Evidence of Male Pheromone in *Conogethes punctiferalis* (Lepidoptera: Pyralidae)[1]

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Abstract *Conogethes punctiferalis* Guenee is a polyphagous insect pest that is difficult to manage because it feeds within plant tissue. Management by mass trapping using semiochemicals, especially pheromones, represents a viable option to control such borers. Herein, pheromonal compounds were extracted from male and female moths and assessed using headspace sampling and electroantennogram response. One-d-old *C. punctiferalis* showed a higher response to volatile solvents than 4-d-old ones, irrespective of sex. The male antenna was found more sensitive than the female for volatile compounds. However, the female response to male headspace extract and abdominal tip extract was very high (i.e., 4.006 mV and 2.217 mV, respectively), which revealed the presence of males producing a female-attracting olfactory cue in *C. punctiferalis*. This was also confirmed by males calling the female before mating by extruding the hair pencils in their abdominal tip. The male pheromone extract when analyzed in gas chromatography–mass spectrometry indicated the presence of methyl acetophenone or 3-ethyl acetophenone.

Key Words acetophenone, calling behavior, *Conogethes punctiferalis*, male pheromone

Conogethes punctiferalis Guenee (Lepidoptera: Pyralidae) is a polyphagous pest that infests 30 crop plants belonging to 23 families (Thyagaraj et al. 2003). It is an important pest not only in South and South East Asia and Australia (Pena et al. 2002) but also as a newly introduced pest in Europe. Although its management is normally attained by the use of chemical pesticides (Renuka et al. 2002, Stanley et al. 2010, Regupathy and Ayyasamy 2014), proper management is achieved only by repeated applications of high concentrations because of the concealed nature of the larvae that feed within the plant tissues. Pest management using pheromones for mass trapping or mating disruption offers a viable alternative for such borers (Breth and Tee 2007). The use of a pheromone is more effective in detecting the infestation and, thus, determines timing of pesticide application (Cruz et al. 2012), leading to a reduction in insecticide usage.

The isolation, identification, and use of sex pheromones of *C. punctiferalis* have been previously reported. The pheromone component of *C. punctiferalis* was first reported by Konno et al. (1982) as (E)-10-hexadecenal. Traps containing 250 μg of

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C. punctiferalis pheromone were reported to significantly reduce the pest in citrus orchards in China (Cai and Mu 1993). The compounds were later identified as (E)-10-hexadecenal and (Z)-10-hexadecenal as the major compounds (Liu et al. 1994). But, the synthetic sex pheromone consisting of (E)-10-hexadecenol and (Z)-10-hexadecenal was not as effective as the crude pheromone extracts (Xiao and Honda 2010). Three compounds, (E)-10-hexadecenal (E10-16:Ald), (Z)-10-hexadecenal (Z10-16:Ald), and hexadecanal (16:Ald), were identified in the female gland extract of *C. punctiferalis* by Jung et al. (2000). Among the various combinations E10-16:Ald and Z10-16:Ald at 70:30 to 80:20 were the most attractive to males in wind tunnel experiments and field trapping experiments in orchards (Jung et al. 2000). The third compound 16:Ald did not show an electroantennogram (EAG) response in a Korean population but elicited response in the *C. punctiferalis* from China (Liu et al. 1994). Apparently, variation in sex pheromonal compositions appears to be far more widespread than previously thought and the composition differs from population to population. The sex pheromone attraction of the Korean population of *C. punctiferalis* varies with that of Chinese and Japanese populations (Boo and Park 2005). In India, E10:16Ald used in pheromone traps resulted in moth catches in castor fields but not in cardamom plantations (Chakravarthy et al. 2013). The (Z)-10 hexadecenol and 16-hexadecenal blend did not attract and trap any male moths (Chakravarthy et al. 2015). Thus, there is a need to isolate and identify the pheromone compound of the *C. punctiferalis* populations in India. Furthermore, a complex combination of the pheromone system of the *C. punctiferalis* consists of E10-16:Ald, Z10-16:Ald for long-range attraction and Z9-27:CH,Z3Z6Z9-23:CH for short-range attraction and final recognition of females by males (Xiao and Honda 2010, Chakravarthy et al. 2015). In addition, E-2-methyl-2-butenoic acid (tiglic acid), a compound extracted from the hair pencils of males, was reported to have a significant role in mate recognition, coupling, and mating success. The present study was designed to extract pheromones of both male and female *C. punctiferalis* by gland excision/solvent extraction and headspace extraction and to determine the biological activity, detect EAG response, and identify the active compounds by using gas chromatography–mass spectrometry (GC-MS) techniques.

