Electrophysiological and behavioural responses of banana pseudostem weevil, *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae) to aggregation pheromone, 2-methyl-4-heptanol and host plant kairomones

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Laboratory bioassays were conducted using an electroantennogram (EAG) and Y-tube olfactometer to study the electrophysiological and olfactory responses of banana pseudostem weevil (BSW), *Odoiporus longicollis* to its aggregation pheromone, 2-methyl-4-heptanol (2M4H) and host plant extract (HPE). The aggregation pheromone and HPE from highly susceptible cultivar Nendran were tested individually and in combination to ascertain the synergistic effect of HPE on 2M4H to the BSW. The peripheral response of antennae to the 2M4H + HPE combination elicited significantly strong amplitude of 4.089 ± 0.043 mV in males in comparison to females over the aggregation pheromone and HPE individually. Dose-dependent responses indicated difference between sexes and concentrations. Behavioural assay using Y-tube demonstrated that both sexes responded to host volatiles, and that males were more sensitive than females. Similarly, both the sexes responded most strongly (64.21% ± 3.91% of males and 55.47% ± 3.06% of females) to the odour sources comprising pheromone with host plant volatiles. Addition of HPE to 2M4H significantly increased EAG activity and also the attraction as indicated in the Y-tube behavioural bioassay; thus, the blend forms a good attractant. The present study provides information useful in developing pheromone-based lure in conjunction with HPE for monitoring and mass-trapping of *O. longicollis*.

Keywords: Aggregation pheromone, banana pseudostem weevil, electroantennogram, host plant extract.

THE banana pseudostem weevil (BSW) is a monophagous pest that causes 10%–90% yield loss with severe infestation occurring at the early vegetative stage of the crop[1]. Widespread occurrence of this pest has been recorded in all banana-growing states of India[2,3] and Southeast Asia[4]. Infested planting materials (suckers), *inter alia*, are the contributing factor for rapid spread of BSW. Female weevil lays eggs inside the air chambers of the leaf sheath through a hole made by its rostrum. The newly hatched larvae feed on tender tissue and make tunnels that prevent the flow of nutrients, often causing death of the plant[2]. Banana pseudostem traps have been used to check and control the pest in banana orchards, but it is labour-intensive and trapping materials may not be available in newly established gardens[5]. The biology and ecology of the weevil have been studied in detail[6,7]. Chemical methods are available but not effective owing to hidden mode of life cycle of the pest[8,9]. The extensive and perhaps indiscriminate application of cyclodiene insecticides (dieldrin, lead heptachlor) has caused resistance in the coleopteran pests and adverse effect on the environment[10]. Organophosphate insecticide substitutes are available but are costly and potentially poisonous to the farmers, and therefore not suitable for small farm-holder production system[11]. Therefore, an effective control method with a view to safeguard the farmers as well as the environment is essentially required. The use of semiochemicals in integrated pest management programmes is increasing, and this intervention has substantial practical usefulness for controlling some coleopteran pests[12,13]. Traps baited with semiochemicals have successfully been used for the management of the boll weevil, *Anthonomus grandis*[14] and the American palm weevil[12]. Therefore, mass trapping of BSWs may be a feasible technique for their management.

2-Methyl-4-heptanol (2M4H) was reported as the male-secreted aggregation pheromone of *Odoiporus longicollis*[15]. Many aggregation pheromones were found working only in combination with host plant tissue. For example, rhynchophorol, the aggregation pheromone of West Indian sugarcane weevil, works only with the host plant sugarcane pieces[16]. Similarly, sordidin, the male-produced aggregation pheromone of *Cosmopolites sordidus* is active with banana stem tissue[17]. The use of plant tissue per se in combination with aggregation pheromone in traps under field conditions is cumbersome and impractical. In the present study, electrophysiological and olfactory responses of BSW to 2M4H and host plant extract (HPE) using electroantennogram (EAG) (model IDAC 232, Syntech, Hilversum, The Netherlands) and Y-tube olfactometer bioassays are reported to develop semiochemical-based attractant trap for BSW.

