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Padmanaban Balakrishnan
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38. Thompson, J. Y., Bayne, S. C. and Swift, E. J., Dental materials citations: Part A, January to June 1996. *Dent. Mater.*, 1996, **12**, 272–286.
39. Kao, C. T. and Huang, H. T., Orthodontic materials. In General Session of International Association for Dental Research, 2003.
40. Uppendar Kumar, A comparative evaluation of growth of micro-organism on the surface of various orthodontic bonding materials. *J. Indian Orthod. Sci.*, 1998, **31**, 47–52.
41. Dexter, S. C. and Maruthamuthu, S., Response of passive alloys with n- and p-type passive films to manganese to biofilms. *Corrosion*, 2001, paper no. 01256, 1–15.
42. Laurent, F., Grossgogeat, B., Reclaru, L., Dalard, F. and Lissac, M., Comparison of corrosion behaviour in presence of oral bacteria. *Biomaterials*, 2001, **22**, 2273–2282.
43. Huang, T. H., Ding, S. J., Min, Y. and Kao, C. T., Metal ion release from new and recycled stainless brackets. *Eur. J. Orthod.*, 2004, **26**, 171–177.
44. Speck, K. M. and Fracker, A. C., Anodic polarization behaviour of Ti–Ni and Ti–6Al–4V in simulated physiological solution. *J. Dent. Res.*, 1980, **59**, 1590–1595.

Received 26 July 2007; revised accepted 5 February 2008

Olfactory responses of banana pseudostem weevil, *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae) to semiochemicals from conspecifics and host plant

A. L. Prasuna[1,]*, K. N. Jyothi[1], A. R. Prasad[1], J. S. Yadav[1] and B. Padmanaban[2]

[1]Pheromone Group, Indian Institute of Chemical Technology, Uppal Road, Hyderabad 500 007, India
[2]National Research Centre for Banana, Tiruchirapalli 620 017, India

Electroantennogram and olfactometer bioassays were conducted to study the olfactory behaviour of banana pseudostem weevil (BSW), *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae), to semiochemicals from conspecifics and host plants. Hexane extracts of whole-body chemicals of male and female weevils, host plant (banana pseudostem sheath) and combinations of weevil extracts and host plant extracts were used as stimuli or odour source in both electrophysiological and behavioural tests. BSW weevils exhibited sex-specific differences in responsiveness towards stimulus extracts in both the assays and males showed greater responsiveness in all the experiments. Female weevils were not responsive to their own body extracts, but showed significant responses towards male extracts. Male weevils were responsive to both male and female extracts. The present study provides electrophysiological and behavioural evidence that the olfactory behaviour in *O. longicollis* weevils is more precisely mediated through the male specific volatiles (aggregation pheromone). These findings also provide necessary information for developing an ecologically safe semiochemical-based control method for *O. longicollis*.

Keywords: Banana pseudostem weevil, electroantennogram, olfactory responses, semiochemicals, volatile extracts.

*For correspondence. (e-mail: prasunai@yahoo.com)

ODOIPORUS LONGICOLLIS Olivier (Coleoptera: Curculionidae), also known as the banana pseudostem weevil (BSW), is one of the main pests in banana (*Musa paradisiaca* L.) plantations in South East Asia and all the banana-growing belts of India[1]. The female BSW punctures the outer leaf sheath of the pseudostem and lays eggs inside the sheath. The emerging larvae feed on the soft tissue of the pseudostem and make extensive tunnels ranging up to 8–10 cm depth until pupation. Extensive infestations of BSW make the pseudostem weak and thus reduce the rate of flowering of the plant and finally result in undersized fruiting or no fruiting at all[1]. It has been estimated that the stem weevil causes 10–90% yield loss depending on the infestation stage and management efficiency. Adult weevils, though scanty feeders, live up to 200 days and often show the tendency to remain in the pseudostem, but exhibit strong flight activity when they move from one host plant to another. The biology, ecology and chemical control of BSW has been previously studied in detail[2]. Because of the long lifespan of adults and endophytic behaviour of the larvae, conventional methods of control, especially chemical control using insecticides proved to be less effective. Additionally, insecticides can be harmful to non-target species and the residues may pollute the environment. Hence, it is necessary to develop alternative control methods that are safe for the environment and highly efficient for the management of *O. longicollis*.

