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Research Article

Attractant Pheromone of the Neotropical Species *Neomegalotomus parvus* (Westwood) (Heteroptera: Alydidae)

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The Neotropical broad-headed bug, *Neomegalotomus parvus* (Westwood), is adapted to various leguminous crops and is considered a pest in common bean and soybean. The chemical communication of this species was studied in order to identify an attractant pheromone. Males and females of *N. parvus* produce several short-chain esters and acids, and their antennae showed electrophysiological responses to five of these compounds, three common to both sexes (hexyl butanoate, 4-methylhexyl butanoate, and hexyl hexanoate), and two female-specific compounds (4-methylhexyl pentanoate and hexyl pentanoate). Both aeration extracts of females and a solution containing five synthetic compounds mimicking the natural blend were attractive to males and females *N. parvus* in a laboratory bioassay. Aspects of the chemical ecology of the broad-headed bugs and the possibility to use pheromone-baited traps in the field for monitoring are discussed.

1. Introduction

Neomegalotomus parvus, or broad-headed bug (Heteroptera: Alydidae), subfamily Alydinae, is native to South America. As other alydines, *N. parvus* is an oligophagous bug that feeds on immature seeds of legumes [1, 2]. The taxonomic status of Neotropical alydine bugs was reviewed by Schaefer and Panizzi [3], Schaffner and Schaefer [4], Schaefer [5], and Schaefer and Ahmad [6]. From these works, Neotropical species formerly classified in the genus *Megalotomus* are now grouped in the genus *Neomegalotomus*, and South American species of *Neomegalotomus* were synonymized as *N. parvus*.

N. parvus has been adapted to various leguminous crops such as lablab beans, *Dolichus lablab* L. [7], pigeon pea, *Cajanus cajan* (L.) Mill., pig bean, *Canavalia ensiformis* (L.) DC., and indigo, *Sesamum indicum* L. [8, 9]. However, it is the common bean, *Phaseolus vulgaris* L. [10, 11] and soybean, *Glycine max* (L.) Merrill [12], that this bug is an economically important pest.

Insect feeding causes direct damage to crops and, in beans, is responsible for reduction of seed mass and high seedling mortality [9, 11]. Santos and Panizzi [12] showed that artificial infestation of soybean plants during the pod-filing stage causes a reduction in seed vigor and viability and has a negative effect on seed quality when infestation is in advanced stage. However, asynchrony between vulnerable stages of soybean seed development and *N. parvus* populations allows soybean crops to usually escape severe injury from this insect in the field [9].

Currently, control of *N. parvus* is exclusively insecticidal, and application timing is not based on the accurate population monitoring. These insects are easily disturbed and highly mobile, so the sampling cloth technique normally used to survey heteropteran populations in the field [13] is not a reliable. In Brazil, sweep-netting is the recommended monitoring method but it is laborious and time consuming; therefore, most growers prefer to use calendar-based application of insecticides. Pheromone-baited traps would be

an alternative, more precise sampling technique to help minimize pesticide applications and increase efficacy.

Semiochemicals of Alydidae have been described for *Alydus* [14, 15], *Megalotomus* [14], *Riptortus* [16–18], and *Leptocorisa* [19] species, and for some of these bugs both adults and nymphs are reportedly attracted. For example, field experiments showed that *Riptortus clavatus* (Thunberg) could be efficiently captured in traps baited with their aggregation pheromone [20–22].

For *N. parvus*, traps baited with live males captured significantly more males that unbaited traps [23]. In addition, it is known from field observations that some bugs are attracted to cow urine [24], and traps baited with cow urine or NH_4OH solutions captured *N. parvus* in the field [25]. Thus, traps are potentially useful for population monitoring of *N. parvus*, but the efficiency of trap-based monitoring could be greatly improved if more specific and powerful attractants were available. The objective of this work was to determine if *N. parvus* males and/or females produce specific compounds that could be used as pheromone.

