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

S. Palanichamy*, B. Padmanaban, M. Mayil Vaganan and S. Uma

ICAR-National Research Centre for Banana, Thayanur Post, Thogamalai Road, Tiruchirappalli - 620 102, Tamil Nadu, India.

*For correspondence (poojabharathi@gmail.com)
Laboratory bioassays were conducted using Electroantennogram and Y-tube olfactometer to investigate the electrophysiological and olfactory response 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 host plant extract from highly susceptible cultivar Nendran were tested individually and in combination to ascertain the influence of HPE on the 2M4H in the behaviour of BSW in the both bioassays. The peripheral response of antennae to 2M4H+HPE combination elicited significantly strong amplitude of $4.089 \pm 0.043$ 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 males were more sensitive than females and 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 and thus the blend forms a good attractant. The present studies provide information useful in developing pheromone based lure in conjunction with host plant extract for monitoring and mass trapping of *O. longicollis*.

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

The 2-methyl-4-heptanol (2M4H) was reported as the male-secreted aggregation pheromone of \textit{O. longicollis}\textsuperscript{15}. Many aggregation pheromones were found working only in combination with
host plant tissue. For example, aggregation pheromone of West Indian sugarcane weevil, Rhynchophorol works only with the host plant sugarcane pieces. Similarly, sordidin, the male-produced aggregation pheromone of Cosmopolites sordidus was also active with banana stem tissue. 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) and Y-tube olfactometer bioassay 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 in the bottom for drainage of water emanating from banana pseudostem and fed with a piece of banana pseudostem of banana cultivar Nendran. The feed was changed as and when it was partially dried up. In the case of grubs, each grub was separately kept in small perforated plastic containers (height 14 cm and dia. 10 cm; Tarsons, Kolkata, India) 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 were segregated into males and females on the basis of rostrum characteristics.

The pheromone, 2M4H was obtained from M/s. Chempure (P) Ltd., Mumbai and 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 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 by¹⁹ was followed in the present investigations. The antenna was mounted on the probe composed of two steel electrodes using an electrically conductive “spectral gel”. The EAG employed for the study was from Syntech, Hilversum, The Netherlands (model IDAC 232). The stimuli used in the experiment were (i) 2M4H+HPE; (ii) 2M4H; (iii) HPE and (iv) control. Filter paper (0.5 x 30 cm) strips were loaded with 10 µl of each stimulus extract and allowed the solvent to evaporate and 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 the stimulus applied with 3 min interval in between simulations for enough antennal receptor recovery. The treatments were presented to the antenna for 6 times along with the control randomly. Every treatment was tested on both male and female antennae. All test and a control stimuli 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 (Fig. 1). The Y-tube olfactometer, with stem 45 cm, arms 7.5 cm at 60° angle and internal diameter 2.0 cm, comprised 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 flow meter (Syntech). The stimuli used in the experiment were (i) 2M4H + HPE vs control (ii) 2M4H vs control and (iii) HPE vs control. Ten µl of volatile stimuli and HPLC grade hexane were separately loaded onto pieces of Whatman No. 1 filter paper (2 x 0.4 cm) and placed on sample and control chambers against the air stream. Five banana pseudostem weevils 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 the sample and the 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.

Activity and preference percentage of the weevils were calculated in each experiment. Activity is the number of weevils moved either left or right arm compared to weevils that did not respond. Preference is the number of active weevils that made choice of either sample or control. The results were obtained as the percentage of activity and of preference using the formula originally described by\(^{20}\) and modified by\(^{19}\).

\[
\text{Per cent activity} = \frac{\text{No. of weevils released} - \text{No. of weevils non-responding}}{\text{Total no. of weevils released}} \times 100
\]

\[
\text{Per cent preference} = \frac{\text{No. of weevils attracted towards the test chamber} - \text{No. of weevils attracted to control}}{\text{Total no. of weevils released} - \text{No. of weevils non-responding}} \times 100
\]

After testing 20 weevils, the olfactometer was turned over around 180\(^{\circ}\) 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 \textit{viz.}, per cent activity and per cent preference were analysed by analysis of variance (ANOVA) and 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-stimulations and at log 4 and 5, the EAG response was indistinguishable and overlapping between sexes. Only at log 3 dilution, distinguishable EAG response was obtained and chosen for further studies (Fig. 2). In case of HPE, 10 µl was found to be optimum dose in perceiving the odour stimulus by the weevils. At 1 µl dose, the perceptivity was very low particularly for females and at 50 µl and above, the sensitivity of the antennae of the weevils was in saturation (Fig. 3).

EAG responses of male and female *O. longicollis* using 2M4H+HPE, 2M4H, HPE and control are summarized in Table 1. 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 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, the 2M4H+HPE combination produced highest absolute EAG response in males (4.089 ± 0.043 mV) than females (3.249 ± 0.072 mV).

The olfactometer bioassay results are given in Table 2. Since significant differences in the migration of male and female in the olfactometer were detected, the activity and preference percentage were separately estimated for males and females. The per cent activity of male was 68.49±3.04, which is not significantly different from female (69.77±3.06) to 2M4H+HPE combination against control and similarly, the per cent activity of male (64.63±4.32) and female (66.23±3.06) was also not significantly different towards HPE. However, the per cent activity of male (67.73±3.94) and female (66.13±2.78) towards 2M4H was significantly different.
The results also demonstrated that the aggregation pheromone, 2M4H alone showed significant preference between males (29.47±3.94) and females (24.83±2.78) whereas the 2M4H+HPE combination and HPE alone did not show significant preference between the sexes against control. But, generally the 2M4H+HPE showed higher preference in both the sexes than to the 2M4H and HPE individually against control. Overall, males responded greatly to pheromone and kairomones 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 preference.

