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SEXUAL BEHAVIOR AND DIEL ACTIVITY OF CITRUS FRUIT BORER *Ecdytolopha aurantiana*

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Abstract—Males and virgin females of the citrus fruit borer *Ecdytolopha aurantiana* Lima, displayed two flight peaks during a 24-hr period, one at dawn and the other at dusk in an orange grove near Gavião Peixoto, São Paulo, Brazil. During the day, when temperatures were highest and relative humidity lowest, most individuals rested on leaves in the lower and middle crown. Moths rapidly moved higher in the crown after sunset, and many were observed flying above the tree canopy. This behavior was mainly associated with mating. Males and virgin females marked with fluorescent powder of different colors were observed in the dark with the aid of a black light. Mating was observed only in the upper crown of citrus trees from 6:00 to 9:00 PM, with a peak (64%) between 7:00 and 8:00 PM. Males of *E. aurantiana* were captured in traps baited either with virgin females or female extracts, suggesting the use of a long-range sex pheromone. At close distance (1–2 cm), males and females displayed a short-range communication behavior, with males exposing hairpencils and vibrating their wings. Females were frequently stimulated to contact the body of a male before copulation. The mean duration of copulation was 1 hr 40 min.

Key Words—Sex pheromone, *Ecdytolopha aurantiana*, attraction, mating behavior, hairpencils, diel periodicity.

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INTRODUCTION

The citrus fruit borer *Ecdytolopha aurantiana* Lima (Lepidoptera: Tortricidae) is a multivoltine species of brown and gray coloration (Lima, 1927; Schultz, 1939, White, 1993). *E. aurantiana* occurs in most neotropical areas, including Brazil (Prates and Pinto, 1988, 1991), Argentina (Schultz, 1939), Costa Rica and Trinidad-Tobago (Pickles, 1936). The larvae of *E. aurantiana* feed on and destroy citrus fruit, guava, *Psidium guajava* L. (Lima, 1927); banana, *Musa acuminata* Colla; coconut, *Cocos nucifera* L. (Meyrick, 1931); cherimolia, *Annona cherimolia* Mill. (Schultz, 1939); litchi, *Lichi chinensis* Sonn. (Lima, 1945); macadamia, *Macadamia integrifolia* Maiden and Betche (White, 1993), and sugar apple (fruta-do-conde), *Annona squamosa* L. (Nakano and Soares, 1995).

E. aurantiana was first observed to cause damage to citrus trees in the State of São Paulo, southeast Brazil, in 1915 (Lima, 1927). By the mid-1980s, this pest had been found in 54 municipalities in the State of São Paulo (Prates and Pinto, 1995) and in 10 other Brazilian States, with consequent reduced citrus production in these regions (Prates and Pinto, 1988, 1991). Yield losses of up to 50% in infested areas were estimated to occur in the State of São Paulo (Garcia et al., 1998), and the latest crop losses throughout the country are estimated at US$ 50 million pr/yr (Anonymous, 2000).

E. aurantiana is a difficult pest to control. Females usually deposit only one egg per fruit, and lay 150–200 eggs during their life (Garcia, 1999). After hatching, larvae pierce the peel and penetrate inside the fruit, where they feed on the pulp (Fonseca, 1934). Once larvae are inside the fruit, their control is impossible, and the fruit becomes unfit for consumption.

The major difficulty in the management of *E. aurantiana* is the lack of an accurate method for monitoring populations. Different sampling methods based on counts of eggs and first-instar larvae on the fruits are costly and inaccurate (Garcia, 1999). A system for adult detection by means of pheromone-based trapping of males would be an alternative method to anticipate the occurrence of this pest, and to control it in a more effective and economic manner, but the mating behavior of *E. aurantiana* has not been previously studied. Our objective was to report the sexual behavior and daily activity pattern of males and virgin females of *E. aurantiana* on citrus trees in an attempt to set up a rational program for the control of this pest.