Semiochemicals or insect behaviour modifying chemicals, which include pheromones, have been proved to provide better and selective pest control or management in the protection of crops and forests[3]. The use of aggregation pheromones, which attract both male and female insects, in association with host volatiles, has led to the development of mass trapping as a control strategy for several weevil species[4,5]. Literature also cites the successful management of weevil populations in cotton, coconut and sweet potato using aggregation pheromones[6–8]. Sordidin, the aggregation pheromone of *Cosmopolites sordidus*, another important pest of banana, closely related to *O. longicollis*, has been identified, synthesized and a commercial formulation has been developed and successfully used for the control of the pest[9–12]. To date, no reports are available providing information either about the existence of aggregation pheromone or about the chemical cues involved in BSW communication. Studies on insect behavioural bioassays and electrophysiology provide better information about the insect semiochemicals[13–15]. In the present study, olfactory behaviour of male and female BSWs towards the whole-body volatile extracts and the banana sheath extract was studied employing electroantennogram (EAG) technique and olfactometer assays as a primary step for developing a semiochemical-based management strategy for the pest.

The *O. longicollis* colony raised from the field-collected insects were obtained from the National Research Centre for Banana, Tiruchirapalli, Trichy, and are being maintained in the laboratory on locally available banana psuedostem variety at 12 h L : 12 h D, 25 ± 1°C and 80% RH conditions in an environmental chamber. Weevils were sexed based on rostrum characteristics to use them in different experiments. Whole-body volatiles of male and female weevils were extracted by dipping 50 males and 50 females in 100 ml HPLC-grade hexane in clean glass sample vials for 48 h at room temperature. To prepare the banana pseudostem extract 50 g of fresh pseudostem leaf sheath was cut into small pieces and soaked in *n*-hexane (100 ml) in a glass vial for 48 h. The supernatant from all the samples was carefully removed with a Pasteur pipette into separate glass vials and stored at 4°C. The extracts were concentrated to approximately 500 μl with the help of rotary evaporator before the experiments.

EAG recording technique was employed for testing the olfactory sensitivity of male and female BSWs against the various hexane extracts of putatively volatile components. The EAG apparatus used for the study was Syntech, Hilversum, The Netherlands[16]. Male and female antennae were excised along with the head capsule and fixed between two stainless steel electrodes with two droplets of 'Spectra gel', which is electrically conductive, applied to the electrodes. The different stimuli used in the experiment were (1) hexane solvent (control), (2) whole-body extract of male weevils, (3) whole-body extract of female weevils, (4) banana pseudostem leaf sheath extract, (5) leaf sheath extract + male whole-body extract, and (6) leaf sheath extract + female whole-body extract. The stimulus cartridges were loaded with filter-paper strips previously impregnated with 10 μl of each stimulus extract after allowing the solvent to evaporate. EAGs were recorded from five insects of each sex for each test stimulus. Control stimulations using filter paper impregnated with 20 μl of hexane solvent were puffed on to the antennae before and after each stimulus presentation. Two puffs of each treatment and control spaced 10 s apart were administered to yield depolarization amplitudes for each replicate weevil. The EAGs from weevils were recorded randomly with hexane extract of a different source and gender of weevils as factors.

A *Y*-tube all-glass olfactometer was used to investigate the behavioural responses of *O. longicollis* males and females to different volatile extracts. The *Y*-tube olfactometer (stem, 15 cm; arms, 7.5 cm at 60° angle; internal diameter 2.3 cm) consisted of two odour-bearing chambers (one for test sample and the other for control or reference), one on each of the arms. An air-delivery unit (Syntech model) was connected to the two arms of the *Y*-tube to draw purified air to pass through the odour sources in the *Y*-tube. Airflow through each of the olfactometer arms was maintained at 250 ml/min.

