is to induce pheromone-producing females under autumnal conditions, such as lower temperatures and shortened day lengths in the laboratory. In this study, we documented similarities in pheromone titers and ratios produced by field-collected and laboratory-in-

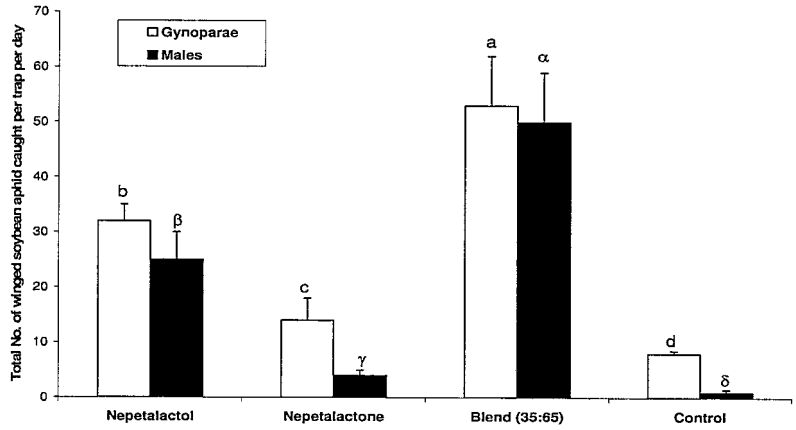

Fig. 3. Total number of male and gynoparous soybean aphids caught in traps with different combinations of identified sex pheromone compounds in 2001. Means with different letters on top of the bars indicate significant differences. ($N = 10$; for gynoparae: $F = 75.42$; df = 3,20; $P < 0.001$; for males, $F = 99.04$; df = 3,36; $P < 0.001$).

duced oviparae. Our results show the reliability of using artificially induced oviparae for aphid pheromone characterization. Similarly, Goldansaz et al. (2004) used laboratory-induced oviparae to identify pheromone components of the potato aphid.

The review of the aphid sensory system by Anderson and Bromley (1987) suggests that the greater abundance of secondary rhinaria on the antennae of alate morphs indicates their involvement in host location and mate selection. Du et al. (1995) reported that the antennae of alate soybean aphids contain placoid sensilla on their secondary rhinaria. Our EAG tests showed that the antennae of both male and gynoparaous soybean aphids posses receptor neurons responding to the two identified pheromone compounds. The fact that male antennae are more specific to (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol indicates a function of this compound in mate finding. The significant EAG and behavioral responses of gynoparous females to (4a*S*,7*S*,7a*R*)-nepetalactone suggest that this compound may be used as a chemical cue to locate overwintering host plants. This function has also been observed in several other aphid species (Campbell et al. 1990, Hardie et al. 1992, 1996). Furthermore, we found that the volatile emission of buckthorn leaves significantly decreased while oviparae were feeding and producing pheromone compounds (J.Z., unpublished data). Further studies will determine if this is a form of host plant defense by not emitting signature volatiles to prevent further damage caused by gynoparae.

The field trapping of both male and gynoparous female soybean aphids with various ratios of (1*R*,4a*S*,7*S*, 7a*R*)-nepetalactol and (4a*S*,7*S*,7a*R*)-nepetalactone has

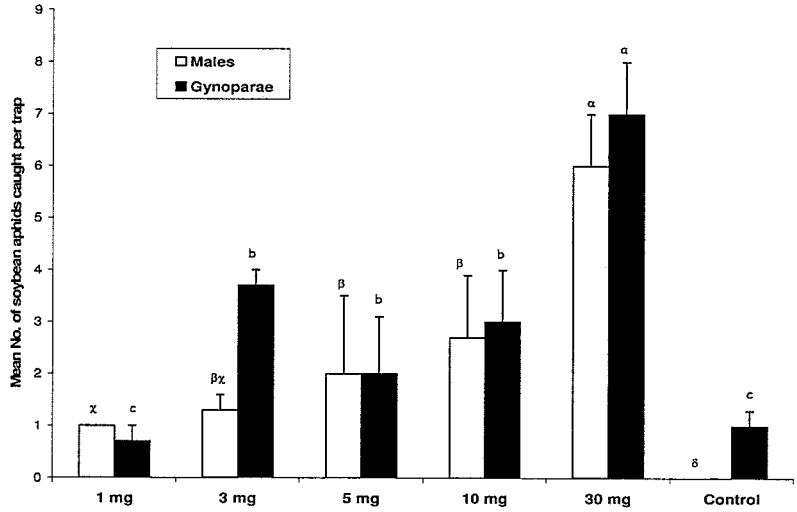

Fig. 4. Mean number of male and gynoparae soybean aphids caught in traps with different doses of the most attractive pheromone blend in 2002. Means with different letters on top of the bars indicate significant differences ($N = 10$; for gynoparae: $F = 34.14$; df = 5,54; $P < 0.001$; for males: $F = 27.57$; df = 4,45; $P < 0.001$).

