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EVIDENCE OF A SEX PHEROMONE AND DAILY CALLING PATTERN OF FEMALES OF *ZAMAGIRIA DIXOLOPHELLA* (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT

Evidence of a sex pheromone released by sapodilla bud borer, *Zamagiria dioxolophella* Dyar (Lepidoptera: Pyralidae), females was obtained under field conditions. Delta type traps baited with 2-d-old virgin females captured wild males in a sapodilla (*Manilkara zapota* (L.) van Royen) plantation. Once the existence of the sex pheromone was demonstrated, the following study was undertaken to describe calling behavior of *Z. dioxolophella* under laboratory conditions. The calling position observed in this moth is similar to that reported for other species of this subfamily. A single period of calling was observed. The calling observed is of the continuous type and was observed only during scotophase. The females initiated calling when 1-d-old. The maximum number of insects calling was observed in 2-3-d-old females between the fourth and eighth h of scotophase. Calling in these females is controlled by a circadian rhythm. The results are discussed in view of the importance of later studies related to the identification of the sex pheromone.

Key Words: *Zamagiria dioxolophella*, calling behavior, sex pheromone

RESUMEN

La evidencia de una feromona sexual liberada por las hembras del barrenador de los brotes del chicozapote, *Zamagiria dioxolophella* Dyar (Lepidoptera: Pyralidae) fue obtenida en condiciones de campo. Trampas tipo delta con dos hembras vírgenes de dos días de edad como atrayentes, capturaron machos silvestres en un rancho de chicozapote (*Manilkara zapota* (L.) van Royen). Una vez demostrada la existencia de dicha feromona, el siguiente paso fue estudiar el comportamiento de llamado de *Z. dioxolophella* en condiciones de laboratorio. La posición de llamado observada en esta palomilla es similar a la reportada para otras especies de esta subfamilia. Un solo periodo de llamado fue observado. El tipo de llamado observado es del tipo continuo y fue observado solamente durante el periodo de oscuridad. Las hembras llamaron desde el primer día de edad. El pico máximo de llamado fue observado de la cuarta a la octava hora de oscuridad en hembras de 2 a 3 días de edad. El llamado en estas hembras está controlado por un ritmo circadiano. Se discuten los resultados en base a la importancia de conocer el comportamiento de llamado para estudios posteriores relacionados con la identificación de la feromona sexual.

Translation provided by the author.

Zamagiria dioxolophella Dyar (Lepidoptera: Pyralidae), the sapodilla bud borer, is found in Mexico, Central America, and possibly South America (Heinrich 1956; Iruegas et al. 2002). Sapodilla fruit in the Soconusco region of Chiapas are attacked by this moth, which is considered an important pest by local producers (Iruegas et al. 2002). The females lay eggs on the sapodilla branch shoots and the larvae feed on the shoots, flower petals, and occasionally on the fruits. Infestation persists throughout the year although the population peak coincides with the peak sapodilla flowering period between Mar and Jun (Iruegas et al. 2002). Currently, control of this species

is based on the use of insecticides. However, chemical control of this species has proved to be difficult due to the cryptic nature of the moth and the fact that no effective method exists to detect the first infestations of this pest, which would facilitate directed applications.

Sexual pheromones have proved to be effective in monitoring programs and, in a few cases, in mating interruption (Walton et al. 2004; Cardé 1990). A sex pheromone has been identified in various species in the Phycitinae subfamily (Millar et al. 1996; Teal et al. 1995; Cork et al. 1991; Zagatti et al. 1991) to which *Z. dioxolophella* belongs, but it is unknown whether the sexual be-

havior of this moth species is mediated by a pheromone. In this study I present evidence of the existence of a sexual pheromone released by female *Z. dioxolophella*. Under laboratory conditions, the calling position, daily calling pattern, calling periodicity during each dark phase and the calling endogenous circadian rhythm is described.

MATERIALS AND METHODS

Zamagiria dioxolophella larvae were collected in sapodilla trees *Manilkara zapota* van Royen, in the following orchards: "El Nayar" (14°49'36"N and 92°20'52"W at 44 masl) and "Cazanares" (14°44'40"N and 92°24'20"W at 20 masl), both located in the municipality of Tapachula, Chiapas, Mexico. In the laboratory, the larvae were held until pupation in 3-L clear plastic cylindrical containers (23 cm height × 14 cm diameter), and allowed to feed upon their host plant (tender young shoots) in controlled conditions at 25 ± 5°C and 65 ± 5% R H with a reversed photoperiod of 16: 8 h (L: D). The photophase was from 17:00 h to 09:00 h and the scotophase from 09:00 h to 17:00 h. Pupae obtained were placed in groups in Petri dishes inside plastic cages (30 × 30 cm) and observed hourly 1 d before emergence. Adults eclosing during the photophase were separated according to sex immediately after emergence.