The BSW colony was raised from field-collected weevils and grubs. The adults were reared in plastic containers (10 l) perforated on the lid for ventilation and at the bottom for drainage of water emanating from banana pseudostem, and fed with a piece of banana pseudostem of cultivar Nendran. The feed was changed when it partially dried up. In the case of grubs, each grub was separately kept in small perforated plastic containers (height 14 cm and diameter 10 cm; Tarsons, Kolkata) with a small piece of banana leaf sheath and it was changed at regular intervals until pupation. Both adults and grubs were nurtured at 25° ± 1°C with 12 h L : 12 h D; and 65%–70% relative humidity conditions. Adult weevils

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were segregated into males and females on the basis of rostrum characteristics[18].

The pheromone 2M4H (obtained from M/s Chempure (P) Ltd, Mumbai) was diluted in hexane (HPLC-grade). Using solvent extraction method, host plant extract (cv. Nendran) was made from 100 g of banana leaf sheaths in hexane. The supernatant was carefully decanted into a round bottom flask and concentrated to 10 µl/g equivalent using a rotary evaporator. In order to choose a suitable dose of 2M4H in relation to maximum response in BSW, the pheromone compound was diluted in five different concentrations from log 1 to 5 in hexane, and HPE was diluted from 1 to 200 µl in hexane and tested.

The methodology described by Prasuna *et al.*[19] was followed in the present study. The antenna was mounted on the probe composed of two steel electrodes using an electrically conductive 'spectral gel'. The stimuli used in the experiment were (i) 2M4H + HPE, (ii) 2M4H, (iii) HPE and (iv) control. Filter paper (0.5 × 30 cm) strips were loaded with 10 µl of each stimulus extract; the solvent was allowed to evaporate and the strips were inserted into the stimulus cartridges. The mounting of antennae was completed within 10 min. The antenna was continuously flushed by humidified and filtered air, except for the stimulus applied with 3 min interval between simulations for enough antennal receptor recovery. The treatments were randomly presented to the antenna six times along with the control. Every treatment was tested on both male and female antennae. All test and the control stimulus responses formed a set of data. The absolute EAG responses were recorded.

A glass Y-tube olfactometer was employed to examine the behavioural responses of both sexes of BSW to 2M4H and HPE (Figure 1). The Y-tube olfactometer, with stem 45 cm, arms 7.5 cm at 60° angle and internal diameter 2.0 cm, comprised of two chambers meant for test and control samples. Purified air was drawn into the arms of the Y-tube through an air delivery unit. The air flow of the Y-tube arms was kept at 250 ml/min using a flow meter (Syntech). The stimuli used in the experiment were: (i) 2M4H + HPE versus control, (ii) 2M4H versus control and (iii) HPE versus control. Ten microlitre of volatile stimuli and HPLC-grade hexane were separately loaded onto pieces of Whatman no. 1 filter paper (2 × 0.4 cm) and placed in the sample and control chambers against the air stream. Five BPWs as a group were introduced at 5 min interval into the Y-tube at the entrance of the stem, enabling them to make a choice between sample and control. Twenty groups of weevils were used in each experiment. Freshly prepared 2M4H/HPE and hexane filter papers were used for each group of weevils.

Figure 1. Y-tube olfactometer used in the bioassay: 1, Air pump; 2, Charcoal filter; 3, Humidifier; 4, Sample/control arm; 5, Y-tube; 6, Weevil release point.

Activity and preference percentage of the weevils were calculated in each experiment. Activity is the number of weevils that moved either to the left or right arm compared to those that did not respond. Preference is the number of active weevils that made the choice of either sample or control. The results were obtained as percentage of activity and of preference using the formula originally described by Hori[20] and modified by Prasuna *et al.*[19]

Per cent activity = [(No. of weevils released – no. of weevils not-responding)/(Total no. of weevils released)] × 100.

Per cent preference = [(No. of weevils attracted towards the test chamber – no. of weevils attracted to control)/(Total no. of weevils released – no. of weevils not-responding)] × 100.

After testing 20 weevils, the olfactometer was turned around by 180° to minimize positional bias. After each experiment, all the weevils were removed and the olfactometer was washed with water thoroughly and rinsed with acetone and oven-dried. Data from EAG experiments and Y-tube bioassay studies, viz. per cent activity and per cent preference were analysed using analysis of variance ANOVA, and least significant difference (LSD) was used to compare the mean values between treatments and sexes.

