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FULL LENGTH ARTICLE

Electrophysiological responses of chafer beetle, *Holotrichia serrata* (F.) (Coleoptera: Scarabaeidae)

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Abstract The chafer beetle, *Holotrichia serrata* F. (Coleoptera: Scarabaeidae) in its larval stage is a serious pest on sugarcane, vegetables, groundnut and coconut in many parts of India. The antennal response of adults to host volatiles and pheromone gland extracts was assessed by electroantennography. Among the preferred host of *H. serrata*, the volatiles from neem, *Azadirachta indica* A. Juss leaf extract elicited higher antennal response than gulmohar *Delonix regia* L. flowers and *Ailanthus excelsa* (Roxb) leaf extracts. The order of response was the same irrespective of the sex. In general the antennal response to pheromone gland and host extracts was higher in males than in females.

1. Introduction

Holotrichia is a genus of the melolonthine scarabs that occurs across the Indian subcontinent and throughout southeast and east Asia (Ward et al., 2002). The chafer beetle, *Holotrichia serrata* F. (Coleoptera: Scarabaeidae) in its larval stage is a serious pest of coconut, (*Cocos nucifera* L.), sugarcane, (*Saccharum officinarum* L.), groundnut, (*Arachis hypogaea* L.) and vegetables in parts of Western and peninsular India (Ganeshaiah and Kumar, 1993). The grubs cause damage by feeding on the roots and the adults emerge with the arrival of monsoon or heavy pre-monsoon showers (Yadav and Sharma, 1995). On emergence at dusk, they aggregate on plants like neem, (*Azadirachta indica* A. Juss), gulmohar, (*Delonix regia* L.), tamarind, (*Tamarindus indica* L.), mahagony, (*Swietenia mahagony* L.), drumstick, (*Moringa oleifera* Lam.) and subabul, (*Leucaena leucocephala* Lam.) for feeding and mating (A.R.V. Kumar Personal communication and Yadav and Sharma, 1995).

Attraction of adult males by females has been documented in *H. serrata* (Ganeshaiah and Kumar, 1993). The pheromone of *H. consanguinea* and *H. reynaudi*, has been isolated and identified as anisole (Leal et al., 1996; Ward et al., 2002). Chemical insecticides are widely used by farmers to manage the grubs of *Holotrichia* (Anitha et al., 2006) and they result in varying degrees of success in grub management. Indiscriminate use of insecticides results in the buildup of residues and cause negative impact on non target organisms. Hence, it is imperative to search for alternative pest control methods. One such option is to exploit the behavioral features of the insect. Ethological control has been successfully applied in the management of scarabs (Leal et al., 1996; Ruther et al., 2000).

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Though *H. serrata* is an important pest, there are no reports on its olfactory response till date. The objective of this study is to investigate the antennal responses of *H. serrata* adults to female pheromone gland extracts and some host plant extracts. Hence, as a primary step to identify physiologically active compounds causing antennal responses, electroantennography was used.

2. Materials and methods

2.1. Test compounds

Chemicals tested for electroantennographic responses of beetles were selected on the basis of a bibliographic search for the compounds eliciting antennal response to Scarabaeidae (Table 1). Chemicals were obtained from Merck (India) Ltd. and used without further purification.

2.2. Solvent extraction of pheromone gland

Adults of *H. serrata* were collected from neem, *A. indica* and *A. excelsa* at Chamaraja Nagar, Karnataka, India. The beetles were sexed by the shape and size of the hind tibial spurs (Musthak Ali, 2001). Pheromone glands from 40 calling females were extruded by gently pressing female's abdomen at the time when they are primed for release of pheromone. Forty pheromone glands of calling females were dissected using INOX 5 forceps and transferred to a glass vial (100 ml capacity) containing 40 ml of a solvent *viz*., benzene, hexane, diethyl ether and dichloromethane. This extract had 40 gland equivalents. The pheromone glands were extracted by soaking them in respective solvents for 12 h at room temperature (25 °C). They were filtered and were concentrated to 5 ml under a gentle stream of nitrogen.

Table 1 Antennal response of *H. serrata* adults to selected compounds.

