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# Reproductive behavior and sex pheromone production in *Eutectona machaeralis* (Lepidoptera: Crambidae)

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## Abstract

The teak skeletonizer, *Eutectona machaeralis* (Walker) (Lepidoptera: Crambidae), is a primary pest of teak trees in plantations and natural forests. However, the biology of this pest is not well studied. We investigated adult emergence, calling behavior, and circadian rhythm of sex pheromone production of this moth in the laboratory. Adult emergence only occurred during scotophase, when females emerged faster than males. Females initiated sexual calling behavior starting 1 d after emergence. This behavior then peaked on the second day of emergence. Sex pheromone release peaked 7 h into scotophase, wherein 2-d-old females elicited the strongest male antennal response to this substance. Our results provide a basis for future investigations of *E. machaeralis* sex pheromone production that may lead to development of novel methods to control this forestry pest.

Key Words: teak skeletonizer; emergence; calling behavior; electroantennogram

## Resumen

El esqueletizador de teca, *Eutectona machaeralis* (Walker) (Lepidoptera: Crambidae), es una plaga principal de los árboles de teca en plantaciones y bosques naturales. Sin embargo, la biología de esta plaga no está bien estudiada. Investigamos la aparición de adultos, el comportamiento de llamada y el ritmo circadiano de la producción de feromonas sexuales de esta polilla en el laboratorio. La emergencia del adulto solo ocurrió durante la escotofase, cuando las hembras emergieron más rápido que los machos. Las hembras iniciaron el comportamiento de llamada sexual comenzando 1 día después de la emergencia. Este comportamiento luego alcanzó su punto máximo en el segundo día de emergencia. La liberación de feromonas sexuales alcanzó un máximo a la 7 hora en la escotofase, en el que las hembras de 2 días de edad provocaron la respuesta antenal masculina más fuerte a esta sustancia. Nuestros resultados proveen una base para investigaciones futuras de la producción de feromonas sexuales de *E. machaeralis* que pueden resultar en el desarrollo de nuevos métodos para controlar esta plaga forestal.

Palabras Clave: esqueleto de teca; electroantenograma

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Teak, *Tectona grandis* L. (Lamiaceae), is among the most valuable tropical timber species native to southeastern Asia where it occurs primarily in India, Sri Lanka, Indonesia, Malaysia, Thailand, Myanmar, and Bangladesh (Moya et al. 2014). In mainland China, it is cultivated in Fujian, Guangdong, Guangxi, and Yunnan provinces. However, teak trees are usually attacked by the larvae of the teak skeletonizer, *Eutectona machaeralis* Walker (Lepidoptera: Pyralidae) syn. *Pyrausta machaeralis* Walker (Lepidoptera: Crambidae), a serious and pernicious pest of this tree species (Nair et al. 1996; Kulkarni et al. 2011). This pest is mainly distributed in the Chinese provinces of Guangdong, Guangxi, and Yunnan (Wu et al. 1979). The teak skeletonizer causes defoliation that adversely affects the growth and vigor of teak trees, causing abnormalities that result in qualitative and quantitative loss in timber production (Beeson

1941; Kulkarni et al. 1996; Nair 2007). In Indian plantations, this pest causes up to 65% of timber losses (Baksha & Crawley 1998; Shukla & Joshi 2001).

Lepidoptera synthetic sex pheromones have been used widely for monitoring, mass trapping, mating disruption, or attracting and killing the adults of economically important moth species (Leal et al. 2003; Ma et al. 2014; Hoshi et al. 2016). However, reproductive behavior (as it relates to sex pheromone production) is a basic component when determining if a pheromone is efficacious, leading to successful production for field application. Currently, no information has been published about the reproductive behavior and sex pheromone gland production of *E. machaeralis* females. Therefore, we initiated studies on characterizing the behavioral response of this species, as well as the circadian rhythm of sex pheromone production, in laboratory studies.