METHODS AND MATERIALS

Insects. Adults of *E. aurantiana* were maintained in the laboratory on an artificial diet developed by Garcia and Parra (1999) at $27 \pm 2°C$, $65 \pm 10\%$ relative humidity, and a 14D:10L photoregime. After emergence, the adults were sexed by the method of Garcia (1999) and transferred to wood cages ($25 \times 25 \times 25$ cm) covered with nylon mesh. Males and virgin females were kept in different cages, with 200–400 individuals per cage. The production of adults in the laboratory varied during the experiment, ranging from 200 to 400 pairs a day. A small portion of insects (ca. 10–20%) was used for continuous rearing in the laboratory.

Greenhouse Experiments. Experiment 1 was conducted to determine the time of day, duration, and insect age at first mating. For each experiment, 10 pairs of *E. aurantiana* were selected soon after emergence and individually placed into transparent plastic cups (200 ml) inverted on Petri dishes (12 cm in diameter). The insects were maintained in a greenhouse at $25 \pm 2°C$; $65 \pm 10\%$ relative humidity, and under natural light. Each Petri dish was lined with a filter paper of the same size, which was moistened daily with distilled water to keep the humidity high inside the plastic cup.

Preliminary assays showed that adults of *E. aurantiana* remained at rest during most of the day and the night, especially from 8:00 AM to 4:00 PM and from 10:00 PM to 5:00 AM. Thus, we observed pairs daily between 4:00 and 10:00 PM for seven days, with behavior recorded at 1-min intervals. To study mating behavior at night, we used a torchlight dimmed by three red sheets of cellophane paper and kept 1 m away from the subject. This experiment was replicated four times (i.e., 10 pairs with four replicates each; $N = 40$) in a completely randomized block design. Data (age at first mating and mating time) were transformed to percentage, and the differences between means were tested for significance by analysis of variance and compared by the Tukey test at 5% level. Tests were conducted on alternate days from November 5, 1998, to January 26, 1999.

Field Cage Experiments. Experiment 2 was designed to analyze the distribution and locomotion of males and virgin females of *E. aurantiana* during the day, as well as to determine the site of mating on the citrus trees. A $6 \times 25 \times 3.5$ m (150 m^2) nylon field cage was constructed, covering six orange trees (6 years old) of the Pêra variety, 2.5–3 m high, in a commercial orange grove located on the campus of Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), Piracicaba-SP, Brazil. The field cage was constructed in such a manner that the trees occupied its central line. Spacing between trees in the line was 4 m. Weeds present in the area covered by the field cage during the experiment were not eliminated.

Diel Distribution and Activity of Adult Moths. After emergence, males and females were sexed by the method of Garcia (1999) and maintained in cages, as previously described. At 2 days of age, males and females were marked with yellow and orange fluorescent powder, respectively. Insects were marked with ink diluted in ethyl alcohol, sprayed with a nasal spray, and dried with an electric fan at low speed (Southwood, 1995). Immediately after marking, the moths were released in the field cage at about 10:00 PM. On the following day, starting at 4:00 AM, observations were made for a period of 30 min at 1-hr intervals for 24 hr. During observations, the location and number of males and virgin females of *E. aurantiana* were recorded for: citrus trees, weeds, and soil and nylon netting.

When on citrus trees, moths were recorded on: leaves, fruit, and branches and trunk. The corresponding height on the tree was also considered: lower crown (0.5–1 m), middle crown (1.1–1.8 m), and upper crown (1.8–3 m). The sites of moth copulation and resting during day were marked with white plastic tape of ca. 20 cm. At night, the marked moths were visualized with the aid of a hand-held black light (model B-160 Spectroline). Movement of the moths was calculated as the difference between the individuals that moved from one place to another in relation to the previous hour. After each hour interval, males and virgin females of *E. aurantiana* were again localized and counted to compensate for individuals that had moved away or had been preyed upon. Therefore, it was possible to calculate the number of males and virgin females that stayed in the same place each hour. Preliminary studies with marked and unmarked adults in the greenhouse did not show any interference of the fluorescent powder with insect longevity or behavior, including mating. Three replicates of 24 hr each were performed, with a total of 250 pairs being released during a 24-hr period on February 9 and 24 and March 9, 1999. Temperature and relative humidity were measured with a thermohygrograph set up inside the field cage in the shade 1.0 m above ground during the assays. Official times of sunrise and sunset were provided by Instituto Astronômico e Geofísico da Universidade de São Paulo, São Paulo-SP, Brazil.

