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AGGREGATION PHEROMONE AND KAIROMONES IN ATTRACTING BANANA PSEUDOSTEM WEEVIL *ODOIPORUS LONGICOLLIS* OLIVIER

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ABSTRACT

A laboratory bioassay was conducted to study the effect of male released aggregation pheromone, 2-methyl-4-heptanol, of banana pseudostem weevil in combination with host plant extract in attracting the sexes of *Odoiporus longicollis* Olivier (Curculionidae: Coleoptera) using 4-arm olfactometer. The results indicated that pheromone and host plant extract combination attracted more weevils than either pheromone or host plant extract individually. There was no variation in the attraction of sexes to semiochemicals. The advantages and disadvantages of using the 4-arm olfactometer over two-choice bioassay is also discussed.

Key words: *Odoiporus longicollis*, banana, 4-arm olfactometer, 2-methyl-4-heptanol, host plant extraxt, repellent, attractant, bioassay, semiochemicals, sex differences

Banana pseudostem weevil (BSW), *Odoiporus longicollis* is one of the important pests of bananas and plantain (Biswas et al., 2015). The BSW causes 100% yield loss depending on the stage at which infestation occurs and the level of management practices (Jeyanthi and Verghese, 1999). Management of BSW is an elusive and complex problem due to the cryptic nature of the weevil. Though chemicals control have been suggested, it is too costly for the poor farmers and indiscriminate use leads to pollution to the handler (Gold et al., 1999). Banana pseudostem trapping was used for monitoring and mass trapping of the pest, but this method was not being adopted by the farmers for lack of effectiveness due to many factors including cultivar used as trapping material and labour requirements (Gold et al., 2002). Gunawardena et al., (1999) identified the male aggregation pheromone of BSW as 2-methyl-4-heptanol (2M4H), which attract both the sexes. Earlier, we have reported the kairomonal activity of banana pseudostem extract (HPE) prepared by microwave oven assisted extraction (Palanichamy et al., 2011). In the present study, the 2M4H and HPE was tested individually and in combination against BSW using a 4-arm olfactometer so as to develop an effective semiochemical-based attractant trap.

MATERIALS AND METHODS

The BSW colony was raised from field-collected weevils and grubs. The adults were brought to the laboratory and placed in a perforated (on the lid for ventilation and in the bottom for drainage of water emanating from banana pseudostem) plastic container (30 L) and fed with a piece of banana pseudostem of banana cultivar Nendran. The banana pseudostem piece was changed as and when it partially dried up. In the case of grubs, each grub was kept individually in small perforated plastic containers (height 14 cm and 10 cm diameter; Tarsons, Kolkata, India) with a small piece (6 x 6 cm) of the banana leaf sheath. The leaf sheath was changed at regular intervals until pupation. Both adults and grubs were maintained in a laboratory set-up with 25±1 °C room temperature having 12 h L: 12 h D and 65-70% RH. Male and female weevils were separated based on their rostrum characteristics (Dutt and Maiti, 1972).

The aggregation pheromone, 2M4H was purchased from M/s. Chempure (P) Ltd., Mumbai and was diluted in hexane (HPLC grade). The host plant extract (cultivar Nendran) was prepared from 100 g of banana leaf sheath pieces in hexane by microwave assisted extraction method (Prasuna et al., 2008; Palanichamy et al., 2011). The supernatant was carefully removed from the flask and reduced to 10 µl/g equivalent using rotary evaporator. The pheromone compound was diluted in hexane (0.01%) and 10 µl each of pheromone and HPE was taken for the bioassay study. A dose response relationship study conducted earlier showed that log 3 dilution was found suitable for pheromone (unpublished data).