Volatile extracts (10 μl) were applied to pieces of Whatman filter paper of the size 1 × 2 cm and placed in one of the *Y*-tube chambers against the air stream and with a control (equal volume of HPLC-grade hexane) in the other. A group of five BSWs (with 5 min interval) were introduced into the *Y*-tube at the entrance of the stem so that they had a choice between the test odour and the control. Twelve groups were used in each experiment. New filter papers with the extracts and hexane were used for every ten insects. The different experiments carried out for behavioural attraction of male and female BSWs were (1) male extract (10 μl) vs hexane (10 μl), (2) female extract vs hexane, (3) leaf sheath extract vs hexane, (4) leaf sheath extract (10 μl) + male extract (10 μl) vs hexane (20 μl), (5) leaf sheath extract (10 μl) + female extract (10 μl) vs hexane (20 μl), (6) female extract (10 μl) vs leaf sheath extract (10 μl) and (7) male extract (10 μl) vs leaf sheath extract (10 μl).

For each experiment we measured 'activity' and 'preference' (choice) of the weevils. Activity is the number of weevils that showed behavioural activity in the olfactometer (trapped in the left or right arm of the *Y*-tube) compared to weevils not responding (not showing any movement towards the arms or up wind). Preference is the number of active weevils choosing one or the other arm (test compound or control/reference) of the olfactometer. The results are expressed as the percentage of activity and percentage of preference, determined using the following formulae with minor modifications from Hori[17].

$$\text{Per cent activity: } \frac{[\text{No. of weevils released} - \text{No. of weevils nonresponding}]}{\text{Total no. of weevils released}} \times 100.$$

Per cent preference: ([No. of weevils attracted towards the test chamber – No. of weevils attracted towards the control/reference]/[Total no. of weevils released – No. of weevils nonresponding]) × 100.

Each experiment was replicated four times using 60 insects and the olfactometer arms were flipped around (180°) to minimize positional effect after testing 20 weevils. All the insects in the *Y*-tube were removed after each experiment and the olfactometer was thoroughly washed, rinsed with acetone and oven-dried for the next experiment. Weevils once used in the experiment were never employed for further experiments. Data from EAG experiments and the per cent preference data obtained from olfactometer studies were analysed by analysis of vari-

Table 1. Electroantennogram values of male and female BSWs to odourous stimuli

Treatment	EAG responses in mV ± SE*,†	
	Males	Females
Control (hexane)	0.66 ± 0.18[a]	0.362 ± 0.04[a]
Whole-body extract of males	1.50 ± 0.14[b]	1.042 ± 0.2[b]
Whole-body extract of females	1.29 ± 0.07[b]	0.446 ± 0.06[a]
Banana sheath extract	1.37 ± 0.29[b]	0.858 ± 0.05[b]
Male body extract + banana sheath extract	3.91 ± 0.39[c]	1.45 ± 0.389[b]
Female body extract + banana sheath extract	2.01 ± 0.30[d]	0.944 ± 0.26[b]

*EAG values between the columns except for control are significantly different.

†Values within columns followed by the different letters in the superscript are significantly different.

Tukey's test for comparison of multiple means: T_α for males 0.424, d*f*: 5, 24; T_α for females 0.424, d*f*: 5, 24.

ance (ANOVA using computer) and differences in pairs of mean values between treatments were examined using Tukey's multiple means comparison test[18].