Female Attractiveness. Experiments 3–6 were designed to provide evidence for a long-range sex pheromone in *E. aurantiana*. Traps (stick Delta traps, Fuji Flavor Co., Tokyo, Japan) were baited with small cages (2.5 × 2.5 × 2 cm) containing two virgin females, two males, a rubber septum with 0.5 ml of female extract (25 female-equivalents), or a rubber septum with 0.5 ml solvent (control). The female extract was obtained on two consecutive days by cutting the last segments of the abdomen (segments VIII and IX) of 250 three-day-old virgin females and dipping (3-5 min) in hexane (5 ml). The time of the extractions was between 6:00 and 8:00 PM, coinciding with the mating time of *E. aurantiana*, previously observed in a greenhouse.

Experiments 3–6 were carried out in field cages, and tested virgin females versus empty trap (control) (experiment 3); virgin females versus males (experiment 4); virgin females versus female extract (experiment 5); and female extract versus solvent (control) (experiment 6). Each assay was carried out separately, and each treatment was replicated three times over two consecutive days in a completely randomized block design, with the treatments being renewed after 24 hr. Traps were placed in the upper crown of the tree (1.9–3 m) with an intertrap distance of 4 m. A total of 328, 290, 350, and 280 two-day-old virgin pairs were released between 4:00 and 5:00 PM on March 15, 22, and 29, and on April 5, 1999. Response to sex pheromone was quantified by counting the males captured in the traps each day. Capture data were transformed to log $(x + 1)$, and the differences between means were tested for significance by analysis of variance, with treatments being compared by the Tukey test at the 5% level of probability.

Attraction of Males to Pheromone Source in the Field. Experiment 7 was conducted in a 1200-ha commercial citrus grove (Ficher Co.) near Gavião Peixoto, SP, Brazil on April 7 and 8, 1999 to confirm the presence of sex pheromone. The experiment was conducted on the Hamlin orange variety, consisting of 12-to 15-year-old, 4-m-high trees spaced 4 m apart, with a 6-m space between rows. Delta sticky traps (Fuji Flavor Co., Tokyo, Japan) containing two virgin females, female extract (40 female-equivalents), and empty traps (control) were used. Each treatment was replicated four times over two consecutive days in a completely randomized block design, with the treatment being renewed after 24 hr. Each trap was placed in the upper third of the crown (2.5–3.5 m), with a 50-m space between traps and between rows and traps. The time when the experiment was started and the method for evaluation were the same as described for the field cage. Similarly, the natural extract of *E. aurantiana* was obtained as described previously, except that it was obtained on only one day from 400 virgin females using 5 ml of hexane. Capture data were transformed to log $(x + 1)$, and the differences between means were tested for significance by analysis of variance, with treatments being compared by Tukey test at the 5% level of probability.

RESULTS

Age, Time, and Duration of Mating. In a greenhouse, about 80% of the mating behavior of *E. aurantiana* was observed on the third and fourth days of life, corresponding to 44.7 and 36.8%, respectively (Figure 1A). Of the 40 pairs studied, 95% mated by the seventh day of life. Most mating behavior (60%) occurred between 7:00 and 8:00 PM, although the mating interval ranged from 5:00 to 9:00 PM (Figure 1B). The mean duration of copulation was 101.5 ± 62.1 min ($X \pm$ SEM; $N = 38$; range: 3–312 min).

Diel Distribution and Activities of Adult Moths. Field cage experiments showed that during the day most *E. aurantiana* moths were found in the lower and middle crown of citrus trees, with the presence of at least 34% and 38% of males, and 37% and 42% of virgin females, respectively (Table 1). This period was characterized by reduced flight activity with the moths usually resting on the surface of leaves, seemingly due to the high temperatures and low relative humidity (Figure 2). In the evening, about 88% and 75% of males and virgin females, respectively, were observed in the upper crown of citrus trees. This locomotion occurred between 6:00 and 7:00 PM, just after dusk (Figure 2). It was in the upper crown of the trees that all copulations took place (Table 1), peaking one hour after dusk, between 7:00 and 8:00 PM (64% of all episodes) (Figure 2).

