academicJournals

Vol. 10(15), pp. 471-481, 15 August, 2015
DOI: 10.5897/SRE2015.6187
Article Number: A3835FB54587
ISSN 1992-2248

http://www.academicjournals.org/SRE

Scientific Research and Essays

*Full Length Research Paper*

# Evidence for the presence of a female produced sex pheromone in the banana weevil *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae)

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Received 10 February, 2015; Accepted 30 July, 2015

**Behaviour-modifying chemicals like pheromones and kairomones hold a great potential in pest management. Evidences from mating behaviour studies of the banana weevil, and from the weevil's responses to their freeze-killed conspecifics, body washes/extracts, live conspecifics (olfactometer studies), and trapped volatiles of mature and immature adults clearly suggest that two types of pheromones are produced in this insect: a female produced sex pheromone and a male produced aggregation pheromone. Both are perceived by olfactory means. The latter has already been isolated by earlier workers and is in use in control programs. Greater successes may however, be recorded with the control of this pest (e.g. in mating disruptions, mass trappings, pest monitoring) if the female-sex pheromone also gets finally isolated, and used in conjunction with good cultural practices.**

**Key words:** Cosmopolites sordidus, sex pheromone, mating disruptions, bioassays, olfactometer studies, gas chromatographic profiles, electroantennogram studies.

## INTRODUCTION

The banana weevil, *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) is recognized as the major insect pest of bananas and plantains (*Musa* spp.) (Ostmark, 1974; Gold et al., 2001). It is known to attack these crops whenever they are cultivated (Zimmerman, 1968; Ostmark, 1974; Pavis, 1988). All four stages of the weevil's life history are associated with the banana plant (Treverrow, 2003). The eggs are usually laid singly and superficially by adult females at the base of the plant, or corm and also in the crop residues Koppenhofer (1993); and upon hatching the larvae which constitutes the most destructive stage of the pest (Jones, 1986) burrows into the stems, weakens them and makes them liable to wind damage (Acland, 1971). Damage to young suckers by a single borer (weevil larva) is almost catastrophic, as the larva eats up a large part of the corm and growing points, setting up secondary rots from which the plant has no chance of recovery (Simmonds and Simmonds, 1953).

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Attacks to older plants however, reduce the vitality and resistance of the plants to drought, leading to the development of poor fruit bunches and stems too weak to resist high winds (Harris, 1947). The fully fed larva normally tunnels to near corm surface to form an oval chamber in which it pupates, just prior to adult emergence (Treverrow, 2003). Emerging adults are nocturnally active, free-living and long-lived (range: 2 - 4 years; Gold et al., 2001).

All species of the genus Musa are attacked by the pest and no banana cultivar (Wolcott, 1933; Simmonds, 1966) is known to have a total resistance to it. Certain cultivars however, are known to be more susceptible to the borers than others (Wolcott, 1933; Viswanath, 1981; Mesquita et al., 1984; Gold et al 2001).

Chemical control with insecticides has been widely used on large commercial plantations and in some areas by small farmers. Prohibitive costs, the harmful effects of insecticides to environment and human health; and the development of resistance by the pest following the use of chemicals (Jones, 1986; Neuenschwander, 1988) have made continual use of chemicals for control of this pest unacceptable. Novel means of control, such as the use of pheromones, in conjunction with good cultural practices currently in use, would help to greatly reduce the losses caused by this pest. Tinzaara et al. (2002) reported that pheromones and other behaviour modifying chemicals (e.g. kairomones) hold a great potential as tools for pest management; particularly for use in pest monitoring, mating disruptions, mass trappings, and even as means for aggregating pests to delivery sites for biological control agents ("lure and kill"). The authors therefore called for further exploits in the synergism between banana plant extracts (kairomones) and the synthetic pheromones in attracting the banana weevil, *C. sordidus*. This study is therefore aimed at investigating this pest for clues about its behaviour-modifying chemicals (specifically for pheromonal involvement in its mating and gregarious behaviour). Such findings (e.g. presence of a sex pheromone) if confirmed, may be exploited for a more effective, novel and integrated control for the pest.

## MATERIALS AND METHODS

All bioassays in this study were conducted in a small fan-ventilated room (approximately 2.5 $m^2$) with controlled temperature and relative humidity conditions (25 ± 2°C and 80 ± 5% respectively). The fluorescent light source in the dark room was covered with a deep red light filter (Kodak Wratten No. 70, transmitting only wavelengths greater than 640 nm, adapted from Budenberg et al. (1993a, b). The dim light made observations in the dark room possible, with no obvious disturbance to responders.

Fresh field-collected weevils that were less than 3 days old in the laboratory were used in all these experiments (bioassays) as either dummies or responders, since they had been found to be more responsive than the laboratory-maintained colonies, from earlier mating trials (Uzakah, 1995). These weevils were always washed thoroughly with distilled water before being used in the experiments. All experiments were conducted between 11 am and 5 pm, that is, two and eight hours respectively after the onset of scotophase in the laboratory, as the activity rhythm for these insects had been shown to be high and virtually uniform during this period (Uzakah, 1995). Duration of observation for each bioassay was 10 min.

