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Behavioral Responses of Banana Pseudostem Weevil, *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae) to Insect and Plant Volatiles

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Abstract *Odoiporus longicollis* (Coleoptera: Curculionidae) is the most important monophagous pest of banana, suggesting specific volatiles from banana pseudostem may be attractive to this pest. A study was conducted to determine the behavioral response of *O. longicollis* adults to male produced chemicals and plant volatiles. Electroantennography (EAG) and behavioral bioassay was conducted using different odor sources collected from male weevils and the host plant. The EAG result showed that, both male and female produced significantly higher antennal response to male volatile (2.231±0.479 and 1.279±0.157 mV) and its combination with healthy (1.877±0.357 and 1.242±0.118 mV) and mechanically damaged pseudostem extract (2.231±0.468 and 1.502±0.234 mV). In the olfactometer study, per cent response of males (ranging from 57.14 to 85.71%) was found to be higher than that of females (ranging from 42.86 to 66.67%) in all the tests. These findings hint a possible role of male produced chemicals along with host plant volatiles in attraction of weevils. Studies are underway to chemically identify the constituents responsible for the attraction of weevils.

Keywords Behavioral bioassay, Electroantennography, *Odoiporous londicollis*, Olfactometer, Semiochemicals.

Introduction

Banana (*Musa* spp.) is an important Fruit commonly grown in tropical and subtropical parts of the world. India is the largest producer of banana in the world and occupies top position among total fruit production in India. It is cultivated in India in 822 thousand ha with 29,221 thousand tons production [1]. Banana is attacked by different insect pests among which, banana pseudostem weevil (BSW) *Odoiporus longicollis* Oliver (Coleoptera: Cureculionidae) is a key pest [2]. BSW is a serious and devastating pest of banana in many parts of the world. Both adults and larvae cause severe damage to banana plants [3]. Currently in India, this weevil is posing a serious threat

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to the banana cultivation in Andaman Islands, Uttar Pradesh, Bihar, West Bengal, Assam, Kerala, Tamil Nadu and Karnataka [4]. BSW distribution and severity of infestation in South Karnataka was not related to geographical location but influenced by the varieties cultivated [5].

Adult BSW are attracted by the volatiles released by the banana plants. Infestation of the weevil normally starts in five-month-old plants [2]. The female weevil lays eggs inside the air chamber of the outer sheath of the pseudostem through holes made by its rostsum. Emerging grubs make extensive tunnels in the pseudostem for feeding and pupate inside the pseudostem to become adults. Early symptoms of the infestation are the presence of small pin-head-sized holes on the stem, exudation of a gummy substance from such holes and fibrous extrusions from bases of leaf petioles. Extensive tunnelling both in the leaf sheath and pseudostem occurs at the advanced stages of infestation. Rotting occurs due to secondary infection of pathogens and a foul odour is emitted. When the true stem and peduncle are tunnelled after flowering, the fruits do not develop properly, presenting a dehydrated condition with premature ripening of the bunch itself. It is estimated that banana pseudostem borer causes 10–90% yield loss depending on the growth stage of the crop and management efficiency [6]. The problem is generally noticed only when the damage is in the advanced stage and grubs are fully grown [7]. The pest status of the banana weevil varies depending on agro-ecological condition [8].

Farmers adopt several control measures, but all have not been effective or validated or integrated with other practices. The endophytic behavior of the larvae and long life span of adults complicates the management of this pest and insecticides failed to reach them. At present, banana pseudostem trapping is used to monitor this pest, but it is difficult to maintain the pseudostem traps for long periods. The banana pseudostem tissue used in conventional trapping becomes less efficient due to rapid dehydration lead to decrease in the attractiveness after few days [9]. Use of semiochemical (pheromone and kairomone) based pest management is a recent trend in horticultural and plantation cropping systems. Several attempts have been made to optimize and develop semiochemical based methods for the management of *O. longicollis* but these were not successful. Most of the insects locate host plants using the plant volatiles. Host attractants as kairomones can be used as attractant for insects either male or female/ both [10]. Both male and female *O. longicollis* weevils use volatile chemicals emitted by the host plant as cues for their location [11]. In many coleopterans, males initiate host location and produce semiochemicals (pheromones) to enhance the attraction of conspecifics towards the host for feeding and mating [12]. Therefore, an attempt was made to determine the behavioral response of *O. longicollis* adults to male produced chemicals and plant volatiles. In this study volatiles collected from the male weevils and plant extracts of healthy and mechanically damaged pseudostems of banana plants were used, since the weevils were observed to get attracted to both healthy banana plants as well as to the cut pseudostem traps in field conditions but the chemicals responsible for attraction were not identified. An air entrainment method was followed for the collection of insect volatiles [13—15] rather than solvent extraction of insects. So that specific volatiles released by the male

Table 1. EAG responses of *O. longicollis* males to different odorous stimuli. Figures within a column followed by a common letter are not significantly different by Tukey post hoc test (Pd"0.05).