During the night, after 8:00 PM, the locomotion and number of matings decreased quickly (ca. 8%; Figure 2). Most moths remained at rest, especially

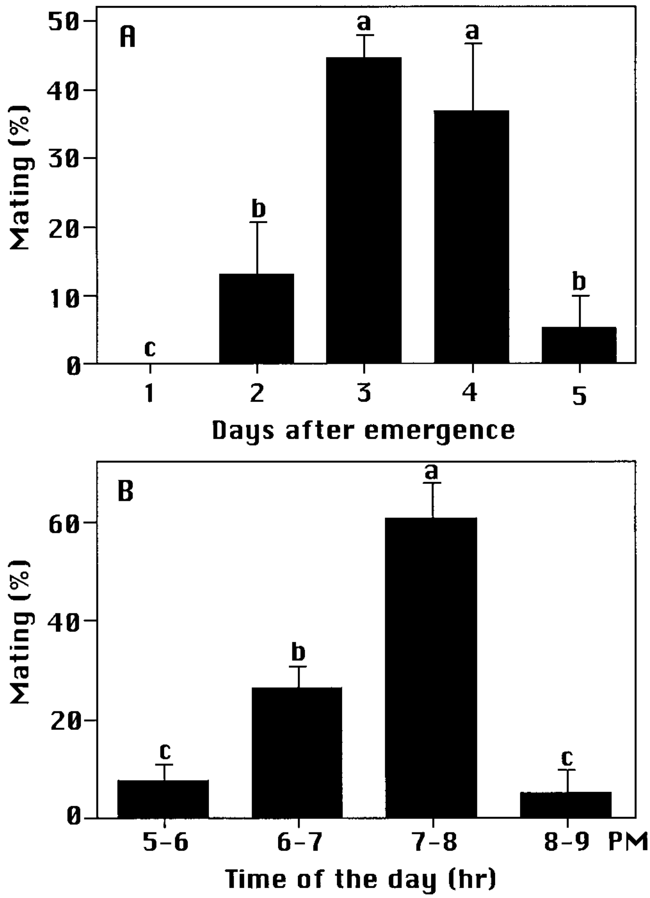

FIG. 1. Mating activities of *E. aurantiana* as function of age (A), and time (B), under greenhouse conditions. Bars with the same letter are not significantly different at the 5% level (Tukey test).

on leaves, occasionally engaging in walks and short flights. Many moths were observed flying for short distances after mating, although these data were not quantified. Adults were never observed feeding, although these data are not conclusive, requiring confirmation in future studies.

Just before dawn, a new peak of flight activity was recorded (Figure 2), and several moths were observed flying into the inner part of trees, especially towards the lower and middle crown of the host tree, reestablishing the same pattern of distribution as observed on the preceding day.

TABLE 1. DAY AND NIGHT DISTRIBUTIONS OF *E. aurantiana* IN CROWN OF CITRUS TREES UNDER FIELD CAGE CONDITIONS

		Height (m)		
Sex and status	Period	0.5–1.0	1.1–1.8	1.9–3.0
Male	Day	10.8 (34.7)[a]	12.1 (38.9)	8.2 (26.4)
	Night	0.5 (1.4)	3.7 (10.1)	32.3 (88.5)[b]
Virgin female	Day	12.2 (37.2)	14.0 (42.7)	6.6 (20.1)
	Night	1.0 (2.5)	8.8 (22.3)	29.7 (75.2)
Mating couples	Night[c]	0 (0)	0 (0)	22.5 (100)

[a] Percentages in parentheses.
[b] Includes individuals of mating couples.
[c] See Figure 2 for time.

Reduced numbers of males and virgin females were recorded on weeds or on the ground/screen during the experiment (Table 2). Although no counts were made, most moths found on the ground were weakened or dead.

Mating Behavior. Males of *E. aurantiana* hovering beside leaves with females in the upper crown of citrus trees were responding to female sex pheromone. Traps containing virgin females or a natural female extract in a solvent (25 female-equivalents) caught on average 8 and 6 male/trap/day in field cages (Figure 3). The same results were obtained in the field in a commercial area, with 13 and 8 males caught per trap per day in traps containing females and natural female extract in a solvent (40 female-equivalents), respectively (Figure 4).