Materials and Methods

Insects. Field-collected *C. punctiferalis* were reared on castor bean, *Ricinus communis* L., in plastic trays. Castor bean is reported as the best option for mass culturing *C. punctiferalis* in Indian conditions (Stanley et al. 2009). Newly-emerged adults were sexed, kept in large cages for oviposition, and provided with a 50% (w/v) sucrose solution. Castor bean inflorescence (panicle) with flowers and immature capsules were kept as an ovipositional substrate with the cut end placed in 500-ml Erlenmeyer flasks containing water. The moths laid eggs singly or in groups in between the warts or just below the style on the ovary of the flowers and on the developing capsules. Newly-hatched first instars were transferred to capsules for rearing. New capsules were given as food once in 4 d (i.e., when dried or eaten by the larvae). Pupae produced were kept for adult emergence.

Behavioral studies. Pupae were kept in clean Petri dish bottoms for adult emergence in a plastic container covered with muslin cloth. The newly-emerged

adults (~20 each of male and female) were introduced into a netted cage (60 × 60 × 60 cm) and provided with castor bean panicles (inflorescence) with flowers and immature capsules as an ovipositional substrate and a cotton pad dipped into a 50% sucrose solution as food. The behavior of adults was visually observed daily from 1800 to 2400 h by using a 40-W red lamp for 5 consecutive days.

Pheromonal extraction. The male and female moths to be used for this part of the study were kept separately immediately after emergence to avoid mating. The moths were placed in the dark for approximately 6 h before extraction to enhance the pheromone yield. Pheromone was extracted by clipping the abdominal tips of 3-d-old unmated males and females, separately in 1 ml of dichloromethane where they remained immersed at room temperature (25°C) for approximately 1 h. The abdomen was gently squeezed with a pair of fine forceps to extrude the last abdominal segments, and the terminal two to three segments were excised with dissection scissors directly into the dichloromethane. Sample tubes containing the solvent with pheromone were immediately capped with screw caps after flushing with N_2 and then stored at −4°C.

An air entrainment method or headspace sampling technique was also used to collect pheromones that were emitted by the adult insect in the sample chamber. A stream of purified air was directed over the insect to emit volatiles, and the emitted pheromones were trapped in a glass tube containing an adsorption media and uniform matrix, which is able to trap small molecules in between its polymer units. The adsorption media used was Porapak Q 80-100 Mesh (ethyl vinyl benzene-divinylbenzene copolymer; Waters Corp., Milford, MA, USA).

The air entrainment apparatus used in this study consisted of two glass cylindrical tubes (entrainment chamber) and one blank tube with a 38-cm length and 5-cm internal diameter (ID) with ground joints on both ends, which can be dismantled. On one side of the cylindrical glass tube, a small glass tube (1-cm length and 0.05-cm ID), packed with 2 g of adsorbent Porapak Q, was fixed. Porapak Q acted as a filter on which volatiles were absorbed and held in place by silanized glass wool. Pure air is made to pass though the entrainment chamber to collect pheromones. Purification of air was achieved by passing the air through glass tubes containing glass beads and then through activated charcoal. All the tubes were connected to a suction pump by means of a flexible silicon tube. When the suction pump was operated, the atmospheric air entered through the molecular sieve, then through activated charcoal, and passed through the air entrainment chamber, where pheromone-releasing insects were kept and finally exchanged through the Porapak Q, where the volatile compounds are absorbed.

Forty 1-d-old males and females, 20 of each sex, were placed separately in the two air entrainment chambers and kept for 3 consecutive days. The moths were kept in the entrainment chamber daily from 1900 to 2400 h for pheromone extraction and, after that, kept in cages and provided with a 50% (w/v) sucrose solution. A blank was also maintained for comparison. The experiment was conducted for 72 h for the collection of pheromone released by the insect. The entrained volatiles were eluted from Porapak Q filters by means of high-performance liquid chromatography–grade dichloromethane. The tube containing Porapak Q was clamped vertically, and the solvent was pipetted in at the top and collected under gravity in a sample tube placed at the base. The sample tubes were immediately capped with screw caps after flushing with N_2 and stored at −4°C until

analysis. Glassware used were kept clean by washing in liquid soap, followed by water, and then rinsed with acetone and dried in an oven at 250°C for 2 h before and after the experiments.

Electroantennography. EAG (Syntech, Hilversum, The Netherlands) studies were conducted with pheromones by using 2-d-old male and female *C. punctiferalis* and with volatile solvents by using 1- and 4-d-old males and females. This EAG consisted of a dual-electrode probe for antenna fixation, a CS-05 stimulus controller, and IDAC 232 box for data acquisition. The antenna was placed so that the tip of the lamella touched one of the electrodes, and the scape was fixed to the other electrode, as suggested by Reinecke et al. (2005). The antenna was fixed between the two electrodes by using Spectra 360 conductive gel (Parker, Orange, NJ, USA). The antenna was flushed continuously with a stream of air filtered through activated charcoal.