These general conditions were rigidly adhered to throughout these investigations, unless otherwise stated. The experiments included the following:

### Responses to freeze-killed weevils

Prior to commencement of experiments, the weevils were thoroughly washed with distilled water and sorted according to sex, using the methods of Longoria (1968) (rostrum punctuations) and Roth and Willis (1963) (curvature of the last abdominal sternite). Weevils of both sexes were freeze-killed individually at -20°C for 30 min, and each was pinned singly through its prothorax into Petri dish (9.5 cm diameter) containing moist sand. Responders (live male or live female weevils) were then introduced singly, so that each Petri dish contained a pair of weevils (a decoy and a live weevil) and the reactions of the live weevils to the decoys were observed. The Petri dishes were covered with similar dishes that had perforations on them to allow for air circulation. Plasticene was used to ensure firm covering for each Petri dish and its cover. The expected responders' reactions were visits to the decoy, arrestments, sniffing (a common practice observed from the weevil's mating behaviour studies, Uzakah, 1995; Uzakah and Odebiyi, 2015) and copulatory attempts.

Each combination (that is, responses of males to male decoys, males to female decoys, females to male decoys and female to female decoys) was replicated eight times (Table 1), and in each case the reactions of responders were recorded. The durations for each observation in this case was 30 min, and no single responder was used more than once.

### Responses to body washes/extracts

Twenty females were immersed in 4 ml of n-hexane for between 24, 48 or 168 h, and the extracts obtained were bioassayed against responding males in the dark room. Sixteen to 37 replications were made (Table 2a). Subsequent extractions were made from both males and females (20 of each sex) using dichloromethane for 24 h (Table 2b). The male and female extracts were then bioassayed against both male and female responders in the dark room, such that all the four possible combinations of weevil responses were investigated, that is, males to male extracts, male to female extracts, females to male extracts and females to female extracts. Each treatment was replicated twenty times (Table 2b).

For all these bioassays, a 10 cm diameter Perspex glass arena with walls 9 cm high was placed on a flat, circular glass plate, the floor of which was lined with Whatman's filter paper. At two opposite ends around the periphery of the arena, were placed cut strips (1.5 $cm^2$) of filter paper, and underneath these were aluminium foil strips of similar dimensions (Plate 1).

One hundred microlitres of test extract and of the control (blank dichloromethane) were applied to these opposite strips of paper respectively and a responding weevil was introduced centrally in the arena, for the determination of its preferred site (that is, its net movement, to either the treated or control site) in each 10-minute bioassay. The aluminium foil strips underneath the strips of paper prevented rapid losses of extracts by seepage through the filter paper, thus ensuring the extracts availability at such sites. In each bioassay, the test extract, blank solvent (or control) and the sex of the responders were unknown to the observer in order to eliminate bias. The order of bioassays was also completely randomized, so as to eliminate positional effects.

**Table 1.** Responses of male and female banana weevils, *C. sordidus* to their freeze-killed conspecifics, with duration of arrestments (in seconds) in parenthesis.

| Bioassay | No. trials | Number of responders | | | |
|---|---|---|---|---|---|
| | | Arrested | Mounting | Sniffing | Copulatory attempts |
| M-M[1] | 8 | 4(8 - 58 s) | 0 | 0 | 0 |
| M-F[2] | 8 | 3(10 - 451 s) | 0 | 0 | 0 |
| F-M[3] | 8 | 4(6 - 92 s) | 1 | 0 | 0 |
| F-F[4] | 8 | 3(12 - 45 s) | 1 | 0 | 0 |

[1]Male response or reaction to freeze-killed male weevil (dummy); [2]Male response or reaction to freeze-killed female weevil (dummy); [3]Female response or reaction to freeze-killed male weevil (dummy); [4]Female response or reaction to freeze-killed female weevil (dummy).

**Table 2a.** Responses of male banana weevils, *C. sordidus* to conspecific female body extracts in n-hexane.

| DOE[1] | n | Mean no. of extra[2] | No. of trials | | | Preferred sites[3] | | |
|---|---|---|---|---|---|---|---|---|
| | | Visits to treatment | Trt | Ctl | Ratio | +ve | -ve | df[4] |
| 7 | 37 | 0.6*** | 78 | 56 | 1.4 | 17 | 3 | 20 |
| 2 | 16 | 1.8** | 72 | 44 | 1.6 | 10 | 1 | 11 |
| 1 | 20 | 1.1* | 47 | 25 | 1.9 | 12 | 1 | 13 |

*P< 0.05; **P < 0.01; ***P < 0.001.Non-parametric Wilcoxon paired sign rank test; [1]Days of extraction (20 females per 4 ml of n-hexane); [2](Number of visits to treatment) minus (Number of visits to control) divided by (Number of trials, that is, n); [3] The 'net movement' of a responder for each 10-minute bioassay. The overall preference for a treated or control site for each bioassay is expressed under a +ve or –ve site respectively; [4] The effective degree of freedom used in the analysis, after subtracting trials with non-visits (zero visits) and/or trials with ties (even number of visits to trt and ctrl) from the total number of trials (n).

**Table 2b.** Responses of male and female banana weevils, *C. sordidus* to overnight extraction of conspecific males and females in dichloromethane.

| Responders | n | Mean no. of extra | No. of trials | | | Preferred sites | | |
|---|---|---|---|---|---|---|---|---|
| | | Visits to treatment | Trt | Ctl | Ratio | +ve | -ve | df |
| **Conspecific males** | | | | | | | | |
| Males | 20 | 1.2** | 44 | 20 | 2.2 | 12 | 2 | 14 |
| Females | 20 | 0.9* | 43 | 25 | 1.7 | 8 | 3 | 11 |
| **Conspecific females** | | | | | | | | |
| Males | 20 | 1.1** | 44 | 22 | 2.0 | 16 | 2 | 18 |
| Females | 20 | $-0.4^{ns}$ | 49 | 56 | 0.9 | 7 | 7 | 14 |

*P < 0.05; ** P < 0.01; ns = non significant at 5%; non-parametric Wilcoxon paired sign rank test. Trt = Treatment (female body extracts); Ctl = Control (blank solvent of n-hexane).