2. Materials and Methods

2.1. Insects Rearing. A laboratory colony of *N. parvus* was established from adults and nymphs field-collected from 2009 to 2011 from beans fields near Embrapa Genetic Resources and Biotechnology, Brasília, DF, Brazil (15° 47′ S and 47° 55′ W). Bugs were reared in 8 L plastic containers, on a diet of green beans pods (*Phaseolus vulgaris* (L.)), branches with flowers, and pods of pigeon pea (*Cajanus cajan* (L.)) Millsp, dried pigeon pea seed, and water at 26° ± 1°C and 65% r.h. a 14 light: 10 dark photoperiod (light 06:00–20:00 h). The food supply was renewed twice a week. Males and females were grouped for mating, with pieces of cotton placed in containers for oviposition. Eggs were conditioned in plastic containers and, after emergence, nymphs were maintained similarly to adults. Males and females used in the experiments were separated after the imaginal molt and cuticular hardening to prevent mating. Sexually mature 8–15-day-old adults were used for all experimental bioassays and volatile collections since at this age insects started to mate.

2.2. Collection of Volatiles. Volatiles were collected ($N = 6$ extracts) from groups of 20–30 males or females *N. parvus*. To minimize emission of defensive compounds [26] the insects were carefully introduced into 1 liter glass containers shortly after the end of scotophase when they were quiescent. Air was drawn into the container through a bed of 4–12 mesh activated charcoal (Fisher Scientific, Pittsburgh, PA, USA), and out of the container through two traps (15 cm × 1.5 cm OD) containing Super Q (100 mg each; Alltech Associates, Inc., Deerfield, IL, USA) by a suction pump (~1 L/min). Insects were fed fresh green beans daily, and aerated continuously for 7–10 d, and a sample taken every 24 h. The Adsorbent traps were eluted with 0.5 mL hexane, and the eluates were stored at −20°C until needed for chemical analysis or behavioral bioassays. Extracts were concentrated under a gentle stream of N_2 to yield a solution of approximately 0.1 bug-equivalent/μL/24 hours of solution (~500 μL) to be tested.

2.3. Analysis and Derivatization of Extracts. For quantitative analysis, 1 μL crude extracts and fractions thereof were analyzed by gas chromatography flame ionization detector ((GC-FID), Shimadzu 17A GC) (Kyoto-Japan) equipped with DB5 column (30 m × 0.25 mm ID, 0.25 μm film; J&W Scientific, Folsom, CA) on a temperature ramp of 50°C/2 min, then 8°C/min to 250°C/10 min. Injections were made in splitless mode. To quantify the pheromone released per insect, 5 aeration samples of females were selected, and 1 μL of isobutyl acetate (1 mg/mL hexane solution) was added as internal standard (IS) at a final concentration of 0.02 μg/mL. One microliter of each sample was injected into the GC in the splitless mode, with helium as carrier gas. Amounts of volatiles released by the insects per day were calculated in relation to the area of the internal standard. Data were collected with Class GC software (Class CG-10 Version 2.01, Shimadzu (Kyoto, Japan)) and were handled using Excel (Microsoft Corporation 2003).

For qualitative analysis, selected extracts were analyzed using an Agilent MSD 5975 instrument equipped with a quadrupole analyzer coupled to a GC 7890, a splitless injector, and helium as the carrier gas. Ionization was by electron impact (70-eV, source temperature 200°C) using the same column and conditions described above for GC-FID analysis. Chemical ionization (CI) MS spectra were obtained using the same GC-MS equipment using methane (CH_4) as the reagent gas with the same column and conditions described above for GC-FID analysis.

Compounds were identified comparing their mass spectra with database spectra (NIST 2008 library), retention indices, and coinjection with authentic standards.

Five aeration samples of females were combined, and concentrated to dryness under gentle N_2 flow, and submitted to alkaline hydrolysis by adding 50 μL of methanol and 50 μL of 2 M NaOH in a 1.5 mL glass conical vial. The sample was kept at room temperature for 2 hours. After, water (100 μL) was added and organic phase was extract with hexane (200 μL tree times). The combined organic phases were concentrated under nitrogen flow to ~50 μL, and the extract was analyzed by GC-MS by electron impact as described above.

2.4. Coupled Gas Chromatography-Electrophysiology. GC-electroantennography (EAG) was used to pinpoint compounds within mixtures that were detected by the antennae of males and females.

