Mysore J. Agric. Sci., 50 (2) : 234-237, 2015

Behavioral and Electrophysiological Responses of the Banana Pseudostem Weevil *Odoiporous longicollis* Olivier to Host Plant Volatiles

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Abstract

Electroantennography (EAG) and behavioral bioassays were conducted to determine the effective host-plant volatile(s) for the attraction of *Odoiporus longicollis* adults. Volatiles from healthy and mechanically damaged pseudostem extract elicited significantly higher antennal response in male (1.187±0.102mV) and female weevils (0.942±0.116mV), respectively. The Y tube olfactometer study revealed that significantly more males were attracted towards the healthy (66.67%) and mechanically damaged pseudostem extract (61.90%), while females showed more attraction towards BSW damaged (57.14%)) and decaying pseudostem extract (57.14%) in addition to healthy plant volatile (52.38%). Hence, these plant volatiles may be used as attractants for the management of the pest.

Banana (*Musa* spp., Musaceae) is one of the most important fruit crops of South East Asian region. India is the largest producer of banana in the world with 822 thousand ha and 29,221 thousand tons production (NHB, 2014). Banana is infested with nineteen insect pests from planting to harvesting in India (Padmanaban *et al*., 2001). Of these, the banana pseudostem weevil (BSW) *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae) is gaining importance as a serious pest causing heavy losses to the growers. Now-a-days, this weevil poses a serious threat to the banana cultivation in the banana growing belts of India (Ravi and Palaniswami, 2002).

Banana pseudostem weevil is a monophagous pest. Adult females of *O. longicollis* lay eggs in the outermost leaf sheath of banana. Larvae hatched from the eggs bore into the living tissue, producing frass-filled tunnels that weaken the affected parts of the host plant and permit invasion of fungal and bacterial pathogens. Mature larvae pupate in cocoons made from plant fibers close to the exit holes. The severity of the loss is greater when infestation occurs at the early vegetative stage (5 months old). It is estimated that banana pseudostem weevil incurs 10-90 per cent yield loss depending on the crop growth stage and management practice (Padmanaban and Sathiamoorthy, 2001).

At present, banana pseudostem trapping is the only method available to monitor this pest. The endophytic behavior of the larvae and long life span of adults complicates the management of this pest as the insecticides were ineffective. Hence, there is an urgent need to explore an alternative ecofriendly practical approach. Use of kairomones is a recent trend in developing semiochemical-based pest monitoring and management, which is one of the viable technologies to combat this pest. Owing to its restricted feeding habit and monophagy, *O. longicollis* may use specific host plant volatiles to find its host for feeding and oviposition. Exploiting these behaviorally active plant volatiles as a potential tool for monitoring and mass trapping purposes offers an ecofriendly management option. In the present study, electroantennographic (EAG) technique was employed to screen different extracts from the banana pseudostem for detecting volatiles with possible semiochemical property.

O. longicollis larvae were collected from damaged banana plants and reared on banana pseudostem pieces under laboratory conditions at 12L: 12D at 25 ± 2^0 C, 70 ± 10 per cent RH at Biocontrol Research laboratories (BCRL), Pest Control (India) Pvt. Ltd. (PCI), Bengaluru. Emerged adults were separated into males and females by rostrum characteristics. They were maintained in separate plastic containers (29cm x 17cm x 33cm). Weevils were provided with freshly cut pseudostem pieces as food, replaced every 5 days.

Solvent extraction method was followed for volatile collection. Pseudostem pieces of 5gm each from healthy plant, BSW damaged plant, mechanically damaged plant and decaying plant were collected and immersed in 10 ml dichloromethane (CH_2Cl_2) (HPLC grade) at 25 ± 2^0 C for 3 days. The solvents were filtered and were concentrated under a gentle stream of nitrogen. On condensing, the sample was stored at -20^0 C until used for bioassay.

Electroantennography (EAG) (Syntech, The Netherlands) bioassay was carried out at BCRL to assess the olfactory sensitivity of both male and female *O. longicollis* adults to different pseudostem extracts. Ten µl of aliquot placed on a filter paper strip (60mm long, 5mm wide Whatman No. 1) inside a glass Pasteur pipette (Dimensions – 5.75, Length – Overall 145.0 mm; tip-47.0 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. Five samples were used, for each sample 5 replicates were performed per sex and each replicate represented one antenna.

The behavioral responses of *O. longicollis* males and females to different pseudostem extracts after EAG screening were further tested in a dual choice Y-tube olfactometer at Insect Behavior Testing Lab (IBTL) of BCRL. The olfactometer consisted of Y-shaped acrylic tube of 6 cm dia. The main tube (stem) of the olfactometer and the two arms were each 30 cm in length at 90^0. The air-delivery unit (model) 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}. The olfactometer study was carried out in a room separated from *O. longicollis* culture. The bioassay was carried out between 11:00 and 1600 h during photophase, which corresponded with the peak mating behavior of *O. longicollis*. Fixed number of pseudostem extracts (40 ìl) were loaded in Whatman filter paper strips of 1 x 3 cm size 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 and DCM were used for each trial (for every 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 m. when a weevil crossed the choice line 10 cm after the division of the base tube and remained there for at least 20 s, it was recorded as a 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. Age and mating status of the weevils were not controlled during the bioassays because of the difficulty of rearing these insects under laboratory conditions.