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## Materials and Methods

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### INSECTS

Larvae of *E. machaeralis* were collected from teak plantation areas in Huizhou, Guangdong Province, China (22.500°N, 114.300°E), during Sep-Nov 2015. Larvae were reared on fresh teak leaves in plastic containers (60 cm × 30 cm × 30 cm) in the laboratory at 25 to 28 °C, 12:12 h (L:D) photoperiod, and 75 to 80 % relative humidity (RH).

### CHARACTERIZATION OF ADULT MOTH EMERGENCE

Male and female pupae were separately maintained in screened cages (40 cm × 30 cm × 30 cm) in 12:12 h (L:D) conditions and observed hourly for adult emergence for 24 h (Zhang et al. 2014). Because emergence occurred during scotophase, observations were conducted with the aid of a red light. Once emerged, moths were removed to separate cages to ensure homogeneity of age. Adults were provided with 10% honey solution soaked in a piece of cotton. In total, 682 male and 604 female *E. machaeralis* were used for this portion of the study.

### CHARACTERIZATION OF CALLING BEHAVIOR

Preliminary observations of 30 female moths indicated that calling behavior only occurred during the scotophase. Therefore, experiments were started during the first complete scotophase after pupal emergence. A dim red light was used for visual observation during this period. Forty newly emerged virgin females were individually placed in transparent plastic cups (11 cm diam × 10 cm ht) and provided with 10% honey solution soaked in a piece of cotton. Calling females are recognized by their characteristic posture of extruding and bending the abdominal tip toward the dorsal surface. The daily onset of calling time (time after lights off) and percentage of females calling daily were recorded. This experiment was repeated 3 times with a total of 120 females observed.

### PHEROMONE GLAND EXTRACTION

To obtain an extract from the sex pheromone gland, females were randomly selected from cages and placed in a freezer at about -20 °C for 1 min that temporarily immobilized them for manipulation. Terminal abdominal segments of each moth were gently pressed with forceps to extrude the sex pheromone gland. Glands were then excised and immersed in distilled hexane (10 µl hexane per abdominal tip) for 40 min. The hexane crude extracts were transferred to individual glass micro-capillary tubes and stored in a freezer (-20 °C) for later use.

### SEX PHEROMONE PRODUCTION AND ELECTROANTENNOGRAM RESPONSE

In the first experiment, sex pheromone gland crude extracts of 2-d-old virgin females ( $n = 30$ ) were obtained hourly from 1 to 10 h during scotophase with the extract solvent concentration set as 1 FE (female equivalent) extracted in 10 µl hexane. Electroantennogram reactions of 2-d-old male *E. machaeralis* antennae to these extracts were conducted during the scotophase with hexane only used as control.

In the second experiment, sex pheromone production of 1- to 6-d-old females (7 h into the scotophase) were conducted with the extract solvent concentration set as 1 FE per 10 µl hexane. Thirty virgin females were used in each trial. Two-d-old male antennae were used to measure electroantennogram responses with hexane as a control. All electroantennogram experiments were conducted during 6:00 to 9:00 PM. Pheromone production and electroantennogram experiments were repeated 3 times over separate days (Ma et al. 2017).

### WIND TUNNEL TESTS

Behavioral tests using male moths were carried out at the same daily time period in a wind tunnel (200 cm × 40 cm × 40 cm) at 24 ± 1 °C and 65% RH. A red light also was used for observation purposes in this portion of the study. Airflow in the tunnel was maintained at 500 ml per min produced by an electric fan and filtered through a 5 cm thick layer of activated charcoal. The pheromone source (referred to as lure) was placed 80 cm upwind from the release platform (37 cm length × 4 cm diam). Groups of 50 males (2- to 5-d-old) were placed in a screened cage (15 cm × 15 cm × 15 cm) and allowed to acclimate in the wind tunnel for at least 2 h prior to testing. The odor source (lure) consisted of a small green rubber septa (8 mm diam, pheromone Technology Co. Ltd., Beijing, China) filled with gland extract or hexane (control), and evaluation started after the solvent completely evaporated. Behavioral observations of male moths included taking off, directional flight, travel distance to lure, landing on lure, and attempting to copulate with the rubber septa on the release platform. Observations were performed for 2 h. This portion of the study consisted of 3 replicates, each on different days.