A GC Perkin Elmer Autosystem XL (NY, USA) was coupled to an EAG detector (Syntech, Inc., Hilversum, The Netherlands). The GC was equipped with a nonpolar DB-5 column (30 m × 0.25 mm ID, 0.25 μm film, J&W Scientific, Folsom, CA, USA), and a splitless injector with helium as the carrier gas (1 mL/min). The column temperature was programmed to 80°C (2 min), then heated to 250°C at 8°C/min, and held for 10 min. The effluent temperature to EAG system was kept at 195°C. The antenna of one male or one female were cut and immediately placed in stainless steel

electrodes, the base of the antenna was placed in the reference electrode and the distal ends of the antenna was placed in the recording electrode, the electric connection was achieved using conductive gel. The electrodes were connected to an Autospike interface box and an AC/DC amplifier IDAC-2 (Syntech, Inc.). Preparations were held in a continuous humidified air flow (1 L/min) with a Stimulus Controller CS-55 (Syntech, Inc.). The females and males antennae of *N. parvus* were tested using a female extracts of *N. parvus* ($N = 5$) containing all compounds identified in the volatiles collections and for a solution containing the synthetic compounds, that showed electrophysiology response from the antenna in crude extract, hexyl butanoate (0.05 mg/mL), (*S*)-4-methylhexyl butanoate (0.005 mg/mL), hexyl pentanoate (0.0075 mg/mL), (*S*)-4-methylhexyl pentanoate (0.002 mg/mL), and hexyl hexanoate (0.0075 mg/mL) ($N = 3$). Only peaks that showed the polarization and depolarization of the antenna were considered as EAG responses, and only compounds that elicited response in all antennas tested ($N = 5$) were considered electrophysiologically active.

2.5. Synthesis of 4-(S)-Methylhexyl Pentanoate and 4-(S)-Methylhexyl Butanoate. (*S*)-4-methyl-1-hexanol (TCI America, Boston, MA, USA) 11.6 mg (0.1 mmol) was treated with butyryl chloride (10.4 μL, 0.1 mmol) in the presence of pyridine (8 μL,/500 μL methylene chloride). The mixture was poured into ice-water, extracted with methylene chloride ($3 \times 100\,\mu$L), the organic extract was washed with 0.1 M HCl, water, and dried with Na_2SO_4. A similar procedure was conducted using valeryl chloride to prepare 4-(*S*)-methylhexyl pentanoate. The structures of synthesized compounds were confirmed by mass spectrometry analysis.

4-(S)-Methylhexyl Butanoate MS. m/z = 129 (12), 115(4), 98 (28), 89 (82), 83(8), 71 (82), 70 (100), 69(49), 57 (58), 56 (36), 55(31), 43(55), 42(15), 41(49).

4-(S)-Methylhexyl Pentanoate MS. m/z = 143(10), 115(5), 103(79), 98(35), 97(6), 85(58), 83(9), 70(100), 69(44), 57(95), 56(34), 55(31), 43(16), 42(13), 41(43).

Source of Compounds. Super *Q* (80/100 mesh) was purchased from Alltech (PA, USA). The sources of chemical as follows: camphene, 6-methyl-5-hepten-2-one, hexanoic acid limonene, undecane, nonanal, dodecane, decanal, tridecane (Sigma Aldrich, Steinheim, Germany), hexyl acetate (TCI-America, portland, USA). Butyl butanoate, pentyl butanoate, hexyl butanoate, hexyl pentanoate, and hexyl hexanoate were provided by Jeffrey Aldrich (USDA-ARS, Invasive Insect and Behavior Laboratory, MD, USA).

2.6. Olfactometer Bioassays. A two-choice olfactometer modified from Borges and Aldrich ("W-olfactometer"; [27]) was used to test the biological activity of *N. parvus* aeration extracts and synthetic compounds. The olfactometer release chamber was a 500-mL three-neck, round-bottom flask (all 24/40 joints, Kontes, Vineland, New Jersey). Two 250 mL rotary evaporator trap adapters (24/40 joints) were attached to each side arm of the release flask (the treatment and control arms). A charcoal (20/40 mesh) filter column (130 mm $\times$ 10 mm ID) was attached to the side arms using two 40 cm long pieces of a silicone tubing (3/16 I.D. $\times$ 5/15 E.D. VWR Scientific Corporation, Darmstadt, Germany), inserted in a "Y" connector of the same diameter and connected to adapters (24/40 joint) on each side arm of the olfactometer. The air was humidified by passage through a container of distilled water between the charcoal filter and the arms of the olfactometer. The middle neck of the flask was connected to the vacuum pump with an adapter, and the air flow was adjusted with a "Clear Flow Rotameter" (Accura Flow Products, Warminster, Pennsylvania 18974-0100) to a flow of 0.8 L/min. The apparatus was positioned horizontally on a countertop in a room with bright fluorescent lights (2×36 W, daylight (6500 K) lamps Sylvania Activa 172, Sylvania, Danvers, MA, USA) during photophase. The temperature in the bioassay room was maintained at $26.0 \pm 1.0^\circ$C. The positions of the olfactometer arms were inverted between control and treatments after each three repetitions to avoid any positional bias. The apparatus was cleaned with fragrance-free liquid soap, rinsed thoroughly with water, and dried at 120°C after every five replicates. The insects were placed in the round-bottom flask (release chamber), and the treatments were placed at the end of the reducing adapter chamber (treatment arms).