Treatments	EAG Response (mV) (Mean±SEM)
Male IVC	2.231 ± 0.479[a]
Healthy pseudostem extract	0.952 ± 0.159[ab]
Mechanically damaged pseudostem extract	0.893 ± 0.165[ab]
Male IVC+ Healthy pseudostem extract	1.877 ± 0.357[ab]
Male IVC+ Mechanically damaged pseudostem extract	2.231 ± 0.468[a]
Control (DCM)	0.511± 0.131[b]
DF	5, 24
F test	5.285
P value	0.002

Table 2. EAG responses of *O. longicollis* males to different odorous stimuli. Figures within a column followed by a common letter are not significantly different by Tukey post hoc test (Pd"0.05).

Treatments	EAG Response (mV) (Mean±SEM)
Male IVC	1.279 ± 0.157[a]
Healthy pseudostem extract	0.506 ± 0.058[b]
Mechanically damaged pseudostem extract	0.481 ± 0.077[b]
Male IVC+ Healthy pseudostem extract	1.242 ± 0.118[a]
Male IVC+ Mechanically damaged pseudostem extract	1.502 ± 0.234[a]
Control (DCM)	0.277 ± 0.051[b]
DF	5, 24
F test	15.251
P value	0.0001

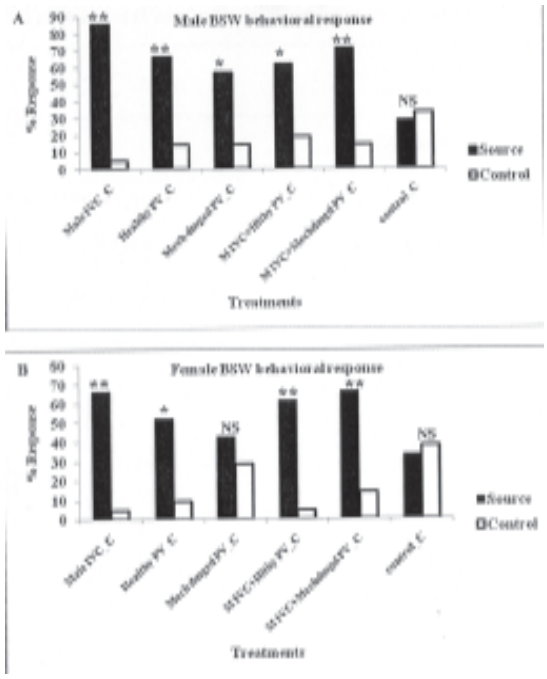

Fig. 1. Behavioral response (%) of *Odoiporus longicollis* (A) Males and (B) Females to volatiles from BSW and Banana plant in a Y-tube olfactometer. Differences between paired bars (χ^2 test) indicated by **P*<0.05, ***P*<0.01, NS=Non-significant. M IVC: Male Insect volatile Collection; PV: Plant volatile; C: Control.

weevils attractive to conspecifics can be identified.

Materials and Methods

Insects

The pupae of banana pseudostem weevils were collected from infested banana plants, maintained in separate plastic containers (7 cm × 5 cm) and daily checked for adult emergence. Emerging adults were sexed based on rostrum characteristics. Male and female weevils were maintained in separate containers (29 cm × 17 cm × 33 cm) in the laboratory under the photoperiod of 12 light (L): 12 dark (D) with 25 ± 2°C and 70 ± 10% RH until use at Biocontrol Research laboratories (BCRL), Sriramanahalli, Bengaluru (13.18´, 77.55´, 980AMSL). Weevils were provided with freshly cut pseudostem pieces as food, replaced every 5 days.

Collection of insect volatiles

Groups of 50 male weevils were maintained separately in 6 cm dia. × 19 cm long glass aeration chambers. A charcoal filtered humidified airstream was pushed through the aeration system at a flow rate of 1.8 L min^{-1} was achieved using a vaccum system. Emitted volatiles were collected daily and trapped in glass tubes with a 30 mg of polymer-conditioned Porapak-Q adsorbent. The weevil volatiles were collected from the Porapak-Q with 1.5 ml of HPLC grade Dichloromethane (DCM) in 15 min. A dilution of 200 weevils were prepared by mixing 4 days volatile collection and were concentrated to 1 ml (20 weevil volatiles per 100 µl of DCM) under nitrogen stream before analysis, so that the volatiles are in diluted form for direct use in different studies. The volatile collection was conducted at 25 ± 2°C temperature and 70 ± 10% RH at Biocontrol Research laboratories (BCRL), Sriramanahalli, Bengaluru. Extracts that were not used immediately were stored at -20°C in a refrigerator. Solvent extraction method was followed for