The common volatile solvents that were usually used for testing the response of insect antenna were subjected to EAG by applying 20 μl on filter paper strips (3 cm × 5 mm) and placed into a 1,000-μl micropipette tip (Tarsons Products Pvt. Ltd., West Bengal, India) and connected to the stimulus controller by silicone rubber tubing. After 10 s of control air, the solvent was blown out with first puff. Another 60 s later, the stimulus was puffed onto the antenna by injecting the vapor phase of the chemical stimuli through a micropipette tip (15 mm) along with the continuous air stream (pulse rate 0.5 s, continuous flow 25 ml s^{-1}, pulse flow 21 ml s^{-1}) to the antenna. The minimum time between each stimulus puff was 120 s. The antennal response to aliquots was recorded from five adults with three replications per antenna. The antennae of 1- and 4-d-old *C. punctiferalis* were tested for response to volatile solvents. Both the male and female abdominal tip extracts and headspace sampling extracts were used to record the response of antenna of 2-d-old *C. punctiferalis* of both sexes by using EAG.

For data analysis on the EAG response for the pheromone gland extracts and headspace extracts, the EAG response in millivolts were corrected for solvent and other background effects by subtracting the averaged EAG responses of the solvent responses recorded before and after each sample. The corrected EAG responses of both male and female antennae were statistically analyzed separately by analysis of variance and, significantly ($P \leq 0.05$) different treatment means were separated by the least significance difference (LSD) (AGRES 1994).

GC-MS. The abdominal tip extracts and the extracts from the air entrainment or headspace sampling of both male and female insects were injected in GC, and its components were identified using Perkin Elmer-Clarus 500 GC and MS equipped with a DB-1 column (30 m × 0.25 mm ID) with helium at the flow rate of 0.5 ml min^{-1} as the carrier gas. The injector was kept at 240°C and the detector at 200°C. The column oven was kept in a temperature program of 60°C with a hold time of 1 min, and then up to 240°C at a rate of 10°C raise and 240°C with a hold time of 2 min. The mass selection (m/z) was 60–650.

Results

Calling behavior and coupling. The insects were found resting on the sides of the cages facing toward the light during the day without any flights, if undisturbed.

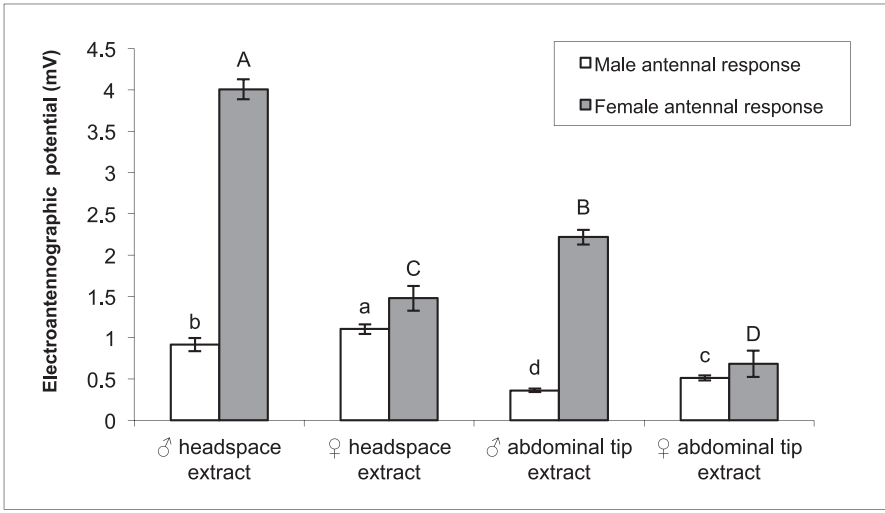

Fig. 1. Electroantennographic response of *C. punctiferalis* to pheromone extracts.

But, after 1930 h, swift flights by both males and females were observed and, at times, landing on the sides of the cages for a few seconds moving their antennae vigorously was also observed. Later, around 2100 h, the insects were found to rest on the substrate with their legs firmly fixed, raising the whole body up and down while extruding the abdominal tips, indicating that the moths were sexually excited. The females exhibited a typical calling posture by bending the abdomen dorsally with extrusion of the apical segments. Calling females were observed 1 d after moth emergence and reached its peak on the second day. Pronounced and clear calling by male moths was also noticed 1 d after emergence by curving the abdomen dorsally and extrusion of hair pencils at the abdominal tip. After 1 to 2 h, the male calling subsided, and the remaining virgin females continued calling and emitting pheromone. This attracts the males, leading to further mating. Copulation lasted for 30 min to 1 h, followed by a significant abdominal constriction of the abdomen by females.

EAG. In general, the antennal response of males to volatiles was more pronounced than that of females, which is evident from the conditioning bioassay conducted using different volatile compounds (Table 1). Both 1- and 4-d-old moths were used for EAG study so as to find the difference in antennal sensitivity with respect to age. It was observed that 1-d-old moths, irrespective of sex, were more sensitive than 4-d-old moths (Table 1). Heptanal was found to elicit a significant response in male antenna of 1-d-old moths. Female moths were found more responsive to pentanol and propyl acetate than any of the compounds tested.

The volatiles/pheromones collected by both the methods (i.e., headspace sampling and abdominal tip extraction) were analyzed by EAG. The response of both sexes of *C. punctiferalis* to male and female headspace sample extracts and abdominal tip extracts is given in the Fig. 1. The analysis of variance of the male

Table 1. Electroantennographic response of 1- and 4-d-old *C. punctiferalis* to different compounds (mean of 30 observations).