Field Evidence of a Sexual Pheromone

The experiment was performed in the "El Nayar" plantation which has 30 ha of sapodilla trees aged between 12 and 15 years, although only 1 hectare was used in this study. Four white delta traps (Pherotech, Delta, BC, Canada) (20 × 21 cm wide and 10.5 cm high) were used. Two 2-d-old virgin females, enclosed in a small wire mesh cage, were placed inside each trap. A drop of natural honey was provided as food *ad libitum*. The bases of the traps were impregnated with glue (Tanglefoot, EUA). A trap without females acted as the control. The traps were hung from the branches at the outermost point of the tree crown at a height of between 2 and 2.5 m. The distance between each trap was approximately 200 m. Traps were revised and rotated daily and the number of captured males recorded; the females and bases with glue were replaced. The experiment was repeated 2 times, from the 10-13 May and from the 13-16 May, each test lasting 3 d. In the second test the traps and control were re-randomized.

Daily Calling Pattern

Twenty recently emerged virgin females were individually placed in the cylindrical containers described above. A fresh, tender young host plant shoot (approximately 15 cm long) with leaves and

flowers was inserted in a plastic vial with cotton soaked in water and placed in each container. The host plant was changed daily after each scotophase. The opening of the containers was covered with gauze to permit circulation of air. A drop of natural honey was placed daily on gauze to ensure that females had food *ad libitum*. The observations began during the first scotophase after emergence and were carried out under the same conditions described for larval maintenance.

The observations undertaken for the calling position indicated that this occurs during the scotophase. Consequently the females were observed every 10 min throughout their first 6 scotophases under a red lamp and in absence of males. If a female called during 2 consecutive observations this was regarded as if the female had called for 20 min, if a female called during only 1 of the 2 observations this was taken as a 10 min calling period (Turgeon & McNeil 1982). The recorded parameters in these observations were, as follows: female age when calling was initiated (scotophases after emergence), the percentage of females calling daily, the daily onset of calling time (time after lights off), number of times that each female called each night and duration of calling of each female. In order to determine if a relationship existed between female age and the time when calling behavior began, a linear regression analysis was conducted with the "Statistica" version 6 (StatSof, 2003) statistical package, after the data were transformed (ln) because there was lack of homogeneity of variance.

Endogenous Calling Periodicity

Pupae were collected from the plantations described above 1-2 d before the experiments and maintained in the laboratory with the same photoperiod as natural conditions (13L: 11D), at 25 ± 5°C and 65 ± 5% R H. Twenty recently emerged females were placed in the cylindrical containers as in the calling daily pattern experiment and observed on the first night to quantify the percentage of females calling. All 20 females were then maintained under continuous light for 5 more d. The females were observed every 30 min for evidence of calling behavior. When calling behavior was observed, they were checked at 10-min intervals until calling behavior ceased. The percentage of females calling, time, and duration of calling of each female were recorded.

RESULTS

Field Evidence of a Sexual Pheromone

In the first experiment performed 10-13 May, a total of 34 males of *Z. dioxolophella* were captured with 4 traps. In the second experiment, carried out 13-16 May, 12 males were captured. Traps without females had no captures.

Calling Position

The sexual calling position in *Z. dioxolophella* females consists of dorsally flexing the abdomen at an approximately 45° angle and then protruding the ovipositor to expose the putative pheromone gland located in the ventral part of the last abdominal segments. Wings were maintained at the sides of the abdomen, lightly touching the surface, when the females were resting. No wing vibration was observed. The antennae were constantly in motion. Once the calling position was adopted it was maintained until calling was over.

