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REPRODUCTIVE BEHAVIOR OF THE FEMALE CAROB MOTH, (LEPIDOPTERA: PYRALIDAE)

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Abstract.—Periodicities of the female reproductive behavior of the carob moth, *Ectomyelois ceratoniae* (Zeller), were investigated in regard to calling, mating, and oviposition. Under varying photoperiods (16:8, 14:10, 12:12 L:D h), female carob moths initiated calling about the midpoint of the scotophase to which they were entrained resulting in a shift to later mean initiation times as the nocturnal period lengthened. Matings were initiated during the fifth and sixth h of scotophase in a 16:8 L:D h light regime; this corresponded with the calling periodicity. Carob moth females laid significantly more eggs in the first hour of scotophase (16:8 L:D h) than in any other hour, after which oviposition declined significantly. Oviposition was greatest from the third through sixth scotophase after which it decreased. Oviposition periodicity was developed by the third scotophase, and peaked during the fourth.

Key Words.—Insecta, Pheromone Behavior, Mating Periodicity, *Ectomyelois ceratoniae*

The carob moth, *Ectomyelois ceratoniae* (Zeller), has occasionally been found in the southern United States. This species was most likely introduced from the Middle East where it is a pest of dates, almonds and pomegranates and was first noticed in California in 1982 (Eichlin 1982). It has since become a serious pest of dates in the Coachella Valley in southern California (Warner 1988, Warner et al. 1990a, b) and is of concern to growers as fewer insecticides are available for controlling this pest. In addition, there is concern that the carob moth may spread northward and threaten the almond and walnut industries in California's Central Valley.

Little information is available regarding behavior of the carob moth. Research has been performed on the effects of abiotic factors on development and diapause (Cox 1976, 1979), however, most research has focused on applied aspects in relation to agricultural crop damage (see Gothilf 1984 and references therein). Due to the recent immigration of the carob moth in the U.S., studies have been initiated to develop semiochemical control of this insect: assessment of male responses to both female sex pheromone and a formate analog (Baker et al. 1991, Todd et al. 1992), and female responses to volatile date odors (Cossé et al. 1994). The goal of this study was to develop a knowledge of the reproductive behavior of the carob moth.

MATERIALS AND METHODS

Insects.—Moths were obtained from date (*Phoenix dactylifera* L.) gardens in the Coachella Valley, California (Lat. 30°30'N, Long. 116°W) in 1985 and maintained year-round in the laboratory for >6 yr with no infusion of wild insects. Larvae were reared on a wheat bran-honey diet (Finney & Brinkman 1967) sup-

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plemented with brewer's yeast and maintained in clear, 4-liter, screened-lid glass jars at 28 ± 2°C with a 16:8 L:D h photoperiod. Additional rearing methods were slightly modified from those of Strong et al. (1968). For the calling and mating studies, pupae were separated by sex and placed in moistened vermiculite-filled cups inside of screen cages (30 by 30 by 30 cm) at the light cycles under which the adult moths were eventually tested. Cups of pupae were removed daily to an empty cage, leaving behind moths of known age. When 3 differing light cycles were used in the calling experiment, pupae harvested each day were separated into three groups with one group set up in each light regime. In the mating experiment, male and female pupae were placed in separate environmental chambers and allowed to emerge. For the oviposition study, pupae were not separated by sex but were otherwise treated as above. All moths were maintained at 23 ± 2° C throughout the course of the study and supplied with 8% sugar water solution ad libitum.