Compounds	Empirical Formula	Mean EAG Response (mV)	
		1-d-old Males	4-d-old Males
Butanol	$C_4H_{10}O$	$5.316^{A} \pm 0.25\ (2.412)^{d}$	$1.835^{D} \pm 0.09\ (1.530)^{e}$
Ethyl butanol	$C_6H_{14}O$	$5.531^{A} \pm 0.28\ (2.456)^{cd}$	$1.953^{C} \pm 0.08\ (1.565)^{d}$
Ethyl propanol	$C_5H_{12}O$	$5.102^{A} \pm 0.32\ (2.366)^{e}$	$1.953^{C} \pm 0.13\ (1.565)^{d}$
Pentanol	$C_5H_{12}O$	$4.322^{B} \pm 0.31\ (2.195)^{g}$	$2.311^{C} \pm 0.16\ (1.676)^{cd}$
Heptanol	$C_7H_{16}O$	$6.354^{A} \pm 0.43\ (2.617)^{b}$	$3.057^{C} \pm 0.28\ (1.887)^{a}$
Heptanal	$C_7H_{14}O$	$7.660^{A} \pm 0.47\ (2.856)^{a}$	$2.670^{C} \pm 0.24\ (1.780)^{b}$
Isoamyl acetate	$C_7H_{14}O_2$	$3.640^{A} \pm 0.35\ (2.035)^{h}$	$1.942^{C} \pm 0.06\ (1.562)^{d}$
Octanol	$C_8H_{18}O$	$4.883^{A} \pm 0.36\ (2.316)^{f}$	$2.424^{C} \pm 0.21\ (1.709)^{c}$
Octanal	$C_8H_{16}O$	$5.748^{A} \pm 0.46\ (2.500)^{c}$	$2.325^{C} \pm 0.09\ (1.682)^{cd}$
Ethyl acetate	$C_4H_8O_2$	$1.722^{B} \pm 0.13\ (1.490)^{i}$	$0.823^{C} \pm 0.06\ (1.523)^{e}$
Propyl acetate	$C_5H_{10}O_2$	$5.950^{A} \pm 0.43\ (2.540)^{c}$	$2.614^{C} \pm 0.09\ (1.764)^{bc}$

All the responses are in negative potential (−mV); Figures in the parenthesis are transformed values.
In a column, square root transformed means followed by a common letter are not significantly different at $P = 0.05$ by LSD.
In a row, means followed by a common capital alphabet are not significantly different at $P = 0.05$ by LSD.

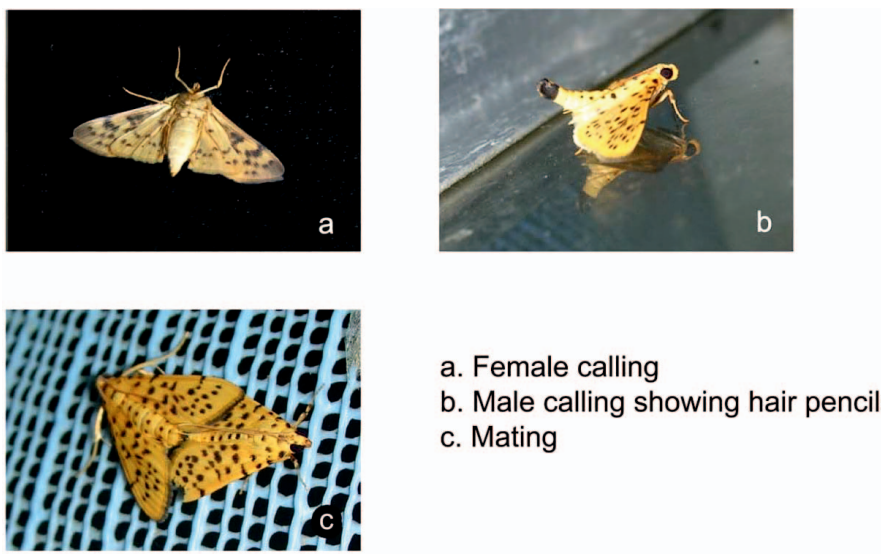

Fig. 2. Calling of male and female *C. punctiferalis* and mating.

Table 1. Extended.

