350 km^3. The minimum area covered by the newly described Youngest Toba ash in the CIOB is estimated to be $\sim 3.2 \times 10^6$ km^2. In the northern sediment cores the ash thickness is comparatively more (up to 13 cm) and decreases (8 cm) in the southernmost cores, with an average of 10 cm. Based on the area covered in the Southern Hemisphere ($\sim 3.2 \times 10^6$ km^2) and average ash thickness (10 cm), the new minimum volume estimate is ~ 160 km^3. This volume is in addition to the earlier volume of 350 km^3 estimated by Rose and Chesner[11] based on the occurrence of ash from the Northern Indian Ocean. Recently, the YTT has been further traced from South China Sea[16] and Arabian Sea[17,19,21]. For the ash volume estimate, we have not considered locations from South China Sea and Arabian Sea, as data are limited to few stations. Therefore, the new occurrence of the YTT in the CIOB, South China Sea and Arabian Sea suggests significant increase in ash volume estimates, and consequent climatic implications need to be reassessed.

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Evidence for a female-produced sex pheromone in the banana pseudostem weevil, *Odoiporus longicollis* Olivier

G. Ravi* and M. S. Palaniswami[†,#]

*Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai 625 104, India
[†]Division of Crop Protection, Central Tuber Crops Research Institute, Thiruvananthapuram 695 017, India

A female-produced sex pheromone in *Odoiporus longicollis* Olivier was detected in a laboratory bioassay system. Both virgin and mated females produce sex pheromone. There was a significant difference in response of males to females, and it varied towards females of different age groups. The calling behaviour of the females and the courtship behaviour of the males have been documented. This report is an evidence on the presence of sex pheromone in *O. longicollis*.

THE banana pseudostem weevil *Odoiporus longicollis* Olivier (Curculionidae : Coleoptera), has become a serious pest on banana and is distributed mainly in Southeast Asia and New Guinea[1,2]. In India though it is distributed all over the country, it is a serious pest in the banana-growing belts of Andaman Island[3], Uttar Pradesh[4], Bihar[5], West Bengal[6], Assam[6], Kerala[7] and Tamil Nadu[8]. The female weevil lays eggs inside the air chamber of the outer sheath of the pseudostem through holes made by its rostrum. Emerging grubs make extensive tunnels in the pseudostem for feeding and pupate in the pseudostem to become adults. Owing to the extensive damage to the pseudostem, it often becomes hollow and weak and bears

[#]For correspondence. (e-mail: palaniswamims@yahoo.com)

either undersized or no fruits depending upon the damage[9]. The problem is generally noticed only when the damage is in the advanced stage and grubs are fully grown. Attempts using conventional methods of pest control were not rewarding due to the concealed mode of life and longevity of the adults (up to 200 days within fallen or even in rotten pseudostems). The biology, ecology and chemical control of *O. longicollis* have been studied in the past[10–13]. In the last few years, efforts are on to evolve integrated pest management (IPM) strategies for efficient management of this pest. Literature on the existence of pheromones is completely lacking. In this work attempts were made to ascertain the presence of sex pheromones in *O. longicollis* so as to develop a pheromone-based IPM strategy against the pest.

Pseudostem weevils collected from Thakkalai area of Kannyakumari District, Tamil Nadu, an endemic area for *O. longicollis*, were cultured on pseudostem[14] (var. Njalipoovan). The insects were reared separately in an environment test chamber (Sanyo MLR350H) maintained at 8 : 16 LD photoperiod at 24 ± 1°C and 60 ± 10% RH. The newly emerged weevils were collected every day and sexed based on rostrum characteristics[9] to maintain them in different age groups for experiments.

The adult weevils were subjected to a behavioural bioassay for the presence of sex pheromone using an all-glass 'Y' tube olfactometer (Figure 1). The olfactometer consisted of a release chamber (100 ml capacity) at one end and two test chambers (100 ml capacity) connected by a glass runway of length 1 m (ID 15 mm) and a 'Y' tube. The other end of the test chambers was connected to a blower through an air inlet chamber fitted with a charcoal filter and an air-flow meter. The blower unit consisted of a battery-operated mini-fan fitted in a glass tube to generate an air current at the rate of 2 m/s. The release chamber and the test chambers were provided with removable glass lids to facilitate insect-bait handling. The release chamber also had a provision for air exit. The movement of the test insect (female weevil) in the test chamber was restricted using a net. This net was provided in the test chamber in such a way that the released males were not prevented from entry into the test chamber.