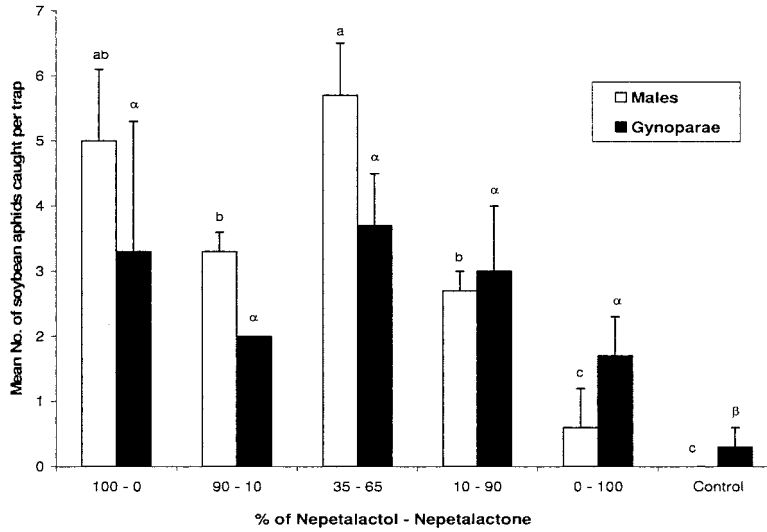

Fig. 5. Mean number of male and gynoparae soybean aphids caught in traps baited with different ratios of the identified sex pheromone compounds at a dose of 10 mg in 2002. Means with different letters on top of the bars indicate significant differences ($N = 10$; for gynoparae: $F = 11.47$; df = 5,54; $P < 0.001$; for males: $F = 47.95$; df = 5,54; $P < 0.001$).

shown the effectiveness of the nepetalactol to male attraction. The attraction of both the alate male and gynoparous female forms of aphids has also been reported for the damson-hop aphid, *Phorodon humuli* (Hardie et al. 1996, Lösel et al. 1996a, b). The specific blend of a 35:65 ratio of these two components in this study represents the actual emitted pheromone ratio from calling oviparae. The ratio was the most attractive blend in 2 yr of field trials. The relatively lower numbers of *A. glycines* caught in pheromone traps in 2002 were caused by the sparse occurrence of soybean aphids during that year (data from Midwest Soybean Aphid Workshop, 5 February 2004). However, the pattern of trap captures was consistent during the 2-yr field-trapping experiments.

The identification of sex pheromones of the soybean aphid provides us with a unique opportunity to explore the possibility of suppressing soybean aphid populations using pheromone-based strategies. For aphids, possible immigration of gravid females into the mating disruption area is negligible, because oviparae are wingless. Mating disruption should be particularly effective against such insects. The suppressive effects should be easily discerned in small

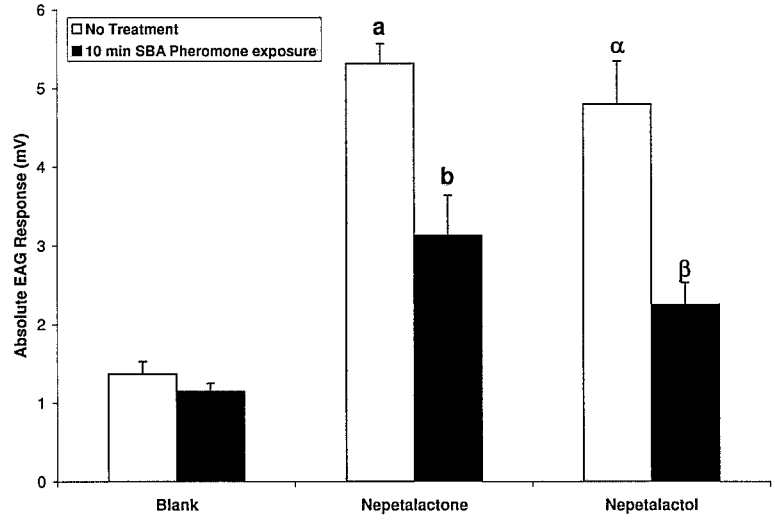

Fig. 6. Comparisons of absolute EAG responses of male soybean aphids pre-exposed with higher dosages of aphid pheromones to those without pre-exposure. Means with different letters on top of the bars indicate significant differences (Student *t*-test, $N = 12$; for nepetalactone: $t = 5.08$, $P < 0.001$; for nepetalactol: $t = 3.54$, $P < 0.005$; for blank: $t = 1.18$, $P > 0.05$).

plots. Furthermore, we also showed the sensory adaptation of soybean aphid antennal responses to the two pheromone compounds.