Periodicity of Pheromone Calling.—Females were held under 16:8, 14:10 and 12:12 L:D h light cycles to investigate the periodicity of calling. Virgin females were individually placed into plastic, air-tight vials (70 mm by 33 mm) in the last hour of photophase; a 10 mm by 10 mm diam piece of moistened dental wick was added to each vial as a water source. Cohorts of females that had been adults for 1, 2, 3, 4 and 5 days were set up for each of the 16:8 and 14:10 L:D h cycles; a single cohort of Day 2 females was set up for the 12:12 L:D h cycle. In this experiment, the term "Day" is used to indicate a 24-h period starting with the first hr of scotophase; this is to avoid confusion between the ambiguous use of "day" for 24 h or for only its photoperiod. Insects labelled as "Day 2" were entering their 2nd complete scotophase as moths and therefore were 25–48 h old. Each cohort consisted of 30 females except for Day 1 for the 16:8 (n = 24) and 14:10 (n = 10) light cycles. Females were moved into a bioassay room that was illuminated by dimmed white and red incandescent lighting (combined lighting level = 0.3 lux). Females were checked for calling (i.e., visible extrusion of the ovipositor/sex pheromone gland and hence, presumed sex pheromone emission) every hr during the scotophase. Observations were made using a flashlight covered by several pieces of red cellophane. This light did not appear to alter the females' behavior. Observations in photophase were made at 2 h intervals until the next scotophase at which point the bioassay was terminated. Data were omitted for any moth which died in the course of the experiment.

Periodicity of Mating Behavior.—As a correlate of the calling periodicity, a mating study was performed. Virgin male-female pairs were placed together in screen mating cages (80 mm by 50 mm, 18 by 14 mesh), the ends of which were closed with plastic petri dishes. Moths were maintained on a 16:8 L:D h cycle and, in the last hour of photophase, a male-female pair (each of Day 3) was introduced into a mating cage; Day 3 moths were chosen because in many moth species, males typically require several days to become sexually mature (Shorey et al. 1968). Pairs were then placed in a bioassay room with dimmed, white incandescent lighting (0.3 lux). Fan-forced air was circulated around the room and another fan continually exhausted the room air outside the building. Moths were observed every 30 min of scotophase until the first pair mated, whereafter they were observed every 15 min until the end of scotophase when the bioassay was terminated. Observations were aided with a red cellophane-covered flashlight,

although at 0.3 lux, there was sufficient light to see pairs coupled. Two replicates were run, 30 pairs per replicate. Data were excluded if either moth of a pairing was dead or moribund at the conclusion of the test.

Periodicity of Oviposition.—Female carob moths (16:8 L:D cycle) were removed from their emergence cage (which contained males of equal age as mating partners) and were set up in screen cages (80 mm by 50 mm) with an open end which was covered with a clear polyethylene sleeve. A cohort consisting of 10 females of known age (Day 2, 3, 4, 5, 6, 7 or 8) was placed in a cage; four cohorts were run for each age of female. The dates used as the oviposition substrate (variety Deglet Noor) have low water content and are preferred by the carob moth in the Coachella Valley over other, moister varieties (C. Kerby, pers. comm.). These dates were of commercial sale quality taken from a recent harvest, rinsed with water to remove elemental sulfur used for mite control, and air-dried. For the experiment, dates were impaled on a bent paper clip tied to a piece of string so they could be easily lowered into and pulled out of the cages. Decaying dates might prove more attractive as oviposition lures because female carob moths are attracted to odors of fermenting or fungus-infested host fruits (Gothilf et al. 1975, Warner 1988, Cossé et al. 1994). However, using them might introduce greater variation into the experiment; hence, non-decaying dates were used. One date was placed in each cage of 10 females and the plastic sleeve was folded over and clipped to minimize moth escape. Cages were transferred to a bioassay room described above. Dates were replaced hourly and deposited eggs were counted and totalled for each of the 8 h of scotophase. Because few eggs were laid on the cage that housed the moths, no attempt was made to count them. At initiation of photophase, females were given another date which was not removed until the end of the 16 h light period; we previously observed little oviposition occurs during photophase. The experiment was terminated at the start of the next scotophase. Females were transferred to vials containing 90% alcohol and later dissected to determine the number of spermatophores in the bursa copulatrix.

Statistics.—Calling periodicity was analyzed with one-way ANOVA with Tukey-Compromise test or two-way ANOVA with Tukey's studentized range tests separations. Mating periodicity was analyzed with a X^2 test for independence using Yate's correction. Oviposition periodicity data were square-root transformed because there was a high variance within the data sets, and then analyzed using two-way ANOVA with Tukey's studentized range test for separation (SAS Institute, 1982).

