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Electrophysiological responses of banana pseudostem weevil, *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae) to methyl jasmonate, 1-hexanol and host plant extract

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The banana pseudostem weevil (BSW), *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae) is one of the most destructive pests of banana and plantains. Our laboratory bioassay studies showed that out of 50 host plants related volatile chemicals screened against BSW, only methyl jasmonate (MeJA) and 1-hexanol was electrophysiologically and behaviourally active. Here, we investigated the influence of host plant extract (HPE) on two semiochemicals, namely methyl jasmonate (MeJA) and 1-hexanol, both independently and in combination, in the electroantennogram (EAG) response in BSW. The results showed that HPE alone elicited greater EAG response only in males suggesting that males involved predominantly in the host plant selection. Addition of HPE to MeJA increased significantly higher EAG response in females and the response was insignificant in males. Similarly, the addition of HPE to 1-hexanol evoked higher response in females but decreased antennal activity was recorded in males compared to HPE alone. The HPE showed additive effect on MeJA and 1-hexanol in their EAG responses only in female BSW. The result also revealed that 1-hexanol+HPE blend exhibited enhanced EAG response compared to 1-hexanol alone in both sexes.

Keywords: Electroantennogram (EAG), Kairomone, Olfactory response, Semiochemical

The banana pseudostem weevil (BSW), *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae) is one of the important pests of bananas and plantains[1]. It is economically more important in low-input small-scale production systems in India and elsewhere in the tropic and sub-tropics[2]. The attack of the pest results in severe crop losses from plant toppling, snapping at crown region of the plant, withering, no flowering and immature fruits to reduced bunch weight[3]. It causes up to 90% yield loss depending on the growth stage of the crop at which the pest attack and level of management practices[4]. The total crop failure has been reported in susceptible cultivar like Nendran[3]. The biology of the pest was studied in detail[5]. The oviposition takes place only in the outermost leaf sheaths and the eggs are placed singly through the females' rostrum in the air chambers of leaf sheath. The emerged grubs feed on soft tissue of pseudostem and make tunnels in the core of the pseudostem. In severe cases, the tunnels may go up to the peduncle and to the collar of the bottom near the rhizome. The flow of nutrients and minerals were thus hampered and the plant become susceptible for topple and as a result, the plants falls prematurely on blow of wind[3,4]. Although chemical control measures were reported[6], the management of BSW is an elusive and complex problem because of the concealed mode of life cycle of the pest[4]. In addition, use of chemical insecticides are not economical to the small-scale producers, pollution to the environment and poisonous to the handler and domestic animals[7].

Banana pseudostem traps have been used for monitoring and control the pest[8], however only 5-15% of population in an area is attracted by these traps[9], moreover the farmers do not prefer the method as it is labour-intensive[8]. Many biocontrol agents for BSW have been identified but due to the problems of mass production, storage and viability forbid their wide range of use[10,11]. As the abovementioned management practices are not feasible on large scale implementation and no candidate natural enemy has so far been identified for BSW, the semiochemicals provide potential solution in management of the pest. Pheromones which constitute a subcategory of the semiochemicals, offers species-specific pest control and have been included in the IPM of many pests[12]. The aggregation pheromone of BSW was reported as 2-methyl-4-heptanol and evaluated in combination with

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pseudostem tissue under field conditions[13,14]. Though the presence of female released sex pheromone in BSW has been reported[15], the same has not yet been identified. The use of natural plant tissue as such with pheromone lure is cumbersome and impractical[16]. In our earlier report, we presented that baiting with male aggregation pheromone (2-methyl-4-heptanol) of BSW in conjunction with pseudostem tissue extract (host plant extract, HPE) in funnel traps attracted more weevils than traps baited with either pheromone or HPE alone[17]. These earlier studies evidenced that identification of the best attractant for *O. longicollis* is the foremost important criteria in developing successful insect traps. Evaluation of chemicals/volatiles for the efficiency of the attraction for a specific insect at field level is very tedious and time-consuming.

