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Full Length Research Paper

Indication of bioactive candidates among body volatiles of gregarious adult locusts *Locusta migratoria manilensis* by electroantennography (EAG) test

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Gregarious adult locusts are believed to release many bioactive volatiles from their bodies for the mediation of their biological characteristics. The determination of these bioactive body volatiles can contribute to the development of new, environmentally benign methods of locust control. An important locust, *Locusta migratoria manilensis* is an extremely destructive agricultural pest in China. Body volatiles of gregarious *L. m. manilensis* adults were found to be involved in mediation of aggregation behavior, but bioactive components in the body volatiles had not been indicated. In this study, four compounds were identified from body volatiles of gregarious *L. m. manilensis* adults using gas chromatography-mass spectrometry analysis: 2-hexanone, butyl acetate and α-pinene from both sexes and 2-heptanone from males only. The electroantennography (EAG) test indicated that gregarious adult males and females had the same EAG response pattern to these compounds. 2-Hexanone, 2-heptanone and butyl acetate elicited dose-dependent responses, which are considered as bioactive candidates among body volatiles. The two ketones, 2-hexanone and 2-heptanone, were the strongest olfactory stimuli for gregarious *L. m. manilensis* adults. The contribution of these EAG-active compounds to the mediation of aggregation or other behaviors still remains to be further investigated.

Key words: *Locusta migratoria manilensis*, body volatiles, GC-MS, EAG, bioactive compounds.

INTRODUCTION

Locusts (Orthoptera) have been regarded as serious agricultural pests since ancient times. Migrating swarms can appear without warning in previously un-infested areas and rapidly devastate crops and pastures (Magor et al., 2008). A single locust swarm can contain billions of individuals and travel hundreds of kilometers each day. Locust outbreaks occur on almost every continent and currently affect the livelihoods of one in ten people worldwide (Simpson and Sword, 2008). Studies on locust biology, with the aim of finding ways to control this pest, are therefore, a long-standing focus for scientific research (Simpson and Sword, 2008).

Locusts can develop into two phases: gregarious and solitarious. Gregarious locusts, especially adults can bring huge damage to agriculture (Simpson and Sword, 2008). Many studies reported that gregarious adult locusts released many bioactive volatiles from their bodies, playing a critical role in the mediation of aggregation behavior, sexual maturation and mating (Pener and Yerushalmi, 1998; Ferenz and Seidelmann, 2003; Hassanali et al., 2005). These studies were mainly focused on the desert locust *Schistocerca gregaria* (Forskål) (Mahamat et al., 1993, 2000; Torto et al., 1994; Seidelmann and Ferenz, 2002; Rono et al., 2008). To some extent, the determination of these bioactive body volatiles can contribute to the development of new, environmentally benign methods of locust control, such as mass trapping and disruption of behaviors (Rochat et al., 2000; Rosa et al., 2006).

However, little is currently known about bioactive body volatiles of an important locust, *Locusta migratoria* (L.) (Orthoptera: Acrididae). It has been indicated that gregarious adults of a subspecies *L. migratoria* migratorioides(Reiche & Fairmaire) mainly emitted aliphatic aldehydes and alcohols, which were involved in

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Supplementary Figure 1. A map showing approximately the distribution of *L. m. manilensis* over the world (the region circled in red ink).

the mediation of aggregation behavior (Niassy et al., 1999). Another subspecies, *L. migratoria manilensis* (Meyen, 1835) is distributed in a different continent from *L. m. migratorioides* and known to frequently cause plague in China (Supplementary figure 1) (Lecoq and Sukirno, 1999; Chen 2000, 2002). Body volatiles of gregarious *L. m. manilensis* adults were found to be involved in the mediation of aggregation behavior (Shi et al., 2010), but bioactive components in the body volatiles had not been indicated. Electroantennography (EAG) is a good tool to identify bioactive compounds and EAG-active compounds are frequently of ecological significance (Van Der Pers, 1981). In this study, the compounds in body volatiles of gregarious *L. m. manilensis* adults (from both sexes) were identified by gas chromatography-mass spectrometry (GC-MS) and the antennal olfactory responses of the adults to these identified compounds were tested by EAG. This work indicate the bioactive candidates among body volatiles of gregarious *L. m. manilensis* adults, providing a basis for further investigations on their contribution to mediation of aggregation or other behaviors.