Mean EAG Response (mV)		Critical	
1-d-old Females	4-d-old Females	Difference (0.01)	*F* Value
$2.821^{B} \pm 0.19\ (1.822)^{e}$	$2.064^{C} \pm 0.18\ (1.600)^{b}$	0.15	1071
$3.974^{B} \pm 0.32\ (2.114)^{bc}$	$0.901^{D} \pm 0.02\ (1.183)^{g}$	0.03	7443
$3.974^{B} \pm 0.25\ (2.114)^{bc}$	$0.904^{D} \pm 0.09\ (1.200)^{f}$	0.025	7506
$4.454^{A} \pm 0.34\ (2.225)^{a}$	$2.046^{D} \pm 0.06\ (1.597)^{b}$	0.033	1993
$4.296^{B} \pm 0.32\ (2.191)^{b}$	$1.719^{D} \pm 0.12\ (1.490)^{c}$	0.025	6957
$4.014^{B} \pm 0.31\ (2.124)^{bc}$	$2.187^{D} \pm 0.22\ (1.640)^{a}$	0.025	6957
$2.828^{B} \pm 0.26\ (1.825)^{e}$	$1.131^{D} \pm 0.08\ (1.277)^{e}$	0.030	2084
$3.461^{B} \pm 0.30\ (1.990)^{d}$	$0.922^{D} \pm 0.16\ (1.192)^{fg}$	0.030	4875
$3.394^{B} \pm 0.31\ (1.972)^{d}$	$1.566^{D} \pm 0.09\ (1.439)^{d}$	0.037	2396
$2.249^{A} \pm 0.09\ (1.652)^{f}$	$1.720^{B} \pm 0.08\ (1.490)^{c}$	0.034	753
$4.454^{B} \pm 0.19\ (2.225)^{a}$	$2.046^{D} \pm 0.19\ (1.597)^{b}$	0.037	7171

and female antennal response analyzed separately showed all the four extracts were significantly different (male response, $F = 114.9$; df = 3,36; $P = 0.000$ and female response, $F = 122.1$; df = 3,36; $P = 0.001$). The response of the female antenna was very high to the male headspace extract, followed by male abdominal tip extract (i.e., 4.006 mV and 2.217 mV, respectively). The antenna of males also showed response to female headspace extract but to a lesser extent (1.103 mV).

GC-MS analysis. No cue compound was identified in GC-MS for female extracts. A prominent peak (RT = 11.54 m), which was noted only in male headspace extract, was taken for identification, with the peak indicating the presence of methyl acetophenone (or) 3-ethyl acetophenone.

Discussion

The calling behavior of insects is used to predict sex, age, and time of pheromone release. Calling is regarded as the external indication of pheromone release. Extension of the proboscis, upward curvature of the abdomen, telescopic extension of the abdominal tip, and rapid fluttering of the wings indicated the readiness of female *C. punctiferalis* for pairing, as revealed in the current study. Such behavior is common to Pyralidae spp. such as *Earias insulana* Boisduval (Tamhankar 1995), *Leucinodes orbonalis* Guenee (Renuka et al. 2000), *Deanolis albizonalis* Hampson (Sujatha et al. 2002), and *Palpita unionalis* (Hubner) (Mazomenos et al. 2002).

A typical male calling, by protruding hair pencils, was also noticed in *C. punctiferalis* (Fig. 2). Male calling, although uncommon, was reported in the lesser

wax moth, *Achroia grisella* F. (Spangler and Hippenmeyer 1998), and *Endoclita excrescens* (Butler) (Eiko et al. 2002). Some nocturnal male moths are reported to produce and release odorous compounds from specialized scales such as hair pencils, hair brushes, and androconias (Kimura and Honda 1999, Costanzo and Monteiro 2007) that are involved in short-range mating behaviors (Dussourd et al. 1991, Heath et al. 1992, Delle-Vedove et al. 2014). Well-developed hair pencils at the base of the abdomen were reported in some Arctinnae; Noctuidae of subfamilies Hadeninae (*Mamestra brassicae* L.), Amphipyrinae, Cuculliinae, and Noctuinae; and in the Pyralidae moth *Ephestia elutella* (Hubner). Males of *Creatonotos transiens* (Walker) were reported to pneumatically expand their coremata to release pheromone, attracting the females and, thus, beginning copulation (Wunderer et al. 1986).

In the present study, pheromone extraction was accomplished both through abdominal tip excision and air entrainment. Abdominal tip excision for extraction of pheromones of *C. punctiferalis* was already done by Chakravarthy and Thyagaraj (1998) and Rajabaskar and Regupathy (2012) in hexane. In order to extract polar compounds, if any, along with the nonpolar volatiles, a medium polar solvent (dichloromethane) was used in the present study, as done by Konno et al. (1982). Although some scientists used 4-d-old moths for pheromone extraction by gland excision (Konno et al. 1982, Chakravarthy and Thyagaraj 1998), Rajabaskar and Regupathy (2012) used 3-d-old moths. The maximum pheromone production of about 13 ng per female was reported in 3-d-old moths (Konno 1986). So, 3-d-old moths were used for pheromone extraction in the present study. Air entrainment/ headspace extraction overcomes this confusion of using 3- or 4-d-old moths for pheromone extraction, as the moths were kept in the entrainment chamber for pheromone extraction consecutively from the second to fourth day of emergence. Headspace extraction is the most reliable method to investigate actual pheromones (Tatsuki and Sugie 1992), with minimum contaminants (Cao et al. 2003).