After each bioassay, the strips of paper and foil, together with the filter paper that lined the arena floor were discarded. The Perspex glass arena and the basal glass plate were thoroughly washed with soap, rinsed with distilled water and then allowed to dry. New and clean set of materials were used in each bioassay.

**Responses to live weevils (olfactometer studies)**

Responses to volatiles emanating from live weevils were studied in a still-air olfactometer, the design of which was slightly modified from that of Phillips and Burkholder (1981) and Budenberg et al. (1993). It consisted of a Perspex glass ring (14 cm diameter, and 9 cm high) placed on a flat, circular glass plate which had two holes (each 1 $cm^2$ in diameter). Each hole was 2.5 cm away on either side of the centre. Directly underneath these holes were placed two small vials (each approximately 2 cm deep), one containing a live insect plus moist cotton wool, and the other moist cotton wool only (control) (Plate 2). Each responder was released at a point equidistant (ca. 6 cm) from the holes and a glass plate was then immediately placed across the Perspex glass ring. The responder's preference for these holes was then determined in each 10-minute bioassay; replicated 20 times (Table 3a). Trial was also repeated with vials holding 5 weevils (emitters) and tested against 5 responders (replicated 20 times) (Table 3b).

The small vials were at the end of each bioassay neatly covered

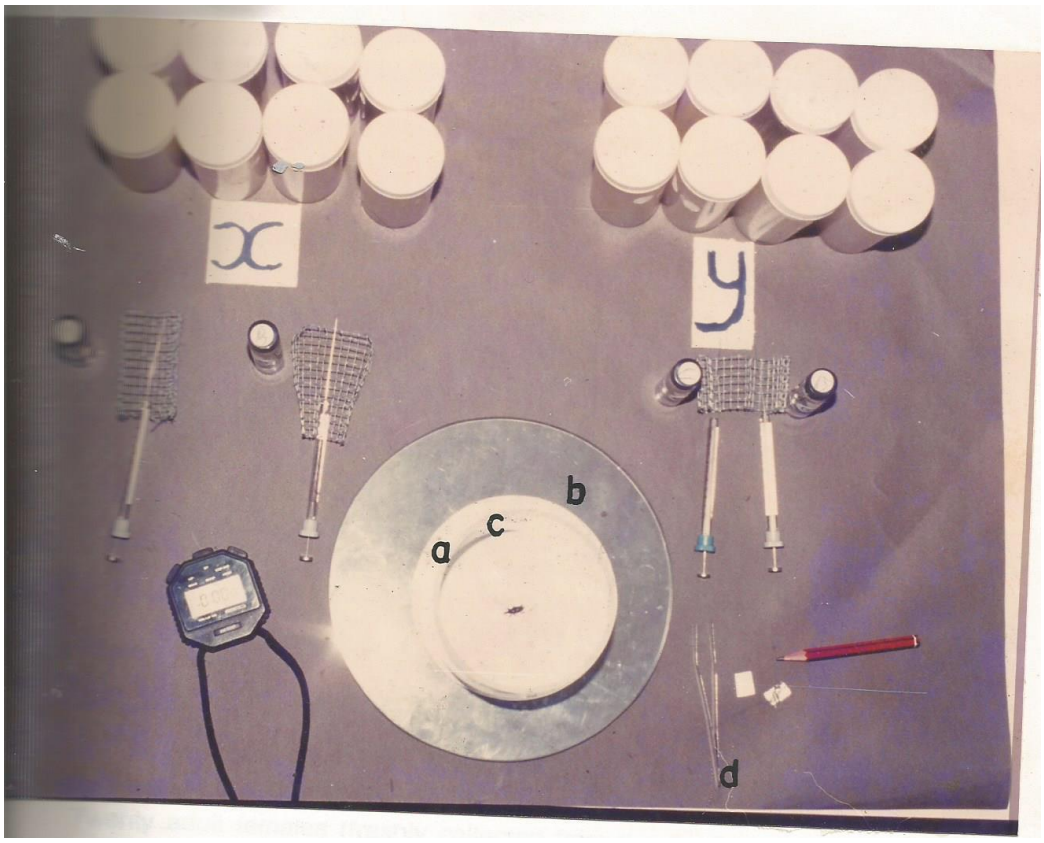


**Plate 1.** The experimental set-up for bioassaying extracts of the banana weevil, *C. sordidus* in the laboratory. x = responding weevils (males); y = responding weevils (females); a = the glass ring arena; b = the basal glass plate of the arena; c =filter paper placed underneath the arena; d = a pair of soft forceps.

