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# PUPAL DEVELOPMENT, LONGEVITY AND BEHAVIOR OF *CARMENTA THEOBROMAE* (LEPIDOPTERA: SESIIDAE)

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Abstract

Pupal duration, longevity, and behavior of males and females of *Carmenta theobromae* (Busck), an insect pest of economic importance in cocoa plantations (*Theobroma cacao* (L.)) were studied. Perforated pods from cocoa farms in Curiepe, Miranda state, Venezuela were collected and taken to INIA-Miranda. *Carmenta theobromae* larvae and pupa were extracted from infested pods. All stages (larvae, pupae, and adults) were kept at 28 ± 5°C, 70 ± 15% RH and a photoperiod 12:12 (L:D). Emergence and daily activity of adults kept in observation chambers was recorded. Pupal development time was 12.78 ± 2.50 d and adult longevity was 5.10 ± 1.96 and 4.39 ± 1.57 d for males and females, respectively. Emergence of adults began between 0730 and 0800 h, and continued until 1400 and 1500 h for females and males, respectively. Adult emergence peaked at 0800 h for both sexes, and the adult sex ratio was 1.2:1 (male: female). Female calling behavior occurred between 1430 and 1930 h, with a pronounced peak at 1730 h, and continued until the third day after emergence. This behavior coincided with more intense flight, walking or wing fluttering in males. The results suggest that *C. theobromae* females release a sex pheromone between 1430 and 1930 h.

Key Words: calling behavior, cocoa, cocoa fruit borer, *Theobroma cacao*

Resumen

Se estudio la duración de la fase pupal, longevidad y el comportamiento de machos y hembras de *Carmenta theobromae* (Busck), un insecto plaga de gran importancia económica en las plantaciones de cacao (*Theobroma cacao* (L.)). Se examinaron y colectaron los frutos que presentaban perforaciones por insectos, en plantaciones de cacao en Curiepe, estado Miranda-Venezuela. Los mismos fueron trasladados al INIA-Miranda, las larvas y pupas fueron extraídas de los frutos colectados. Todas las fases (larva, pupa y adultos) se mantuvieron a 28 ± 5°C, humedad relativa 70 ± 15%, y fotoperíodo de 12:12 horas (luz:oscuridad). Se registró la emergencia y la actividad diaria de adultos mantenidos en cámaras de observación. El tiempo de desarrollo de la pupa fue de 12,78 ± 2,5 días, mientras que la longevidad de los adultos fue de 5,10 ± 1,96 y 4,39 ± 1,57 días para el macho y la hembra, respectivamente. La emergencia de adultos se inicia entre las 07:30 y 08:00 horas, y se extiende hasta las 14:00 y 15:00 horas, en hembras y machos respectivamente. El mayor pico de emergencia de adultos ocurre a las 08:00 horas para ambos sexos y se obtuvo una proporción sexual de 1,2:1 (machos: hembras). El comportamiento de llamado sexual de la hembra se presentó entre las 14:30 y 19:30 horas, con un pico de llamado a las 17:30 horas, manteniendo este comportamiento hasta el tercer día de vida. Esta conducta coincidió con la mayor actividad de vuelo, caminata o aleteo de los machos. Los resultados sugieren que la hembra de *C. theobromae* emite la feromona sexual entre las 14:30 y 19:30 horas.

Translation by the authors.

The cocoa fruit borer, *Carmenta theobromae* (Busck) (Lepidoptera: Sesiidae) is one of the major insect pests of cocoa in Venezuela (Sánchez & Capriles de Reyes 1979). It is distributed in the principal cocoa producing areas in Aragua and Miranda states in the central region of the country (Sánchez & Marín 1987) and has been reported from Colombia and Panamá (Eichlin 1995).

The larvae produce perforations and internal galleries in the pericarp of infested fruit, and the damage leads to secondary damage due to phytopathenogenic fungal infections (such as *Phytophthora* sp.) that cause the fruit to rot. Characteristic reddish brown piles of excrement, with the appearance of ground coffee, held together by a mass of silken threads can be observed in the en-

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try holes of larvae. Larvae have been found on the branches, stems, buds and greenpoint cushion galls of cocoa plants (Sánchez & Capriles de Reyes 1979).