All the extracts elicited antennal response in both male and female BSW. Among different extracts, healthy pseudostem and BSW damaged pseudostem extract elicited significantly higher response ($F_{4, 20}$=4.652; P=0.008) in male antennae than control. While extracts of decaying pseudostem and mechanically damaged pseudostem were found on par. When female antenna was exposed to above mentioned extracts, mechanically damaged pseudostem extract elicited significantly higher response ($F_{4, 20}$= 6.256; P=0.001) than other extracts and control (Table I).

Dual choice Y-tube olfactometer studies were conducted to determine the behavioral responses of male and female *O. longicollis* adults to different fractions of banana pseudostem extracts. The results indicated that, significantly higher number of males were attracted towards the healthy pseudostem (÷2=7.118, p=0.008) and mechanically damaged pseudostem exracts (÷2=8.067, p=0.005) compared to control. The male responses towards BSW damaged (÷2=0.333, p=0.564) and decaying pseudostem extracts (÷2=3.267, p=0.071) were not statistically different from respective control (Table 2). Similarly, male weevil attraction towards healthy (÷2=6.231, p=0.013), BSW damaged (÷2=7.143, p=0.008) and decaying pseudostem extracts (÷2=5.400, p=0.020) were significantly higher than the control. Response of males toward mechanically damaged pseudostem extract

TABLE I

EAG response (Mean (±SEM)) of BSW male and female antennae against banana pseudostem extracts

Treatments	EAG Response (mV) (Mean±SEM)	
	Male	Female
Healthy pseudostem extract	1.187±0.102[a]	0.578±0.070[abc]
BSW damaged pseudostem extract	1.274±0.102[a]	0.867±0.080[ab]
Decaying pseudostem extract	1.001±0.092[ab]	0.528±0.096[bc]
Mechanically damaged pseudostem extract	1.058±0.119[ab]	0.942±0.116[a]
Control (DCM)	0.730±0.056[b]	0.377±0.081[c]
DF	4, 20	4, 20
F test	4.652	6.256
P value	0.008**	0.001**

Note : Figures within a column followed by a common letter are not significantly different by Tukey post hoc test ($p<0.05$)

TABLE II

Behavioral responses of O. longicollis males to plant volatiles

Treatments	Number of response		Chi square value	‡Responded individual (N)	p	
	Male	Female				
Healthy vs Blank	14	3	7.118a	17	0.008	*
BSW damaged vs Blank	7	5	0.333b	12	0.564	NS
Mechanically damaged vs blank	13	2	8.067c	15	0.005	*
Decaying vs Blank	11	4	3.267c	15	0.071	NS
Blank vs Blank	6	7	0.077d	13	0.782	NS

‡ Response between source and control is significantly different at $p<0.05$, NS- non-significant different. ‡ n= 21, df=1

TABLE III

Behavioral responses of O. longicollis females to plant volatiles

Treatments	Number of response		Chi square value	‡Responded individual (N)	p	
	Male	Female				
Healthy vs Blank	11	2	6.231a	13	0.013	*
BSW damaged vs Blank	12	2	7.143b	14	0.008	*
Mechanically damaged vs blank	9	6	0.600c	15	0.439	NS
Decaying vs Blank	12	3	5.400c	15	0.020	*
Blank vs Blank	8	6	0.286b	14	0.593	NS

‡ Response between source and control is significantly different at $p<0.05$, NS- non-significant different. ‡ n= 21, df=1

(÷2=0.600, p=0.439) was not statistically different compared to control (Table III).

In this study, both male and female weevils responded to all pseudostem extracts, which may be used to identify and locate the hosts for feeding, mating and for oviposition by both male and female, respectively. Gunawardena and Dissanayake (2000) suggested that the host volatiles have additional function as an oviposition stimulant for females in addition to other function. Both male and female *O. longicollis* weevils use volatile chemicals of banana for locating their mates (Prasuna *et al.,* 2008) and for oviposition (Abera, 1997 and Gold *et al.,* 2001). Sahayaraj and Kombiah (2009) suggested that *O. longicollis* use decayed pseudostem as cues for food location. Palanichamy *et al.* (2011) suggested that microwave assisted pseudostem extract can be used as kairomones for attracting weevils particularly in the fields where, female population preponderates male. Hence, major volatiles released by the host plants can be used to develop semiochemical-based-management practices for *O. longicollis*.

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(*Received :* May, 2016 *Accepted :* June, 2016)