Compound	Average EAG response (mv)[a]	
	Male	Female
Methanol (1)	−0.80 ± 0.02^{cd}	−0.62 ± 0.04^{c}
Ethyl acetate (2)	−1.40 ± 0.03^{a}	−0.81 ± 0.04^{b}
Isoamyl acetate (2)	−0.42 ± 0.03^{e}	−0.21 ± 0.03 ^{e}
Ethyl iso butyrate (1)	−1.19 ± 0.05^{b}	−0.81 ± 0.05^{b}
Ethyl butyrate (2)	−0.91 ± 0.04^{c}	−0.65 ± 0.06^{c}
Propyl acetate (1)	−1.35 ± 0.06^{a}	−1.31 ± 0.05^{a}
Dodecane (1)	−0.73 ± 0.09^{d}	−0.19 ± 0.05^{e}
Tridecane (2)	−0.34 ± 0.03^{e}	−0.11 ± 0.01^{e}
Nonane (1)	−0.48 ± 0.01^{e}	−0.24 ± 0.02^{e}
Linalool (2)	−0.66 ± 0.03^{d}	−0.40 ± 0.03^{d}

Columns with the same letter are not significantly different at $p = 0.05$. DMRT numbers within brackets after compound names refer to references.
(1) CPCRI (2006).
(2) Jonas Bengtsson et al. (2009).
[a] EAG responses are averages of ten antennae and corrected for background responses before and after the sample replicate.

2.2.1. Solvent extraction of host (leaf/flower)

Leaves @ 100 g each of host plants *viz*., *A. indica, A. excelsa* from the middle peripheral area of the canopy of over 30 year old trees and flowers @ 100 g from gulmohar, *D. regia* were collected and immersed in 300 ml of dichloromethane (Merck) at room temperature (24 ± 2 °C) for 24 h. The solvent was filtered through Buchner funnel using Whatman filter paper 40 and concentrated to 5 ml in a rotary flash vacuum evaporator. The concentrated extracts were diluted with hexane (HPLC grade) in ratios of 1:0, 1:1 and 1:5 to evaluate dose response.

2.3. Electroantennography

All electrophysiological studies were conducted on adult beetles (females and males) 1–3 days old. These beetles were maintained in plastic bins of 5 × 20 cm dia, filled with soil (approx. 1 kg).

Electroantennogram measurements were made using a commercially available electroantennographic detection system (Syntech, Hilversum, The Netherlands) consisting of a dual electrode probe (PRG 2) for antenna fixation, a CS-05 stimulus controller and an IDAC 232 box for data acquisition. The antennal club was fanned and fixed with the tip of the lamella to one of the electrodes and the scape was fixed to the other electrode as suggested by Reinecke et al. (2005). The antenna was flushed continuously with a stream of activated charcoal filtered air.

The volatile compounds (5 µl) were applied on filter paper (Advantec 5C (110 mm), Japan) strips of 3 cm × 5 mm and were placed inside the pipette tips (Tarsons-India 100–1000 µl) which were connected to stimulus controller by a silicone rubber tube. The stimulus was puffed onto the antenna by injecting the vapor phase of the micro tip pipette 15 mm upstream from the antennae in the continuous air stream (pulse time 0.5 s, continuous flow 25 ml/s, pulse flow 21 ml/s). The minimum delay between the stimulus puffs was 120 s. Antennal response to aliquots was recorded from ten adults with three replications per antenna. The sequence of exposure of each stimulus to each antenna was randomly defined.

EAG responses of pheromone gland and plant volatile extracts were recorded after correcting for solvent and other background effects by subtracting the averaged EAG responses of the solvent responses recorded before and after each sample as described previously by Visser (1979). Corrected EAG responses were statistically analyzed by an analysis of variance (ANOVA). Statistical significance of the difference among treatments was compared by Duncan's multiple range test (DMRT) (Duncan, 1955).

3. Results and discussion

3.1. EAG responses to volatiles

All the tested compounds selected based on bibliographic search for scarabaeid response elicited significant EAG responses in *H. serrata* (Table 1). The non aromatic esters, ethyl acetate and propyl acetate elicited higher response in both male (−1.40 and −1.35 mV, respectively) and female (−.81 and −1.31 mV, respectively) beetles while the hydrocarbon alkanes *viz*., tridecane and nonane elicited the lowest responses.

Among the compounds tested, esters, on average, were better than alcohols and alkanes in eliciting *H. serrata* antennal response.