### STATISTICAL ANALYSES

One way analysis of variance (ANOVA) was conducted to assess temporal pattern of mean female calling data, male electroantennogram response to sex pheromone gland extracts, and antennal responses. Means were separated by Tukey's HSD test. Male electroantennogram responses to sex pheromone gland extracts were normalized using electroantennogram software (Ockenfels Syntech GmbH, Buchenbach, Germany). All analyses were conducted using SPSS 17.0 software (IBM Company, Chicago, Illinois, USA) and differences were considered significant at  $P < 0.05$ .

## Results

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### CHARACTERIZATION OF ADULT MOTH EMERGENCE

The majority of moth emergence (78%) occurred during the first 7 h of scotophase. A higher proportion of females than males emerged during the first 4 h, and the eclosion rate of males was greater during the following 5 to 9 h period (Fig. 1). No adults emerged at 12 h as the scotophase advanced to photophase. Adult emergence reached its peak at day 6 (Fig. 2).

### CHARACTERIZATION OF CALLING BEHAVIOR

Peak calling behavior (55%) was greatest for 2-d-old females compared with the rest of the ages tested (Fig. 3). After this age, calling behavior diminished to 10% at 5 d. We also observed 2 peaks in calling behavior (2 and 8 h) during scotophase (Fig. 4).

### SEX PHEROMONE PRODUCTION AND ELECTROANTENNOGRAM RESPONSE

There were significant differences in the electroantennogram response of males to sex pheromone extracts obtained at different hours during the scotophase ( $F = 16.82$ ;  $df = 10$ ;  $P < 0.05$ ). The extract from females collected at 7 h evoked the strongest electroantennogram response that indicated peak production of pheromone had occurred (Fig. 5). Electroantennogram response of males to the pheromone also varied with age of the females ( $F = 34.64$ ;  $df = 6$ ;  $P < 0.05$ ) (Fig. 6). Extracts from 2- to 4-d-old