EAG results obtained from stimulating male and female antennae with *n*-hexane, whole-body extracts of weevils, host plant extract and combinations of weevil body extracts and leaf sheath extracts are presented in Table 1. All the stimuli tested elicited significant EAG responses from male and female BSWs in comparison to control (*n*-hexane; Figure 1) indicating the presence of certain antennally active constituents in these extracts. Males produced significantly higher EAG deflections than female weevils in all the tests except for control. (Two-factor ANOVA without replication keeping gender of weevils and hexane extracts of different source as factors – columns: $F = 7.36$; $P < 0.05$; d$f = 1$; rows: $F = 2.49$, $P > 0.05$; d$f = 5$.) Analysis of results with Tukey's test for multiple means comparison revealed significant differences in EAG responses of males to different treatments (Table 1). Male extract in combination with banana sheath extract produced larger EAGs in males than any other stimulus source. The highest absolute EAG response recorded with male antenna against male body extract + banana sheath extract was 4.25 mV (Figure 1 *c*). EAG responses of females to their own body extracts were found to be low compared to control. Male body volatile extracts elicited stronger EAGs ranging up to 1.45 mV (Figure 1 *d*) in female antenna. Tukey's test for multiple means comparison revealed significant differences among the treatments in female insects (Table 1). EAG technique is generally used to measure the summed electrophysiological response (voltage changes) of many olfactory receptors present all along the antenna of insects to stimulus chemicals[19,20]. The EAG responses reported here represent the total electric potential generated in several olfactory receptors of BSW antenna responding simultaneously to a puff of many odourous chemicals present in the whole-body extracts of male and female weevils. The sex-specific differences in sensitivities to each stimulus suggest that male *O. longicollis* antennae are more sensitive in perceiving the chemicals than female weevils.

The results of all the olfactometer experiments are presented in Table 2. Since we found significant differences in the movement of male and female weevils in the olfactometer during the experiments, we determined per cent activity and per cent preference separately for each experiment. Per cent activity of males (ranging from 75 to 98.3%) in all the tests was found to be higher than that of females (ranging from 35 to 63.33%). Analysis of per cent preference data with two-way ANOVA without replication between groups resulted significant sexual dimorphism in per cent preferences of BSW to various extracts ($F = 6.54$, $P = 0.04$). One-way ANOVA followed by Tukey's test for means provided information about the preferences of actively involved male and female weevils towards the odour sources. *O. longicollis* males are attracted towards their own body extracts as well as female extracts and banana sheath extracts. Female BSW are not attracted to their own body volatiles and active females showed more orientation towards control/reference in experiments with female body volatiles vs hexane (control) and leaf sheath extract (reference). The number of active female weevils that moved towards the test chamber (female body volatiles) was smaller than those that moved towards the control/reference. The per cent preference of females thus resulted in negative values (Table 2). Female body volatiles had some influence on female responsiveness only when mixed with leaf sheath extract. However, leaf sheath alone elicited significant behavioural responses in female weevils. Both sexes showed almost similar preference towards male extract combined with leaf sheath. However, male weevil preferences in the rest of the experiments are significantly higher than female weevils. Both the activity and preference studies provided useful information about the behavioural responses of weevils towards odour sources. Activity studies indicated the general olfactory responsiveness of male and female weevils, and the per cent preference studies provided information about the specific responsiveness or choice of active weevils towards odour sources.

Significant responses of male and female *O. longicollis* weevils towards male extract during EAG and olfactome-

Table 2. Per cent activity and preference of male and female *Odoiporus longicollis* towards volatile extracts in different experiments

Sl. no.	Experiment	Per cent activity		Per cent preference*,†	
		Male	Female	Male	Female
I	Whole-body extract of female vs hexane	80	61.66	60.8[ab]	–89.18[a]
II	Whole-body extract of male vs hexane	98.33	63.33	72.88[a]	60.22[b]
III	Banana sheath extract vs hexane	75.0	58.33	68.19[a,ab]	48.50[c]
IV	Male extract + banana sheath extract vs hexane	100	60.00	63.33[ab]	61.11[b]
V	Female extract + banana sheath vs hexane	75.00	45.00	64.44[a,ab]	18.5[d]
VI	Male extract vs leaf sheath	91.00	35.00	61.0[ab]	17.9[d]
VII	Female extract vs leaf sheath	88.00	60.00	58.33[ab]	–66.66[e]

*Values of per cent preference between the columns are significantly different. (Two-way ANOVA without replication $F = 6.5$; $P < 0.05$; F critical = 5.98.)

†Values within columns followed by different letters in the superscript are significantly different.