Non aromatic esters – saturated open chain compounds were found to elicit significant EAG response from both males and females of *H. serrata*. The amplitude of the responses varied with the carbon chain length of the compound. Ethyl acetate a C4 elicited higher response in males. The female antenna was more responsive to propyl acetate. In either case the response decreased with an increase in the carbon chain length. In general the antennae of both male and female beetles were more responsive to esters followed by alcohol and hydrocarbon alkane. The aromatic esters, ethyl acetate, ethyl butyrate and ethyl iso-butyrate were identified as kairomones to coconut red weevil, *Rhynchophorus ferrugineus* (Olivier) (Gries et al., 1994). Perez et al. (1997) reported that ethyl acetate and ethyl butyrate aided as a synergist to metalure a pheromone of West Indian sugarcane weevil, *Metamasius hemipterus sericeus*.

Among the sexes, the response of males to all the compounds tested was higher as compared to the females. The antennal response was similar in both the sexes when exposed to propyl acetate. The difference between the two antennae was the highest for dodecane. In general, the absolute EAG response reflects the number of activated receptor neurons and their degree of activation. In our observations, the response of the male antenna was often stronger than the female antenna. This is in contrast to the results of Reinecke et al., 2005 where the response of female *Melolantha melolantha* was stronger than that of males. There exists sexual dimorphism in the antennal morphology of *H. serrata*. The physical properties with respect to electrical conductivity and resistance are also known to vary between male and female antennae (Reinecke et al., 2005). The difference in the physical property between males and females may be the cause for the lower amplitude of EAG recorded in females as compared to males.

3.1.1. Influence of pheromone gland extracts

A comparison of the antennal response of both sexes to female pheromone gland extracts in various solvents was made. Extracts of pheromone glands in diethyl ether (−4.43 mV) when exposed to male antennae elicited significantly higher response as compared to the extracts made in dichloromethane or hexane which were at par (−3.40 and −3.31 mV, respectively) (Fig. 1). The antennal response to the gland extracts made by benzene was significantly low. A similar trend in response to the extracts was observed in female antennae. Female antennal response was limited to a maximum of 20% to that of the male antennae, on average.

Among the solvents used to obtain extracts from the pheromone gland, diethyl ether, dichloromethane and hexane caused the maximum EAG amplitude in the male antennae of *H. serrata*. Extraction of abdominal glands of *H. consanguinea* beetles in dichloromethane and ether yielded a clean profile of anisole and indole; the compounds responsible for attraction of *H. consanguinea* males (Leal et al., 1996). Pheromone glands of *H. reynaudi* extracted in hexane yielded indole, anisole and phenol (Ward et al., 2002). The antennae of *H. serrata* females respond to their gland extracts but at a lower level as compared to males irrespective of the solvents used for extraction. This indicates that there is a possibility of both sexes

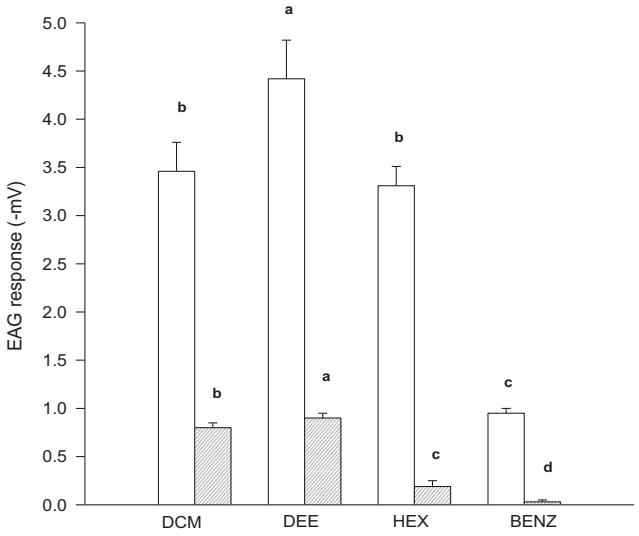

Figure 1 Responses (+SE) of male (white bars) and female (grid bars) *H. serrata* antennae to female pheromone gland extracted in dichloro methane (DCM), diethyl ether (DEE), hexane (HEX) and benzene (BENZ) in electroantennographic experiments. The responses were reduced by mean responses to respective solvents. Bars capped with different letters indicate significant difference at $p = 0.05$ with DMRT.