RESULTS

Calling Periodicity.—Female carob moths initiated calling in the fourth through sixth h of scotophase (16:8) and fourth through seventh h (14:10) for all five age cohorts of females; Day 2 females on the 12:12 light cycle initiated calling in the fifth h (Fig. 1). ANOVA of the 14:10 and 16:8 cohorts revealed a significant difference among the groups for initial onset of calling (two-way ANOVA, $F = 9.80$; df = 9, 220; $P < 0.0001$). The 16:8 moths called significantly earlier than the 14:10 females ($F = 61.97$; df = 1; $P < 0.0001$), however, there was also a significant difference between cohorts within a light cycle ($F = 3.51$; df = 4; $P < 0.01$). The interaction of age × light cycle was not significant ($F = 2.08$; df = 4; $P > 0.05$). In the 16:8 groups, as the age of the female cohort increased,

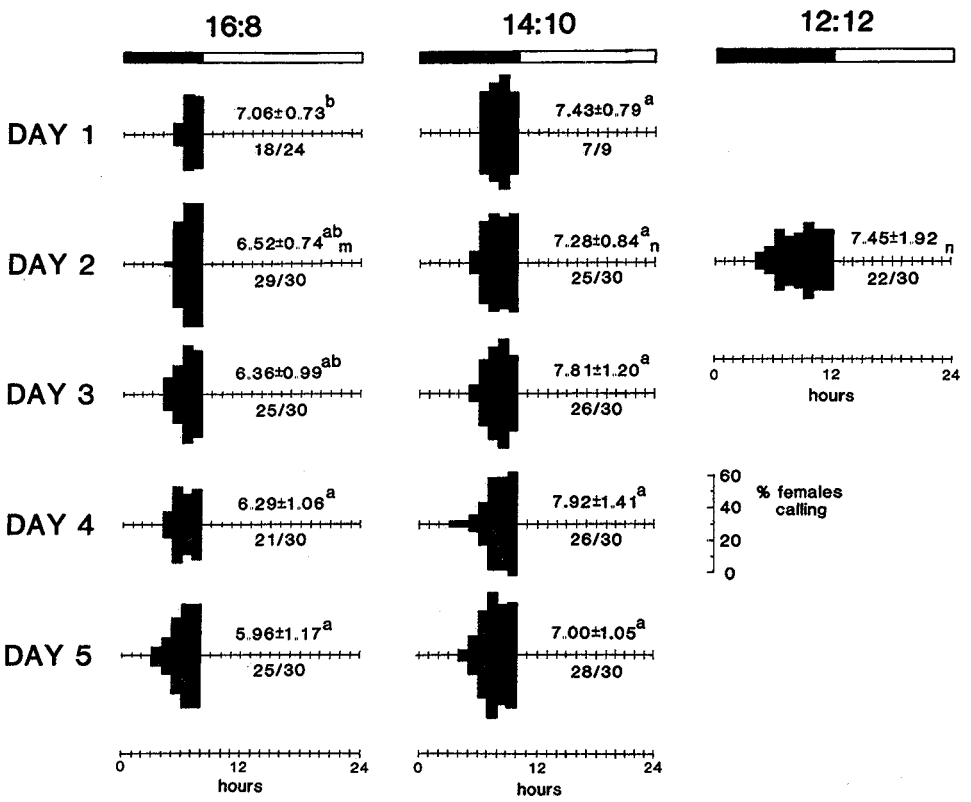

Figure 1. Percentage of calling female carob moths at 16:8, 14:10 and 12:12 L:D hr photoperiod regimes. Bar above symmetric histograms indicates dark:light cycle. Average initial onset of calling (x ± SD) is indicated above the tick-marked axis; # females observed calling at least once/# females alive at experiment termination is indicated below. 16:8 versus 14:10 data were analyzed by two-way ANOVA. Means having none of the superscript letters ($^{a\,b}$) in common indicate significant differences within a column (Tukey's Studentized Range test). Day 2 females were compared with a one-way ANOVA. Means having none of the subscript letters ($_{m\,n}$) in common indicate significant differences across the row (Tukey-Compromise test).

their time of initial onset of calling occurred significantly earlier in the scotophase (Fig. 1). This behavior was not exhibited, however, with the 14:10 cohorts. Additionally, all calling terminated during the first hr of photophase and no calling was observed during the remainder of the photophase.