Insect suppression by the use of mass trapping through combinations of sex pheromones and host plant volatiles has shown increased success in controlling major agricultural pests (Kobayashi et al. 1981, Smit et al. 2001, Baker and Heath 2004). Mass trapping of the autumnal generation soybean aphids (including gynoparae as they seek to find winter host plants and males flying from soybean fields to locate oviparae) may be a useful control strategy using this newly identified sex pheromone. The subsequent year's population may be reduced by interfering with the preceding fall aphid generation's ability to locate mates or buckthorn plants. Although the mass-trapping technique against the soybean aphid may be more laborious than mating disruption, further research may prove its effectiveness.

Acknowledgments

This project was funded by National Science Foundation-SBIR Program 0450032 to J.Z. Technical support from J. Jones, J. Heath, and B. Ellingson is appreciated. We also appreciate the support from the ISU-IPRT.

References Cited

Anderson, M., and A. K. Bromley. 1987. Sensory system, pp. 153–162. *In* A. K. Minks and P. Harrewijn (eds.), Aphids: their biology, natural enemies, and control. Elsevier, Amsterdam, The Netherlands.

Baker, T. C., and J. J. Heath. 2004. Pheromones—function and use in insect control, pp. 407–460. *In* L. I. Gilbert, K. Iatro, and S. Gill (eds.), Comprehensive insect physiology, biochemistry, pharmacology and molecular biology. Elsevier, Amsterdam, The Netherlands.

Birkett, M. A., and J. A. Pickett. 2003. Aphid sex pheromones: from discovery to commercial production. Phytochemistry 62: 651–656.

Boo, K. S., M. Y. Choi, I. B. Chung, V. E. Eastop, J. A. Pickett, L. J. Wadhams, and C. M. Woodcock. 2000. Sex pheromone of the peach aphid, *Tuberocephalus momonis,* and optimal blends for trapping males and females in the field. J. Chem. Ecol. 26: 601–609.

Campbell, C.A.M., G. W. Dawson, D. C. Griffiths, J. Pettersson, J. A. Pickett, L. J. Wadhams, and C. M. Woodcock. 1990. Sex attractant pheromone of damson-hop aphid *Phorodon humuli* (Homoptera, Aphididae). J. Chem. Ecol. 16: 455–465.

Clark, A. J., and K. L. Perry. 2002. Transmissibility of field isolates of soybean viruses by *Aphis glycines.* Plant Dis. 86: 1219–1222.

Dawson, G. W., D. C. Griffiths, L. A. Merritt, A. Mudd, J. A. Pickett, L. J. Wadhams, and C. M. Woodcock. 1990. Aphid semiochemicals - a review, and recent advances on the sex pheromone. J. Chem. Ecol. 16: 3019–3030.

Dawson, G. W., J. A. Pickett, and D.W.M. Smiley. 1996. The aphid sex pheromone cyclopentanoids: synthesis in the elucidation of structure and biosynthetic pathway. Bioorg. Med. Chem. 4: 351–361.

Du, Y.-J., F.-S. Yan, X.-L. Han, and G.-X. Zhang. 1995. Olfaction in host selection of the soybean aphid Aphis Glycine. Acta Entomol. Sinica. 37: 385–391.

Goldansaz, S. H., S. Dewhirst, M. A. Birkett, A. M. Hooper, D.W.M. Smiley, J. A. Pickett, L. Wadhams, and J. N. McNeil. 2004. Identification of two sex pheromone components of the potato aphid, *Macrosiphum euphorbiae* (Thomas). J. Chem. Ecol. 30: 819–834.

Guldemond, J. A., A.F.G. Dixon, J. A. Pickett, L. J. Wadhams, and C. M. Woodcock. 1993. Specificity of sex pheromones, the role of host plant odor in the olfactory attraction of males, and mate recognition in the aphid *Cryptomyzus.* Physiol. Entomol. 18: 137–143.

Hardie, J., S. F. Nottingham, G. W. Dawson, R. Harrington, J. A. Pickett, and L. J. Wadhams. 1992. Attraction of field-flying aphid males to synthetic sex pheromone. Chemoecology 3: 113–17.