responding to female released pheromones. The aggregation of both male and female adults of *H. serrata* in the field was documented by Ganeshaiah and Kumar (1993). Pheromone detectors have been found in male and female antennae of *Anomala octiescostata* (Leal et al., 1994). The aggregation of both adults (male and female) of *H. consanguinea* to anisole (the female produced pheromone) was reported by Leal et al. (1996). Females of black chafer, *H. loochooana loochooana* release anthranilic acid (Yasui et al., 2003) and it attracts both sexes (Arakaki et al., 2003). The response to pheromone extracts is lower in *H. serrata* females than in its male counterparts. A similar observation in Japanese beetle revealed that pheromone detection by females was low compared to males as the number of pheromone detecting sensilla placodea in female antennae was lower than the males (Kim and Leal, 2000). We confirm a similar phenomenon operating in *H. serrata* also.

3.1.2. Influence of host plant extracts on beetles

All the three host extracts tested elicited antennal response. A comparison of the antennal response to individual extracts revealed a significant difference between the male and female antennae for every extract used in the experiment. In general male antennae were more sensitive to plant extracts than female antennae. Among the extracts, the neem leaf elicited the highest response followed by the *D. regia* flower and *A. excelsa* leaf. The order of response remained the same, independent of sex (Fig. 2).

Dose–response measurements were made individually for the host extracts to determine the response thresholds for both sexes. All the three host extracts tested show dose dependent responses from the antennae of both sexes. The differences between the doses tended to be strong and significant, besides varying with the level of concentration. In the case of sexes,