The bioassay was conducted uniformly between 1600 and 1800 h in a room maintained at stand rearing condition. The internal state of the weevils was kept as constant as possible. Before using them in a behavioural bioassay, they were examined for presence of all appendages (antenna, leg, etc.) and held in groups of 10 to 20. In the olfactometer, the adult weevils were observed to show phototrophic behaviour; thus to avoid visual cues and interference, the experiments were conducted in dim light. Each experiment was repeated at least ten times. Individual insects once used in the bioassay were not used again. This was done to prevent prior exposure to

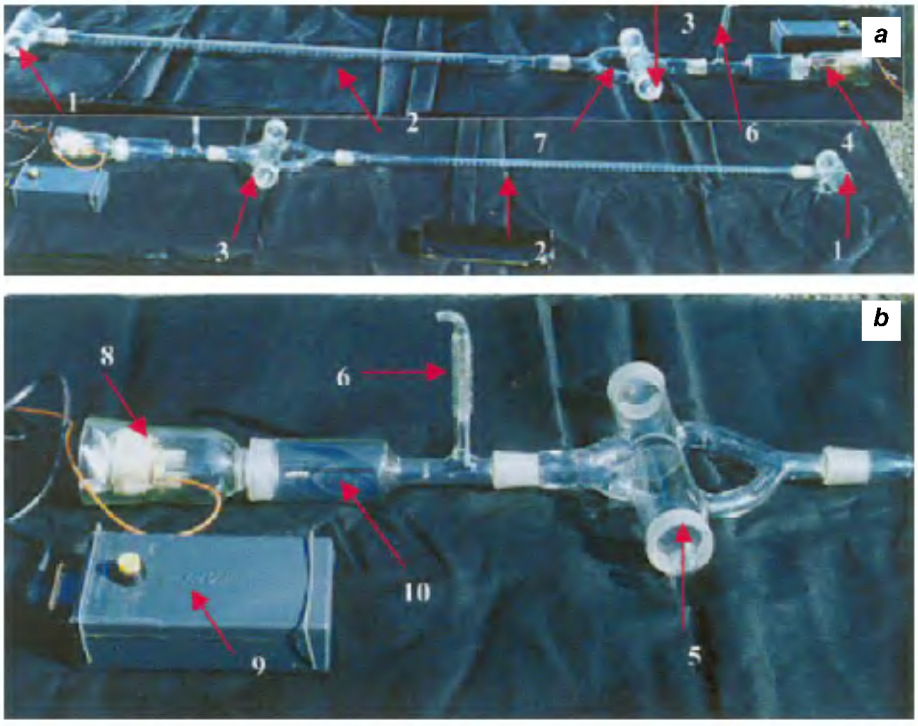

Figure 1. ***a***, Two units of olfactometer; ***b***, Air-blow unit connected to the test chamber and 'Y' tube. 1, Release chamber with air-exit hole; 2, Wind tunnel or runway connecting test chamber and release chamber through 'Y' tube; 3, Test chamber (control); 4, Air-blow unit; 5, Test chamber (with test insect); 6, Air-flow meter; 7, 'Y' tube connecting runway and test chamber; 8, Mini fan; 9, Battery; 10, Air-filter chamber (with activated charcoal).

the stimulus influencing the response. Prior to being exposed to the stimuli of the test chamber, the weevils were acclimatized in the release chamber by allowing a reasonable time (15 min) with a piece of pseudostem as food to settle on. The entire olfactometer excepting the blower and the air inlet tube was washed thoroughly with dichloromethane, followed by soap solution (Labolin®), and finally after rinsing with acetone, the unit was oven-dried between experiments to make the olfactometer odour-free.

Presence of a sex attractant among the *O. longicollis* adults was investigated in the olfactometer by exposing either of the sexes to the air current carrying the odour of the opposite sex. The preliminary studies indicated the presence of an air-borne sex attractant in the females. Based on the observation, the olfactory assay was continued with females of different age groups. Female weevils from pre-emergence resting stage, less than 10, 20, 30, 40, 50 and 60-day-old adults, were tested for their attractiveness to the males. Among the two test chambers, the one with a female of a particular age group with a piece of pseudostem (banana sheath) was considered as treatment and in the other test chamber a piece of pseudostem alone served as control. The attraction of the males (20 numbers) to the female was recorded by computing an index called 'attraction index (AI)'[15].

$$\text{AI} = \frac{\text{No. of males responding to the female} - \text{No. of males responding to control}}{\text{No. of males released} - \text{No. of males responding to control}}.$$

In the above formula, 'number of males responding to control' refers to number of males that enter the control chamber with the pseudostem piece. In addition to AI, 'approach latency' was also recorded. The approach latency is defined as the time taken by an individual male from the release chamber to reach the female in the test chamber through the 1 m wind tunnel/runway in a given test.