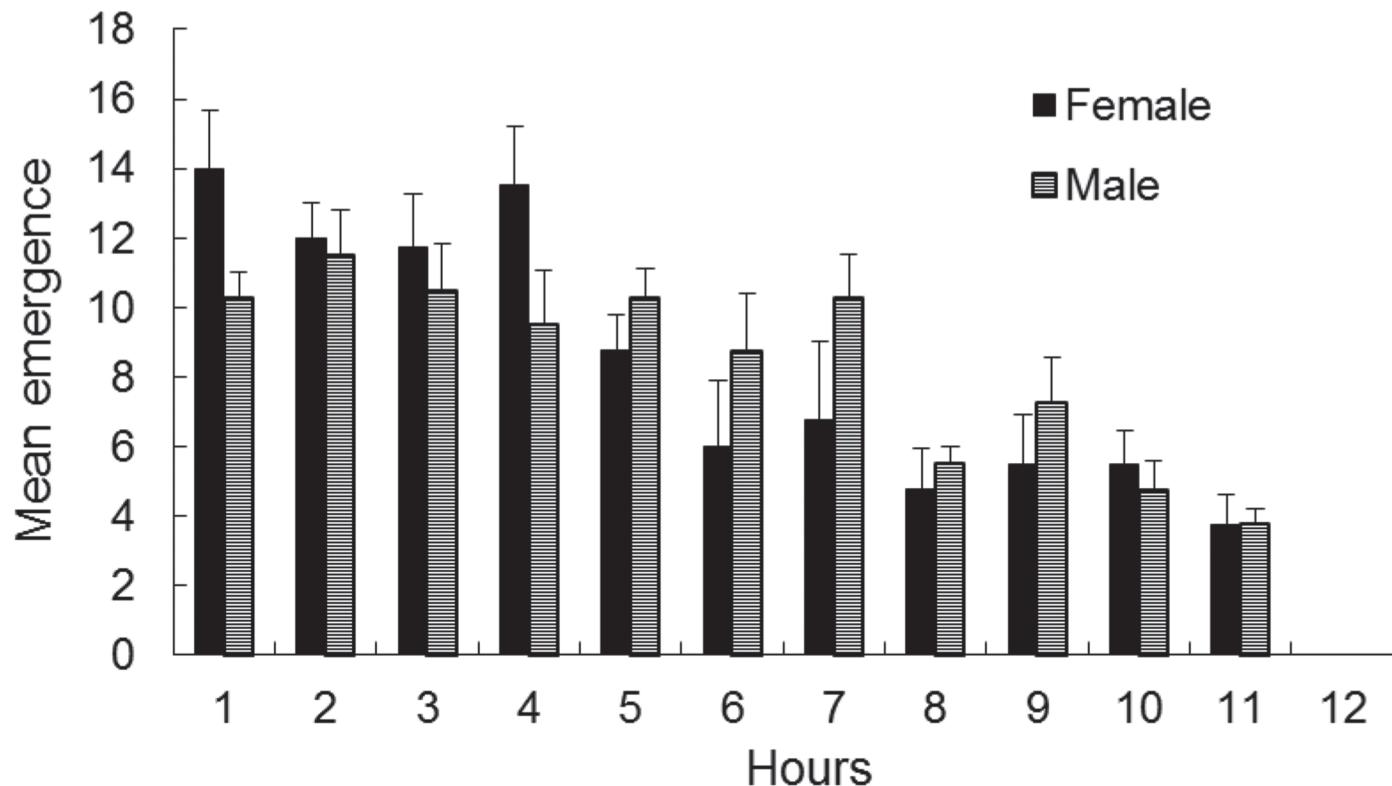


Fig. 1. Hourly emergence of *Eutectona machaerilis* adults in the laboratory during scotophase.

females evoked significantly greater electroantennogram response from males compared with extracts from 1- or  $\geq$  5-d-old females.

#### WIND TUNNEL TESTS

There was no difference between sex pheromone gland extract and controls with respect to male moth take off. However, male directional flight and number of landings on the odor source (i.e., pheromone extract) was significantly greater than the hexane control ( $df = 3, P < 0.05$ ) (Fig. 7).

#### Discussion

In our study, we showed that *E. machaerilis* 1-d-old females started calling during the first h of scotophase. This suggests that females of this species are reproductively mature upon emergence. We also found that the percentage of females calling continued for the first 2 d after emergence then steadily decreased through d 6. These results are in contrast to many moth species, such as *Mamestra configurata* Walker (Howlader

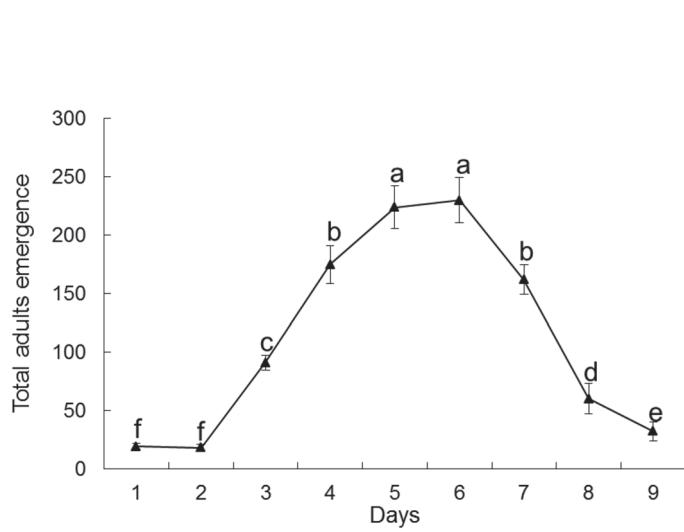


Fig. 2. The influence of age on daily emergence of *Eutectona machaerilis* adults (mixed sex) in the laboratory during scotophase. Values with the same letter are not significantly different ( $P > 0.05$ ).