The preliminary knowledge on degree of responses of a specific insect to various chemicals/volatiles will facilitate to identify the best attractant. As the antennae are responsible for the perception of volatile chemicals, measuring the physiological responses of olfactory receptors of insect antennae is the most reliable way to shortlist the semiochemicals that to be used as an attractant in an insect trap. It has been proved that electroantennogram (EAG) is the best technique to monitor physiological responses of olfactory receptors in BSW[14]. Preliminary bioassay screening of fifty host plant related volatile chemicals using EAG and Y-tube olfactometer revealed that only methyl jasmonate (MeJA) and 1-hexanol were physiologically and behaviourally active in BSW. In the present study, we tried to determine the olfactory sensitivity of male and female BSW to the MeJA, 1-hexanol and HPE singly and in combination using EAG technique to identify a suitable combination of semiochemical to be used as attractant for BSW.

Materials and Methods

Insect culture

The adult weevils and their grubs were collected from banana plantations in lower Pulney hills of Dindigul district, Tamil Nadu, which is an endemic area and used for raising BSW colony. Adults were brought to the laboratory and cultured in plastic containers (30 L capacity, height 40 cm, top 36 cm and bottom 27 cm diameters) (Tarsons, Kolkata) with perforated lid for ventilation and for collection of water emanating from banana pseudostem. The weevils were fed with pseudostem of banana cv. Nendran with fortnightly changes of pseudostem pieces. In the case of grubs, each one was kept separately in small perforated plastic containers (14×10 cm and height/dm) with a small piece of banana leaf sheath. The leaf sheath was changed at weekly intervals until pupation. Both adults and grubs were maintained in the laboratory at 25±1°C with 12 h L: 12 h D and 65-70% RH conditions. Male and female weevils were separated based on their rostrum characteristics[5].

Preparation of odour stimuli

The host plant extract was prepared from 100 g of banana leaf sheaths soaked in hexane (HPLC grade) and kept on a shaker overnight to facilitate extraction. The clear supernatant was carefully transferred into a flask and reduced to a volume of 1.0 mL (10 µl/g equivalent)[18]. Similarly, the MeJA and 1-hexanol (M/s. Sigma-Aldrich, Bengaluru) were diluted with hexane.

Dose response relationship

In order to choose a suitable dose evoking maximum response in BSW, MeJA and 1-hexanol were diluted in five different concentrations from log 1 to 5 (v/v). The HPE (10 µL/g equivalent) was taken in five different quantities of 1, 10, 50, 100 and 200 µL and the EAG responses were recorded.

Electroantennogram recordings

The methodology described by Prasuna *et al.*[18] was followed in the present investigation. Antennal responses of adults of both genders to the MeJA, 1-hexanol and HPE and different combinations of the three stimuli were analyzed using EAG apparatus (Syntech, Hilversum, The Netherlands). The antennae of the adults (5-10 days old) were excised at the base using micro scissors. The antenna was fixed in between two stainless steel electrodes with two droplets of 'spectra gel', which was electrically conductive, applied on the electrodes and the antenna mounting process was completed within 10 min. The different stimuli used in the experiment were: MeJA+1-hexanol+HPE; MeJA+1-hexanol; MeJA+HPE; 1-hexanol+HPE; MeJA; 1-hexanol; HPE and control (solvent). The stimulus cartridges were loaded with filter paper (0.5×3.0 cm) strips previously impregnated with 10 µL of MeJA or 1-hexanol or HPE after allowing the solvent to evaporate. In the case of combination stimuli, a 10 µL of HPE was added to strip containing 10 µL MeJA/1-hexanol. The antenna was continuously bathed by humidified filtered air except the stimulus applied. At least 3 min time was elapsed between stimulations to allow the antenna for adequate receptor recovery. The treatments were presented to the antenna in a random basis. Each treatment was assayed on both male and female antennae for six times along with the control and antennal responses were recorded.

Statistical analysis

Data from EAG experiment was analysed by analysis of variance (ANOVA) and difference in pairs of mean values between treatments were examined using least significant difference (LSD) multiple mean comparison test.

Results

The dose-response relationship studies carried out against male and female BSW are presented in Fig. 1 A and B. The results of both semiochemicals revealed that log dilutions 1 and 2 exhibited overstimulation and saturation of antennal receptors occurred and at log 4 and 5, the amplitude of EAG response was weak and overlapping between sexes. Only at log 3 dilution, distinguishable EAG responses between genders was obtained. The optimum dose of HPE was found to be 10 µL 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 weevils antenna was in saturation (Fig. 1C). Hence, for further electro-physiological recordings, the appropriate log 3 concentration of MeJA and 1-hexanol and 10 µL quantity of HPE were chosen. Moreover, at these concentrations, the degree of antennal response as well as the amplitude of responses between genders was comparable and the concentration was near the threshold for a high response.