A four-arm olfactometer (Syntech, Netherlands) was used to test the behavioural responses of male and female BSW to its aggregation pheromone, 2M4H and HPE. Apparatus consists of an exposure chamber composed of 10 mm thick grey and opaque aluminium floor, with a 5 mm thick glass ceiling. The floor pointed star-shaped walls of the exposure chamber was constructed by four grey aluminium crescents 90^{O} arc of radius 170 mm and depth 5 mm, glued to the floor. A thin strip of foam gasket was fixed on the top of the aluminium floor arc to make an airtight seal when the glass ceiling was placed. To make it further airtight vacuum grease was applied (SD Fine Chemicals Ltd, Mumbai) over rubber gasket. Each point of the star ran into an aluminium tube (inside radius 5 mm) which came through outside of the floor at an angle of 45^{O}. The glass ceiling was held down firmly on four sides by a clamp. Air was filtered through an activated charcoal filter (Model Syntech), before being branched off to separate but identical lines leading to each corner of the olfactometer. A flow meter (0-2 L/min.) was used to control the flow of air. The amount of air flow was determined by fine adjustments in the flow meter. Air from the flow meter was passed into a 300 ml jar containing distilled water for increasing the humidity and then passed into a second 300 ml jar containing 10 µl each of 2M4H and/or HPE. The air was drawn from this jar directly to the corners of the exposure chamber. Finally, air flows exited through a tube placed in the center by a small vacuum pump connected with the exit tube, which could be disconnected, with a plexiglass vial containing test insects. The vial containing gauze has retained the insect and allows air to pass through when the vacuum pump was connected.

The behaviour of a set of ten male and female BSW was recorded separately in the four-arm olfactometer for four different odour complex including control (treatments) with ten replications. Less than twenty days old virgin weevils were used in the experiments. Before using them in the experiment, they were checked for appetite and starved for at least three hr (Ravi and Palaniswami, 2002). The activity of each insect was recorded for 10 min and each insect was used only once in all the tests. The experiment set up was covered by a black cloth for making darkness. All the experiments were conducted at 25±2 ^{O}C and RH 65-70%. To avoid any distortion of the results due to possible differences in the shape or intensities of the airflows running in different arms, the whole apparatus was washed and the test complex moved to a different arm after every ten insects tested. Two-way ANOVA was performed using computer software and the least significant difference was used for mean comparison between treatments and sexes at 95% confidence level.

RESULTS AND DISCUSSION

The response of BSW to different odour stimuli viz., 2M4H + HPE, 2M4H, HPE and control was presented in Table 1. The results showed that 2M4H + HPE combination attracted a maximum mean of 4.300 ± 0.386 male and 3.900 ± 0.348 female respectively. The aggregation pheromone, 2M4H alone attracted a mean of 2.200 ± 0.133 male and 1.900 ± 0.366 female weevils. Interestingly, HPE alone attracted a mean of 2.300 ± 0.153 male and 1.700 ± 0.260 female weevils. The response of male towards the 2M4H + HPE combination was significantly different from both 2M4H and HPE alone. Similarly, the response of male to 2M4H and HPE was significantly not different implies that both the compounds influenced the BSW to the same degree. In fact, the response of female to the same treatment also showed similar results indicated that 2M4H required synergism from the host plant odour to stimulate behavioural response in BSW.

Table 1. Male and female *O. longicollis* attracted to odour source (two-way ANOVA)

	Odour field			
Sex	2M4H +HPE	2M4H	HPE	Control
Male	4.30 ± 1.14^{a}	2.20 ± 0.63^{b}	2.30 ± 0.67^{b}	0.00^{c}
	(11.72)	(8.44)	(8.63)	(0.91)
Female	3.90 ± 1.20^{a}	1.90 ± 0.88^{b}	1.70 ± 0.82^{b}	0.00^{c}
	(11.27)	(7.75)	(7.30)	(0.91)
CD (P=0.05)	(T X S)	(T)	(S)	
	1.16	0.82	0.58	

Values in parentheses square root transformed. Data on non-responded weevils excluded. Mean followed by the same letter in a column not significantly different (p=0.05). Least significant difference was performed for mean comparison; Abbreviations: T = Treatment; S = Sex; Mean ± = SE and n = 10.