Daily Calling Pattern

Female calling was initiated on the first night after emergence (85%). The maximum calling peak was observed on the second and third night, with 95% of the females calling in both cases, and the number calling diminished on the fourth and sixth nights (Fig. 1). There was considerable calling pattern variation regarding how long individual females called each night. The mean calling period duration in the 20 individually caged females throughout 6 d was 303 ± 7 min (mean \pm SE) (range of 10 to 400 min) (Fig. 2). Females showed just 1 calling period. The calling behavior observed corresponds to the continuous type and occurred only during the scotophase (Fig. 2). One to 2-d-old females initiated calling activity 4 h after turning off the lights. The daily onset of calling showed a significant positive correlation with age ($n = 106$; $df = 1, 104$; $F = 20.05$; $P = 0.001$; $R^2 = 0.16$) (Fig. 3). The oldest females called earlier, 3-6-d-old females calling 1 h earlier on average. The mean calling duration for these females was 6 h while the youngest called for a mean of 4.5 h (Fig. 4). The highest percentage of calling was observed between the third and eighth h of scotophase in 3-6-d-old females. The mean calling duration was clearly influenced by age. One-d-old females called for less time (234 min) than 5-d-old females (335 min).

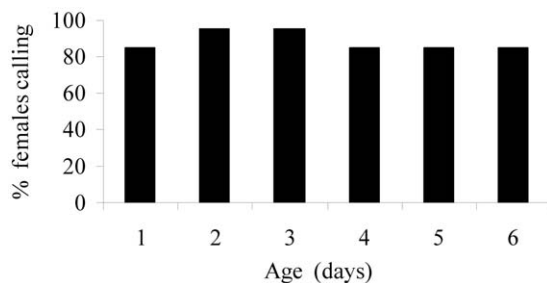


Fig. 1. Percentage of *Z. dioxolophella* females calling daily during the 6 nights of the experiment exposed to a photoperiod of 16 L: 8 D at 25°C and $60 \pm 5\%$ RH ($n = 20$).

Endogenous Calling Periodicity

Maintaining females to a 13L: 11D photoperiod until the first night and then placing them in continuous light affected calling behavior. On the first night after emergence the percentage calling was 95%, but when placed under continuous light this percentage decreased from the second until the sixth day with the exception of the fifth day where there was a small increase. However, the females called every 24 h during continuous light, presenting a circadian calling rhythm (Fig. 5).

DISCUSSION

The existence of a sexual pheromone released by *Z. dioxolophella* females attractive to conspecific males was demonstrated with the capture in the field of wild males by caged 2-d-old virgin females. In the majority of moth species, females adopt a specific body position, known as the calling position, to release the sexual pheromone. This behavior varies between Lepidoptera species in the characteristic position of the abdomen, wings, antennae, legs and other body parts (Mozuraitis 2000). The calling position observed in *Z. dioxolophella* is characteristic of the Phycitinae subfamily (Richards & Thomson 1932). The extension of the abdomen upwards in order to expose the cuticle of the pheromone gland is the main characteristic of the calling position in this moth species. The calling position in Phycitinae females is very similar to that described for Phylonorictidae females (Mozuraitis 2000) only differing in that in the former case the antennae are raised and continue moving whereas in the latter case they are placed alongside the wings and are motionless. Only 1 calling position was observed in this study while in other species such as *Diatrea considerata* Heinrich (Osorio & Cibrián 2000) 2 different positions were observed.

The daily individual calling pattern of *Z. dioxolophella* females varied considerably regarding calling duration time in contrast to the number of calling periods whereby all females presented just 1 calling period. In this study, *Z. dioxolophella* females initiated calling on the first night after emergence from the pupae. Similar results were described for *D. abietella* females (Fatzinger & Asher 1971). Other species such as *P. unionalis* (Mazomenos et al. 2002) called from the second night after emergence onwards. Studies carried out on different Pyralidae species have established that a correlation exists between ovary development and calling behavior (Swier et al. 1976) and that females which call on the first night after emergence are reproductively mature (West et al. 1984). Matthews & Matthews (1988), mention that insects which mate quickly after emergence generally have a short life span. Our laboratory results on calling have to be corroborated.