To know the changes in male courtship behaviour with reference to concentration of the female-produced sex pheromone, 20-day-old virgin females were studied for their attractiveness when they were more than one number in the test chamber. The scent produced by a single female was assumed as one female equivalent. Here the response of the males in terms of their courtship behaviour was recorded.

The olfactory assay conducted with *O. longicollis* showed evidence for an air-borne sex attractant from the females which attracted the males for courtship. In the olfactometer when females (20 numbers) were placed in the release chamber and a male, either mated or unmated, was introduced into the test chamber, the air current carrying the male odour caused no behavioural change in the females (Table 1). The females were neither attracted nor stimulated by the male odour. However, when the males (20 numbers) were kept in the release chamber and a virgin female introduced in the test chamber, the males showed excitement and courtship behaviour. The behavioural response of the males when exposed to the female scent was distinct as *long-range courtship behaviour* or *short-range courtship behaviour*. The former included (i) lifting of the head, (ii) holding the antennae out at right angles to the body across the fume, and (iii) proceeding to walk or run into the test chamber having the caged female. The latter included (i) moving around the female, (ii) antenna-to-antenna contact, (iii) downward bending of the posterior end of the abdomen, and (iv) mounting. The males exhibited behavioural responses to the scent of virgin as well as mated females, and the behaviour of the males was the same towards virgin and mated females.

In a particular experiment the courtship response observed among the males to the odour of a female was categorized as 'positive' if even one individual of the 20 males tested showed either of the seven courtship behaviours listed above, and if none of the courtship behaviours was observed, then it was categorized as 'no response'.

This is a report of the long-range and short-range courtship behaviour observed in the male *O. longicollis*. However, extension of antennae, zig-zag approach and copulatory attempt are some of the courtship responses already reported in other Coleopteran groups, namely *Attagenus megatoma*[16], *Trogoderma* species[17] and *Dendroctonus*[18].

The females were also found to elicit eight characteristic calling behaviours (Figure 2). The calling behaviour of the female observed included (i) knocking the substrate with rostrum, (ii) vibrant antennal beating on the substrate, (iii) laying close to the ground to rub the entire abdomen on the floor, (iv) raising the abdomen off the substrate with full extent of the hind leg and stretching the abdomen, (v) dilation of body segments with wings kept loosely above the abdomen, (vi) bending the abdomen and partly exposing the concealed ovipositor through

Table 1. Response of male and female of *Odoiporus longicollis* in the olfactometer assay

Release chamber[a]	Test chamber[b]	Type of response
Female (virgin/mated)	A piece of banana sheath	No response
Female (virgin)$^+$	Male (virgin)$^+$	No response
Female (virgin)$^+$	Male (mated)$^+$	No response
Female (mated)$^+$	Male (virgin)$^+$	No response
Female (mated)$^+$	Male (mated)$^+$	No response
Male (virgin)$^+$	Female (virgin)$^+$	Positive response
Male (virgin)$^+$	Female (mated)$^+$	Positive response
Male (mated)$^+$	Female (virgin)$^+$	Positive response
Male (mated)$^+$	Female (mated)$^+$	Positive response

$^+$, A piece of banana sheath as food; [a]A group of 20 insects was used as respondents; [b]One insect was used as an attractant.

the genital opening, (vii) extruding the ovipositor and curving down to the substrate accompanied by rapid protrusion and retraction of the ovipositor, and (viii) pressing the substrate with the extruded ovipositor. The calling behaviours listed above were also exhibited in a mixed fashion.

This study provides an evidence for the presence of sex pheromone in the females of *O. longicollis*. There are reports of sex pheromones in other Curculionids[19–21]. The female calling behaviour in *O. longicollis* has been recorded in this study in the family Curculionidae. Literature on calling behaviour of females is scanty. However extending the ovipositor to expose the cuticle overlying the pheromone gland by the calling female has been documented elsewhere in the members of the families Noctuidae[22] and Torticidae[23].