Semiochemicals including pheromones and host plant kairomones are dose dependent \textit{i.e.}, insects perceive the odours of pheromones and kairomones in particular concentrations and also genders responded differently to different concentrations of semiochemicals\textsuperscript{15,21,22}. Therefore, dose-dependent stimulation of weevils to the aggregation pheromone and the host plant kairomones were 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 \textit{O. longicollis}\textsuperscript{19}. In a laboratory bioassay, host plant volatiles and the synthetic pheromone tested individually and in mixture strongly attracted the banana rhizome borer, \textit{C. sordidus}\textsuperscript{23}. Host plant extract enhanced the level of response of \textit{C. sordidus} to the synthetic aggregation pheromone Cosmolure\textsuperscript{(R)}\textsuperscript{24}. Additive effect of host plant on pheromone attraction has been reported and however, increasing of host plant tissue to pheromone beyond a particular size did not significantly increase weevil response\textsuperscript{25}. In our studies also, the host plant extract from highly susceptible cultivar Nendran with the aggregation
pheromone (2M4H) was found effective on weevil olfactory response as it induced strong EAGs in males and females. Since, male weevil responded more to the HPE, it means male weevils might involve in the host plant selection. After reaching the host, it might release aggregation pheromone and lure both male and female weevils. Banana pseudostem traps are being used to check the pest in the banana plantation. It is believed that the volatile chemicals (kairomones) emanating from the cut surface of banana stem attract the weevils. Identification of kairomones from the pseudostem therefore will help in developing pheromone based lures for the management of the weevil.

The EAG and behavioural studies revealed that male produced aggregation pheromone, 2-methyl-4-heptanol, plays predominant role in the host finding behaviour of male weevils and the host plant-associated kairomones also played in increasing the attraction of the aggregation pheromone. Female weevils responding to aggregation pheromone might release sex pheromone and attract males for mating. In support, the present study results showed that the EAG response of females was low towards all stimuli in comparison to males and also possible presence of female produced sex pheromone in *O. longicollis* was suggested\(^\text{26}\). Many volatiles were identified from pseudostem of cv. Gitumo, a highly susceptible banana to *C. sordidus* and 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 minor component 1, 8-cineole was found attractive to *C. sordidus*\(^\text{27,28}\). 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 detection, monitoring and management programme that at present depend on visual symptoms like gummy exudation and pin head hole on the pseudostem, which are evident only at 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\textsuperscript{29}. This ultimately will reduce the use of harmful pesticides in the field, which will be beneficial to farming community, environment and beneficial insects.


Table 1. Electroantennogram responses of male and female *O. longicollis* to 2-Methyl-4-heptanol and host plant extract

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (hexane)</td>
<td>0.221 ± 0.036&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.328 ± 0.035&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>2M4H + HPE</td>
<td>4.089 ± 0.043&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.249 ± 0.072&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2M4H</td>
<td>2.473 ± 0.079&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.994 ± 0.090&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>HPE</td>
<td>3.255 ± 0.125&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.270 ± 0.043&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*EAG values between the columns except in control are significantly different. †Values within columns followed by the different letters in the superscript are significantly different. Least significant difference (LSD) performed for comparison of multiple means.
Table 2. Per cent activity and per cent preference of banana pseudostem weevil, *O. longicollis* towards different odour stimuli

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>2M4H + HPE vs control</td>
<td>68.49&lt;sup&gt;a&lt;/sup&gt; ± 3.04</td>
<td>69.77&lt;sup&gt;a&lt;/sup&gt; ± 3.06</td>
<td>64.21&lt;sup&gt;a&lt;/sup&gt; ± 3.91</td>
<td>55.47&lt;sup&gt;a&lt;/sup&gt; ± 3.06</td>
</tr>
<tr>
<td>2M4H vs control</td>
<td>67.73&lt;sup&gt;b&lt;/sup&gt; ± 3.94</td>
<td>66.13&lt;sup&gt;b&lt;/sup&gt; ± 2.78</td>
<td>29.47&lt;sup&gt;b&lt;/sup&gt; ± 3.94</td>
<td>24.83&lt;sup&gt;b&lt;/sup&gt; ± 2.78</td>
</tr>
<tr>
<td>HPE vs control</td>
<td>64.63&lt;sup&gt;b&lt;/sup&gt; ± 4.32</td>
<td>66.23&lt;sup&gt;b&lt;/sup&gt; ± 3.06</td>
<td>40.23&lt;sup&gt;b&lt;/sup&gt; ± 4.32</td>
<td>32.24&lt;sup&gt;b&lt;/sup&gt; ± 3.06</td>
</tr>
</tbody>
</table>

*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. Least significant difference (LSD) performed for comparison of multiple means.*
Figure Legends

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.
Figure 2. Responses of male and female banana pseudostem weevil, O. longicollis to different doses of 2-methyl-4-heptanol.
Figure 3. Responses of male and female banana pseudostem weevil, O. longicollis to different doses of host plant extract.
Figure 1