In small pods, the larvae perforate the internal part of the fruit and destroy the recently formed grains, or halt their growth producing early ripening. The pod then dries attached to the tree. However, this type of damage is not frequent (Sánchez & Marín 1987). In physiologically ripe fruits, the galleries only reach the pericarp, the interior of the fruit being protected by the lignified mesocarp, which acts as a barrier to the larvae, allowing the fruit to continue its growth, reach maturity, and be successfully harvested if fungal infections do not occur. Nevertheless, in many insect damaged fruits, the grains cohere into a solid mass, symptoms thought to be due to the colonization by phytopathenogenic microorganisms. When pupation occurs, the larvae move towards the surface of the pericarp, where the larval exuviae or pupal remains can be observed. (Sánchez & Capriles de Reyes 1979; Delgado 2004).

Fruit borers have traditionally been controlled by synthetically produced chemical insecticides (organochlorides, organophosphates, and others) that have secondary effects on the environment (Sánchez & Capriles de Reyes 1979). These strategies can be substituted by environmentally friendly methods such as ethological control (use of pheromones and other behavioral manipulation techniques) and biological control (use of natural enemies such as predators, pathogens, and parasitoids).

In Lepidoptera, the release of pheromones is associated with calling behavior, during which behavior one of the sexes produces the sex pheromone, triggering a response in the opposite sex. The pheromone producing gland in most lepidopteran females is located in the last ventral abdominal segments, between the seventh and eighth, or the eighth and ninth segments (Hagaki & Conner 1988; Scoble 1992; Raspotnig et al. 2003). During calling behavior, female moths assume a typical position whereby the abdomen extends and curves dorsally, accompanied by the protrusion of the eighth and ninth abdominal segments, facilitating the release of the volatile pheromone. This behavior may take place over a few hours. *Thaumetopoea pityocampa* (Denis & Schiffermuller) (Thaumetopoeidae) calls for 6.4 h (Zhang & Paiva 1998), and *Hylesia metabus* (Cramer) (Saturniidae) calls for 7.5 h (Fornés & Hernández 2000), with 2 types of calling behavior. Calling behavior can last much longer; for example, *Lymantria dispar* L (Lymantriidae), has been recorded calling up to 16.5 h (Webster & Yin 1997).

In spite of the importance of *C. theobromae* as a pest of cocoa, information on its biology and behavior is scarce. In Venezuela, Delgado (2004), reported that the pupal phase of this insect lasts for 8.6 ± 2.2 d, but was unable to determine the duration of the egg and larval phases due to the impossibility of obtaining fertile eggs.

In this study, we report the duration of the pupal and adult phases, as well as the period of activity in *C. theobromae* males and females. In addition, we characterize the calling behavior of females. These results will aid isolation of the sex pheromone.

## MATERIALS AND METHODS

### Insect Sampling

A total of 55 field sampling efforts were made, with an average of 400 pieces of cocoa pods collected per sampling effort. All sampling was done in Curiepe, Municipio Brión, in the Barlovento region, Miranda state, Venezuela, during Jan-Jul 2005, Dec 2005, Jan-Jul 2006, Dec 2006, and Jan-Jul 2007. Cocoa pods showing symptoms of infestation by *C. theobromae* larvae were collected and taken to the Instituto Nacional de Investigaciones Agrícolas (INIA-Miranda) in plastic bags. Larvae and pupae were extracted from pods with disinfected penknives and placed individually inside a section of a healthy mature cocoa pod. Each pod with a larva was then placed in a 350-mL polyethylene container. Pods were changed every 2 to 3 d. Pupation trays (600 or 900 $cm^2$) were prepared by placing a sheet of rectangular foam and moist tissue paper of the same dimensions on them. Field collected pupae and those obtained from the larvae reared in the laboratory were transferred onto the trays, with 42-70 pupae per tray depending on tray size, and each pupa was covered with a transparent plastic 30-mL cup. The collected material was maintained in a 7.5 $m^2$ room at the INIA-Miranda, at a temperature of 28 ± 5°C, 70 ± 15% RH and a photoperiod 12:12 (L:D).