MATERIALS AND METHODS

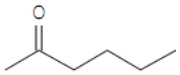
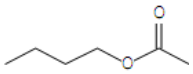
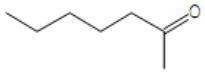
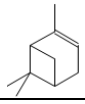
Insects

Locusts (*L. m. manilensis*) were reared in crowded conditions (30 to 40 individuals/cage) on maize leaves (*Zea mays*) in cylindrical wire mesh cages (18 cm diameter × 45 cm) in the Key Laboratory of Biological Control, Ministry of Agriculture, China Agricultural University. Cages were maintained under constant illumination at 30±2°C and 70% relative humidity.

Collection of body volatiles

The adult males and females (10 to 12 days after emergence) were sampled separately. 50 live adults at a time were placed in a cylindrical glass bottle (17 cm diameter × 27 cm) connected to a trap made from a glass tube (6 mm diameter × 75 mm) filled with activated charcoal (80 mesh; 0.5 g) held between two glass wool

Table 1. Compounds identified, by means of GC-MS, from body volatiles of gregarious *L. m. manilensis* adults.

Retention time (min)	Compound	Structure	The relative amount (% TIC)*	
			Male	Female
4.8	2-Hexanone		24.67 ± 0.05	33.64 ± 0.15
5.5	Butyl acetate		22.23 ± 0.02	6.48 ± 0.01
8.6	2-Heptanone		7.38 ± 0.12	—
10.4	α-Pinene		45.72 ± 0.08	59.88 ± 0.16

*Mean ±SE. TIC = Total ion current.

plugs. A vacuum pump was used to draw charcoal-purified air from the bottle over the activated charcoal filter in the trap at a rate of 300 ml/min. After 12 h, the trapped volatiles were eluted using 5 ml of redistilled, analysis grade dichloromethane (Beijing Chemical Reagents Company, Beijing, China), concentrated to 0.5 ml in a nitrogen flow and stored at -20°C. A control sample was collected from an empty bottle using the same method. Before each collection, the charcoal trap was cleaned by means of extraction with redistilled analysis grade dichloromethane and activated at 200°C for 3 h. Locusts were starved for 6 h to purge their alimentary canals with the intention to prevent fecal pollution of body volatiles. Three replicate collections from each sex were taken.

Identification of body volatiles by GC-MS

Volatiles were analyzed using a gas chromatograph coupled with a mass spectrometer (GC-MS, SHIMADZU GCMS-QP2010). The gas chromatograph was equipped with a capillary column (DB-5 ms, 30 m × 0.25 mm diameter × 0.25 µm film thickness), using nitrogen gas as the carrier and a 1 µl splitless mode injector at temperatures of 250°C and temperature regimes of 40°C for 5 min, rising to 80°C at a rate of 2°C/min and then to 240°C at a rate of 8°C/min. The ion source temperature was 250°C. Mass spectra were acquired at electron impact (EI) mode of 70 eV and a scanning scope from 20 to 650 m/z. The relative amount of each component was calculated from the total ion current. The amounts reported are an average (and standard error) of the three replicate collection samples. All compounds were identified by comparison with mass spectra data from the NIST05 MS library. Impurities were excluded by comparison between the GC-MS test samples and the control sample. Final identifications were confirmed by comparison of spectra and retention times to those of four authentic standards: 2-hexanone (99.9%, Supelco, USA); butyl acetate (99.9%, Supelco, USA); 2-heptanone (98%, Acros, Belgium); and α-pinene (98%, Aldrich, USA).