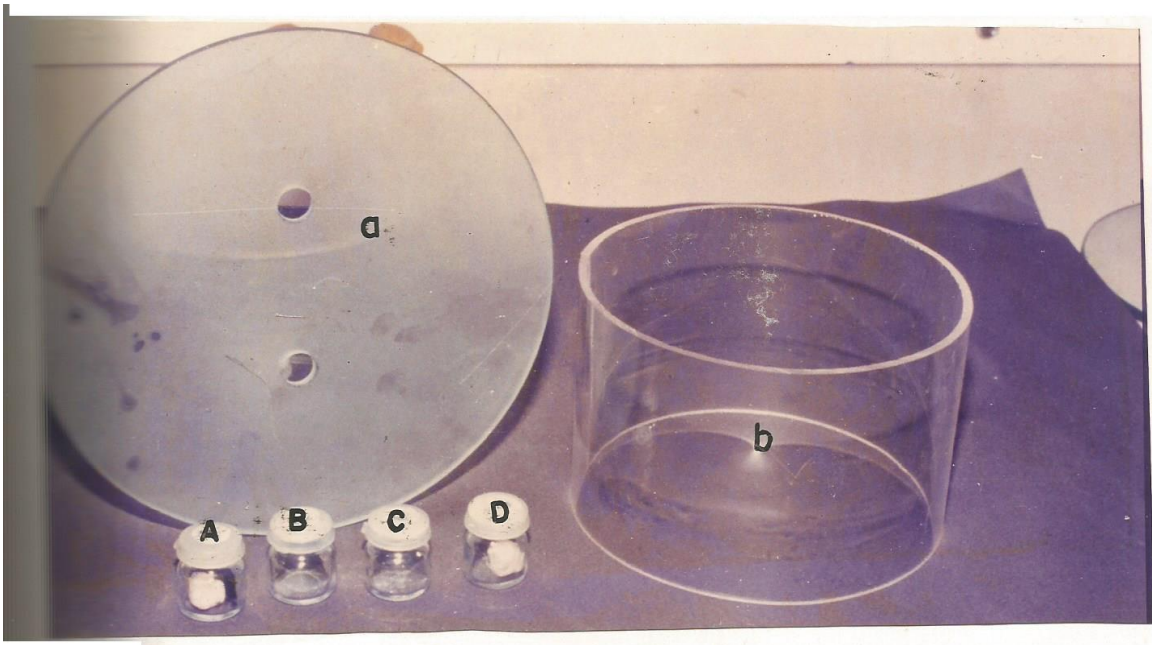


**Plate 2.** A Close-up of the olfactometer set-up used for the study of the banana weevil, C. sordidus pheromones in the laboratory. a= perforated basal glass plate of arena, b= the glass ring arena, A, B, C, D = glass containers for emitting weevils.

**Table 3a.** Responses of male and female banana weevils, *C. sordidus* to their conspecifics (using 1 responder against 1 emitter).

| Responders | n | Mean no. of extra | No. of trials | | | Preferred sites | | |
|---|---|---|---|---|---|---|---|---|
| | | Visits to treatment | Trt | Ctl | ratio | +ve | -ve | df |
| | | **Conspecific males** | | | | | | |
| Males | 20 | 0.3$^{ns}$ | 7 | 2 | 3.5 | 5 | 1 | 6 |
| Females | 20 | 0.6* | 18 | 6 | 3.0 | 5 | 1 | 6 |
| | | **Conspecific females** | | | | | | |
| Males | 20 | 0.4$^{ns}$ | 11 | 4 | 2.8 | 6 | 1 | 7 |
| Females | 20 | 0$^{ns}$ | 5 | 5 | 1.0 | 2 | 3 | 5 |

* P < 0.05; ns = non significant at 5%; Non-parametric Wilcoxon paired sign rank test.

**Table 3b.** Responses of male and female banana weevils, *C. sordidus* to their conspecifics (using 5 responders against 5 emitters).

| Responders | n | Mean no. of extra | No. of trials | | | Preferred sites | | |
|---|---|---|---|---|---|---|---|---|
| | | Visits to treatment | Trt | Ctl | ratio | +ve | -ve | df |
| | | **Conspecific males** | | | | | | |
| Males | 20 | 0.1$^{ns}$ | 35 | 33 | 1.1 | 8 | 9 | 17 |
| Females | 20 | 1.8*** | 51 | 15 | 3.4 | 15 | 2 | 17 |
| | | **Conspecific females** | | | | | | |
| Males | 20 | 1.0* | 40 | 20 | 2.0 | 13 | 4 | 17 |
| Females | 20 | 0.3$^{ns}$ | 29 | 34 | 0.9 | 6 | 8 | 14 |

*P < 0.05; *** P < 0.001; ns = non significant at 5%; Non-parametric Wilcoxon paired sign rank test.

**Table 4a.** Responses of male and female banana weevils, *C. sordidus* to trapped adult volatiles of their conspecifics[a].

| Responders | n | Mean no. of extra | No. of trials | | | Preferred sites | | |
|---|---|---|---|---|---|---|---|---|
| | | Visits to treatment | Trt | Ctl | ratio | +ve | - ve | df |
| | | **Conspecific males** | | | | | | |
| Males | 20 | 1.8*** | 56 | 21 | 2.7 | 16 | 2 | 18 |
| Females | 20 | 2.1*** | 56 | 15 | 3.7 | 13 | 1 | 14 |
| | | **Conspecific females** | | | | | | |
| Males | 20 | 0.9** | 37 | 20 | 1.9 | 11 | 1 | 12 |
| Females | 20 | 0.7$^{ns}$ | 41 | 27 | 1.5 | 7 | 4 | 11 |

** P < 0.01; *** P < 0.001; ns = non significant at 5%; Non-parametric Wilcoxon paired sign rank test. [a] trapping set-up contained moist cotton wool.

with their plastic caps, while the glass rings, basal and cover glass plates were thoroughly washed with soap and distilled water.

**Responses to trapped volatiles of field collected adults**

Fresh field-collected adults were immediately washed and sexed and 20 of each sex were respectively put into two 0.4 L cylindrical glass flasks which contained damp cotton wool. Air from a compressed air cylinder was passed through the flasks (200 ml/min for 24 to 48 h) via glass tubes (8 × 0.4 cm internal diameter) each of which contained approximately 10 g of activated charcoal plus glass wool.