A single *N. parvus* adult (male or female) was gently introduced into the release chamber of the Y-tube olfactometer with the aid of an artist's paint brush (Camel Hair, number 1), and its pattern of behavior (response) was recorded for 10 min/replicate. The duration of each bioassay replicate was monitored using a stopwatch. Prior to testing, the insects were allowed to acclimate for a short period (ca. 3 min) in the release chamber while assembling the treatment chambers. The first choice of the insect was recorded, that is, the first arm of olfactometer that the insect chose, entered and remained in for at least 100 sec. The test insects were used only once during the bioassays.

The bioassay procedures described above were used to compare the biological activity of aeration extracts of females, and synthetic standards prepared in proportions matching that produced by *N. parvus* females. For aeration extracts, the solution of test stimulus was 1 individual equivalent/24 hours (IE) spotted on a strip of filter paper (1.5 cm long and 0.5 cm wide); controls consisted of filter papers treated with hexane. Forty-five bioassays were performed for each sex (males and females). Bioassays were conducted using a 5 μL of a synthetic solution containing the five synthetic compounds that showed EAG responses ($N = 35$ for females and $N = 40$ form males) (hexyl butanoate (0.005 mg/mL), (*S*)-4-methylhexyl butanoate (0.0005 mg/mL), hexyl pentanoate (0.00075 mg/mL), (*S*)-4-methylhexyl pentanoate (0.0002 mg/mL), and hexyl hexanoate (0.00075 mg/mL)).

2.7. Statistical Analysis. Choices made by the insects in the bioassays were analysed using logistic regression. The fitted model contained a factor for the side (left or right) on which the stimuli were presented to control for this variability. We tested the hypothesis of no preference (50% first choice to

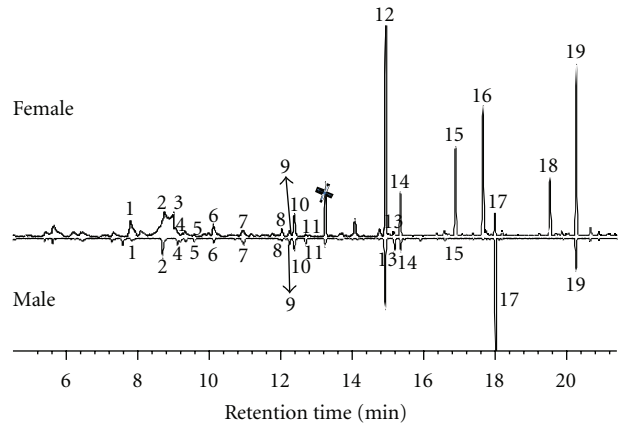

Figure 1: Gas chromatogram profile of an air-entrainment extract of males and females of *Neomegalotomus parvus*. (1) Camphene, (2) 6-methy-5-hepten-2-one, (3) hexanoic acid, (4) butyl butanoate, (5) hexyl acetate, (6) limonene, (7) (*E*)-2-octen-1-ol, (8) pentyl butanoate, (9) undecane, (10) nonanal, (11) unknown compound, (12) hexyl butanoate, (13) dodecane, (14) decanal, (15) 4-methylhexyl-butanoate, (16) hexyl pentanoate, (17) tridecane, (18) 4-methylhexyl-pentanoate, and (19) hexyl hexanoate.

each vibratory signal) using a chi-square Wald test. All tests were conducted using the *R* programming language [28].

3. Results

3.1. Chemical Analysis. The chemical analysis of males and females of *N. parvus* extracts obtained from volatile collection showed quantitative and qualitative differences between the extracts. Quantification of extracts showed that females produce higher amounts of several compounds (Table 1, Figure 1) compared to males and females also release some specific compounds that were not found in the extracts of males, such as hexyl pentanoate and 4-methylhexyl pentanoate.