EAG analyses

EAG responses from isolated adult antennae to the compounds identified earlier were measured by means of an EAG system (Syntech, The Netherlands). Each locust was kept individually without food supply for 2 h, after which one of its antennae was excised. Then an incision was made at the distal end of the antenna, after which the antenna was connected to two silver electrodes (recording electrode and reference electrode) using Spectra 360 conductive gel (Parker, Orange, N. J. USA). A Syntech PC-based signal processing system was used to amplify and process EAG signals. The signals were analyzed using Syntech EAG 2000 software.

Authentic standards of test compounds were dissolved in mineral oil at five different concentrations (0.5, 5, 10, 15 and 20 µg/µl). A 4 µl aliquot of each test solution was applied to a piece of filter paper (1 × 2 cm), which was placed in a 10 cm length glass Pasteur pipette. For the blank control, only 4 µl mineral oil was applied. For the reference stimulus, 4 µl of a solution was made up from authentic standard hexanal (99%, TCI, Japan) dissolved in mineral oil at 20 µg/µl. The tip of each pipette was then inserted into a small hole (2 mm diameter, 14 cm from the outlet) of a main airflow tube (8 mm diameter × 15 cm) in which a continuous, charcoal-filtered moistened airflow was blown (at 150 ml/min) onto the prepared antenna. A 0.5 s puff of charcoal-filtered airflow was injected through the large end of the Pasteur pipette, transporting the volatile compound from the filter paper to the antenna for stimulation, using an electronically controlled stimulus flow controller (CS-05, Syntech, The Netherlands).

Each compound was tested three times at each dose on an antenna. The following protocol was adopted: 80 µg hexanal (reference stimulus), mineral oil (blank control), test compound (2, 20, 40, 60 and 80 µg, sequentially), 80 µg hexanal (reference stimulus) at 1 min intervals between stimulations. For each test compound, EAG responses were recorded from four antennae from different individuals of each sex. To compensate for variation in responses and declines in antennal sensitivity during a measuring session, the absolute EAG response to 80 µg hexanal was scaled to a value of 100. Values between two 80 µg hexanal calibration references were estimated by linear interpolation. The dose-response line charts were drawn using SPSS software package version 17.0 (SPSS Inc., Chicago, IL).

RESULTS

Identification of body volatiles by GC-MS

Four compounds were identified from adult body volatiles: 2-hexanone (aliphatic ketone), butyl acetate (aliphatic ester), 2-heptanone (aliphatic ketone) and α-pinene (aliphatic alkene) (Table 1; Figure 1). 2 Hexa-