collection of banana pseudostem extract. To prepare the banana pseudostem extract, 5 g each of fresh pesudostem and mechanically damaged pseudostem were cut into small pieces and soaked in 10 ml dichloromethane (CH_2Cl_2) (HPLC grade) separately in a glass vial at 25 ± 2°C for 65 h. The solvent extract from both the samples were carefully transferred to separate glass vials. The extracts were concentrated under a gentle stream of nitrogen. On condensing, the sample was stored at -20°C until used for bioassay. The antennal and behavioral responses of weevils to male volatiles, plant volatiles and their combinations were conducted using Electroantennography (EAG) and Y-olfactometer at Insect Behavior Testing Laboratory (IBTL), BCRL, Bengaluru.

Electroantennography (EAG)

Electrophysiological responses of male and female *O. longicollis* antennae to different volatile extracts collected from weevils and plants were recorded using Syntech EAG system. Ten µl of aliquot placed on a filter paper strip (60 mm long, 5 mm wide Whatman No. 1) inside a glass Pasteur pipette (5.75, Length – Overall 145.0 mm; tip-0.47 mm) was used for stimulus delivery. This was connected to the stimulus controller by silicone rubber tubing. After 10 seconds, the solvent was blown out with first puff. Another 60 seconds later, the stimulus was puffed on to the excised antenna by injecting the vapour phase of the chemical stimuli through a polysterene tube along with a continuous air stream (pulse rate 0.5 s, continuous flow 25 ml s^{-1}, pulse flow 21 ml s^{-1}) to the antenna. For each sample dilution, 5 replicates were performed per sex and each replicate represented one antenna.

Laboratory bioassay

The test volatile extracts were further subjected to a dual choice Y-tube olfactometer to determine the behavioral responses of *O. longicollis* males and females. The oriented movements of weevils to different volatile samples were recorded. The olfactometer consisted Y-shaped acrylic of 6 cm dia. The main tube (stem) of the olfactometer and the two arms were each 30 cm in length at 90°. The air-delivery unit was connected to the two arms of the Y-tube to draw purified air to pass through the odor sources in the Y-tube. Airflow through each of the olfactometer arms was maintained at 0.5 L min^{-1}. 40 µl of volatile extracts in DCM were loaded in Whatman filter paper strips (1 × 3 cm) and were placed in one of the Y-tube chambers and the other chamber served as control (equal volume of HPLC-grade DCM). New filter papers with the extracts in DCM were used for each trial (3 weevils). The position of treatments was alternated after each trial, to avoid directional bias. A group of 3 BSWs were introduced into the base tube of the olfactometer and the behavior was observed for 15 min. Totally 21 weevils were used for each treatment in 7 batches, each batch with 3 weevils. When a weevil crossed the choice line 10 cm after the division of the base tube and remained there for at least 20s, it was recorded as choice for the odor source in that arm. If the weevils stayed in the common tube or at the junction of the two arms and did not make a choice during this time were considered a non-responding individual and were excluded from the statistical analysis.

Statistical Analysis

Electroantennogram responses were compared using a one-way analysis of variance (ANOVA) followed by a honestly significant different (HSD) Tukey test (SPSS 16.0 version software). Results of all olfactometer bioassays were analyzed with a binomial test (χ^2 test; SPSS 16.0 version software) and means were compared by Tukey HSD test ($p<0.05$).

Results and Discussion

All the stimuli tested elicited significant EAG responses from both male and female antennae in comparison to control indicating the presence of certain antennally active constituents in the extracts. Tested males produced significantly higher EAG deflections than female weevils in all the tests except for control (Tables 1 and 2).

Statistical analysis of results with Tukey's post hoc test revealed significant differences in EAG responses of males to different treatments. Male volatile alone and its combination with mechanically damaged pseudostem extract elicited significant antennal

Table 3. Responses of male and female BSW adults to select semiochemicals in Y-tube olfactometer. Control: DCM, Statistically significant differences using binomial test at *p<0.05; **p<0.01 and NS=Non-significant.

Odor sources	Frequencies Male	Female
Male IVC vs. Control		
Male IVC	18**	14**
Control	01	01
Not decided	02	06
Total	21	21
Healthy plant extract vs. Control		
Healthy plant extract	14**	11*
Control	03	02
Not decided	04	08
Total	21	21
Mechanically damaged plant extract vs. Control		
Mechanically damaged plant extract	12*	09^{NS}
Control	03	06
Not decided	06	06
Total	21	21
Male IVC+Healthy plant extract vs. Control		
Male IVC+ Healthy plant extract	13*	13**
Control	04	01
Not decided	04	07
Total	21	21
Male IVC+ Mechanically damaged plant extract vs. Control		
Male IVC + Mechanically damaged plant extract	15**	14**
Control	03	03
Not decided	03	04
Total	21	21
Control vs. Control		
Control	06^{NS}	06^{NS}
Control	07	08
Not decided	08	07
Total	21	21

responses in males than any other stimulus and these two were statistically on par (2.231±0.479 mV and 2.231±0.468 mV, respectively). The EAG responses of male BSW antennae to the combination of male volatile with healthy pseudostem extract (1.877±0.357 mV) was higher than the response to healthy pseudostem extract (0.952±0.159 mV) and mechanically damaged pseudostem extract when tested alone (0.893±0.165 mV) (Table 1).