Tukey's test for the comparison of multiple means: T_α for males 9.11; d*f*: 6, 14; T_α for females 5.66; d*f*: 6, 14.

Negative (preference) values indicate higher preference of weevils towards control/reference than towards treatment.

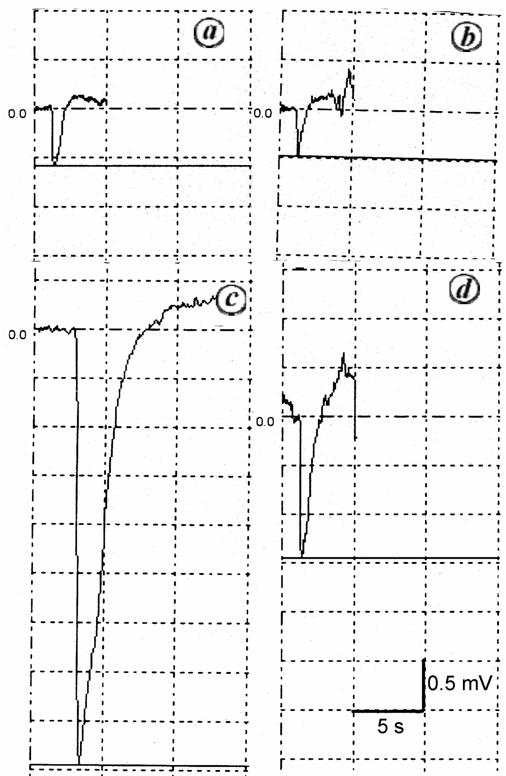

Figure 1. Representative electroantennograms elicited from male and female *Odoiporus longicollis*. ***a***, Male to hexane; ***b***, Female to hexane; ***c***, Male to male extract + banana sheath extract; ***d***, Female to male body extract. Scale – Vertical: 0.5 mV/div; Horizontal: 5 s/div.

ter experiments suggest that volatile compounds emitted by male weevils play a major role in weevil aggregation behaviour. Males of many coleopteran species were reported to produce aggregation pheromones[21]. The chemical identification of aggregation pheromones has been reported in at least nine families of Coleoptera and in many species of the family Curculionidae[22,23].

Results obtained with male *O. longicollis* weevils in EAG experiments followed by olfactometer bioassays towards female volatile extracts also suggest a female-based chemical communication system in BSW. Additionally, volatile extract from female weevils generated comparatively weaker or no EAGs from female antennae and no preferences in females in olfactometer studies. These results suggest that female BSW hexane extracts do not influence the chemical communication of female weevils. Ravi and Palaniswami[24], in their studies with live BSW, also reported that females of all the age groups are not responsive to live females (virgin as well as mated) in *Y*-tube olfactometer studies. However, they found positive response of males towards live females of different ages, suggesting evidence for the presence of female sex pheromone in BSW.

Male-produced volatiles and the combination of banana sheath extract + male body volatiles were highly attractive to male *O. longicollis* weevils in *Y*-tube olfactometer studies. It is interesting to note that these stimuli also elicited largest EAG responses in male weevils. Thus the study demonstrates that though EAGs do not indicate whether the stimulus compound is an attractant or repellent, they certainly provide evidence for a group of compounds that are behaviourally important for the pest. Male weevils showed significantly lower attractiveness towards the volatile extracts of female body extracts and banana sheath extract than the male body extracts. The results clearly indicate that the male-produced volatiles had greater influence on olfactory orientation of male BSW. Response of males to male-produced volatiles may be an adaptive feature as males in groups are more effective than individual males in attracting females for mating[25]. The host-plant extract (banana sheath extract) alone was found to produce considerable influence on weevil olfactory response, as it produced measurable EAGs and preferences in male and female BSWs in EAG and olfactometer assays. This suggests that both male and female *O. longicollis* weevils use volatile chemicals emitted by the host plant as cues for their location. However, EAG response in female weevils was found to be significantly higher only when stimulated by male odours and with a combination of male + host plant odours. These results suggest that as in many coleopteran species, *O. longicollis* males initiate host location and after reaching the host plant produce semiochemicals (pheromones) to enhance the attraction of conspecifics towards the host for feeding as well as for mating[26].