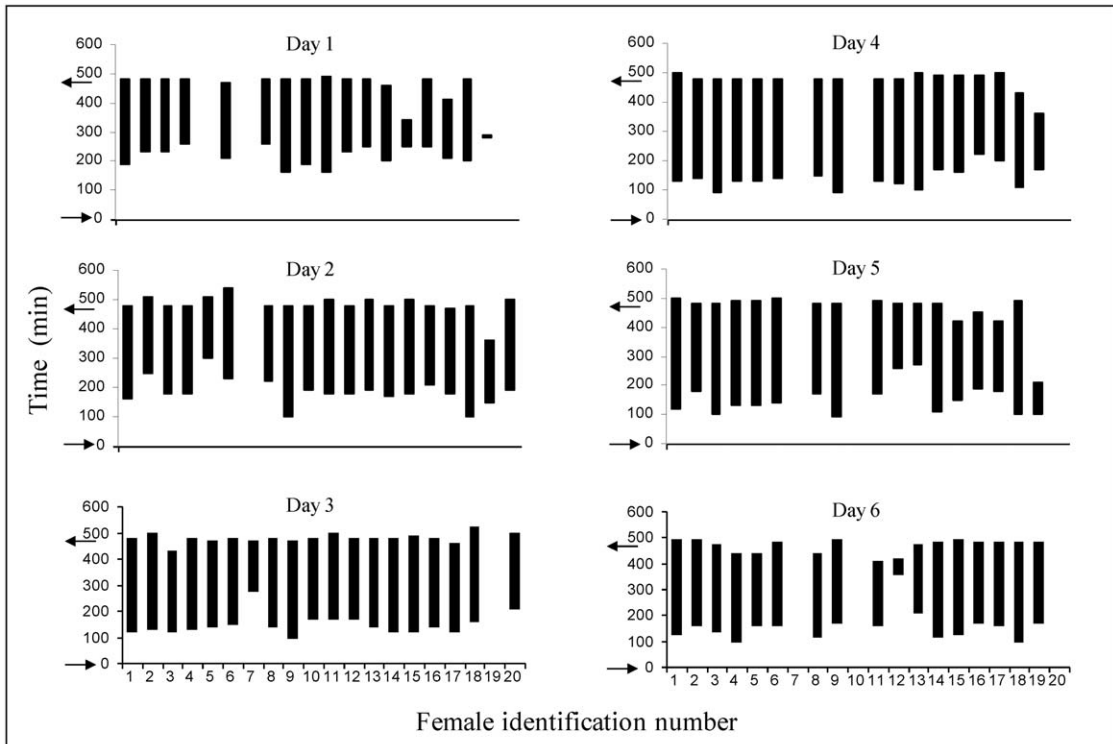


Fig. 2. Daily calling pattern of individually caged 1-6-d-old *Z. dioxolophella* virgin females. Each number represents an individual female. The arrows indicate the start (inwards) and end (outwards) of the scotophase ($n = 20$).

rated in the field. In our study, the maximum calling peak was observed on the second and third night in contrast to *P. unionalis*, in which this occurred on the fourth night (Mazomenos et al. 2002). In both cases, this behavior decreased after

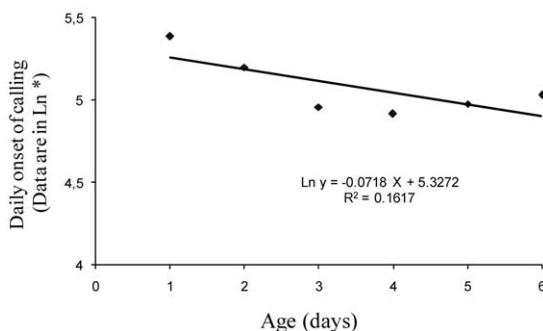


Fig. 3. Relationship between *Z. dioxolophella* female age and the daily onset of calling time under laboratory conditions ($n = 106$; $df = 1, 104$; $F = 20.05$; $P = 0.001$; $R^2 = 0.16$). The averages of each day are displayed. *The data are in Ln values. The corresponding values are: d 1 (Ln of 218 min = 5.38 ± 0.04), d 2 (Ln of 181 min = 5.19 ± 0.06), d 3 (Ln of 142 min = 4.95 ± 0.05), d 4 (Ln of 136 min = 4.91 ± 0.06), d 5 (Ln of 145 min = 4.97 ± 0.07), d 6 (Ln of 153 min = 5.02 ± 0.07) ($n = 20$).

the maximum peak. Similar results have been reported in species not belonging to the Pyralidae family, for example *Mamestra configurata* Walker (Howlader & Gerber 1986) and *Heliothis zea* (Boddie) (Raina et al. 1986). As in our study, the older *P. unionalis* females called earlier (Mazomenos et al. 2002). Howlader (1985) established that in *M. convecta* both daily onset of calling and calling duration varied with age in females. Some *Z. dioxolophella* females continued calling several min after the light photoperiod was initiated. Prolonged calling in the oldest females may be partly due to isolation of the females (Lawrence & Bartell 1972) and/or attributable to the fact that under laboratory conditions the lights are abruptly turned on and off with no gradual increase or decrease in light as in natural conditions.