These glass tubes containing the activated charcoal and glass wool were placed at both ends of each glass flask. The contents of these capillary tubes at the inlet of this set-up cleaned the air entering into the flask, while the ones at the outlet trapped the volatiles from the weevils (replicated 15-20 times) (Table 4a and b). Trapped volatiles were then eluted with 4 ml of dichloromethane (Aldrich, HPLC grade), and the extracts obtained were then bioassayed against responding males and females using the same procedures and set-up as in (2) above. The experiment was repeated with trapped adult volatiles collected from similar sets of weevils but from cylindrical glass flasks or trapping-chambers which contained no damp cotton wool. Trials were replicated 15 to 20 times (Tables 4b and a respectively).

**Table 4b.** Responses of male and female banana weevils, *C. sordidus* to trapped volatiles of their adult conspecifics:[a]

| Responders | n | Mean no. of extra | No. of trials | | | Preferred sites | | |
|---|---|---|---|---|---|---|---|---|
| | | Visits to treatment | Trt | Ctl | ratio | +ve | -ve | df |
| | | **Conspecific males** | | | | | | |
| Males | 15 | 1.1* | 38 | 22 | 1.7 | 10 | 3 | 13 |
| Females | 15 | 1.6** | 43 | 19 | 2.3 | 9 | 2 | 11 |
| | | **Conspecific females** | | | | | | |
| Males | 15 | 0.9* | 40 | 27 | 1.5 | 9 | 2 | 11 |
| Females | 15 | $0.4^{ns}$ | 24 | 18 | 1.3 | 7 | 5 | 12 |

* P < 0.05; *** P < 0.001; ns = non significant at 5%. Non-parametric Wilcoxon paired sign rank test; [a] Trapping set-up without moist cotton wool.

**Table 5.** Responses of mature male and female banana weevils, C. sordidus to trapped volatiles of their immature conspecifics:[a]

| Responders | n | Mean no. of extra | No. of trials | | | Preferred sites | | |
|---|---|---|---|---|---|---|---|---|
| | | Visits to treatment | Trt | Ctl | ratio | +ve | -ve | df |
| | | **Immature conspecific males** | | | | | | |
| Mature males | 15 | $0.5^{ns}$ | 24 | 16 | 1.5 | 5 | 2 | 7 |
| Mature females | 15 | $0.3^{ns}$ | 26 | 22 | 1.2 | 6 | 5 | 11 |
| | | **Immature conspecific females** | | | | | | |
| Mature males | 15 | 1.4* | 48 | 27 | 1.8 | 8 | 2 | 10 |
| Mature females | 15 | $0.1^{ns}$ | 16 | 15 | 1.1 | 4 | 3 | 7 |

* P < 0.05; ns = non significant at 5% level; Non-parametric Wilcoxon paired sign rank test; [a]Traping set-up without moist cotton wool.

### Responses to trapped volatiles of immature adults

Immature or newly emerged adults (ages between 1 - 10 days old) collected from suckers, were quickly washed and sexed. Twenty females and twenty males were next respectively put into two 0.4 ml cylindrical glass flasks, according to sex, and their volatiles were trapped as above (4). The immature weevils were very tender and delicate, so the trapping duration here was reduced to 24 h only, and no damp or cotton wool was introduced in the chamber, in order to prevent entanglement/concealment in the wool, during the 'brief' trapping period.

Extracts of these immature-adults' volatiles were bioassayed against responding mature males and females, using the same procedures and set-up as in (2) and (4) above; replicated 15 times (Table 5).

### Gas chromatography (GC) studies

Extracts of trapped volatiles of freshly collected field adults, and also from the immature weevils (using 20 weevils per 4 ml dichloromethane, in each case) were used in the Gas chromatographic (GC) studies). For each extract three microlitres (3 µl) was injected into the GC (Hewlett Packard 5890) using a fused silica capillary column (SPBTM-1)(30 m × 0.32 mm × 0.25 µm film thickness). The temperature program used was: initial temperature 40°C for two minutes, 5°C/minute rise to the final temperature 250°C and final time 20 min. Actual dilute extracts (unconcentrated) were used in this study as the concentrated ones produced too many peaks.

## RESULTS

### Responses to freeze-killed weevils

The responders' reactions to freeze-killed weevils are presented in Table 1. The expected behavioural reactions from responders (particularly of males to freeze-killed females) were arrestments, mounting, sniffing or copulatory attempts (or genitalia contacts). However, no records of sniffing or copulatory attempts were made in this study. There were few instances of female climbing over the dummies, but there was nothing to suggest that males were in any way sexually attracted or aroused by the presence of these dummies, since they failed to sniff or to attempt mating with these dummies. The few arrestments that live males made around female dummies did not result in any distinct behavioural activity. The arrestments recorded for the different treatments were not in any way different from one another (Table 1). The longest period of arrestment of 451 s by male

responder to freeze-killed female, did not even result in mounting, sniffing or copulatory attempts.

### Responses to body washes/extracts

The results of male responders to female body extracts are shown in Table 2a while Table 2b gives the responses of both males and females to body extracts of males and of females.

There was no significant response of males to the female body extracts. The response seemed stronger with increase in days of extraction (DOE) as indicated by the decreasing level of probability with increasing DOE (Table 2a). Table 2b however, showed that in addition to the significant male responses to female extracts, both males and females responded significantly to male body extracts. Female responders, however, did not respond significantly to female body extracts.