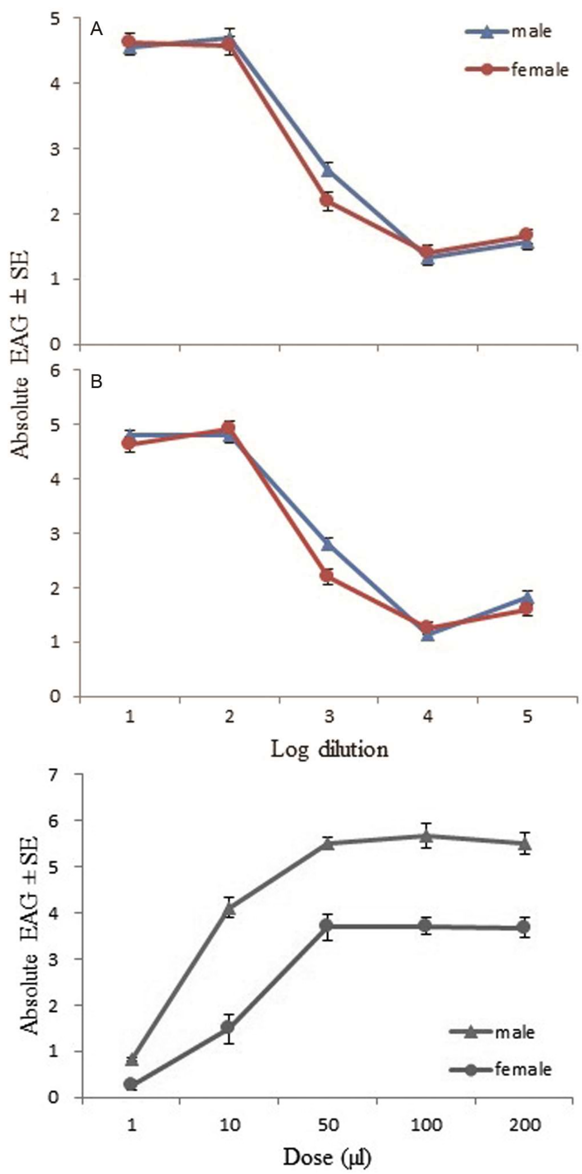

Fig. 1 — Dose response (mean ± SE) curve of *Odoiporus longicollis* to (A) MeJA; (B) 1-hexanol; and (C) HPE for male and female

The EAG responses of male and female BSW to seven different odour stimuli are presented in Table 1. The results revealed that all the stimuli individually and in combinations evoked more EAG responses in BSW in comparison to control indicating some antennal active compounds present in the stimuli. In general, all the stimuli aroused higher response in male as compared to female BSW. Among the various combination of stimuli tested, the HPE alone elicited higher response in male (4.214±0.029 mV) followed by MeJA+HPE blend (4.185±0.054 mV) in male. Though HPE exhibited weak EAG response in female (1.467±0.048 mV), the combination of HPE with MeJA resulted significantly higher antennal response (3.776±0.043 mV) in female. The other combinations such as 1-hexanol+HPE elicited a medium level of

Table 1 — Electrophysiological response of *Odoiporus longicollis* to EAG active semiochemicals MeJA and 1-hexanol and host plant extract

Odour stimuli	EAG response (mV)*†		
	Male	Female	Mean
MeJA+1-hexanol +HPE	2.625±0.127^{e}	1.826± 0.067^{h}	2.226^{F}
MeJA+1-hexanol	1.473±0.088^{i}	1.204± 0.069^{j}	1.339^{G}
MeJA+HPE	4.185±0.054^{a}	3.776±0.043^{b}	3.981^{A}
1-hexanol+HPE	3.558±0.157^{d}	3.327±0.106^{c}	3.442^{B}
MeJA	2.641±0.130^{e}	2.514±0.086^{f}	2.578^{D}
1-hexanol	2.622±0.106^{f}	2.361±0.085d^{g}	2.491^{E}
HPE	4.214±0.029^{a}	1.467±0.048^{i}	2.840^{C}
Control	0.228±0.014^{k}	0.221±0.026^{k}	0.225^{H}
Mean	2.693^{A}	2.087^{B}	