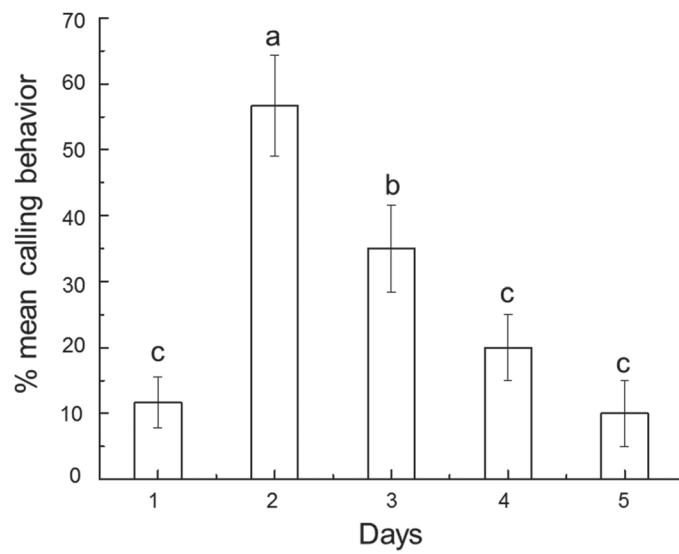
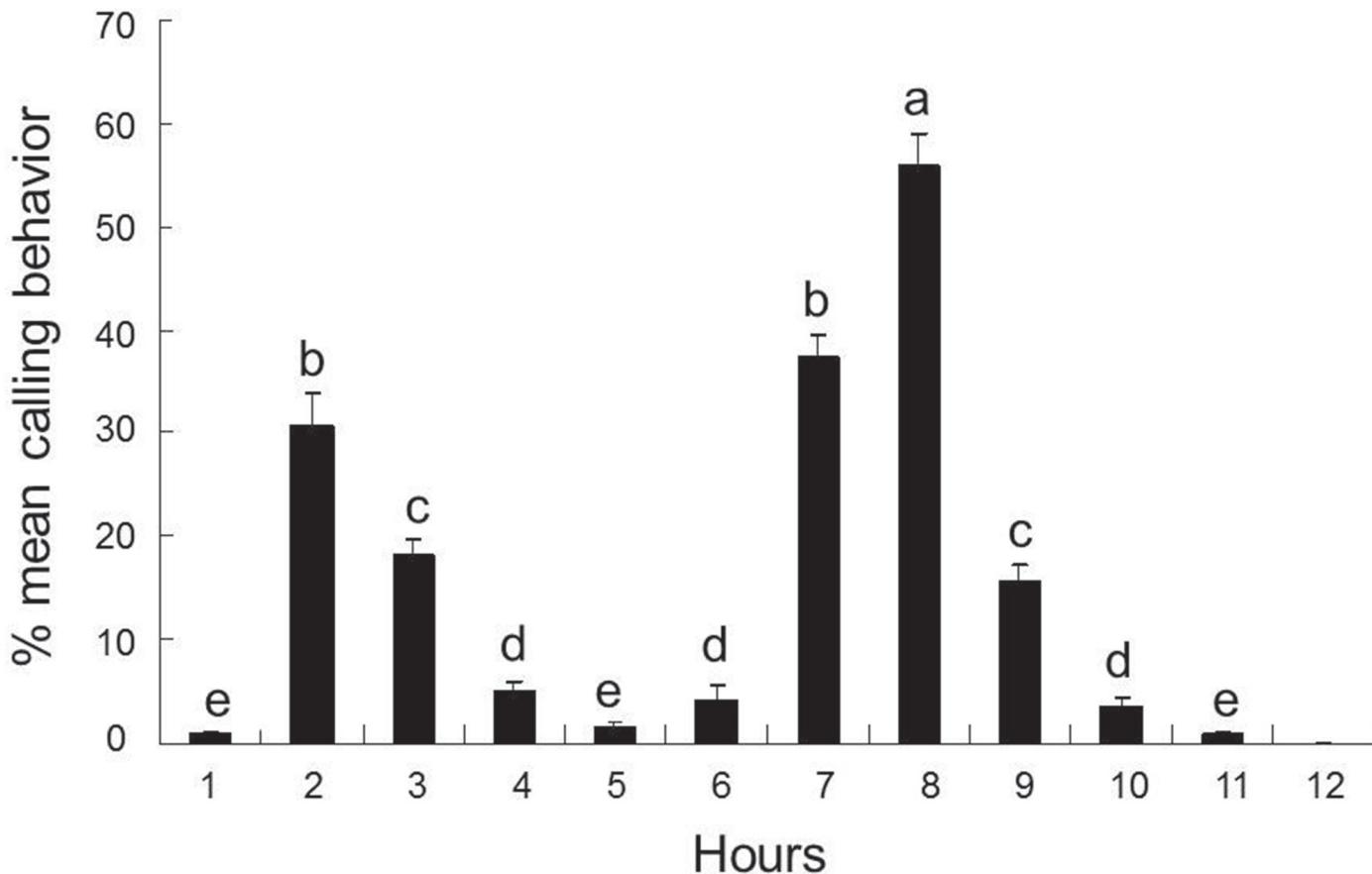