The mass spectra and the retention times of compounds **15** and **18** (Figure 1) did not match those of any compound from the database and/or the literature. The mass spectra of **15** (*m/z*, relative abundance): 129(11), 115(4), 98(28), 89(78), 83(9), 71(76), 70(100), 69(47), 57(53), 56(34), 43(49), 41(42), and compound **18**: 143(9), 115(5), 103 (76), 98(35), 97(6), 85(57), 83(7), 70(100), 69(30), 57(89), 56(32), 55(28), 43(15), 42(12), 41(40) suggested ester homologues. CI-MS analysis of the female crude extract showed that compounds **15** and **18** had a molecular adduct ions ($[M+H]^+$) at 187 and 201, thus providing additional evidence that these two compounds could have a similar chemical structure differing by one methyl group. In order to obtain more information about the chemical structure of these two esters, a pooled female extract was submitted to alkaline hydrolysis. The GC-MS analysis of the hydrolyzed crude extract showed that the peaks corresponding to the esters disappeared and new peaks were generated, one of which matched the synthetic standard of 4-methyl-1-hexanol. These results, combined with retention index data (Table 1), suggested that compounds 15 and 18 could be 4-methylhexyl butanoate and 4-methylhexyl pentanoate, respectively. Indeed, the mass spectra and retention indices of synthetic (*S*)-4-methylhexyl butanoate and (*S*)-4-methylhexyl pentanoate matched those of esters found in female crude extract.

The other volatile compounds identified from both males and females are common to Alydidae; mainly short-chain esters and acids, and one alcohol [(*E*)-2-octen-1-ol] that is a common defensive compound in several Pentatomidae (Table 1).

3.2. Coupled Gas Chromatography-Electrophysiology. In GC-EAG experiments the antennae of *N. parvus* males and females responded to only five components present in the extract of females (Figure 2) that were identified as hexyl butanoate, 4-methylhexyl butanoate, hexyl pentanoate, 4-methylhexyl pentanoate, and hexyl hexanoate. When a blend containing these five synthetic compounds (hexyl butanoate, (*S*)-4-methylhexyl butanoate, hexyl pentanoate, (*S*)-4-methylhexyl pentanoate and hexyl hexanoate) was tested, the antennae of males and females responded in a similar way as to aeration extracts of females.

3.3. Bioassays. In W-olfactometer bioassays with aeration extract of females, both *N. parvus* males and females were significantly attracted to the extract treatment arm of the olfactometer (Figure 3). Similarly, males and females were significantly attracted to the treatment arm containing the five-component synthetic blend active in EAG experiments (Figure 4).

4. Discussion

Males and females of *N. parvus* produce several short chain esters and acids, most of which were previously reported for others species of Alydidae from the metathoracic scent glands [14, 15, 17, 18, 22] (Table 2); however, this is the first report of pentanoates from Alydidae. The antennae of *N. parvus* showed electrophysiological responses to five of these esters, three common to both adult sexes (hexyl butanoate, 4-methylhexyl butanoate and hexyl hexanoate), and two female-specific compounds (4-methylhexyl pentanoate and hexyl pentanoate). Both males and females were attracted to aeration extracts of females, and to the synthetic blend of the five EAD-active compounds in proportions mimicking those of the compounds produced by females.

Interestingly, in the Alydidae either the male [17, 18] or female [15–19] can emit the attractant pheromone, depending on the genus. In the rice alydid bug, *Leptocorisa chinensis* (Dallas), although there were no detectable qualitative differences in aeration extracts of males versus females, only males were attracted to a 5 : 1 blend of *(E)*-2-octenyl acetate and octanol [19]. In the alydid *R. clavatus*, the attractant pheromone is produced by males and attracts females, males, and nymphs, plus an egg parasitoid [17]. Furthermore, it has been established for *R. clavatus* that two of the three essential pheromone components ((*E*)-2-hexenyl (*E*)-2-hexenoate and (*E*)-2-hexenyl (*Z*)-3-hexenoate) are produced in the enlarged lateral accessory glands of males that are attached to the metathoracic scent gland reservoir. However, the third

Table 1: Amounts of the compounds identified in extracts obtained from air-entrainment of males and females of *N. parvus* ($N = 5$).