The pheromone component 2M4H works only in the presence of host plant odour as in the case of many Coleopteran pests. For example, the pheromone of sugarcane weevil, *Metamasius hemipterus,* Rhynchophorol found to be field active only in the presence of fresh sugarcane pieces or pseudostem of banana (Cerda et al., 1999) and sordidin, the male aggregation pheromone of banana rhizome weevil, *Cosmopolites sordidus* attract more weevils in the presence of pseudostem pieces (Alpizar et al., 2002). Additive effect of host plant volatiles on pheromone of *C. sordidus* was also reported (Tinzaara et al., 2007). In our earlier studies, the extract prepared by microwave oven assisted extraction method elicited more electroantennogram response in female than the extracts obtained by both solvent extraction as well as air-entrainment method (Palanichamy et al., 2011). The bioassay methods, which tell us which extracts fractions or compounds from insects or host plants are biologically active. Without an accurate bioassay, it is impossible to select the active compounds from a complex mixture of compounds produced by the insect or host plant (Tumlinson, 1990). The behavioural assays to study insect attraction to specific odours are tedious, time consuming and often require a large number of replications. Olfactometer was usually employed to test the behavior of walking insects, whereas, the wind tunnel allows to evaluate the behavior of both walking and flying insects (Faccoli and Hentry, 2003). Olfactometer test can usually be conducted with one or two odour sources at a time. We used a four-arm olfactometer in which four distinct odour sources can be created in a chamber in which an individual or group of weevils can freely walk (Vet et al., 1983).

Both male and female BSW responded exceptionally well in the olfactometer towards different odour sources in our experiment. It was evident from the fact that when no odour was offered (control), most of the weevils were remain in the central release chamber during the observation time (10 min) and those that do walk into an arm do not show a significant preference for a particular arm. In other words a great majority of the weevils entered the arm carrying odour source, thus this olfactometer is suited to test the attractiveness of the individual odours. By placing the odour stimuli in a different chamber for different replicates, the possibility that the results are due to positional effect is ruled out. However, since male and female weevils were released in the group separately, both sexes may influence each other's choice. Since male weevil's releases aggregation pheromone and the female weevils reportedly release sex pheromone (Ravi and Palaniswami, 2002), the pheromones might affect the results of this experiment. In addition, the males/females could have left marking pheromones, which have made the test odour less attractive to subsequent test insects reducing their response. However, only ten BSW males/females were tested consecutively in the olfactometer then the whole apparatus was washed and the new odour sources placed in another arm. Moreover, as using 2M4H + HPE complex the behavioural parameters were significantly different from those observed with other odours suggest that the presence of at least one of the volatiles emanating from the HPE causes attraction of BSW. The host plant kairomone for banana corm weevil, *Cosmopolites sordidus* was identified as 1, 8-cineole. However, though both *C. sordidus* and *O. longicollis* shared the same host, 1, 8-cineole was found physiologically active and behaviourally inactive for *O. longicollis*. Identification of one or more constituents from banana plant which should show both physiologically and behaviourally active to *O. longicollis* may be useful in developing semiochemical-based attractant for monitoring and control of *O. longicollis*. The whole experimental set up is covered with thick black cotton cloth to ensure that the insects have no visual cues. The main advantages of the 4-arm olfactometer is included multiple odours can be tested at the same time and insects can be released in groups led to saving experimental and rearing time (Turlings et al., 2004). The major disadvantages of using 4-arm olfactometer were relatively high cost and inconvenient in cleaning and drying the entire device between each replication. The possibility of the attractive odours adsorbed on the glass and Teflon parts rendered the arms attractive even after an odour source was removed. Despite these disadvantages, the 4-arm olfactometer is highly efficient, practical and also promises to be a useful tool for olfactometric studies.

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