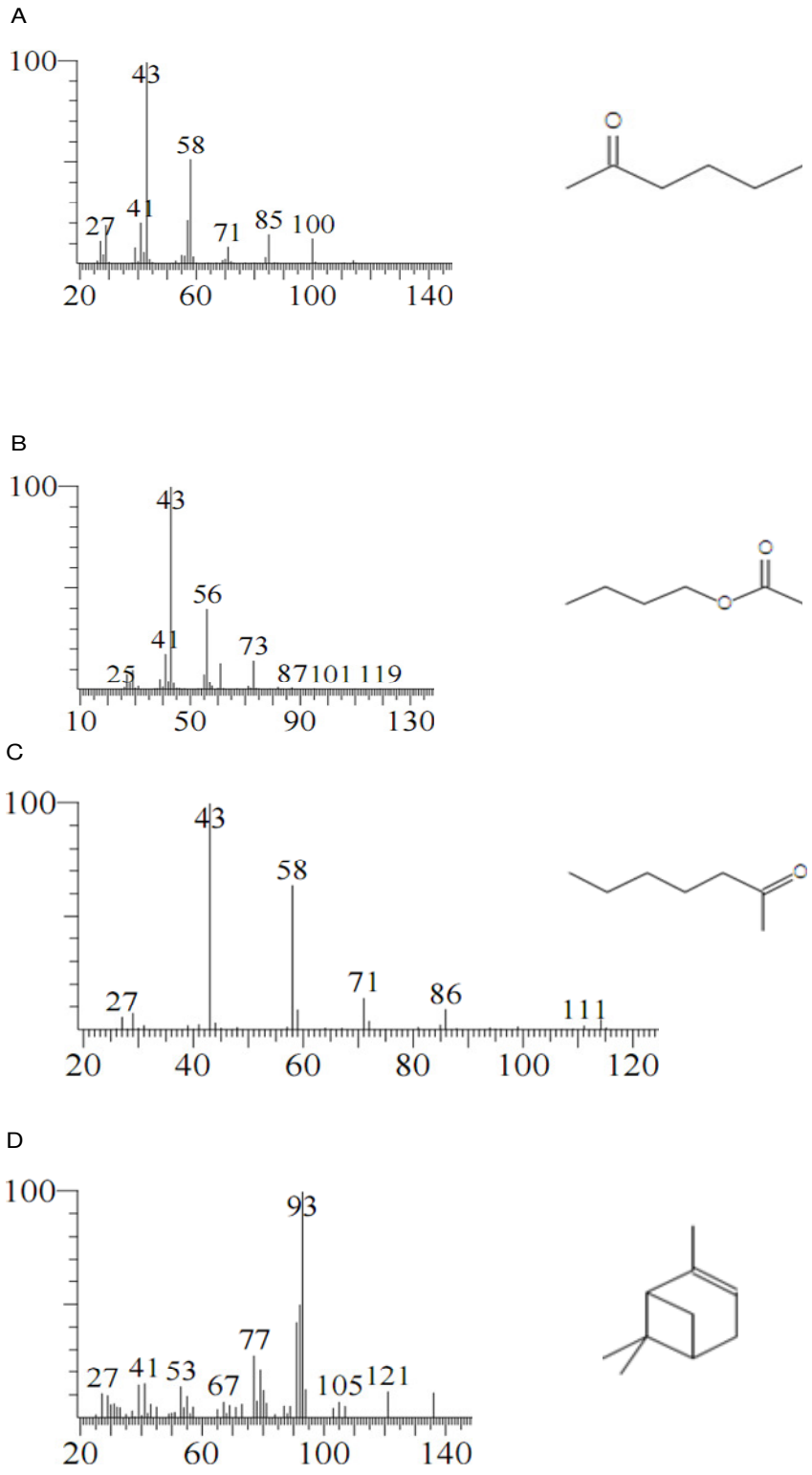


Figure 1. Mass spectra of the four compounds identified from body volatiles of gregarious *L. m. manilensis* adults. (A) 2-Hexanone; (B) Butyl acetate; (C) 2-Heptanone; (D) α-Pinene.

none, butyl acetate and α-pinene were identified from both sexes, but 2-heptanone was identified only from males. In each sex, the relative amount of α-pinene was the largest, followed by 2-hexanone and butyl acetate. 2-