### Duration of the Pupal Phase and Adult Longevity

A bioassay was conducted with part of the specimens collected in 2005 and 2006. With 267 recently formed pupae we recorded daily the dates of adult eclosion and their subsequent death. We placed 138 male and 121 female adults in transparent plastic 350-mL cups. The insects were fed with a 1:9 honey:water solution and adult longevity was recorded. In order to differentiate moth sex, weight differences between male and female pupae were determined from a group of 10 male and 10 female pupae inside their cocoons. Cocoons were weighed 2 d after their formation, and reweighed on adult emergence, with a digital electronic balance (Mod Explorer 0.0001 accuracy, Ohaus Corporation, Switzerland). The weight of each pupa was then calculated by subtracting the weight of the pupa + cocoon from the weight of the cocoon after adult emergence.

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### Adults Emergence

These observations were made with another group of specimens collected during 2005. The time of emergence of adult males and females was recorded starting at 0700 h at 30-min intervals for 24 h.

### Adult Behavior and Diel Periodicity

Following a method similar to that used by Fornés & Hernández (2000) and Bergh et al. (2006), we placed recently emerged male and female adults, reared from specimens obtained during 2006 and 2007, in observation chambers, 30 × 30 × 30 cm, with a metal frame and transparent plastic walls. Each chamber represented a replicate, with a total of 38 bioassays, 114 females and 117 males (Table 1). Observations of the activity period, calling behavior, and mating were made until the death of the individuals. At the beginning of each bioassay, a section of a branch of a cocoa tree with at least 5 leaves attached was placed in the chamber. Observations were made every 30 min during 24 h. Adults were fed with a 1:9 honey:water solution. Flight, walking, or wing fluttering behavior of males and females, and female calling behavior were observed. In order to determine the diel periodicity of the behavior observed of the insects, another group of chambers, 36 with males and 33 with females were observed during 96 h from the time of emergence, following a method similar to that used by Bergh et al. (2006).

### Statistical Analysis

Descriptive statistics were used to calculate the average duration of pupae and adults. The pupal weight of males and females was compared by a Student's *t*-test for independent samples (Infostat 2004). Adult emergence; flight, walking or wing fluttering by males, and flight, walking or wing fluttering by females, were analyzed by circular statistics (Batschelet 1981; Fisher 1993). The time that each activity was recorded (time unit) was transformed into an angular direction, with the equation $a_i = [(360°)(X)]/k$, where $a_i$ is the transformed time unit, X, is the time (in h) and k (24 h) is a constant that represents the total time of the units in the circle (Zar 1999). The null hypothesis (Ho) tests whether the data are uniformly distributed around a circle. In addition, flight, walking or wing fluttering in males, and calling behavior in females were correlated with an angular-angular correlation in the Oriana 2.02e program (Kovach Computing Services 2008).

TABLE 1. PLACEMENT OF *C. THEOBROMAE* ADULTS IN THE OBSERVATION CHAMBERS USED FOR THE BEHAVIOR BIOASSAYS.

| Groups of adults/chamber | Number of chambers | ♀ | ♂ |
|---|---|---|---|
| 4 ♀ & 2 ♂ | 5 | 20 | 10 |
| 2 ♀ & 3 ♂ | 8 | 16 | 24 |
| 4 ♀ & 5 ♂ | 2 | 8 | 10 |
| 3 ♀ & 6 ♂ | 4 | 12 | 24 |
| 2 ♀ & 2 ♂ | 3 | 6 | 6 |
| 7 ♀ & 5 ♂ | 2 | 14 | 10 |
| 3 ♀ & 2 ♂ | 5 | 15 | 10 |
| 5 ♀ & 5 ♂ | 2 | 10 | 10 |
| 1 ♀ & 1 ♂ | 5 | 5 | 5 |
| 4 ♀ & 4 ♂ | 2 | 8 | 8 |
| Total | 38 | 114 | 117 |

## RESULTS AND DISCUSSION

### Duration of the Pupal Phase and Adult Longevity

The mean duration of the pupal phase was 12.78 ± 2.5 d and adult longevity of *C. theobromae* was 5.10 ± 1.96 d for males and 4.39 ± 1.57 d for females (Table 2). Only 1 mating was observed, so it was not possible to study the egg or larval stages. Female pupae were heavier than male pupae ($t = 10.26$, $df = 18$, $P < 0.001$). Delgado (2004) recorded the duration of the pupal stage as 8.6 ± 2.2 d for *C. theobromae*, which is much shorter than that observed in the present study. However, rearing conditions were not reported by Delgado (2004).