### Responses to live weevils (olfactometer studies)

Females responded significantly ($P < 0.001$) to 'concealed' live males. Similarly, males responded significantly ($P < 0.01$) to 'concealed' females. However, there was no significant response from males to 'concealed' males and from females to 'concealed' females (Table 3).

### Responses to trapped adult volatiles

Males responded significantly to the trapped adult volatiles of both males and females, however, females responded significantly to only the trapped adult male volatiles and not to the trapped adult female volatiles (Table 4a and b).

### Responses to trapped volatiles of immature adults

Males responded significantly to trapped volatiles of immature females. The responses of males to the trapped volatiles of immature males, and of females to the trapped volatiles of immature males or immature females were not significant (Table 5).

### Gas chromatographic (GC) studies

The GC profiles of the banana weevil volatiles are shown in Figures 1 and 2. The figures clearly showed these compounds to be relatively volatile, since some peaks were recorded within the first five minutes of start of the run (that is, at such low temperature of 40°C). Combined gas chromatography and electroantennogram (GC-EAG) studies however, could not be done to establish the physiologically active peaks.

## DISCUSSION

Our results strongly suggest that two pheromones are produced in this insect - a female produced sex pheromone and a male produced aggregation pheromone. The apparent absence of discreet behavioural responses (sniffing, copulatory attempts) by responding males to freeze-killed females suggest that at death, female banana weevils cease to be sexually attractive to the males.

This could be due to cessation in the production of the mating stimulant (pheromone) by females at death, or sudden loss of it soon after death of the female. This pheromone may be relatively volatile, as can be seen from the chromatograms (Figures 1 and 2), so it is likely to be lost after death. The male indifference to the female could, however, also be due to the absence of a specific (and perhaps a complementary) movement by the freeze-killed (still) females, perhaps necessary for mating in this insect. Dean et al. (1969) reported that in the tsetse fly, *Glossina morsitans orientalis* Vanderplank (Diptera: Glossinidae) the male flies appeared sexually activated only after movement of the female. Langley et al (1975) similarly reported that in the laboratory, the male *G. m. morsitans* Vanderplank was aroused by movement of other individuals. They, contrary to the results obtained in this study, also reported that dead tsetse flies of this species were sexually attractive to mature males and that the attractiveness of the females was not diminished by low temperature storage or vacuum drying. Selander (1978) and Tiles et al. (1988) similarly reported repeated matings with freeze-killed females in the pine weevils.

The fact that a male virtually had to contact a female, and sniff her abdominal tip with his antennae in order to perceive the pheromone (Uzakah, 1995; Uzakah and Odebiyi, 2015), suggests that the female pheromone in *C. sordidus* is only active within a very short range. This observation seem to be in agreement with that of Cross and Mitchell (1966), who suggested that female boll weevils produced a weak secondary pheromone which males perceived by olfactory means over distances of less than 5 cm.

The observed lack of strong behavioural responses (e.g. sniffing, copulatory attempts, entanglements etc.) to freeze-killed males by responders, as was usually observed with live weevils, also seem to suggest that males at death, similarly cease production of the pheromone (aggregation pheromone), and that the pheromone is also a volatile and not a contact pheromone.

The more significant responses observed by responding males to extracts obtained from prolonged exposures (48 and 168 h of extraction) of the female to the solvent than to those obtained from short extraction