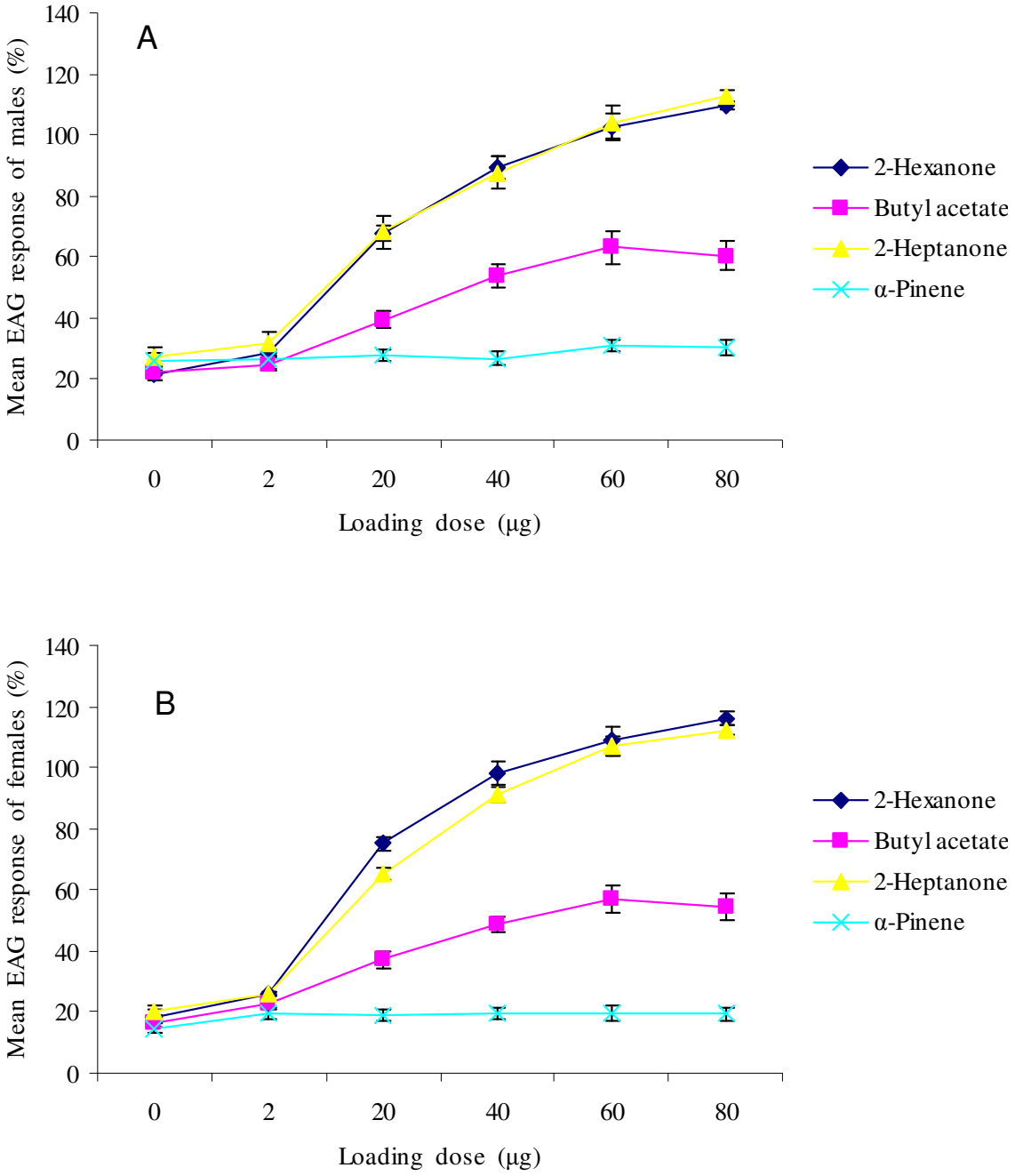


Figure 2. Mean ±SE EAG responses of gregarious *L. m. manilensis* adult males (A) and females (B) to the four compounds (2-hexanone, butyl acetate, 2-heptanone and α-pinene) from body volatiles of the locust adults. For each compound, six doses were tested, that is, 0, 2, 20, 40, 60 and 80 µg.

Heptanone was the least component in male.

EAG analyses

The same EAG response pattern was found between adult males and females. 2-Hexanone, butyl acetate and 2-heptanone elicited dose-dependent responses, which increased as loading doses increased, but a plateau was found in the responses to butyl acetate at a dosage of 60 µg. Among the four test compounds, the two ketones (2-hexanone and 2-heptanone) elicited the strongest responses of adult males and females, whereas α-pinene elicited the weakest responses. At a low test dose (2 µg), however, none of the responses were strong. These results can be seen in Figure 2.