In conclusion, olfactory behaviour of *O. longicollis* is clearly under the influence of chemicals produced by the weevils themselves and the host plant. Though the results indicate the existence of a female-produced sex pheromone system in *O. longicollis*, the major olfactory activity of both male and female weevils is found to be mediated through the male-produced chemicals. These findings confirm the male-based aggregation pheromone system in BSW, the existence of which has not been reported earlier. The results help in chemical identification and planning the synthesis of aggregation pheromone. The utility of synthetic aggregation pheromone in integrated management of BSW is ideal as it attracts both male and female weevils to a point source. This ultimately reduces the amount of insecticides required for the control, and due to its specificity also increases the efficiency of beneficiary insects in the field.

1. Dutt, N. and Maiti, B. B., Bionomics of the banana pseudostem weevil, *Odoiporus longicollis* (Coleoptera: Curculionidae). *Indian J. Entomol.*, 1972, **34**, 20–30.
2. Padmanaban, B., Sundararaju, P. and Sathiamoorthy, S., Incidence of banana pseudostem borer, *O. longicollis* Oliv. (Coleoptera: Curculionidae) in banana peduncle. *Indian J. Entomol.*, 2001, **63**, 204–206
3. Welter, S. C., Pickel, C., Miller, J., Cave, F., Van Steenwyk, R. A. and Dunley, J., Pheromone mating disruption offers selective management options for key pests. *Calif. Agric.*, 2005, **59**, 16–22.
4. Alpizar, D., Fallas, M., Oehlschlager, A. C., Gonzalez, L. M., Chinchilla, C. M. and Bulgarelli, J., Pheromone mass trapping of the West Indian sugarcane weevil and the American palmweevil (Coleoptera: Cucculionidae) in palmito palm. *Fla. Entomol.*, 2002, **85**, 426–430.
5. Weissling, T. J., Giblin-Davis, R. M., Gries, G., Gries, R., Perez, A. L., Pierce, H. D. and Oehlschlager, A. C., Aggregation pheromone of palmetto weevil *Rhynchophorus cruentatus* (F.) (Coleoptera: Cucculionidae). *J. Chem. Ecol*., 1994. **20**, 505–515.
6. Dickerson, W. A., Ridgeway, R. L. and Planner, F. R., Southeastern boll weevil eradication program, improved pheromone trap, and program status. In Proceedings, Beltwide Cotton Research and Production Conference, Dallax, TX, National Cotton Council, Memphis, TN, 24–27 June 1987, pp. 335–337.
7. Chinchilla, C., Oehlschlager, A. C. and Bulgarelli, J., A pheromone based trapping system for *Rhynchophorus palmarum* and *Metamasius hemipterus*, ASD Oil Palm Paper, Costa Rica, 1996, vol. 12, pp. 11–17.
8. Downham, M. C. A., Smith, N. E. J. M., Laboke, P. O., Hall, D. R. and Odongo, B., Reduction of pre-harvest infestations of African sweetpotato weevils *Cylas brunneus* and *C. puncticollis* (Coleoptera: Apionidae) using pheromone mating disruption technique. *Crop Prot.*, 2001, **20**, 163–166.
9. Budenberg, W. J., Ndiege, I. O. and Karago, F. W., Evidence for volatile male-produced pheromone in banana weevil *Cosmopolites sordidus*. *J. Chem. Ecol*., 1993, **19**, 267–272.
10. Jayaraman, S. *et al.*, Synthesis, analysis and field activity of sordidin, a male produced aggregation pheromone of *Cosmopolites sordidus*. *J. Chem. Ecol*., 1997, **23**, 1145–1161.