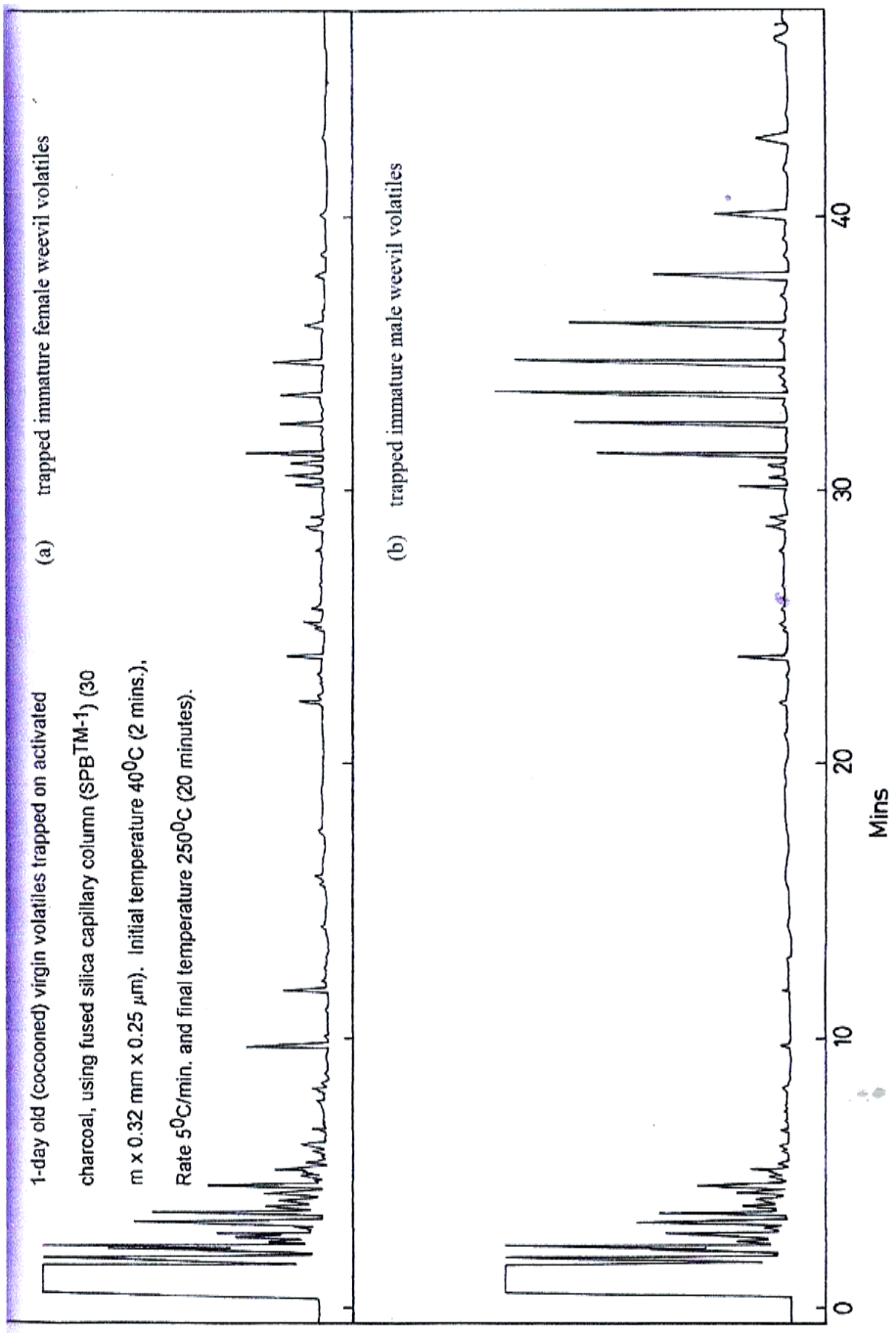


**Figure 1.** Gas chromatographic profiles of trapped volatiles of immature banana weevils, *C. sordidus.*

period (24 h) (Table 1) confirm that the pheromone is produced internally and so not present on the surface of the female (that is, a volatile and not a contact pheromone). Perhaps the more thorough extraction resulted in the dissolution of internal lipids and facilitated the extraction of internal sources (which were not possible with the less thorough extraction). Further evidence of this is obtained from the various responses of responders to trapped volatiles of mature and immature adults, and also from the still-air olfactometer studies, where significant responses by live males to live females were observed.

The significant responses by responders (males and females) to males in all these bioassays (except to the freeze-killed males) are consistent with the view that the males of this insect produced a volatile aggregation