DISCUSSION

The distribution (i.e., observed average number) and behavior of males and virgin females of *E. aurantiana* were synchronized and varied with respect to

TABLE 2. DISTRIBUTION OF *E. aurantiana* IN DIFFERENT PARTS OF CITRUS TREES OVER A 24-HR PERIOD

Place	Males	Virgin females	Total number (% mean)
Citrus			
Leaves	25.9 (88.7)	33.9 (90.8)	59.8 (89.7)
Fruits	0.7 (2.6)	0.4 (1.2)	1.1 (1.9)
Branches/trunk	0.5 (1.8)	0.8 (2.2)	1.3 (2.0)
Weeds	0.2 (0.7)	0.5 (1.4)	0.7 (1.1)
Soil/Netting	1.8 (6.2)	1.1 (4.4)	2.9 (5.3)

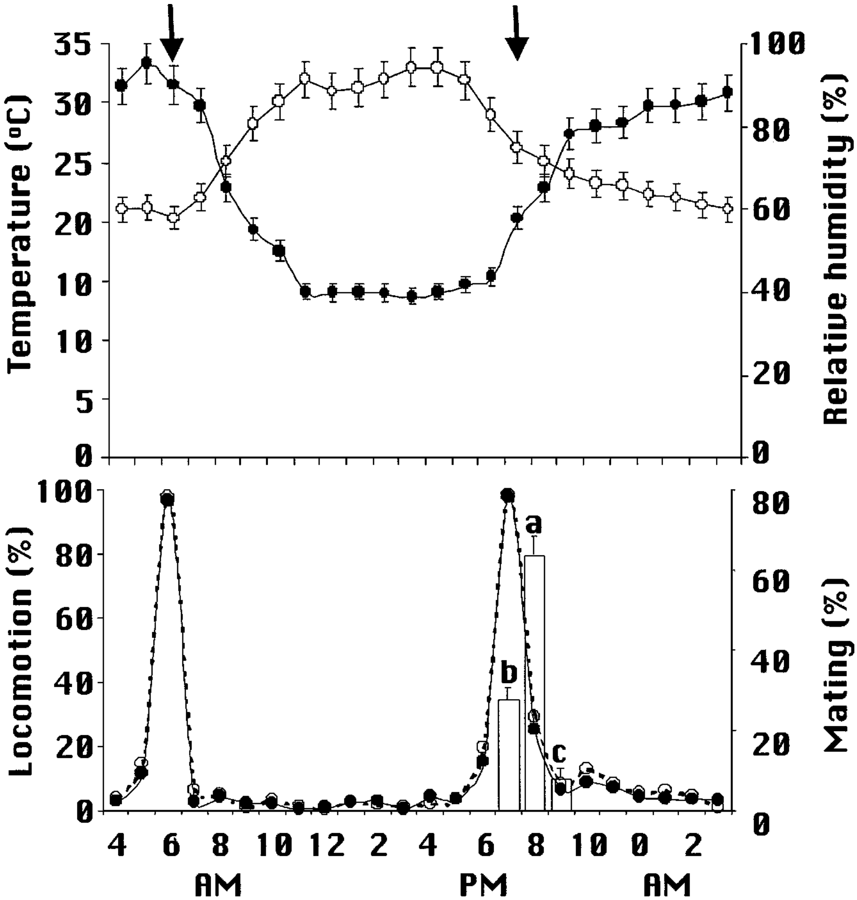

FIG. 2. Diel periodicity of locomotion and mating in adult *E. aurantiana* in relation to air temperature and relative humidity. (Top) Average temperature (—○—) and relative humidity (—●—) with SEM (bars); (Bottom) females (—○—) and males (—●—) locomotion. Arrows indicate time of sunrise and sunset. Bars with the same letter are not significantly different at the 5% level (Tukey test).

location in the crown of citrus trees and time of day. About 1 hr before dawn, many moths started to fly, and flight activity peaked just before dawn, as also reported for many other tortricids (Lewis and Taylor, 1964; Dreisig, 1986; Quiring, 1994). For *E. aurantiana*, the increase in flight activity during this period was not associated with mating or oviposition. Temperature increased quickly during the morning, and probably moth flight activity was due to the search for microhabitats with a reduced risk of hygrothermal stress. When temperature