The life cycle from egg to adult of other Sesiidae, such as *Synanthedon pictipes* (Grote & Robinson), *Carmenta haematica* (Ureta), *Carmenta mimosa* Eichlin & Passoa, *Chamaesphecia hungarica* (Tomala) can vary both with the species and the environmental conditions (Cleveland et al. 1968; Cordo et al. 1995; Forno et al. 1991; Gassmann & Tosevski 1994).

TABLE 2. AVERAGE DATA FOR *C. THEOBROMAE* PUPAE AND ADULTS UNDER LABORATORY CONDITIONS, COLLECTED FROM THE BARLOVENTO REGION, MIRANDA STATE, VENEZUELA.

| | Average Duration (d) | | Average Weight (mg) | |
|---|---|---|---|---|
| Phase | N | (Mean ± SD[1]) | n | (Mean ± SD)[2] |
| PUPA | | | | |
| Emerged | 383 | 12.78 ± 2.5 | | |
| ♂ | 196 | 13.35 ± 2.2 | 10 | 37.27 ± 5.5 |
| ♀ | 187 | 12.18 ± 2.4 | 10 | 63.68 ± 6.3 |
| ADULT | | | | |
| ♂ | 138 | 5.10 ± 1.96 | — | — |
| ♀ | 121 | 4.39 ± 1.57 | — | — |

[1]SD, Standard deviation.
[2]Weight of pupae with significant differences between the sexes (Student's *t*-test, $t = 10.26$; $df = 18$; $P < 0.001$).

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Adult Emergence, Behavior, and Diel Periodicity

In *C. theobromae*, male and female emergence starts between 0730 and 0800 h, and continues until 1530 h (Fig. 1). The highest emergence peak occurs at 0800 h for both sexes. Two lesser peaks were also observed for both sexes at 1100 and 1300 h. A total of 509 males and 419 females emerged representing a sex ratio of 1.2:1 (males: females). The emergence of both sexes showed a significant diel periodicity with Watson's $U^2$ values of 19.926 and 16.739 ($P < 0.005$) for males and females, respectively (Table 3).

Like most Sessidae, this fruit borer is diurnal (Scoble 1992). The activity peak is at 1730 h for males, and 1300 h for females, which falls off as the scotophase is reached (Fig. 2). Additionally, Watson's $U^2$ values were significant (Table 3), indicating a diel periodicity for the behaviors analyzed in both males and females. Calling behavior is performed by the female and consists in the typical elevation and dorsal curvature of the abdomen, accompanied by the protrusion of a cylindrical-tubular telescope-like structure from the last abdominal segments. This behavior is similar to that described for the females of several other species including: *Plodia interpunctella* (Hübner) and *Ephestia cautella* (Walker) (Pyralidae) (Coffelt et al. 1978), *Manduca sexta* (Linnaeus) (Sphingidae) (Hagaki & Conner 1988), and *H. metabus* (Saturniidae) (Fornés & Hernández 2000).

Females exhibited calling behavior between 1430 and 1930 h, with most females calling at 1730 h. This coincided with the period of greatest flight, walking, or wing fluttering in males, and the angular-angular correlation coefficient was significant ($r = 0.92$; $n = 14$; $P < 0.05$).

During scotophase individuals of both sexes were inactive and resting. Only 1 mating was observed in a chamber that contained 3 males and 2 females. Mating behavior in the moth *Hylesia nanus* (Lepidoptera: Saturniidae), has been reported to only occur under certain sex densities (Santos et al. 1988). We tested this phenomenon by using variable sex densities in the observation chambers, but mating activity was not observed. Calling behavior occurs during sunset, and mating behavior could occur at the same time. Thus, it is possible that the light gradient is the main factor affecting this behavior. It will be very interesting to test this hypothesis.

Our results indicate that *C. theobromae* females emit a sex pheromone during calling, and that males actively respond to this semiochemical.