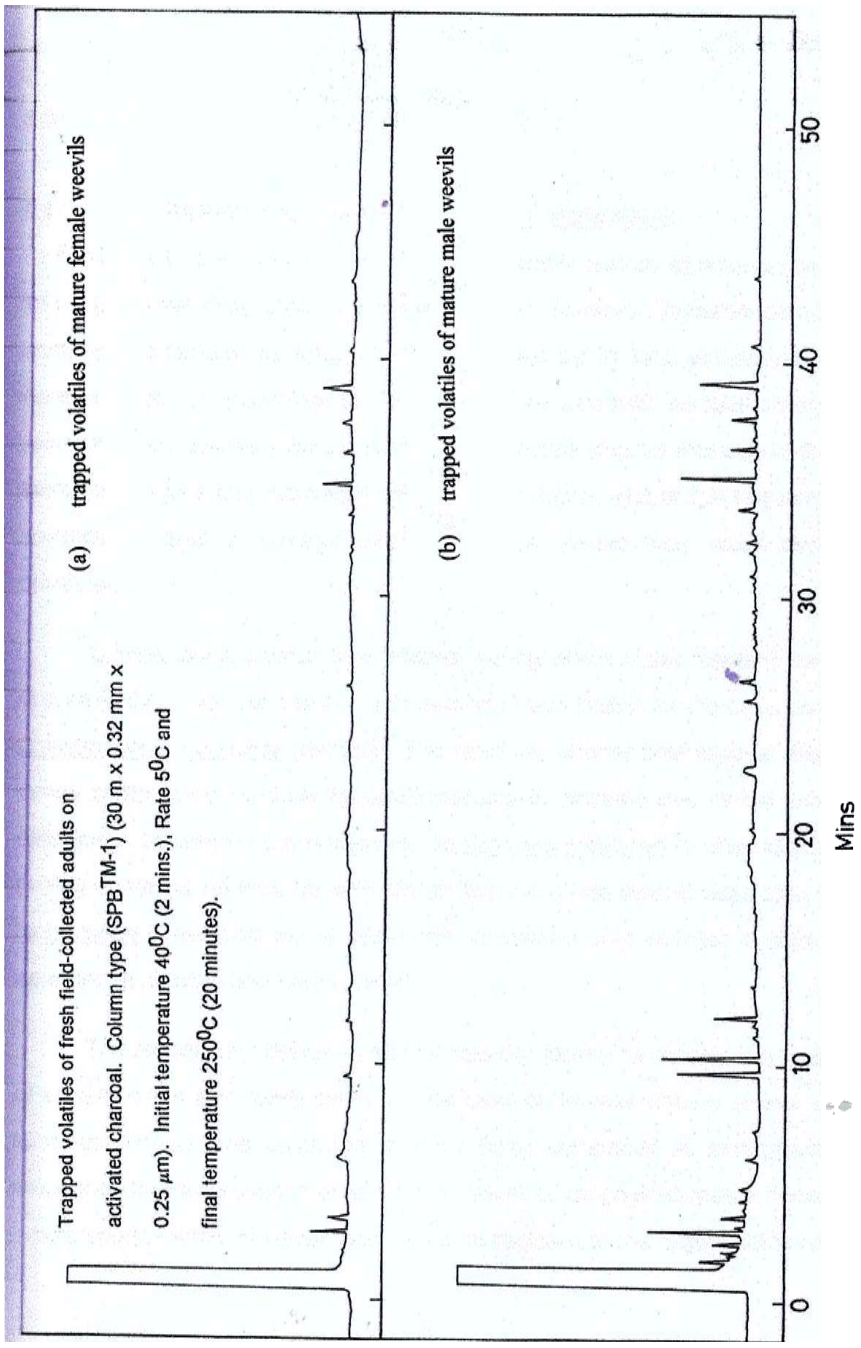


**Figure 2.** Gas chromatographic profiles of trapped volatiles of adult banana weevils, *C. sordidus.*

pheromone. However, the failure of earlier workers to detect the female sex pheromone was probably due to the fact that the female pheromone is produced in small amounts. Short range pheromones are common in insects. In the rove beetle (Coleoptera: Staphylinidae), Peschke (1983) reported that the cuticular pheromone was detected only over a short distance of 2.2 mm, but that the reception mechanism was by olfaction. Humphries (1967) reported that the mating behavior of the hen flea, *Ceratophyllus gallinae* (Schrank) Insecta: Siphonaptera), is only initiated if the male came into contact with female and received some specific stimulus from her abdomen through his maxillary palps.

The presence of two types of pheromones (a male produced aggregation pheromone and a female sex pheromone) has been reported in other weevils (Tinzaara

et al., 2002). For instance, Selander (1978) and Tiles et al. (1986) confirmed the presence of two types of pheromones in the pine weevil, *Hylobius abietis* (L), while the findings of Phillips and Burkholder (1981), and Sharma and Deora (1980) showed this for the rice weevil, *Sitophilus oryzae* (L). Hedin et al. (1979) also confirmed this for the boll weevil, *Anthonomus grandis* Boheman.

The female pheromone of the banana weevil, compared to that of the male was apparently a weak one, and so only effective over a short range and essentially for mating purposes; while the male one, necessary for aggregating the sexes was evidently more effective over longer ranges. A similar observation was made by McKibben et al. (1977) who reported that male boll weevils produced 1 µg of the pheromone per day while the females produced approximately 0.01 µg per day, confirming the weakness of the female weevil pheromones. The finding was supported by later work by Hedin et al. (1979) for this weevil. According to these authors, this was perhaps the reason for the failure of earlier workers to find the pheromone of the female weevil.

The significant responses made by adult males to trapped volatiles from immature females (approximately 1 - 10 DAE), and the apparent lack of responses by responders to those of immature males (Table 5) suggests that the female pheromone is produced at an earlier stage than that of the male during the life of the insect. The production of these pheromones in these weevils is apparently linked with age at sexual maturity. Previous laboratory studies (Uzakah, 1995), reveal that female *C. sordidus*, were not sexually mature until about 2 weeks after emergence. It would be interesting to investigate the ages at which these weevils commenced the production of these pheromones.

Selander (1978) and Tiles et al. (1988) observed that virgin females of the pine weevils, *Hylobius abietis* (Coleoptera: Curculionidae) produced a sex pheromone that attracted males. They also observed a strong attraction between virgin males and adult males, and so inferred that males at this stage perhaps produced the sex pheromone. Attraction between immature males and adult males of the banana weevils was also observed in this study, but it was not found to be statistically significant (Table 5).

Comparisons of the GC profiles for the trapped volatiles of immature weevils (that is, male vs. female; Figure 1); and also those of mature weevils (males vs females; Figure 2) clearly revealed qualitative differences - the males in both cases produced higher peaks for the compounds that were common to both sexes. Combined gas chromatography and electroantennogram (GC-EAG) studies could not be done to ascertain the physiologically active peaks in these chromatograms, neither was GC-MS (that is, gas chromatography cum mass spectrometry) studies embarked upon to help characterize the peaks found in these chromatograms.

The male aggregation pheromone of *C. sordidus* has since been isolated, synthesized and even field-tested (Tinzaara et al., 2011, Alpizar et al., 2012). This synthetic pheromone, named sordidin or Cosmolure+ (depending on manufacturers), have been found to be successful in mass trappings; capturing 18 times more weevils than the conventional pseudostem traps in Uganda (Tinzaara et al., 2011); although Alpizar et al (2012) highlighted a range of 2½ to 8-fold increases under different conditions.

Sex pheromones in like manner, are also widely and successfully used against several crop pests particularly, against lepidopterous pests of fruits, vegetables and forests (Srivastava and Dhaliwal, 2012). Mason and Jansson (1991) even reported its potential for the Coleoptera (that is, as a mating disruptant in the sweet potato weevil, *Cylas formicarius* (Coleoptera: Apionidae). Same may be applicable with the banana weevil sex pheromone, and thus help to bring about delays, reductions or even prevent propagation of the weevil's population. This potential benefit of the banana weevil sex pheromone, if tested and proven, may be combined with sordidin plus other good cultural practices for a more holistic, novel and effective control of this serious pest of Musa.

## Conflict of Interest

The authors have not declared any conflict of interest.

## ACKNOWLEDGEMENTS

This work was funded by the Royal Norwegian Ministry for Research Cooperation and Development (NORAD), and was carried out at the International Centre for Insect Physiology and Ecology (ICIPE) Nairobi, using the facilities of the Behavioural and Chemical Ecology Research Unit. The contributions of all my Supervisors and other key contributors are highly appreciated.

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