Dose-response experimental results of 2M4H revealed that at log dilutions 1 and 2 exhibited over-stimulation, while at log dilutions 4 and 5, the EAG response was indistinguishable and overlapping between sexes. Only at log dilution 3, distinguishable EAG response was obtained and chosen for further studies (Figure 2). In case of HPE, 10 µl was found to be the optimum dose in perceiving odour stimulus by the weevils. At 1 µl dose, the perceptivity was very low, particularly for females and at 50 µl and above, sensitivity of the antennae of the weevils was desensitized (Figure 3).

Table 1 provides a summary of EAG responses of male and female *O. longicollis* using 2M4H + HPE, 2M4H, HPE and control. All the test stimuli evoked significantly higher responses in male and female *O. longicollis* in comparison with control, implying presence of antennal receptor components. Male weevils elicited significantly higher response than females in all the test stimuli, except control, and within test stimuli, the 2M4H + HPE combination produced higher EAG response in comparison to 2M4H and HPE tested individually in both sexes.

Comparison between responses of males and females, though all the three test stimuli produced significant EAG, showed that the 2M4H + HPE combination produced highest absolute EAG response in males (4.089 ± 0.043 mV) than females (3.249 ± 0.072 mV).

Table 2 gives the olfactometer bioassay results. Since significant differences in the migration of males and females in the olfactometer were detected, the activity and preference percentages were estimated separately for males and females. The per cent activity of males was 68.49 ± 3.04, which is not significantly different from females (69.77 ± 3.06) to 2M4H + HPE combination against control. Similarly, the per cent activity of males (64.63 ± 4.32) and females (66.23 ± 3.06) was also not significantly different towards HPE. However, the per cent activity of males (67.73 ± 3.94) and females (66.13 ± 2.78) towards 2M4H was significantly different.

The results also demonstrate that 2M4H alone shows significant preference between males (29.47 ± 3.94) and females (24.83 ± 2.78), whereas the 2M4H + HPE combination and HPE alone do not show significant preference between the sexes against control. Generally, the 2M4H + HPE combination shows higher preference in both the sexes than the 2M4H and HPE individually against control. Overall, males responded well to the pheromone and kairomone combination in EAG test and to the pheromone in the behavioural assays. Furthermore, between males and females only the pheromone alone showed significant difference in olfactory orientation in terms of per cent activity and per cent preference.

The response of insects to the semiochemicals including pheromones and host plant kairomones are dose-dependent, i.e. insects perceive the odours of pheromones and kairomones in particular concentrations and genders respond differently to different concentrations of semiochemicals[15,21,22]. Therefore, dose-dependent stimulation of weevils to the aggregation pheromone and host plant kairomones was performed and the doses of 2M4H and HPE, which exhibited distinguishable antennal response and perceptivity between genders of insects, were used for EAG and behavioural bioassays.

In Y-tube olfactometer studies, the mixture of banana extract and male weevil body wash volatiles was found highly attractive to male *O. longicollis*[19]. In a laboratory bioassay, host plant volatiles and the synthetic pheromone tested individually and in the mixture strongly attracted the banana rhizome borer, *Cosmopolites sordidus*[23]. HPE enhanced the level of response of *C. sordidus* to the synthetic aggregation pheromone Cosmolure$^{+(R)}$ (ref. 24). Additive effect of host plant on pheromone attraction has been reported; however, increasing host plant tissue to pheromone beyond a particular size did not significantly increase weevil response[25]. In our study also, HPE from the highly susceptible cultivar Nendran with 2M4H was found effective in weevil olfactory response, as it induced strong EAGs in males and females. Since male weevils responded more to HPE, they might be involved in the host plant selection. After reaching the host, they release aggregation pheromone and lure both male and female weevils. Banana pseudostem traps are

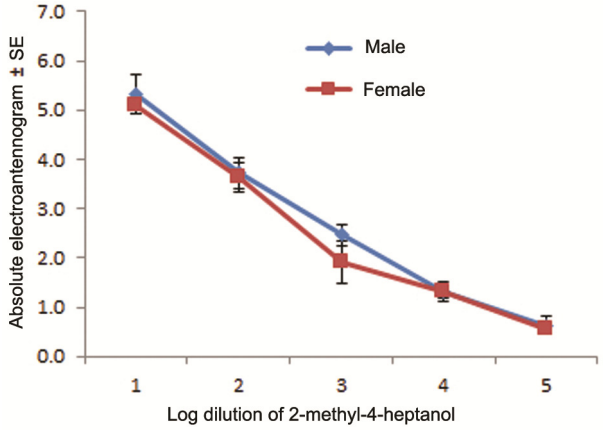

Figure 2. Responses of male and female, *Odoiporus longicollis* to different doses of 2-methyl-4-heptanol.