[*Values followed by different small letters in the superscript are significantly different for interaction except for control. †Values followed by the different capital letters in the superscript are significantly different for sex. ¶Values followed by different capital letters in italics are significantly different for treatments. Mean comparison by least significant difference (LSD)]

EAG response in female (3.558±0.157 mV) when compare to HPE alone (1.467±0.048 mV). However, decreased antennal response in male of BSW was noticed in HPE with 1-hexanol combination as compared to HPE alone. Similarly, 1-hexanol+HPE blend showed higher activity in both the sexes as compared to 1-hexanol alone.

Discussion

Banana pseudostem traps are being used to monitor BSW in the field and considered as one of the good package of practices. It has been proved that volatiles emanating from the cut surface of the pseudostem attract banana weevils. The volatiles collected from the banana pseudostem consist of mono and sesquiterpenes[19]. The laboratory bioassay studies revealed that the major components present in the pseudostem did not elicit any physiological and behavioural activities in corm weevil, *Cosmopolites sordidus*[20]. Between the two minor components of pseudostem (∞-caryophyllene and 1,8-cineole) only 1, 8-cineole elicited electrophysiological and behavioural activities[21]. However, the complex activity of plant volatiles makes the banana pseudostem as a good attractant for BSW. In our results also, the HPE elicited a strong response in male and a weak response in female BSW indicating its strong kairomonal activity only in male. In phytophagous insects only males purposefully search host plants for females as females likely to visit feeding and ovipositional sites, for possible encounters for mating opportunities on host plants[22]. There are reports on synergistic effect of host plant/volatiles on semiochemicals particularly on pheromones[23], which encouraged us to study and develop a semiochemical based attractant for BSW. As we have identified two semiochemicals *viz*., MeJA and 1-hexanol from screening of fifty synthetic analogues of plant volatiles as attractants to BSW based on physiological and behavioural activities, in the present investigation the efficacy of HPE on these two semiochemicals was evaluated. In our dose-response study of MeJA and 1-hexanol, the medium concentration (log 3) was well correspond to attraction whereas the higher concentrations (log 1 and 2) correspond to repellency or behavioural inhibition. It is reported that synthetic plant volatile chemicals act as insect attractants at low concentration and repellent at higher concentrations[24]. Similarly, aggregation and sex pheromones, which are essential components of IPM method, used in push-pull strategies to repel the target pest from a protected host with high concentration while luring them towards an attractive trap with low concentration[25]. MeJA, a phytohormone plays a major role in signaling during plant-herbivore and plant-plant interactions[26] whereas, 1-hexanol belongs to the category of green leaf volatiles, which is constitutively produced or during herbivore attack and involved in the host finding behaviour of insects[27].

As in many Coleopteran pests, addition of host plant material increased the pheromone activity[24]. In our result, the HPE enhanced the activity of MeJA and 1-hexanol only in female weevils. These findings suggest that BSW may rely on MeJA and 1-hexanol in particular dose and can be used to monitor the pest under field conditions in conjunction with HPE. The combination of MeJA and 1-hexanol did not elicit more response than it did individually as these two chemicals showed hindering effect on each other. It is reported that addition of n-hexanol with n-heptanol did not elicit higher EAG activity when compared to individual EAG responses in *O. longicollis*[14].

Interestingly, it is observed that in general the responses were more in males than in females irrespective of stimuli. Thus, it is inferred that the gender difference in responses to various stimuli indicated the possible difference in the morphology of antennae of male and female BSW. The antennae of male are small and rough whereas in female they are big and smooth[28]. Further examination of the behaviour response of BSW to these EAG active semiochemicals in combination with HPE may help in designing semiochemical based attractant trap for BSW.

Conclusion

The investigation clearly demonstrated that HPE has the potential to be used as attractant in traps to lure male population and in conjunction with MeJA to capture both the sexes of BSW. However, further behavioural studies required to confirm the behavioural responses of BSW to HPE and MeJA+HPE combination.

Conflict of interest

The authors report no conflict of interest.

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