Compounds	Retention index (DB-5)*	(ng/24 hours/insect)	
		Males	Females
(1) Camphene	954	0.02 ± 0.01	1.7 ± 0.02
(2) 6-Methyl-5-hepten-2-one	980	1.12 ± 0.85	1.05 ± 0.78
(3) Hexanoic acid	981	0.59 ± 0.37	1.19 ± 0.74
(4) Butyl butanoate	995	1.11 ± 0.73	0.01 ± 0.01
(5) Hexyl acetate	1008	1.26 ± 0.91	1.37 ± 0.79
(6) Limonene	1035	0.06 ± 0.01	1.2 ± 0.52
(7) (*E*)-2-Octen-1-ol	1059	0.06 ± 0.02	0.01 ± 0.01
(8) Pentyl butanoate	1093	1.23 ± 1.17	0.66 ± 0.16
(9) Undecane	1100	2.14 ± 0.98	1.67 ± 0.92
(10) Nonanal	1104	1.28 ± 0.51	1.48 ± 0.45
(11) Unknown compound	1165	0.14 ± 0.13	0.33 ± 0.12
(12) Hexyl butanoate	1193	2.15 ± 1.92	43.58 ± 10.12
(13) Dodecane	1200	2.21 ± 1.49	2.89 ± 1.48
(14) Decanal	1207	1.68 ± 0.49	2.08 ± 0.64
(15) 4-methyl hexyl-butanoate	1262	0.11 ± 0.07	4.49 ± 1.64
(16) Hexyl pentanoate	1288	—	6.84 ± 2.11
(17) Tridecane	1300	15.06 ± 14.05	5.11 ± 2.21
(18) 4-methylhexyl-pentanoate	1364	—	0.49 ± 0.15
(19) Hexyl hexanoate	1386	1.20 ± 0.84	1.50 ± 0.52

* Retention index was calculated using the retention time obtained in GC-FID analysis using a DB-5 column with a temperature program of 50°C/2 min, then 8°C/min to 250°C/10 min.