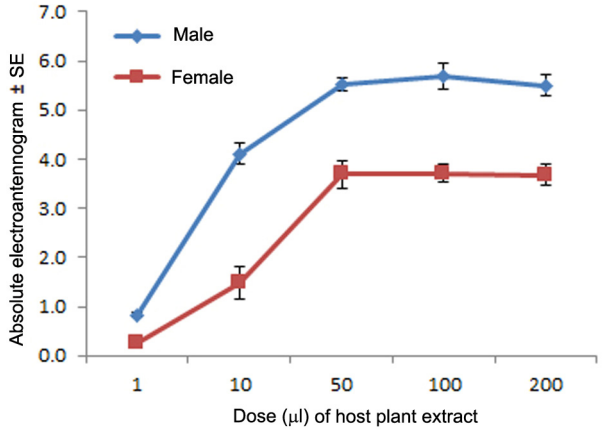

Figure 3. Responses of male and female, *O. longicollis* to different doses of host plant extract.

Table 1. Electroantennogram (EAG) response of male and female *Odoiporus longicollis* to 2-methyl-4-heptanol (2M4H) and host plant extract (HPE)

	EAG responses (mV ± SE)*†	
Treatment	Male	Female
Control (hexane)	0.221 ± 0.036^{f}	0.328 ± 0.035^{f}
2M4H + HPE	4.089 ± 0.043^{a}	3.249 ± 0.072^{b}
2M4H	2.473 ± 0.079^{c}	1.994 ± 0.090^{e}
HPE	3.255 ± 0.125^{b}	2.270 ± 0.043^{d}

*EAG values between the columns except in control are significantly different. †Values within columns followed by different letters in the superscript are significantly different. Least significant difference (LSD) was performed for comparison of multiple means.

Table 2. Per cent activity and per cent preference of *O. longicollis* towards different odour stimuli

Treatment	Per cent activity		Per cent preference*,†	
	Male	Female	Male	Female
2M4H + HPE versus control	68.49[a] ± 3.04	69.77[a] ± 3.06	64.21[a] ± 3.91	55.47[a] ± 3.06
2M4H versus control	67.73[a] ± 3.94	66.13[b] ± 2.78	29.47[b] ± 3.94	24.83[c] ± 2.78
HPE versus control	64.63[b] ± 4.32	66.23[b] ± 3.06	40.23[b] ± 4.32	32.24[b] ± 3.06

*Values of per cent preference between the columns are significantly different. Two-way ANOVA was performed with six replications. †Values within columns followed by different letters are significantly different. LSD was performed for comparison of multiple means.

being used to check the pest in banana plantations. The volatile chemicals (kairomones) emanating from the cut surface of banana stem may attract the weevils. Identification of kairomones from the pseudostem therefore will help in developing pheromone-based lures for the management of the weevils.

The EAG and behavioural studies revealed that male-produced aggregation pheromone (2M4H), plays a predominant role in the host-finding behaviour of male weevils, and the host plant-associated kairomones are also essential for increasing the attraction of the aggregation pheromone. Female weevils responding to the aggregation pheromone might release the sex pheromone and attract males for mating. In support, the present study results show that the EAG response of females is low towards all stimuli in comparison to males. Also, the possible presence of female-produced sex pheromone in *O. longicollis* has been suggested[26]. Many volatiles were identified from pseudostem of cv. Gitumo, a highly susceptible banana to *C. sordidus*. The major components, viz. α-pinene, β-pinene, β-myrcene, limonene, α-cubebene, α-copaene, α-cedrene, β-caryophyllene and α-humulene were not attractive, but the minor component 1,8-cineole was found attractive to *C. sordidus*[27,28]. This result showed that one or few minor component(s) present in the volatiles from banana pseudostem may be attractive to BSW. Development of suitable trapping techniques will improve the detection, monitoring and management programme which at present depends on visual symptoms like gummy exudation and pinhead hole on the pseudostem, which are evident only at the terminal stage of infestation. Isolation of pure kairomones from the host plants and/or identification of pheromones may be useful for monitoring and management purposes[29]. This ultimately will reduce the use of harmful pesticides in the field, which will be advantageous to the farming community, environment and beneficial insects.

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