In *Z. dioxolophella*, the calling circadian rhythm is endogenously based, demonstrated by the fact that the females called at 24-h intervals over 5 d under continuous light after having called during 1 night in a normal photoperiod. This has also been reported for other moths belonging to this subfamily such as *Anagasta kuhniella* Zeller (Traynier 1970), although in that species the moths were kept in continual darkness. In insects, the circadian rhythm is regulated

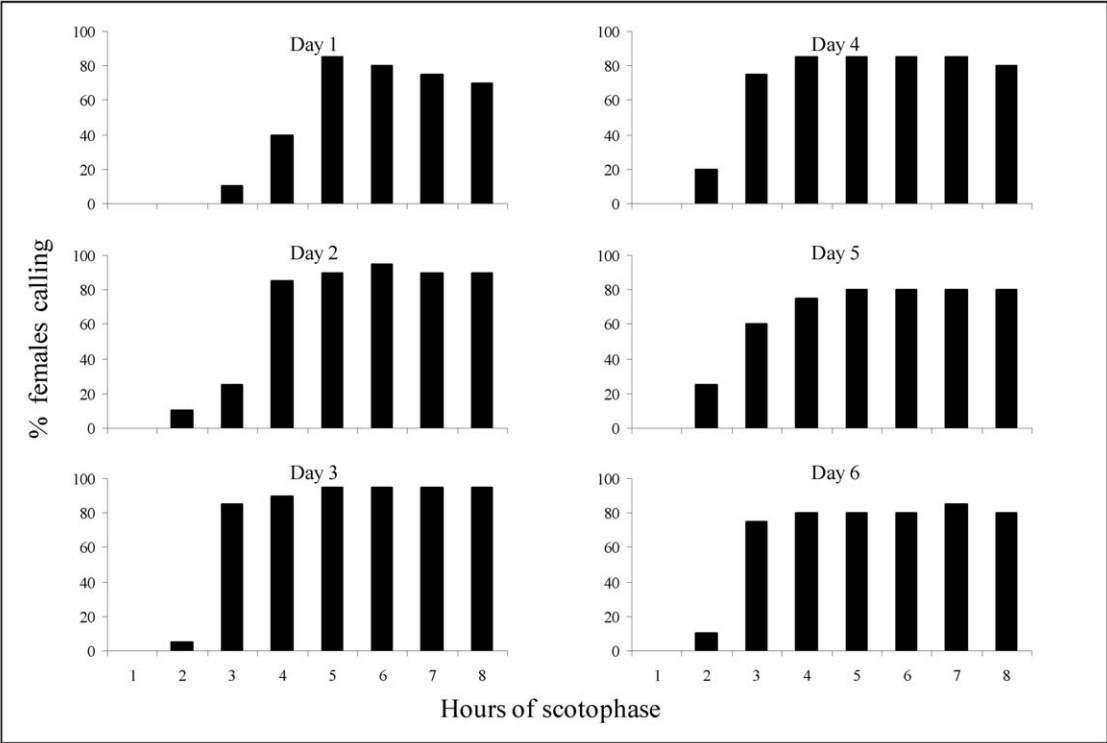


Fig. 4. Percentage of *Z. dioxolophella* virgin females calling each h during 8 h in scotophase. The females were observed during 6 consecutive scotophases ($n = 20$).

by specific neurons and peripheral tissue cells (Giebultowicz 2000), synchronizing the time of the day males search for a mate and thus increasing communication efficiency (Levine 2004).

The importance of knowing female age and calling time is due to the fact that in many moth species sexual pheromone release (calling) by females is correlated with the time of male maximum response, and in accordance with a daily rhythm regimen (Mazomenos et al. 2002 and cited authors; Raina & Menn 1987). Currently,

the sexual pheromone of *Z. dioxolophella* is being researched by using glandular extracts analyzed by gas chromatography coupled with mass spectrometry in 2-3-d-old females calling between the fourth and eighth h of darkness.

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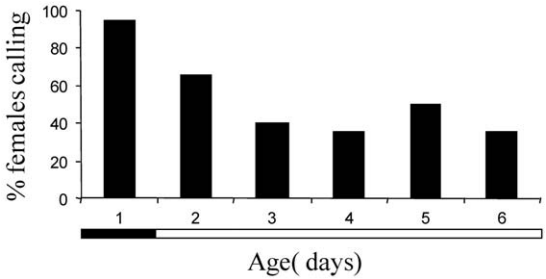


Fig. 5. Percentage of calling *Z. dioxolophella* females during 1 scotophase subject to 13L: 11D photoperiod (black bar) and then submitted to continual light for 5 d (white bar) ($n = 20$).

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