A similar pattern throughout adult life as regards the diel periodicity of flight, walking, or wing fluttering in these insects is shown in Fig. 3A. Males were always more active between 1630 and 1900 h when females were least active in flight or movement, or when they performed calling behavior (Fig. 3B). The circular analysis confirms the existence of statistically significant differences in the diel periodicity of insect behavior during the first 3 d of evaluation (Table 3). Additionally, the behaviors observed diminished considerably with the age of the insects. For example, 51.5% of females were observed calling during the

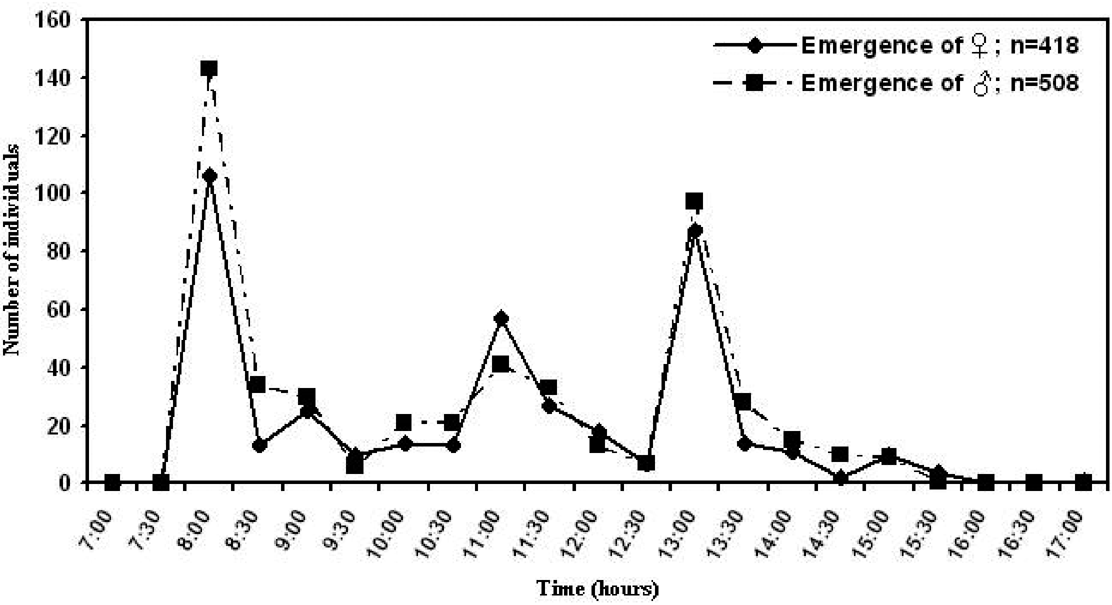


Fig. 1. Daily emergence of *C. theobromae* adults collected from the Barlovento region, Miranda state, Venezuela.

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TABLE 3. CIRCULAR DISTRIBUTION ANALYSIS FOR THE DETERMINATION OF THE DIEL PERIODICITY OF BEHAVIORS OF *C. THEOBROMAE* ADULT MALES AND FEMALES.

| | Parameters | Circular Distribution Analysis | |
|---|---|---|---|
| Variable | *n*[1] | $U^2$[2] | *P*[3] |
| Emergence ♂ | 508 | 19.926 | <0.005 |
| Emergence ♀ | 418 | 16.739 | <0.005 |
| Flight, walking or wing fluttering ♂ (Fig. 2) | 117 | 11.694 | <0.005 |
| Flight, walking or wing fluttering ♀ | 114 | 10.379 | <0.005 |
| Calling ♀ (Fig. 2) | 114 | 5.954 | <0.005 |
| Calling ♀ (Fig. 3) | | | |
| Day 1 | 33 | 8.457 | <0.005 |
| Day 2 | 33 | 9.952 | <0.005 |
| Day 3 | 33 | 6.012 | <0.005 |

[1]*n*: number of individuals in each activity.
[2]Watson's $U^2$ goodness of fit test for one sample.
[3]Probability.

first day, this increased slightly to 57.6% on the second day, but then decreased to 30.3 and 10.6%, on d 3 and d 4, respectively, (Fig. 3B).