Hardie, J., J. R. Storer, F. J. Cook, C.A.M. Campbell, L. J. Wadhams, R. Lilley, and L. Peace. 1996. Sex pheromone and visual trap interactions in mate location strategies and aggregation by host-alternating aphids in the field. Physiol. Entomol. 21: 97–106.

Kobayashi, M., T. Wada, and H. Inoue. 1981. A comparison of communication disruption technique and mass-trapping technique for controlling moths using sex pheromone of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Proc. Japan/USA Symp. On IPM., 32–40.

Lösel, P. M., M. Lindemann, J. Scherkenbeck, J. Maier, B. Engelhard, C.A.M. Campbell, J. Hardie, J. A. Pickett, L. J. Wadhams, A. Elbert, and G. Thielking. 1996a. The potential of semiochemicals for control of *Phorodon humuli* (Homoptera: Aphididae). Pest. Sci. 48: 293–303.

Lösel, P. M., M. Lindemann, J. Scherkenbeck, C.A.M. Campbell, J. Hardie, J. A. Pickett, and L. J. Wadhams. 1996b. Effect of primary-host kairomones on the attractiveness of the hop-aphid sex pheromone to *Phorodon humuli* males and gynoparae. Entomol. Exp. Appl. 80: 79–82.

Midwest Soybean aphid Workshop, February 5, 2004. http://www.ipm.uiuc.edu/fieldcrops/insects/soybean_aphids/workshop/index.html.

Park, K. C., and J. Hardie. 1998. An improved aphid electroantennogram. J. Insect Physiol. 44: 919–928.

Peterson, C. J., L. T. Nemetz, L. M. Jones, J. R. Coats. 2002. Behavioral activity of catnip (Lamiaceae) essential oil components to the German cockroach (Blattodea: Blattellidae). J. Econ. Entomol. 95: 377–380.

Pettersson, J. 1970. An aphid sex attractant. I. Biological studies. Entomol. Scand. 1: 63–73.

Pettersson, J. 1971. An aphid sex attractant. II. Histological, ethological and comparative studies. Entomol. Scand. 2: 81–93.

Pickett, J. A., L. J. Wadhams, C. M. Woodcock, and J. Hardie. 1992. The chemical ecology of aphids. Annu. Rev. Entomol. 37: 67–90.

Ragsdale, D. W., D. J. Voegtlin, and R. J. O'Neil.. 2004. Soybean aphid biology in North America. Ann. Entomol. Soc. Am. 97: 204–208.

Smit, N.E.J.M., M.C.A. Downham, P. O. Laboke, D. R. Hall, and B. Odongo. 2001. Mass-trapping male *Cylas spp.* with sex pheromones: a potential IPM component in sweetpotato production in Uganda. Crop Protect. 20: 643–651.

Soybean aphid watch 2005. http://www.ncpmc.org/alerts/soybeanaphid.cfm.

Takahashi, S., M., Inaizumi, and K. Kawakami. 1993. Life cycle of the soybean aphid *Aphis glycines* Matsumura, in Japan. Jap. J. Appl. Entomol. Zool. 37: 207–212.

van-den-Berg, H., D. Ankasah, and A. Muhammad. 1997. Evaluating the role of predation in population fluctuations of the soybean aphid *Aphis glycines* in farmers' fields in Indonesia. J. Appl. Ecol. 34: 971–984.

Voegtlin, D. J., S. E. Halbert, and G. Qiao. 2004. A guide to separating *Aphis glycines* Matsmura and morphologically similar species that share its hosts. Ann. Entomol. Soc. Am. 97: 227–232.

Wang, R. Y., and S. A. Ghabrial. 2002. Effect of aphid behavior on efficiency of transmission of Soybean mosaic virus by the soybean-colonizing aphid, *Aphis glycines.* Plant Dis. 86: 1260–1264.

Zhang, A., D. Amalin, S. Shirali, M. S. Serrano, R. A. Franqui, J. E. Oliver, J. A. Klun, J. R. Aldrich, D. E. Meyerdirk, and S. L. Lapointe. 2004. Sex pheromone of the pink hibiscus mealybug, Maconellicoccus hirsutus, contains an unusual cyclobutanoid monoterpene. Proc. Natl. Acad. Sci. U.S.A. 101: 9601–9606.

Zhang, G. X., and T. S. Zhong. 1982. Experimental studies on some aphid-cycle patterns. Sinozoology 2: 7–17.

Zhang, G. X., and T. S. Zhong. 1983. Economic Insect Fauna of China, Fasc. 25: Homoptera: Aphidinea, part 1. Science Press, Beijing, China.

Received for publication 23 February 2005; accepted 3 January 2006.