When Day 2 females were analyzed across the three light cycles, the 16:8 females called significantly earlier than either of the other two groups (one-way ANOVA; $F = 4.37$; df = 2, 73; $P < 0.01$). The 14:10 and 12:12 groups were statistically indistinguishable (Fig. 1).

Mating Periodicity.—There was no difference between the 2 replicates for hour of mating initiation ($X^2 = 3.13$; df = 7; $P > 0.05$) or frequency of mating between pairs ($X^2 = 0.30$; df = 1; $P > 0.05$) so the data were pooled. Mating occurred in 43 of 55 (76.4%) of the pairings; five pairs were excluded due to death or moribund condition of one of the partners. Mating times were not randomly distributed throughout the scotophase ($X^2 = 72.04$; df = 7; $P < 0.001$) and carob

moths started mating in the fourth h of scotophase (N = 3). There was a significant increase of initiated matings during the fifth and sixth h (N = 20 and 13 respectively; these values are statistically similar). After this, the number of matings initiated within the seventh (N = 5), and eighth h (N = 2) of scotophase decreased significantly. The number of pairs observed *in copula* during each h was 3, 21, 35, 34 and 23 for the fourth, fifth, sixth, seventh and eighth h respectively. Pairs remained in the coupled position for 2.35 ± 0.84 h.

Oviposition Periodicity—There was a very pronounced nocturnal periodicity and age effect for carob moth female oviposition behavior ($F = 11.03$; df = 83, 168; $P < 0.0001$, Table 1). Two-way ANOVA revealed highly significant effects for hour of scotophase ($F = 69.21$; df = 8; $P < 0.0001$), age of female ($F = 23.74$; df = 6; $P < 0.0001$) and the interaction of these variables ($F = 2.37$; df = 48; $P < 0.0001$). Carob moths laid the greatest number of eggs during the first hour of scotophase (Table 1). There is a statistically significant decrease for each of the following 2 h periods followed by a diminishing of the behavior to near zero by the sixth h. Virtually no eggs were laid during the 16 h photophase. Considering the effect of age, egg deposition rose significantly by Day 3, peaked with Day 4 females whereafter egg production decreased to significantly lower levels by Day 7 (Table 1).

Virtually all females were mated at the end of their second full scotophase (Day 3) as indicated by the presence of spermatophores, and the number of matings increased with age with a mode of one spermatophore for Day 3 thru Day 6 females, and two spermatophores beyond Day 6 (Table 1). This increase with age was significant across the group ($F = 34.56$; df = 6, 248; $P < 0.0001$) with Day 7 and 8 females having significantly more spermatophores than Day 3 through 6 females (which were all statistically similar). Day 2 had significantly fewer spermatophores compared to every other group (Table 1).

Discussion

Female carob moths exhibit periodicities in their reproductive behavior which may be useful in developing field control methods for the insect. The only previous reference to female reproductive behavior of the carob moth was that of Cox (1976) in which he states that calling occurred "when it became darker" and that oviposition occurred "during twilight and dark periods." Carob moth calling was initiated near the mid-point of the scotophase over a range of ages and light regimes from short (16:8) to longer night (12:12) (Fig. 1). When observed as a group, carob moth females continued calling until the photophase, whereupon they abruptly ceased.

When virgin carob moth females were placed with males under a 16:8 L:D cycle, matings were initiated at the same period (fifth to sixth h scotophase) as the initiation and rise in calling of 16:8 females (Fig. 1).

Overall, most carob moth oviposition in this study occured during the first 3 h of scotophase with the highest number of eggs deposited during the first h (16:8 light regime) (Table 1). Oviposition decreased sharply by the mid-point of the scotophase and was virtually zero during the photophase. An ontogeny of oviposition may occur where a periodicity develops by Day 3, production peaks at Day 4 and then drops off afterward. The lower performance of the Day 2 and Day 3 females may be partly due to some females still being virgins. This pattern

Table 1. Hourly mean (SD) egg output and number of spermatophores detected for groups of 10 female carob moths (four replicates) at differing ages.