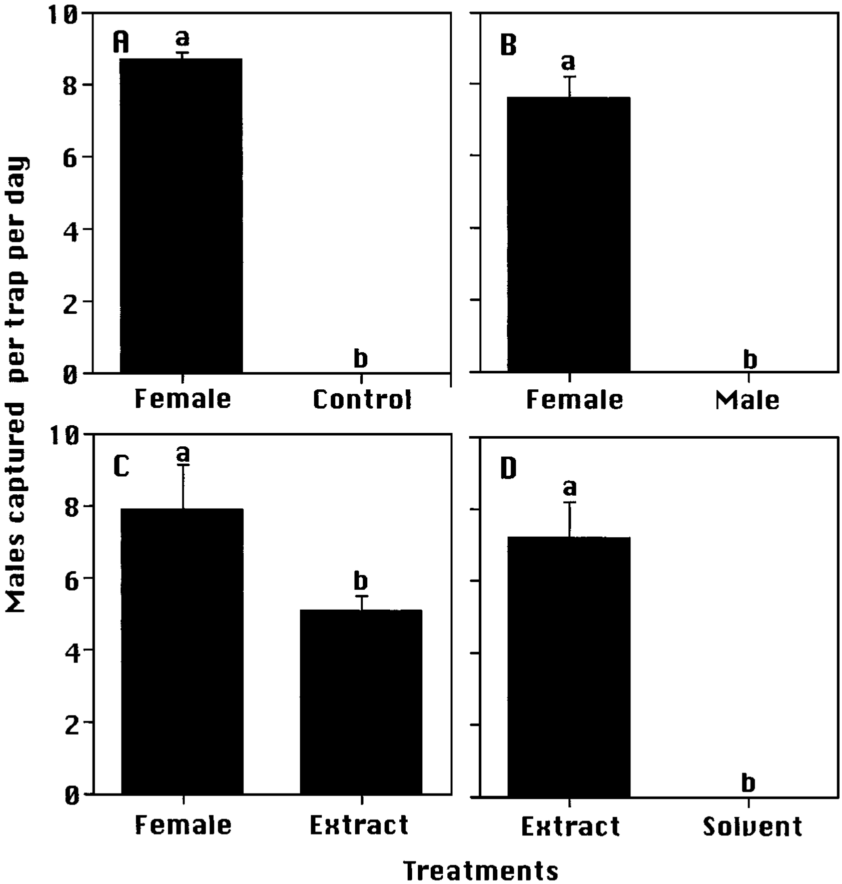

FIG. 3. Trap catches of male *E. aurantiana* in field cage experiments 3–6 baited with females (A), males (B), natural female extract in a solvent (C), or solvent alone (D). Bars with the same letter in each experiment are not significantly different at the 5% level (Tukey test).

was highest and relative humidity lowest, moths spent most of the day resting on leaves in the lower and middle crown of their host tree. Garcia (1999) reported that the viability and longevity of *E. aurantiana* is much reduced when the moth is exposed to temperatures above of 32°C and relative humidity below 50%.

During the day, some moths flew among the leaves of the tree or adjacent ones. However, these flights were sporadic and covered a short distance. Probably, they were motivated by the incidence of sunlight and the presence of different ant species and predators. Escaping predators may also explain the larger