DISCUSSION

In this study, aliphatic ketones, an aliphatic ester and an aliphatic alkene were identified in the body volatiles of gregarious *L. m. manilensis* adults. A previous study indicated that gregarious adults of *L. m. migratorioides* mainly emitted aliphatic aldehydes and alcohols (Niassy et al., 1999). Thus, body volatile emissions of gregarious *L. migratoria* adults may be dominated by aliphatic compounds, unlike those of the gregarious adult desert locust, *S. gregaria*, which are mainly composed of aromatic compounds (Mahamat et al., 1993, 2000; Torto et al., 1994; Seidelmann and Ferenz, 2002; Rono et al., 2008).

In this study, α-pinene, the largest relative amount of compound in body volatiles of both *L. m. manilensis* adult males and females, could not elicit dose-dependent EAG responses. In contrast, the least amount of compound in male body volatiles was 2-heptanone; not identified in female, and elicited dose-dependent EAG responses. Therefore, the large relative amount of one volatile compound does not mean importance to antennal olfactory response. However, the bioactivity may depend on the functional groups of chemicals. In this study, the two ketones (2-hexanone and 2-heptanone) elicited the strongest EAG responses of adult males and females, whereas α-pinene elicited the weakest responses. Although the olfactory system of *L. m. manilensis* adults may be sensitive to changes in the functional group of the stimuli, this depends on the dosage of the stimuli. At a low test dose (2 μg), the responses to all four test compounds were not strong.

In this study, three components (2-hexanone, 2-heptanone and butyl acetate) elicited dose-dependent EAG responses in both sexes. This suggests that these three compounds are bioactive candidates among body volatiles of gregarious *L. m. manilensis* adults, possibly involved in the mediation of aggregation behavior (Shi et al., 2010). Fecal volatiles of gregarious *L. m. manilensis* adults also were thought to mediate aggregation behavior, but no common component was found in the body and fecal volatiles (Yu et al., 2007; Shi et al., 2010). It is possible that EAG-active compounds in body volatiles could have a synergistic effect with such compounds present in the fecal volatiles (Pener and Yerushalmi, 1998). Although in this study 2-hexanone and butyl acetate, as well as α-pinene were identified in both sexes of *L. m. manilensis* adults, 2-heptanone was identified only in adult males. This is possibly consistent with the results of a previous study indicating that the aggregating signals in body volatiles of gregarious *L. m. migratorioides* adult males contain more active components than those of conspecific females (Niassy et al., 1999). Certainly, male-specific 2-heptanone possibly plays its special role. In the desert locust *S. gregaria*, male-specific body volatiles can be used to induce aggregation, accelerate maturation or repel other conspecific males for mate guarding (Mahamat et al., 1993, 2000; Torto et al., 1994; Seidelmann and Ferenz, 2002; Rono et al., 2008). In several Coleoptera species, aggregating or sex signals are produced only by males (Ramirez-Lucas et al., 1996; Sánchez et al., 1996; Wakefield et al., 2005; Vidal et al., 2010).

In other insects, 2-heptanone has been found to have important bioactivity. For example, in ants, secreted by the mandibular glands, it functions as a nestmate identifier and an alarm signal (Moser et al., 1968; Hernández et al., 2006). In honeybees, it serves as an alarm pheromone or sometimes as a 'forage-marking' pheromone (Vallet et al., 1991; MacLean and Schmolz, 2004; Hunt, 2007). Butyl acetate identified in this study was ever reported to be a component of aggregation pheromone in a pest beetle *Strategus aloeus* (Rochat et al., 2000). However, it remains to be seen whether the EAG-active compounds found in this study function as aggregating signals for *L. m. manilensis* adults or are involved in the mediation of other behaviors. Field bioassay may be important, because this can access to natural law, providing a powerful basis for the development of new, environmentally benign methods of locust control. In addition, it is a mixture of body volatiles to function as olfactory stimulus in wild environment, which may elicit a different response pattern of *L. m. manilensis* from that elicited by only a single component.

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