In general, the response of male antennae to volatile solvents was found to be more pronounced than that of the female antennae. The antennal response of male *C. punctiferalis* to (E)-10-hexadecenal is reported to be very high, up to 4.5 mV (Konno et al. 1982). A decrease in antennal sensitivity with an increase in age is also noticed, which is also a common phenomenon in other insects (Seabrook et al. 1987, Den-Otter et al. 1991). Of the two extraction techniques used in the study, the headspace extract showed a significant response (double response) compared with the solvent extraction by abdominal tip excision. So, headspace extraction is better than the solvent extraction. Cao et al. (2003) used both of these techniques to extract pheromones of *Tyta luctuosa* (Denis & Schiffermüller) and found a greater response in headspace extraction than gland excision.

In the present study, females responded strongly to male pheromones in EAG, although the male antenna was found to be more sensitive than female antenna for general volatile compounds. Thus, the presence of male-produced female attractant is evident from this study. The male calling by bending the abdominal tip and extruding the hair pencils observed in the present study added support to this conclusion. Although male pheromones are poorly understood in lepidopterans, their presence is reported. For example, Birch and Hefetz (1987) listed 45 species of lepidopterans identified to have male pheromones. The main components of male pheromones include aromatic alcohols, acids, and aldehydes in Noctuidae,

pyrrolizidine ketones in Danainae, and pyrrolizidine lactones in Ithomiinae. The strong female attraction to the male headspace extracts observed in the present study open up a new area for further studies, which will lead in the use of this compound in pest management.

Acetophenone is identified as the male pheromone of *C. punctiferalis* in the present study. Earlier, 3,4-dimethoxy acetophenone was reported as a male pheromone produced from the androconial organ of *Amauris niavius* (Linnaeus) and *A. tartarea* Mabille (Lepidoptera: Nymphalidae) (Schulz et al. 1993). The pheromone component of Pyralidae males includes cyclic alcohols, aldehydes, unsaturated aldehydes, and lactones. The male hair pencil scent of *C. punctiferalis* was identified by Kimura and Honda (1999) as E-2-methyl-2-butenoic acid (tiglic acid), and males with hair pencils from which the pheromone was washed away by hexane showed significantly lower mating success than control males. Later, Kimura et al. (2002) observed the involvement of tiglic acid in inter- and intraspecific male recognition in the final steps of courtship behavior. However no orientation behavior or attraction was found in *C. punctiferalis* females by tiglic acid. Because a significant response is obtained for male extracts (acetophenone) from females, this study indicates the use of male pheromone for further exploration, development, and assessment is necessary. However, further studies are needed for confirmation of the male pheromone and its biological activity by means of attraction to female moths in field conditions, which will help in pest management.

In conclusion, chemical signaling by males is no less important than by females in the courtship of some lepidopterans (Eisner and Meinwald 1987, Birch et al. 1990). The volatile chemicals disseminated by males from the androconial organs have been reported to generally play a much more significant role than those of females during a sequence of mating behavior (Honda 2005, Delle-Vedove et al. 2014). The presence of a male pheromone is evident from the study, and the use of male pheromone in plant protection (i.e., detection, surveillance, and forecasting) can be done only if a long-range female-by-male attraction is found. Biologically, the male pheromone serves to inhibit the activity of other males, thus eliminating competition for mating. Hence, a possibility arises for the artificial disruption of chemical communication of moths with their own weapon (Girichanov 1999). Hirai (1982) showed that components from the glands of *Pseudaletia unipunctata* (Haworth) males can repel both sexually active males and females of *P. separata* Walker, *Leucania loreyi* (Duponchel), *L. striata* Walker, *Spodoptera litura* F., and *Etiella zinckenella* (Treitschke). In the future, the use of the male pheromone as documented in this study could lead to a management strategy for *C. punctiferalis*.

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References Cited

AGRES. **1994.** Statistical Software Version 3.01. Pascal International Software Solutions, Bellevue, WA, USA.

Birch, M.C. and A. Hefetz. 1987. Extrusible organs in male moths and their role in courtship behavior. Bull. Entomol. Soc. Amer. 33: 222–229.

Birch, M.C., G.M. Poppy and T.C. Baker. 1990. Scents and eversible scent structures of male moths. Annu. Rev. Entomol. 35: 25–58.

Boo, K.S. and K.C. Park. 2005. Insect semiochemical research in Korea: Overview and prospects. Appl. Entomol. Zool. 40: 13–29.

Breth, I.D. and E. Tee. 2007. Progress in managing internal lepidopteran pests in tree fruit. New York Fruit Quart. 15: 19–22.

Cai, R.X. and Z.L. Mu. 1993. Trap trials of *Dichocrosis punctiferalis* Guenee with sex pheromone in citrus orchards. China Citrus. 22: 33.

Cao, W., R.E. Charlton, J.R. Nechols and M.J. Horak. 2003. Sex pheromone of the Noctuid moth, *Tyta luctuosa* (Lepidoptera: Noctuidae), a candidate biological control agent of field binweed. Environ. Entomol. 32: 17–22.