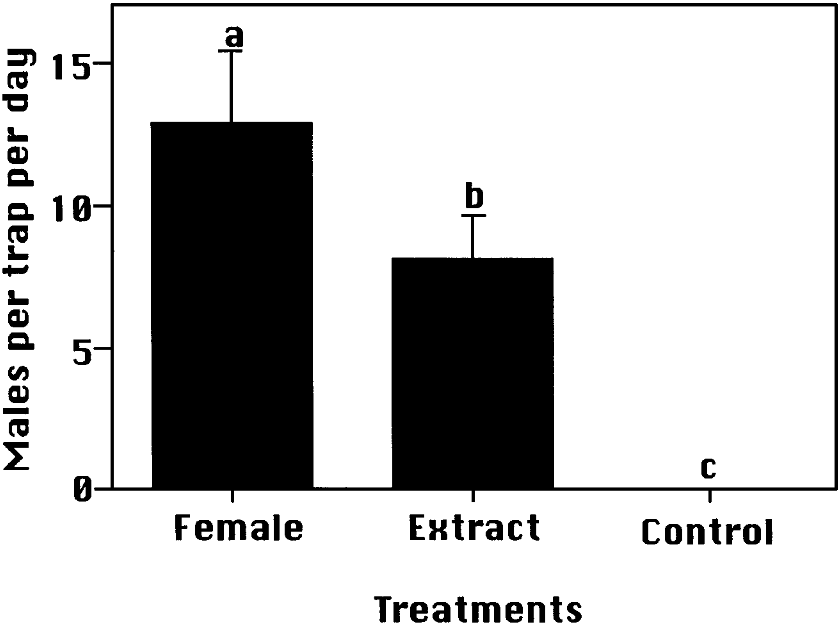

FIG. 4. Captures of male *E. aurantiana* in experiment 7 in traps baited with live virgin females, pheromone gland extract, or solvent controls. Bars with the same letter are not significantly different at the 5% level (Tukey test).

numbers of males and virgin females frequently found resting on leaves rather than on other parts of the trees. The large numbers of ants foraging on branches for nectar, oils, and honeydew throughout the day probably explain the escape behavior of the moths. Although this was not quantified, on several occasions various species of spiders also were observed preying on *E. aurantiana* adults on the leaves.

The second major peak of *E. aurantiana* activity started at dusk and continued for about 2 hr between 6:00 and 8:00 PM. Several moths were observed flying above the host tree canopy at dusk, as also reported for many other tortricids, an activity usually associated with oviposition and mating (see Pointing, 1961; Dustan, 1964; Turgeon, 1985; Turgeon et al., 1987; Quiring, 1994). Redfern and Di Giácomo (1991) and Garcia (1999) reported that oviposition of *E. aurantiana* is concentrated during the twilight period, with the latter author detecting about 60% of the eggs during this period.

In the present study, the flight activity of *E. aurantiana* in the evening was primarily associated with sexual behavior. In our experiment, we did not observe oviposition because we only used males and virgin females. Most of the mating activity occurred about 1 hr after dusk and only in the upper crown of the host tree. This is an original finding concerning tortricid moths since there are no previous studies describing the exact site of copulation on host trees (Howell, 1995). The fact that the mating site may be related to the calling ability of females, or more probably to a better release of sex pheromone by them, may explain the capture of males in traps placed in the upper crown of trees. It was not possible to determine why copulation pairs were not observed at other sites on the tree even after repeated observations, although a likely hypothesis is that staying on the top of the tree represents an adaptive advantage. In addition, capture of tortricid moths with fermented products (Yothers, 1927), pheromone baited traps (Lewis and Macaulay, 1976; Riedl et al., 1979; McNally and Barnes, 1981; Ahmad and Al-Gharbawi, 1986; Weissling and Knight, 1995), and light traps (Borden, 1931; Hamilton and Steiner, 1939) has been reported to be more effective when the traps are set on tree tops.

The presence of males close to virgin females just before mating was due to long-distance attraction elicited by a female sex pheromone. Observations in greenhouses suggest that visual or short-range communication signals may play a role. Usually, males approached females within a distance of 1–2 cm, then presented a stereotyped sequence of behaviors, including vibration of the wings and hairpencil display toward the female, which frequently attracted the female towards them. The female then contacted the body or the end of the abdomen of the male, and this stimulus caused the male to turn quickly and attempt copulation. When copulation was successful, male and female remained attached through the abdomen in opposite direction for ca. 1 hr and 40 min. A similar courtship was described for the oriental fruit moth *Grapholita molesta* (Busck) (Baker and Cardé, 1979). On the other hand, mean copulation time varies widely among tortricids. Copulation time is 40–60 min in *Cydia pomonella* (L.) (Howell, 1995) and about 29 min in *G. molesta* (Dustan, 1964), while in *Zeiraphera canadensis* Mut. & Free, it is 4 hr and 20 min (Turgeon et al., 1987).

In summary, this study determined the time when mating takes place, and thus, when pheromone glands should be extracted for pheromone identification. In addition, observations that mating occurs on the top of trees from 6:00 to 9:00 PM are of importance not only for the design of trap experiments, but also for future utilization of sex pheromones for monitoring citrus fruit borer populations.

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