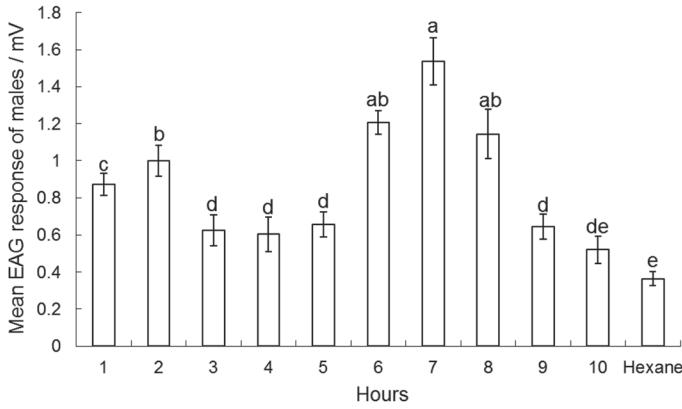
Fig. 3. The influence of age on calling behavior in *Eutectona machaerilis* females. Means with the same letter are not significantly different ( $P > 0.05$ ).



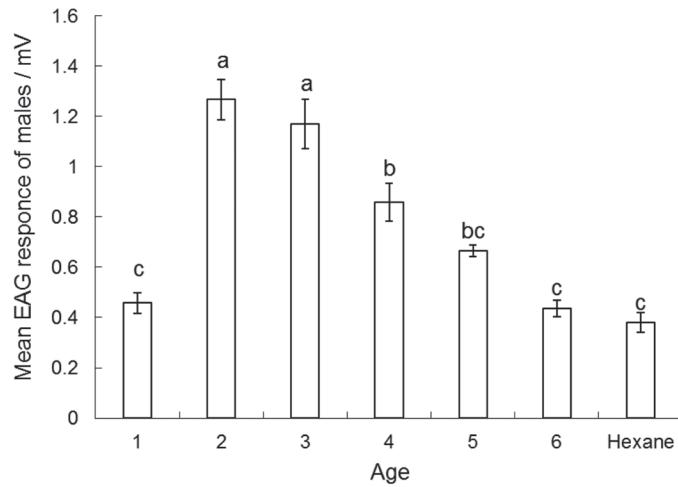
**Fig. 4.** Mean percentage of *Eutectona machaeralis* females that exhibited calling behavior during scotophase. Means with the same letter are not significantly different ( $P > 0.05$ ).