Chakravarthy, A.K. and N.E. Thyagaraj. 1998. Evaluation of selected synthetic sex pheromones of the cardamom shoot and capsule borer, *Conogethes punctiferalis* Guenee (Lepidoptera: Pyralidae) in Karnataka. Pest Mgmt Hort. Ecosyst. 4: 78–82.

Chakravarthy, A.K., K.R.M. Bhanu, N.E. Thyagaraj, B. Doddabasappa and P.R. Shashank. 2013. Responses to host plants and pheromone components of shoot and fruit borer, *Conogethes punctiferalis* (Guenee) (Lepidoptera: Crambidae) infesting castor and cardamom. In: Abstracts International Conf on Chemical Ecology, 19–23 August 2013, Melbourne, Australia. International Society of Chemical Ecology, Berkeley, CA, USA. p 101. https://www.chemecol.org/programs/2013%20abstracts.pdf

Chakravarthy, A.K., V. Kammar, D. Lokeshwari, A.T. Rani, T. Nagaraj and V.V. Rajan. 2015. The black-spotted yellow shoot and fruit borer, *Conogethes* spp. (Crambidae: Lepidoptera)—A global perspective. Curr. Sci. 109: 1016–1018.

Costanzo, K. and A. Monteiro. 2007. The use of chemical and visual cues in female choice in the butterfly *Bicyclus anynana*. Proc. Royal Soc. B. 274: 845–851.

Cruz, I., M.L.C. Figueiredo, R.B. Silva, I.F. Silva, C.S. Paula and L.E. Foste. 2012. Using sex pheromone traps in the decision-making process for pesticide application against fall armyworm (*Spodoptera frugiperda* [Smith] [Lepidoptera: Noctuidae]) larvae in maize. Int. J. Pest Manag. 58: 83–90.

Delle-Vedove, R., B. Frerot, M. Hossaert-Mckey and L. Beaudoin-Ollivier. 2014. Courtship behavior of the castniid palm borer, *Paysandisia archon*: Potential roles of male scents and visual cues in a day-flying moth. J. Insect Sci. 14: 52.

Den-Otter C.J., T. Tchicaya and A.M. Schutte. 1991. Effects of age, sex and hunger on the antennal olfactory sensitivity of tsetse flies. Physiol. Entomol. 16: 173–182.

Dussourd, D.E., C.A. Harvis, J. Meinwald and T. Eisner. 1991. Pheromonal advertisement of a nuptial gift by a male moth (*Utetheisa ornatrix*). Proc. Natl Acad. Sci. USA. 88: 9224–9227.

Eiko, K., H. Toshitaka, S. Takashi and K. Hiroshi. 2002. Copulation and male calling in the swift moth, *Endoclita excrescens* (Butler) (Lepidoptera: Hepialidae). Appl. Entomol. Zool. 37: 163–169.

Girichanov, I. 1999. Male pheromones in relation to the origin of the pheromone system in Lepidoptera. Last accessed 23 July 2016. (http://community.fortunecity.ws/greenfield/porton/875/PHEROMON.htm).

Heath, R.R., P.J. Landolt, B.D. Dueben, R.E. Murphy and R.E. Schneider. 1992. Identification of male cabbage looper sex-pheromone attractive to females. J. Chem. Ecol. 18: 441–453.

Hirai, K. 1982. Directional flow of male scent released by *Pseudoletia seperata* Walker and its repellent effect on adults and larvae of four Noctuid and one Phycitinae moth. J. Chem. Ecol. 8: 1263–1270.

Honda, K. 2005. Mating behavior of butterflies, Pp. 302–349. *In* Honda K. and Y. Kato (eds.), Biology of Butterflies. University of Tokyo Press, Tokyo.

Jung, J.K., K.S. Han, K.S. Choi and K.S. Boo. 2000. Sex pheromone composition for field-trapping of *Dichocrocis punctiferalis* (Lepidoptera: Pyralidae) males. Korean J. Appl. Entomol. 39: 105–110.

Kimura, T. and H. Honda. 1999. Identification and possible functions of the hairpencil scent of the yellow peach moth, *Conogethes punctiferalis* (Lepidoptera: Pyralidae). Appl. Entomol. Zool. 34: 147–153.

Kimura, T., J. Sakai and H. Honda. 2002. Microstructure and pheromone producing function of male hair pencils in the yellow peach moth, *Conogethes punctiferalis* (Lepidoptera: Pyralidae). Entomol. Sci. 5: 237–347.

Konno, Y. 1986. Time lag between sex pheromone content and the calling behavior in the yellow peach moth, *Conogethes punctiferalis* (Guenee), Lepidoptera: Pyralidae. Appl. Entomol. Zool. 21: 622–624.

Konno, Y., K. Arai and Y. Matsumato. 1982. (E)-10-hexadecenal, a sex pheromone component of the yellow peach moth, *Dichocrosis punctiferalis* Guenee (Lepidoptera: Pyralidae). App. Entomol. Zool. 17: 201–217.