Dogwood borer females, maintained at a constant temperature of 25°C, a photoperiod 16:8 (L:D) and 69% RH, showed an emergence peak between 0600 and 0700 h. Calling behavior started between 1900 and 2100 h (start of the scotophase at 2200 h), and peaked at 2100 h with the calling period lasting an average of 1.4 h on the first day, and 2.4 h on the second day after emergence (Bergh et al. 2006). In *Thaumetopoea pityocampa*, calling behavior occurs from the end of the photophase to the end of the scotophase, with an average duration of 6.4 h, when subjected to a natural photoperiod 14:10 (L:D), registered between sun-

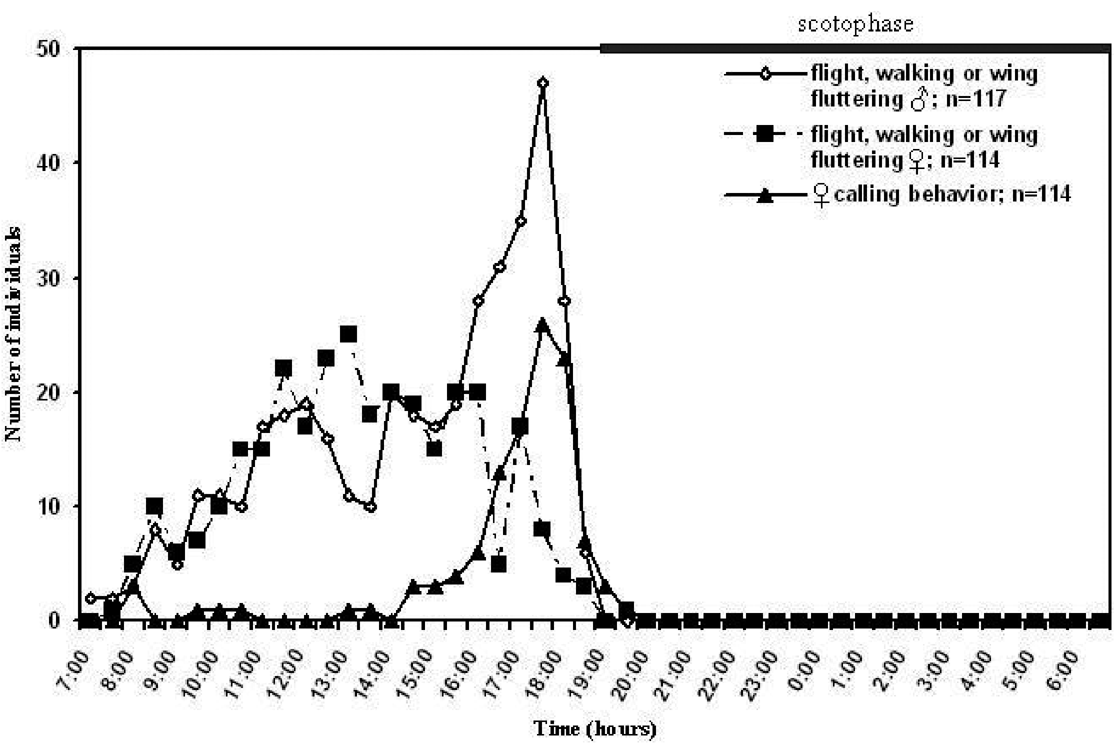


Fig. 2. Ethogram of *C. theobromae* adults, collected from the Barlovento region, Miranda state, Venezuela. Photoperiod 12:12 (L:D). Start of scotophase: 1900 h.