11. Alpizar, D., Fallas, M., Oehlschlager, A. C., Gonzalez, L. M. and Jayaraman, S., Weevil aggregation pheromone for mass trapping banana weevil in banana and plantain. In Abstracts of the XIV International Plant Protection Congress, Jerusalem, Israel, July 1999, p. 100.
12. Tinzaara, W., Tushemereirwe, W. K. and Kashaija, I., Efficiency of pheromones and trap types in the capture of the banana weevil *Cosmopolites sordidus* Germar in Uganda. *Ugandan J. Agric. Sci*., 2000, **5**, 91–97.
13. Metcalf, R. L., Ultramicrochemistry of insect semiochemicals. *Mikrochim. Acta*, 1998, **129**, 167–180.
14. Rochat, D., Gonzalez, A. V., Mariau, D., Villanueva, A. G. and Zagatti, P., Evidence for male-produced aggregation pheromone in American weevil *Rhynchophorus palmarum* (L.) (Coleoptera: Cucculionidae). *J. Chem. Ecol*., 1991, **17**, 1221–1230.
15. Jaffe, K. *et al.*, Chemical ecology of palm weevil *Rhyncophorus palmarum* (L.) (Coleoptera: Curculionidae): Attraction to host plants and to male produced aggregation pheromone. *J. Chem. Ecol*., 1993, **19**, 1703–1720.
16. Yadav, J. S., Prasad, A. R., Jyothi, K. N., Prasuna, A. L., Hafeez-ur-Rehman, Sudhakaran, K. and Vijaykumar, S., Development and optimization of pheromone dose, dispenser type, lure age and trap design on trapping of male sugarcane inter node borer, *Chilo sacchariphagus indicus* (Lepidoptera: Pyralidae). *Int. Pest Control*, 2007, **49**, 78–84.
17. Hori, M., Onion aphid (*Neotoxoptera formosana*) attractants in the headspace of *Allium fistulosum* and *A. tuberosum* leaves. *J. Appl. Entomol*., 2007, **131**, 8–12.
18. Montgomery, D. C., *Design and Analysis of Experiments*, John Wiley, New York, 1984, 2nd edn.
19. Nagai, T., Summation and gradient characteristics of local electroantennogram esponse of the European corn borer, *Ostrinia nubilalis*. *Pestic. Biochem. Physiol*., 1984, **24**, 32–39.
20. Wibe, A., How the choice of method influences on the results in electrophysiological studies of insect olfaction. *J. Insect Physiol*., 2004, **50**, 497–503.
21. Rao, S., Cosse, A. A., Zilkowski, B. W. and Bartelt, R. J., Aggregation pheromone of the cereal leaf beetle: Field evaluation and emission from males in the laboratory. *J. Chem. Ecol*., 2003, **29**, 2165–2175.
22. Lacey, E. S., Ginzel, M. D., Millar, J. G. and Hanks, L. M., Male produced aggregation pheromone of Cerambycid beetle *Neoclytus acuminatus*. *J. Chem. Ecol*., 2004, **30**, 1493–1507.
23. Tafoya, F., Lopez-Collado, J., Stanley, D., Rojas, J. C. and Cibrain-Tovar, J., Evidence of an aggregation pheromone in males of the cactus weevil *Metamasius spinolae* (Coleoptera: Curculionidae). *Environ. Entomol.*, 2003, **32**, 484–487.
24. Ravi, G. and Palaniswami, M. S., Evidence for a female-produced sex pheromone in the banana pseudostem weevil *Odoiporus longicollis* Oliver. *Curr. Sci*., 2002, **83**, 893–898.
25. Weldon, C. W., Influence of male aggregation size on female visitation in *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). *Aust. J. Entomol*., 2007, **46**, 29–34.
26. Xiao-ling Sun, Qing-yin Yang, Jonanthan Sweeny and Chang-qi Gao, A review: Chemical ecology of *Ips typographus* (Coleoptera: Scolytidae). *J. For. Res*., 2006, **17**, 65–70.

ACKNOWLEDGEMENT. We thank the National Agricultural Technology Programme (ICAR) for providing funds to carry out this work.

Received 29 June 2007; revised accepted 28 February 2008