Analysis of results with Tukey's post hoc test revealed significant differences among the treatments in antennal responses of female BSWs. The female antennae produced significantly higher EAG response to male volatile extract (1.279±0.157 mV) and combination of male volatile extract with mechanically damaged and healthy pseudostem extract (1.502±0.234 mV and 1.242±0.118 mV, respectively), were statistically at par. The healthy and mechanically damaged pseudostem extracts when given individually without male volatile extract elicited significantly lower EAG response in female antennae and they were statistically on par (Table 2).

The EAG result of both male and female weevils showed that, both sexes produced higher antennal response to male volatile and combination of male volatile with the plant extracts compared to plant extracts alone. Others also reported that body extracts of male weevils in combination with banana sheath extract produced larger EAGs in both males and female *O. longicollis* than banana sheath extracts alone [11]. All the odorous stimuli of banana pseudostem extracted by different methods elicited higher EAGs in male compared to female, indicating that male perceived pheromones/kairomones may be involved in the process of mate/host plant sciection [10]. The study also shows that males showed more antennal activity than females.

The responses of *O. longicollis* males and females in Y-tube olfactometer to different odor sources are shown in Table 3. The attraction of *O. longicollis* male to the male weevils volatile extract (P=0.0001, χ^2=15.211), healthy pseudostem extracts (p=0.008, χ^2=7.118) and to the combination of male volatile extract with mechanically damaged pseudostem extract (p=0.005, χ^2=8.000) was found highly significant than control. Similarly, the extracts of mechanically damaged pseudostem alone (p=0.020, χ^2=5.400) and combination of male volatile with healthy pseudostem extract (p=0.029, χ^2=4.765) was also more attractive to male weevils compared to DCM solvent as control (Table 3). On the other hand, male volatile (p=0.001, χ^2=11.267) and the combination of male volatile with healthy and mechanically damaged pseudostem extracts (p=0.001, χ^2=10.286 and p=0.008, χ^2=7.118, respectively) were highly attractive to female weevils

than healthy pseudostem extract (p=0.013, χ^2=6.231). The attraction of females to the mechanically damaged pseudostem extract (p=0.439, χ^2=0.600) was statistically non-significant (Table 3). Ambrogi and Zarbin reported that combination of male extract and soybean stem was more attractive to males and female of *Sternechus subsignatus* Boehman, weevils than soybean stem alone [13]. The attraction of the guava weevil, *Conotrachelus psidii* Marshall, males and females to the natural headspace extract of males was significant as well as the attraction to the combination of host plant headspace extract with the synthetic pheromone component. On the other hand, neither the synthetic compound nor the host plant volatiles alone were attractive to males and females [15]. Others have also observed that in the laboratory, volatiles from pseudostem tissue had an additive effect on attraction of *Cosmopolites sordidus* Germar, weevils to the pheromone but the effect was not significant in the field [9].

The per cent response of male and female weevils to different odor sources in each experiment was calculated due to significant differences was observed in the movement of weevils in the olfactometer bioassay. In all the tests, per cent response of males (ranging from 57.14 to 85.71%) was found to be higher than that of females (ranging from 42.86 to 66.67%). On the other hand, both sexes showed similar type of response to all the odor sources except mechanically damaged pseudostem extract was more attractive to males but not to the females (Fig. 1). These results were in confirmation with other workers [11]. The percent activity of both male and female was more towards the conbination of male body extract and banana sheath extract than to the banana sheath extracts per se. These findings lead to the conclusion that, male volatiles along with plant volatile as a possible role in the host plant as well as male location.

The electrophysiological and olfactometer studies revealed that the plant volatiles alone are not effective for attracting the *O. longicollis* weevils but their combination with male produced volatiles is found to have additive effect in attracting the weevils under the laboratory conditions. Male volatile and its combination with plant extracts produced considerable EAGs and behavioral responses in male and female BSWs. However, male weevils showed higher EAGs and olfactory responses to all the odor stimuli than females. Therefore, male weevils are more responsive to odors and male produced chemicals with plant volatiles play a role in the attraction of both sexes. Since, analysis of the extracts of *O. longicollis* males via GC-EAD showed that, two peaks of male extracts consistently activated the antennae of male and female weevils. Studies are underway to chemically identify the constituents responsible for the attraction of weevils.

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