In general, many insect species have been found to produce and emit sex pheromone during their lifespan, some at the time of emergence and many when they attain sexual maturity (after a certain age)[19]. The experiment examining the attraction of males to females of various age groups provided evidence for the change in intensity of response (Table 2). The females of pre-emergence resting stage as well as females of up to 4-day-old were non-attractive to the males. This may probably be due to sexual immaturity of the individuals. The female begins calling males only five days after emergence. Twenty-day-old females elicited maximum

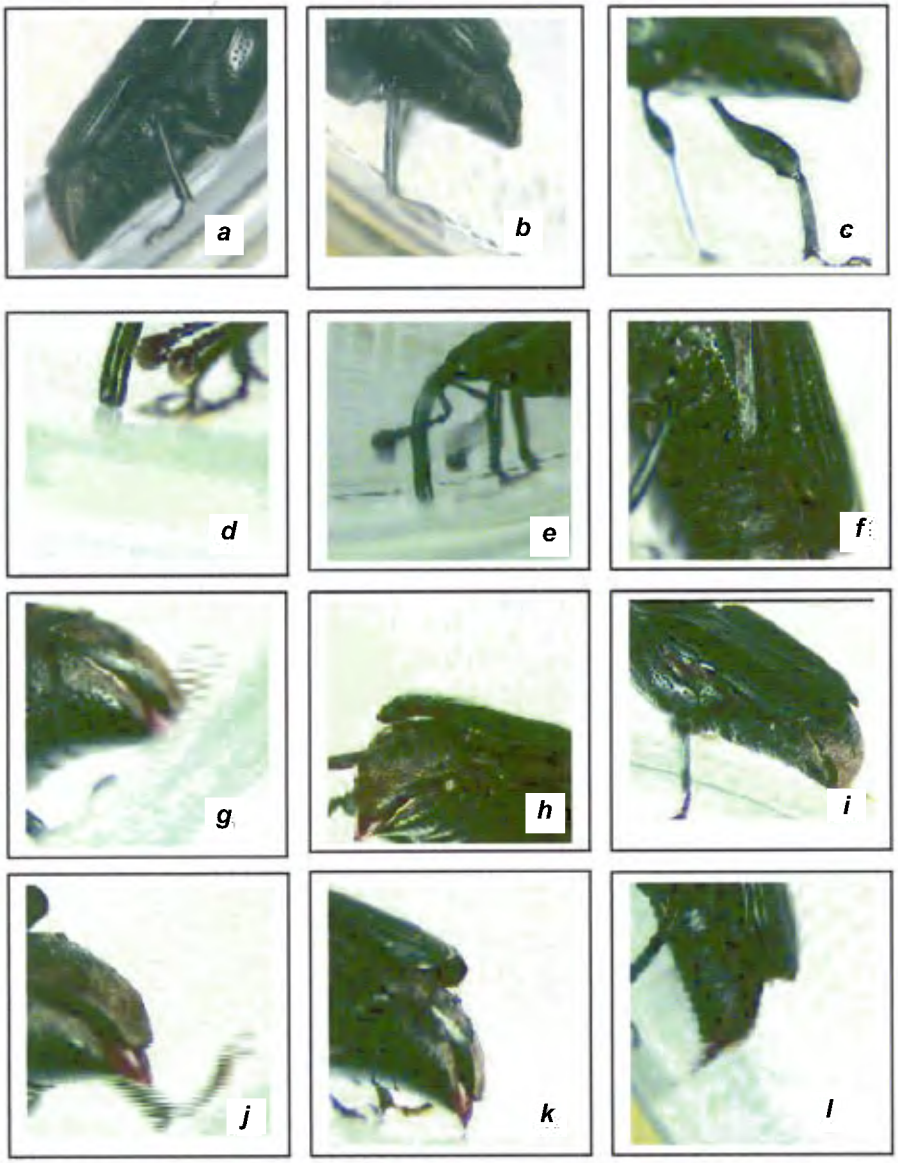

Figure 2. Calling behaviour of female *O. longicollis*. ***a***, Rubbing entire length of abdomen on the substrate; ***b*** and ***c***, Raising abdomen to full extent of the hind leg; ***d*** and ***e***, Knocking the substrate with rostrum and vibrant antennal beating; ***f***, Loosely held wings, ***g*** to ***j***, Protrusion of the ovipositor; ***k***, Thrusting the substrate with ovipositor; and ***l***, Rapid protrusion and retraction of the ovipositor.