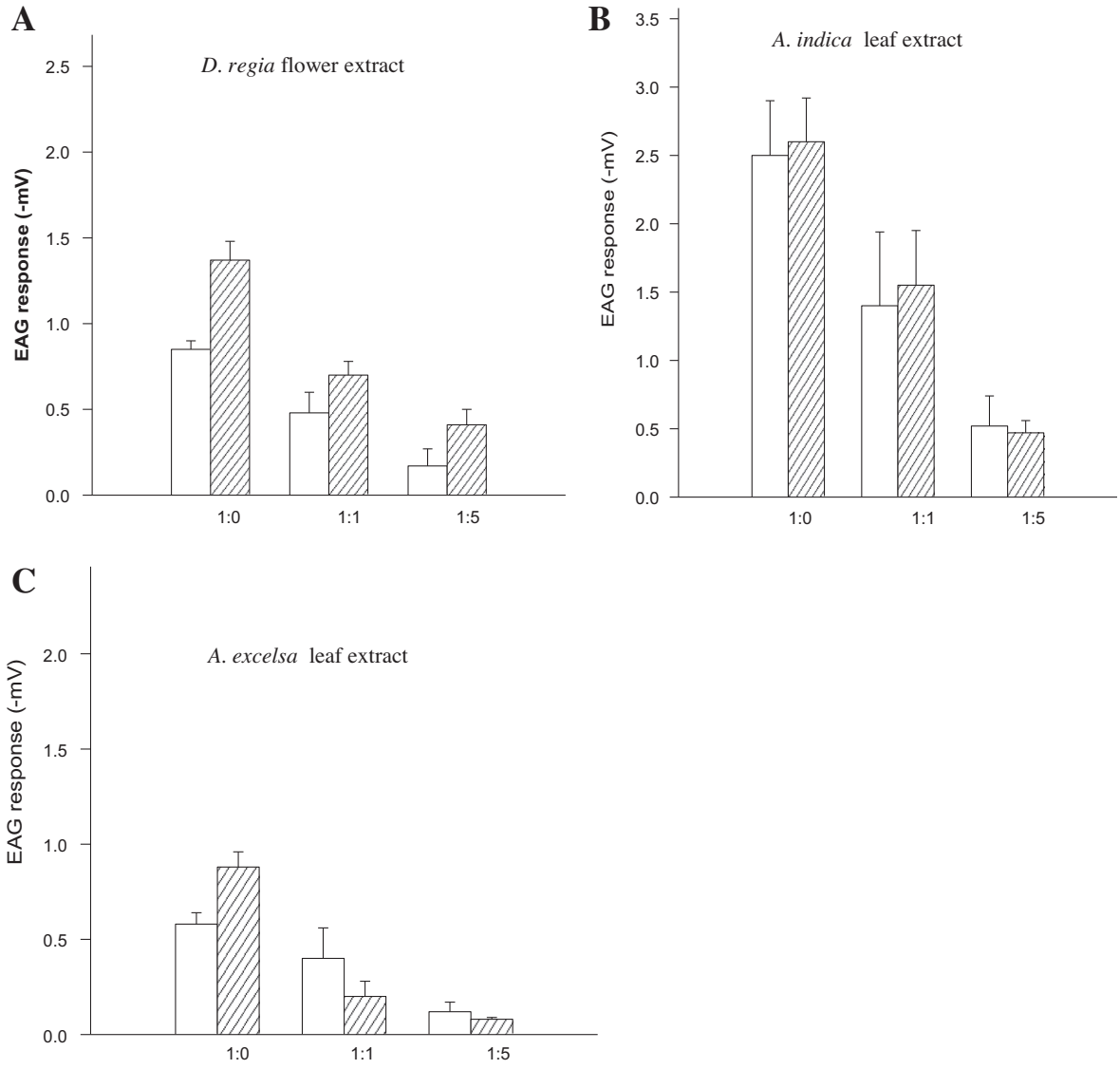

Figure 2 Dose dependent antennal responses (+SD) of female (white bars) and male (girded bars) *H. serrata* to host plant extracts (A. Gulmohar, *D. regia* flower extract, B. Neem, *A. indica* leaf extract, C. *A. excelsa* leaf extract). The responses for all the doses were reduced by mean responses to equivalent amounts of respective solvents.

only *D. regia* flower extracts showed a significant difference between male and female antennae whilst the neem leaf and *A. exelca* extracts had no significant differences between the female and male antennae (Table 2 and Fig. 2).

Our observations revealed that the adults of *H. serrata* preferred to aggregate on *A. excelsa* and on the flowers of Gulmohar in addition to neem. These host species were also recorded by other authors (Veeresh, 1977) in addition to a record of neem, acacia, ber, guava (Pal, 1977); *B. monosperma* (Yadav and Sharma, 1995) and tamarind, *T. indicus* (Anitha et al., 2006).

Both adult male and female antennae responded to host volatiles. But there was sexual dimorphism in the olfactory perception of the host volatiles by *H. serrata*. The male antenna is more sensitive to host extracts. Perhaps, this indicates the necessity of finding the host first for their mate location, apart from using the female produced pheromone as the cue. The use of green leaf volatiles as a sexual kairomone has been observed in *M. melolantha* and *H. consanguinea* males (Reinecke et al., 2005; Yadav and Yadav, 2004). A combination of the synthetic sex attractant (R,Z)-5-(Idecenyl) dihydro-2(3H)-furanone with a 3:7 mixture of phenethyl propionate (PEP) and eugenol caught significantly more *Popillia japonica* (Klein et al., 1981).

Though the response of female antennae of *H. serrata* to host volatiles *viz*., neem, gulmohar and *Ailanthus* is low, the results show that both are able to perceive the host volatiles at varying degrees and are dose dependent in both the sexes. The female antennae of *M. melolantha* in general responded less to green leaf volatiles (GLV) as compared to male antennae (Reinecke et al., 2002, 2005). As both males and females respond to flower and plant/floral volatiles, they can be categorized as aggregation kairomones as suggested by Ruther et al. (2000). The lures attracting not only males (like most sex

Table 2 Influence of *H. serrata* beetle sex on electroantennographic response to host volatiles – statistical parameters after two way ANOVA (factors: Dose and sex).

Plant extract	Factor	MS	F	P level
Gulmohar flower	Dose	1.380	141.54	0.000
	Sex	0.640	65.70	0.02
	Dose × sex	0.90	6.1	0.01
Neem leaf	Dose	8.868	53.4	0.000
	Sex	0.002	0.014	ns
	Dose × sex	0.002	0.01	ns
Ailanthus leaf	Dose	0.836	104.7	0.000
	Sex	0.003	0.33	ns
	Dose × sex	0.128	16.1	0.00

pheromones) but also the gravid females are a vital tool to manage scarab pests.

The study is in agreement with earlier studies on the role of sex pheromones and host volatiles in attracting the phytophagous scarab beetle. The host volatiles and pheromone gland extracts confirm the physiological response in the antennae of *H. serrata*. Further it has to be confirmed by behavioral assay and field studies. This study demonstrates the need for a better understanding of synergy between host volatiles and pheromones in developing them as a tool for pest management.

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