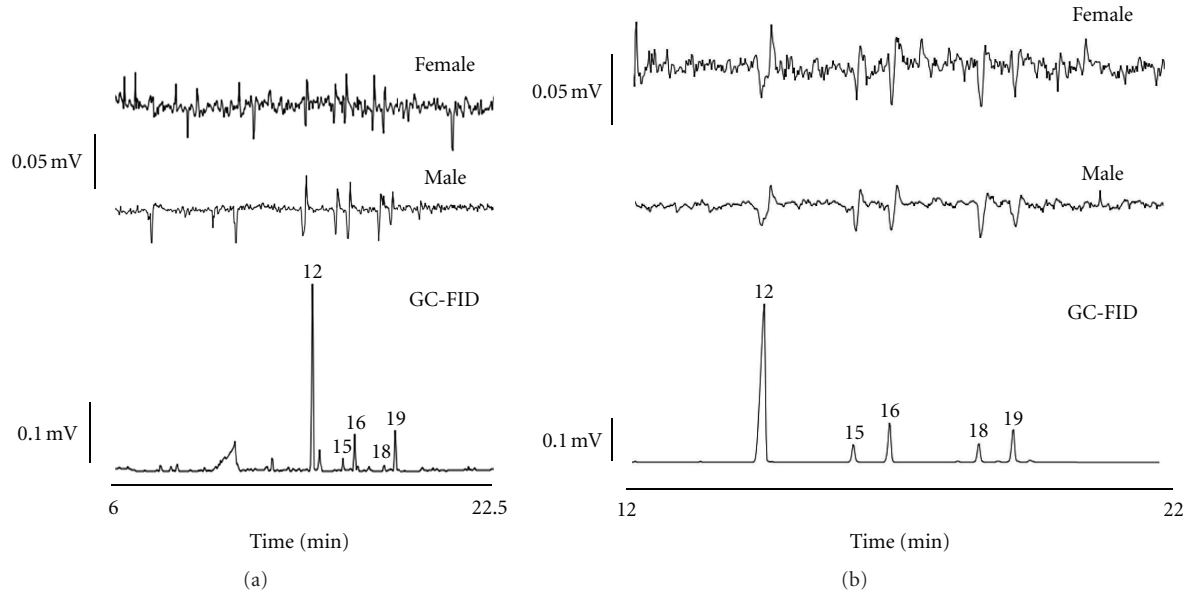

Figure 2: (a) A typical response to crude extracts of female aerations of antenna of *Neomegalotomus parvus* female and male in GC-EAG analyses. (b) A typical response to a synthetic mixture of compounds found in *N. parvus* aerations and scent glands of antenna of *N. parvus* female and male in GC-EAG analyses. (12) hexyl butanoate, (15) 4-methylhexyl-butanoate, (16) hexyl pentanoate, (18) 4-methylhexyl-pentanoate, and (19) hexyl hexanoate. In (b) the synthetic mixture tested was (12) hexyl butanoate (0.05 mg/mL), (15) 4-*S*-methylhexyl-butanoate (0.005 mg/mL), (16) hexyl pentanoate (0.0075 mg/mL), (18) 4-*S*-methylhexyl-pentanoate (0.002 mg/mL), and (19) hexyl hexanoate (0.0075 mg/mL).

Table 2: Principal compounds found in metathoracic scent gland and volatile collection of Alydidae species.

Compounds	*M. scutellaris* [18]	*R. serripes* [17]	*R. clavatus* [21, 22]	*M. quinque-spinosus* [14]	*A. eurinus* [14, 15]	*A. pilosulus* [14]	*L. chinensis* [19]	*N. parvus* (this work)
Butanal					MF	MF		
Nonanal							MF	
2-Methyl-butanal				MF				
Methyl propanal				MF				
(*E*)-2-Hexenal	MF	MF		MF	MF	MF		
(*E*)-2-Octenal	F						MF	MF
Octanol							MF*	
(*E*)-2-Hexen-1-ol	MF				MF	MF		
(*E*)-2-Octen-1-ol	MF						MF	MF
2-Methyl-butanoic acid				MF				
Butanoic acid	M			MF				
Hexanoic acid	M	M			MF	MF		MF
(*E*)-2-Hexanoic acid		M						
(*Z*)-3-Hexanoic acid		M						
(*E*)-2-Octanoic acid	M							
Hexyl-acetate								MF
Octyl acetate							MF	
(*E*)-2-Octenyl acetate							MF*	
(*Z*)-3-Octenyl acetate							MF	
Isobutyl 2-methyl-propanoate				MF				
2-Methyl-butyl 2-methyl-propanoate				MF				
2-Methyl-butyl butanoate				MF	MF			
(*E*)-2-hexenyl butanoate	M	M						
Butyl butanoate				MF	MF	MF		
(*E*)-2-Methyl-butenyl-butanoate					F*			
2-Methyl-butyl-butanoate					F*			
Butyl-butanoate								MF
Pentyl-butanoate								MF
Hexyl-butanoate					MF	MF		MF*
4-Methyl-hexyl-butanoate								MF*
(*E*)-2-Hexenyl-butanoate	F	F						
Hexyl-pentanoate								F*
4-Methyl-hexyl-pentanoate								F*
Butyl-hexanoate				MF				
2-Methyl-butyl-hexanoate				MF				
(*E*)-2-Hexenyl-(*Z*)-3-hexenoate	M	M	M*					
(*E*)-2-Hexenyl-(*E*)-2-octenoate		M						
(*E*)-2-Hexenyl-(*E*)-2 hexenoate		M	M*			MF		
(*E*)-2-Hexenyl-(*Z*)-2-hexenoate		M	M*					
Hexyl hexanoate								MF*
Tetradecyl-2-methyl-propanoate			M*					
Octadecyl-2-methyl-propanoate			MF					

Note: compounds labeled with F are female specific, labeled with M are male specific, and labeled with MF were identified in both gender. Compounds labeled with asterisk are those for which attraction function were confirmed in laboratory or field assays. Aldrich et al. [18], Leal et al. [17], Endo et al. [21], Yasuda et al. [22], Aldrich and Yonke [14], Aldrich et al. [15].

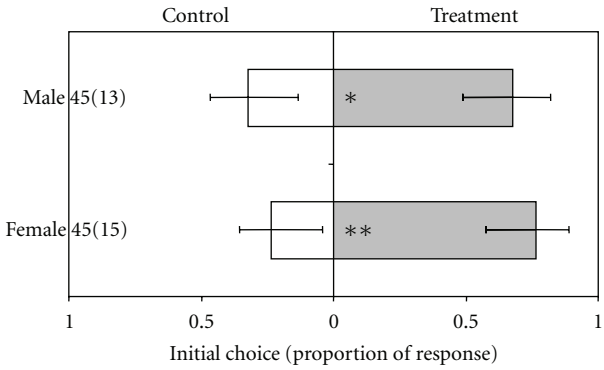

Figure 3: Initial choice (mean ± confidence interval (CI) 95%) of *Neomegalotomus parvus* males and females to crude extract of female aeration. Analyses of initial choices were carried out by logistic regression and χ^2 Wald test. *indicates $P < 0.05$ and **indicates $P < 0.01$. Numbers at left indicate total number of insects tested and numbers in brackets indicate the number of insects that did not make a choice after 5 minutes.