Table 2. Response of male *O. longicollis* (20-day-old) to females of different age groups

Age of female in the test chamber*	Attraction index	Approach latency (min)	
		Range	Mean ± SE
Pre-emergence resting stage	0.000[f]	NR	–
Up to 4 days old	0.000[f]	NR	–
5 days old	0.639[e]	4.0–7.0	5.81 ± 0.406
10 days old	0.772[b]	3.0–6.0	4.63 ± 0.189
20 days old	0.938[a]	1.0–4.0	2.54 ± 0.365
30 days old	0.752[bc]	3.0–7.0	4.81 ± 0.278
40 days old	0.683[de]	2.0–8.0	5.79 ± 0.309
50 days old	0.748[bcd]	3.0–7.0	4.67 ± 0.282
60 days old	0.691[cde]	4.0–6.0	5.12 ± 0.148
SE ±	0.032	–	–
C.D. (0.05)	0.064	–	–

Age indicated is days after emergence from the pre-emergence resting stage; SE, Standard error; NR, No response.

Table 3. Courtship response of *O. longicollis* male to the females of different numbers

Age of female in test chamber*	Length of test (min)		Behaviour observed[a]
	Range	Mean ± SE	
One female	1.0–4.0	2.59 ± 0.297	Long-range courtship behaviour followed by short-range courtship behaviour.
Two females	1.0–6.0	2.67 ± 0.139	Long-range courtship behaviour followed by short-range courtship behaviour.
Four females	1.0–4.0	1.79 ± 0.088	Long-range courtship behaviour followed by short-range courtship behaviour in quick succession.
Eight females	3.0–7.0	3.26 ± 0.281	Early onset of short-range courtship behaviour.
Sixteen females	5.0–16.0	11.32 ± 0.416	Onset of short-range courtship behaviour bypassing long-range courtship behaviour.

*Virgin female; [a]A group of 20 males (20-day-old) was used as respondents and all of them showed the same response; SE, Standard error.

male responses. A maximum attraction index of 0.938 (± 0.740) was observed in 20-day-old females and the approach latency was also comparatively shorter (mean, 2.54 min). When the females were of more than one month old, the attraction remained more or less constant.

Change in the courtship behaviour in the males when they were exposed to scent of different female equivalents was also documented (Table 3). In response to an odour strength of one female equivalent, the males showed both long-range and short-range courtship behaviours. The first male reached the female within 1 min and the last one reached at 4 min (mean, 2.54 ± 0.365 min). When the fume strength was increased to four female equivalents, the courtship behaviours followed the same sequence but occurred slightly faster. The mean approach latency recorded was 1.79 min. When the odour concentration increased to eight female equivalents, interestingly, there was a quick onset of close-range courtship behaviour among the males. The males were observed to mount on other males on their way to the test chamber while in the connecting wind tube/runway itself, thus taking more time to reach the females (mean approach latency = 3.26 min). The scent from a group of 16 females made the males aggressive, they showed sudden onset of close-range courtship behaviour without expressing any of the three distant courtship behaviours. The males remained in the release chamber for a long time (mean approach latency = 11.32 min ± 0.416). This may be due to confusion caused by the higher concentration of the female odour. The change in male behaviour with increase in female pheromone concentration is of interest in terms of its possible implications for management of the pest. The observation envisages the use of pheromones both at a low dose to attract the males and at a higher dose as a mate-confusing technique, and in the long run the perpetuation of the species may be contained or reduced.

In the recent past, the use of pheromones as one of the eco-friendly tactics in the pest management programme has assumed a greater dimension as a novel technique in monitoring and mass trapping insect pests of crop plants. So far, around 15 compounds consisting of sex pheromones and aggregation pheromones are in use against different pest species. Recent concerns on environmental safety and pesticide residues on banana fruit have caused a shift in pest control towards the utilization of naturally-occurring biological attractants such as pheromones[20,24–26]. This offers a distinct advantage in efficiency and safety compared to strategies based solely on chemical pesticides. *Sordidin*, a commercial aggregation pheromone against *Cosmopolites sordidus*, the rhizome weevil of banana identified by Budenberg *et al.*[27], is popular among banana farmers[28]. The isolation, characterization, synthesis and formulation of the sex pheromone of *O. longicollis* reported in this study will be a boon to banana farmers as an effective monitoring and mass-trapping technique against the pseudostem weevil.

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