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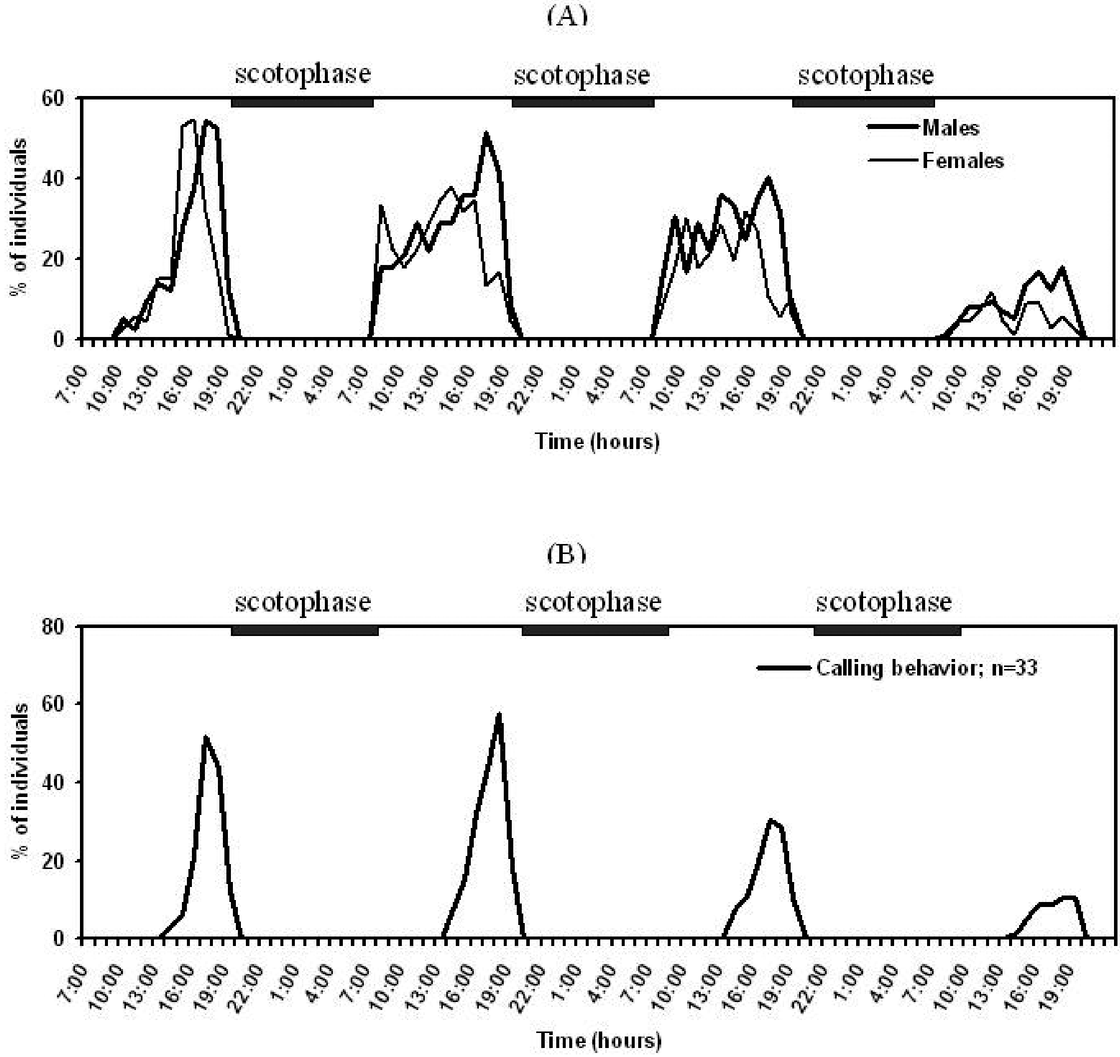


Fig. 3. Diel periodicity of the behavior of *C. theobromae* adults under laboratory conditions. (A), flight, walking or wing fluttering in males and females. (B), female calling behavior

rise at 0645 h and sunset at 2035 h (Zhang & Paiva 1998). In *Lymantria dispar*, maintained at 17°C with a photoperiod of 16:8 (L:D), calling behavior lasts on average 16.5 h, and when females are submitted to constant illumination, calling is continuous (Webster & Yin, 1997; Zhang & Paiva, 1998).

In this study, the results obtained for *C. theobromae* are somewhat similar to those reported by Bergh et al. (2006) for the sesid *S. scitula* in that adults emerged during the morning, and female calling behavior peaked at the end of the photophase, but differs considerably from the behavior patterns observed for *T. pityocampa* and *L. dispar*, both of which belong to different families.

In conclusion, the pupal phase in *C. theobromae* has an average duration of 12.78 d. Adult males, with longevity of 5.10 d, live longer than females. Male and female adult emergence, flight, walking, wing fluttering behaviors, and calling behavior in females all show a marked diel periodicity. Female calling behavior takes place over 5 h with a peak at 1730 h. This characteristic calling behavior in the females, together with simultaneous and more intense flight, walking, or wing fluttering activity in males, indicates that *C. theobromae* females are responsible for the synthesis and release of the sex pheromone in this species. Calling behavior starts on the first day and peaks on the second day of adult emergence, and ex-

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tends until the fourth day, but with a reduced number of females engaged in this activity. The results obtained in this study will facilitate the design of experiments for the isolation and identification of the components of the sex pheromone produced by *C. theobromae* females, and investigations into its potential use as a future control approach for this pest.

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