& Gerber 1986) and *Copitarsia consueta* Walker (both Lepidoptera: Noctuidae) (Rojas & Cibrián-Tovar 1994), where females are not reproductively mature, or capable of calling, immediately after emergence; these observations also have been repeated by other investigators working with a number of other moth species including *Grapholitha molesta* (Busck) (Lepidoptera: Tortricidae), *Holamelina lamae* (Freeman) (Lepi-

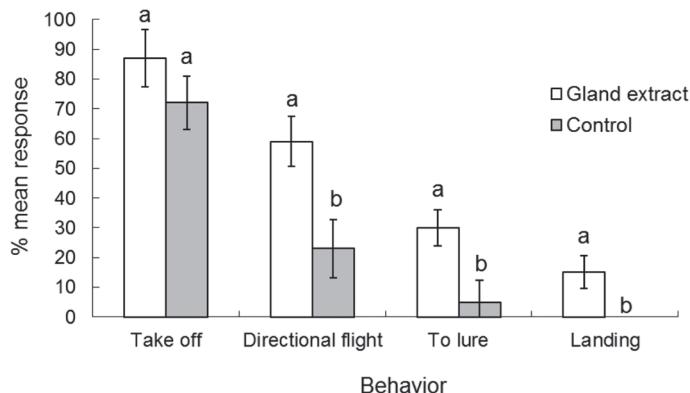
doptera: Arctiidae), and *Sesamia nonagrioides* (Lef.) (Lepidoptera: Noctuidae) (Baker & Cardé 1979; Schal & Cardé 1986; Babilis & Mazomenos 1992). These authors noted that calling behavior was lowest in newly emerged females but increased with advanced age. Undoubtedly, differences in calling behavior are influenced by a variety of physiological factors inherent among lepidopteran species.



**Fig. 5.** Mean electroantennogram response of male *Eutectona machaeralis* to sex pheromone gland crude extract obtained from 2-d-old virgin females at different hours during scotophase. Means with the same letter are not significantly different ( $P > 0.05$ ).



**Fig. 6.** Mean electroantennogram response of male *Eutectona machaeralis* to sex pheromone gland crude extract obtained from 1- to 6-d old virgin females at 7 h into scotophase. Means with the same letter are not significantly different ( $P > 0.05$ ).



**Fig. 7.** Mean behavioral responses of *Eutectona machaeralis* males to sex pheromone gland crude extracts and the control in a wind tunnel. Means with the same letter are not significantly different ( $P > 0.05$ ).

Also, previous authors have reported a positive correlation between female calling and amount of sex pheromone production in several lepidopteran species including *Zamagiria dixolophella* Dyar (Lepidoptera: Pyralidae) (Castrejón-Gómez & Rojas 2006; Castrejón-Gómez 2010), *Estigmene acrea* Drury (Lepidoptera: Arctiidae) (Mazo-Cancino et al. 2004), *Cerconota anonella* Sepp. (Lepidoptera: Oecophoridae) (Da Silva et al. 2006), and *Helicoverpa assulta* Guenée (Lepidoptera: Noctuidae) (Kamimura & Tatsuki 1993). In our study, electroantennogram response of male *E. machaeralis* was significantly stronger to 7 and 8 h pheromone extracts of 2-d-old females compared with other time intervals with the exception of 6 h where there was no difference (Fig. 5). Generally, production of sex pheromone in moths is controlled by a pheromone biosynthesis activating neuropeptide (Raina et al. 1989; Tillman et al. 1999). But an alternative physiological pathway also has been previously described by Gadenne (1993) and Picimbon et al. (1995), wherein sex pheromone production was mediated by juvenile hormone in female *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae).

A variety of factors obviously influence the ability of females to attract a mate. It is likely that the physiological stage of the insect, in combination with ambient environmental conditions (i.e., temperature, wind speed, and relative humidity), may guide calling behavior. Additionally, many types of herbivorous insects are attracted to host plant volatiles. In fact, the males of some species are attracted to females only in the presence of host plant odor. For example, males of the leek moth (*Acrolepiopsis assectella* Zeller [Lepidoptera: Acrolepiidae]) are attracted to females in the presence of odors from leek (*Allium porrum* L. [Amaryllidaceae]) (Bernays & Chapman 1994). We also have observed that the teak skeletonizer only feeds on teak, so host plant volatiles might act as additional chemical cues that may potentiate calling behavior of female moths. The fact that mating behavior of this moth species occurs on the host plant is an important finding relative to sex selection and mate finding.

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