Liu, M.Y., Y. Tian and Y.X. Li. 1994. Identification of minor components of sex pheromone of yellow peach moth, *Dichocrosis punctiferalis* Guenee (Lepidoptera: Pyralidae) and field trials. Entomol. Sinca. 1: 15–155.

Mazomenos, B.E., M. Konstantopoulou, D. Stefanou, S. Skareas and L.C. Tzeiranakis. 2002. Female calling behavior and male response to the synthetic sex pheromone components of *Palpita unionalis* (Lepidoptera: Pyralidae). IOBC/WPRS Bull. 25: 1–10.

Pena, J.E., H. Nadel, M. Barbosa-Pereira and D. Smith. 2002. Pollinators and pests of *Annona* species, Pp. 197–222. *In* Pena J.E., J. Sharp and M. Wysoki (eds.), Tropical Fruit Pests and Pollinators: Biology, Economic Importance, Natural Enemies and Control. CABI Publication, Wallingford, UK.

Rajabaskar, D. and A. Regupathy. 2012. Calling behavior and attractive response of cardamom shoot and capsule borer, *Conogethes punctiferalis* Guenee (Lepidoptera: Pyralidae) to the female crude extract and synthetic blend. J. Entomol. 9: 296–301.

Regupathy, A. and R. Ayyasamy. 2014. A Guide on Crop Pests. Namrutha Publications, Chennai, India. 398 pp.

Reinecke, A., J. Ruthar and M. Hilker. 2005. Electrophysiological and behavioral responses of *Melolantha melolantha* to saturated and unsaturated aliphatic alcohols. Entomol. Exp. Appl. 115: 33–40.

Renuka, S., A. Regupathy and K. Chozhan. 2000. Studies on the sex pheromone release behavior of *Leucinodes orbonalis*. In: Entomo Congress 2000; 2000 November 5–7; Trivandram, Kerala, India: Publisher. p X–X.

Renuka, S., A. Regupathy and D. Rajabaskar. 2002. Bio-efficacy of profenofos (Curacron 50EC) against cardamom thrips *Sciothrips cardomomi* (Ramk), XIV. In: Rethinam P., H.H. Khan, V.M. Reddy, P.K. Mandal and K. Suresh, editors. Proc. PLACROSYM; 2000 December 12–15; Hyderabad, India: Publisher. p 491–492.

Schulz, S., M. Boppre and R.I. Vane-Wright. 1993. Specific mixtures of secretions from male scent organs of African milkweed butterflies (Danainae). Phil. Trans. R. Soc. B. 342: 161–181.

Seabrook, W.D., C.E. Linn, L.J. Dyer and H.H. Shorey. 1987. Comparison of electro-antennograms from female and male cabbage looper moths (*Trichoplusia ni*) of different ages and for various pheromone concentrations. J. Chem. Ecol. 13: 1443–1453.

Spangler, H.G. and C.L. Hippenmeyer. 1998. Binaural phonotaxis in the lesser wax moth, *Achroia grisella* (F.) (Lepidoptera: Pyralidae). J. Insect Behav. 1: 117–122.

Stanley, J., S. Chandrasekaran and G. Preetha. 2009. *Conogethes punctiferalis* (Lepidoptera: Pyralidae): Its biology and field parasitisation. Indian J. Agric. Sci. 79: 906–909.

Stanley, J., S. Chandrasekaran, G. Preetha and S. Kuttalam. 2010. Toxicity of diafenthiuron to honey bees in laboratory, semi-field and field conditions. Pest Manage. Sci. 66: 505–510.

Sujatha, A., S.M. Zaheruddeen, A. Arjunarao and B. Nagalingam. 2002. Electroatennogram and wind tunnel studies on olfactory responses of mango fruit borer *Deanolis albizonalis* (Hampson) to female sex pheromone. Pest Manage. Hort. Ecosys. 8: 83–89.

Tamhankar, A.J. 1995. Sex pheromone gland and calling behavior of female spiny bollworm *Earias insulana* (Boisduval). Entomon. 20: 1–4.

Tatsuki, S. and H. Sugie. 1992. Rice insect pheromone identification and utilization in monitoring, Pp. 347. In: Heinrichs E.A. and T.A. Miller (eds). Rice Insects: Management Strategies. Springer-Verlag, NewYork.

Thyagaraj, N.E., P.K. Singh and A.K. Chakravarthy. 2003. Bioecology of cardamom shoot and fruit borer, *Conogethes punctiferalis* Guenee. Curr. Res. 32: 3–4.

Wunderer, H., K. Hansen, T.W. Bell, D. Schneider and J. Meinwald. 1986. Sex pheromones of two Asian moths (*Creatonotos transiens*, *Creatonotos gangis* Lepidoptera: Arctiidae): Behavior, morphology, chemistry and electrophysiology. Exp. Biol. 46: 11–27.

Xiao, W. and H. Honda. 2010. Non-polar body waxes enhance sex pheromone activity in the yellow peach moth, *Conogethes punctiferalis* (Guenee) (Lepidoptera: Crambidae). Appl. Ent. Zool. 45: 449–456.