	Hour of scotophase								16 h photophase			
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th–24th	Avr. for each day	Avr. no. spermatophores	*n*
Day 2	6.3	7.0	7.5	7.5	5.5	2.5	3.8	1.3	1.0	42.3 bc	0.30 c	(40)
	(8.5)	(10.7)	(9.7)	(8.1)	(1.9)	(1.3)	(2.6)	(1.0)	(0.8)	(38.7)	(0.46)	
Day 3	31.5	18.3	18.3	11.3	14.3	5.3	2.5	4.5	1.3	105.0 ab	1.10 b	(38)
	(8.2)	(10.3)	(9.7)	(7.4)	(6.8)	(1.9)	(2.1)	(2.6)	(1.0)	(32.0)	(0.51)	
Day 4	43.5	41.0	31.3	16.0	8.5	7.3	6.3	8.3	2.5	164.8 a	1.26 b	(35)
	(16.7)	(40.2)	(28.7)	(11.7)	(6.8)	(3.9)	(1.3)	(4.7)	(1.3)	(88.7)	(0.70)	
Day 5	44.5	15.6	10.8	9.0	8.0	4.3	2.0	4.3	0.5	99.0 abc	1.53 b	(38)
	(21.0)	(7.4)	(9.2)	(4.8)	(1.2)	(2.2)	(1.4)	(2.8)	(1.0)	(30.3)	(0.60)	
Day 6	46.8	18.0	9.0	7.0	8.8	4.0	1.5	3.3	0.3	98.5 abc	1.54 b	(35)
	(25.4)	(11.2)	(6.4)	(5.6)	(9.0)	(3.6)	(1.0)	(1.7)	(0.5)	(56.4)	(0.70)	
Day 7	31.0	13.0	5.3	3.3	2.3	2.0	2.8	1.0	0.3	60.8 bc	2.32 a	(37)
	(12.9)	(7.8)	(3.4)	(1.0)	(2.5)	(1.8)	(4.9)	(1.4)	(0.5)	(24.8)	(0.94)	
Day 8	32.5	11.8	2.8	3.0	0.8	1.5	0.3	1.0	0	53.5 c	2.17 a	(35)
	(17.2)	(6.3)	(1.0)	(0.2)	(1.0)	(0.6)	(0.5)	(1.4)	(0)	(25.4)	(1.07)	
Mean for each h	33.7 a	17.8 b	12.1 c	8.1 c	6.9 cd	3.8 de	2.7 ef	3.4 e	0.9 f			
	(19.7)	(18.5)	(14.6)	(7.3)	(6.2)	(2.9)	(2.7)	(3.3)	(1.1)			

Overall means having no letters in common are significantly different for hourly oviposition (row), daily egg output (column) and spermatophores (column). Oviposition data were analyzed with two-way ANOVA with Tukey's studentized range test separation. Spermatophore data were analyzed with one-way ANOVA with Tukey's compromise test separation.

of oviposition is found in at least five other species of pyralid moths (Bell 1981, Andrews et al. 1980). As the females age and egg production decreases, the number of spermatophores per female continues to increase suggesting that lack of sperm is probably not an explanation for decreased oviposition (Table 1).

The information presented here may be of importance to date growers in their attempts to control the carob moth. Because females oviposit most heavily right after sunset, insecticide sprays during this time might increase the chances of killing egg-laden females. Similar studies with cotton pests documenting their nocturnal behavior patterns have resulted in changes in control strategies in that some crop-dusting is performed at night when the insects are more likely to contact pesticide (UC Press, 1984). In contrast, trying to control carob moths by targeting the mating behavior might be difficult because mating periodicity in moths is dependent on abiotic variables other than photoperiod (e.g., temperature, Baker & Cardé 1979, Kanno 1981). Also control methods may be ineffective for interrupting the mating behavior of the carob moth because the actual mating location in the date agrosystem is unknown. Therefore, targeting the ovipositing female may be the more effective control strategy because females are in the date canopy laying their eggs.

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