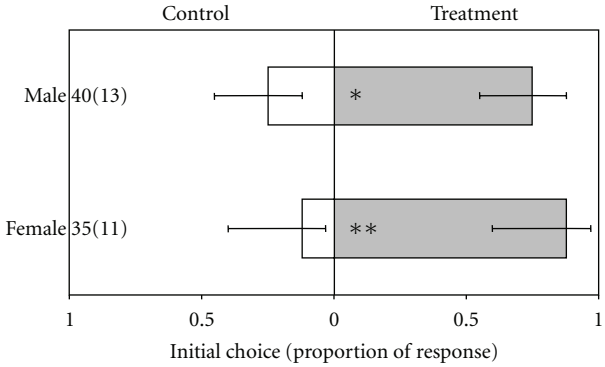

Figure 4: Initial choice (mean ± confidence interval (CI) 95%) of *Neomegalotomus parvus* males and females to 5 uL of a solution containing the synthetic authentic standards found in females extracts (hexyl butanoate (0.005 mg/mL), 4-(*S*)-methylhexyl-butanoate (0.0005 mg/mL), hexyl pentanoate (0.00075 mg/mL), 4-(*S*)-methylhexyl-pentanoate (0.0002 mg/mL), hexyl hexanoate (0.00075 mg/mL). Analyses of initial choices were carried out by logistic regression and χ^2 Wald test. * indicates $P < 0.05$ and ** indicates $P < 0.01$. Numbers at left indicate total number of insects tested and numbers in brackets indicate the number of insects that did not make a choice after 5 minutes.

essential component (myristyl isobutyrate) is produced from cells in the abdominal sternum of males [15].

Of the known alydid pheromone systems, *N. parvus* attractant pheromone appears most similar to that of *Alydus eurinus* (Say), where females produce a pheromone from the nonsexually dimorphic lateral accessory glands of the metathoracic scent gland complex with essential components being (*S*)-(-)-2-methylbutyl butanoate and *(E)*-2-methyl-2-butenyl butanoate. Although the chirality of 4-methylhexyl hexanoate and 4-methylhexyl pentanoate in *N. parvus* has not been unequivocally established ((*R*)-4-methyl-1-hexanol was not available), the biological activity of a blend containing synthetic esters with *S* absolute configuration combined with the fact that only (*S*)-(-)-2-methylbutyl butanoate was active for *A. eurinus* is suggestive that the 4-methylhexyl esters in *N. parvus* may also have *S* configuration. Dissections of adult *N. parvus* by one of us (J. Aldrich) indicated that the lateral accessory glands are enlarged in females and contain the key pheromone components (unpublished data). In *A. eurinus*, the female-produced pheromone attracts males, and to lesser extents females and nymphs; further tests are needed to establish whether the *N. parvus* pheromone will exhibit a similar pattern in the field.

Interestingly, a communication system similar to that of alydids occurs in plant bugs (Miridae) where sex pheromones consisting of aliphatic esters are produced in metathoracic scent glands of females [29–31], and in certain seed bugs (Lygaeidae) where males produce chemically similar aggregation pheromones from sexually dimorphic metathoracic scent glands [32].

In summary, *N. parvus* females produce two specific compounds that were not identified in male extracts and these compounds are pentanoates, the first time observed in Alydidae species, the other esters identified, hexanoates, and butanoates, were previously reported for others species of Alydidae. Five of these esters, hexyl butanoate, (*S*)-4-methylhexyl butanoate, hexyl pentanoate, (*S*)-4-methylhexyl pentanoate, and hexyl hexanoate stimulate an electrophysiological and behavioral response from males and females, indicating a possible function as aggregation pheromone. The identification of an attractant pheromone for *N. parvus* may lead to a more effective monitoring system for this pest, to more accurately guide insecticidal programs against this pest. Further field testing is necessary to make this potential application a reality.

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