SEX PHEROMONE BIOLOGY OF THE ADULT TOMATO PINWORM, *KEIFERIA LYCOPERSICELLA* (WALSINGHAM)[1,2]

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Abstract

Laboratory and field tests established that the adult tomato pinworm, *Keiferia lycopersicella* (Walsingham), is crepuscular (evening) in its mating habits. Approximately 75% of males captured in traps baited with female pheromone extracts and placed in tomato fields in February were caught during the twilight period (5-8 PM EST). Females exposed to an abrupt-transition photoperiod in the laboratory exhibited maximum calling activity 0.5 h after lights-out, but males were responsive to female extracts throughout the night. Virgin pairs mated only during hours 1-3 of the laboratory scotophase.

Females called most and males were most responsive to the pheromone in a laboratory olfactometer during the third night after eclosion. Males were more responsive when bioassayed with dim light from both above and below the olfactometer than when there was light only from below.

The tomato pinworm (TPW), *Keiferia lycopersicella* (Walsingham), has been a serious pest of tomato in southern California for many years (Oatman 1970), and since 1970 has become economically important in Florida (Wolfenbarger 1974, Wolfenbarger et al. 1975). Poe et al. (1975) suggested that several changes in cultural practices and insecticide use are probably responsible for its present status in Florida.

Generally TPW infestations are combated with multiple applications of insecticides; however, Oatman (1970) has demonstrated that, in the absence of insecticides, parasites can suppress larval pinworm populations. He further suggested that parasites must be integrated in some manner with other measures to achieve economic control. Exploitation of lepidopteran sex pheromones may provide a means of suppressing moth species without impairing the effectiveness of their natural enemies.

Methods and Results

The research colony originated from insects collected on tomato at Immokalee, FL. The insects were reared to pupation on tomato plants in a greenhouse at 27±4°C, 60-90% RH and the prevailing natural photoperiod. Soon after pupation they were placed in environmental cabinets at 28±1°C, 60-65% RH and 14L:10D photoperiod. The pupae were segregated by sex, and the resulting moths were collected daily, held in age-segregated groups in 2.3-liter clear plastic boxes, and fed a 10% sucrose solution.

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[1]Lepidoptera: Gelechiidae.

[2]Mention of a commercial or proprietary product in this paper does not constitute an endorsement of that product by the USDA or University of Florida.

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PERIODICITY OF PHEROMONE-RELEASING BEHAVIOR

The pheromone-releasing behavior (calling) of female moths was observed in environmental cabinets maintained as described, but with a scotophase background illumination of ca. 1.3 lux (Spectra Illumination Meter Model F 200-TV-B). The virgin females emerged individually in clear plastic 7-dram snap cap vials and were provided with 10% sucrose in water throughout the experiment.

Preliminary study had indicated that females exhibited calling behavior only during the scotophase. Therefore, we observed and recorded the behavior of each female at 1-h intervals from 1 h before to 1 h after the scotophase. The study was conducted for 6 nights following the day of eclosion (which occurs during the morning hours). From 38 to 105 females were observed each hour each night. The hourly percentages of 3-night-old females that exhibited pheromone-release behavior (wings slightly spread, abdomen elevated, pheromone gland everted) are diagrammed in Fig. 1.

Only 26% of the females were calling at 1 h into the 1st night. This increased to 39% on the 2nd night, and 58% on the 3rd night and decreased to ca. 45% from the 4th to 6th nights. The mean percent (for all hours each night) of females exhibiting calling behavior was 13.2, 15.0, 25.6, 23.2, 20.8, and 14.8 for nights 1 to 6, respectively. The maximum number of calling females was observed during the 1st h of the scotophase of all nights; a secondary increase occurred ca. 4-6 h later. Females did not call in advance

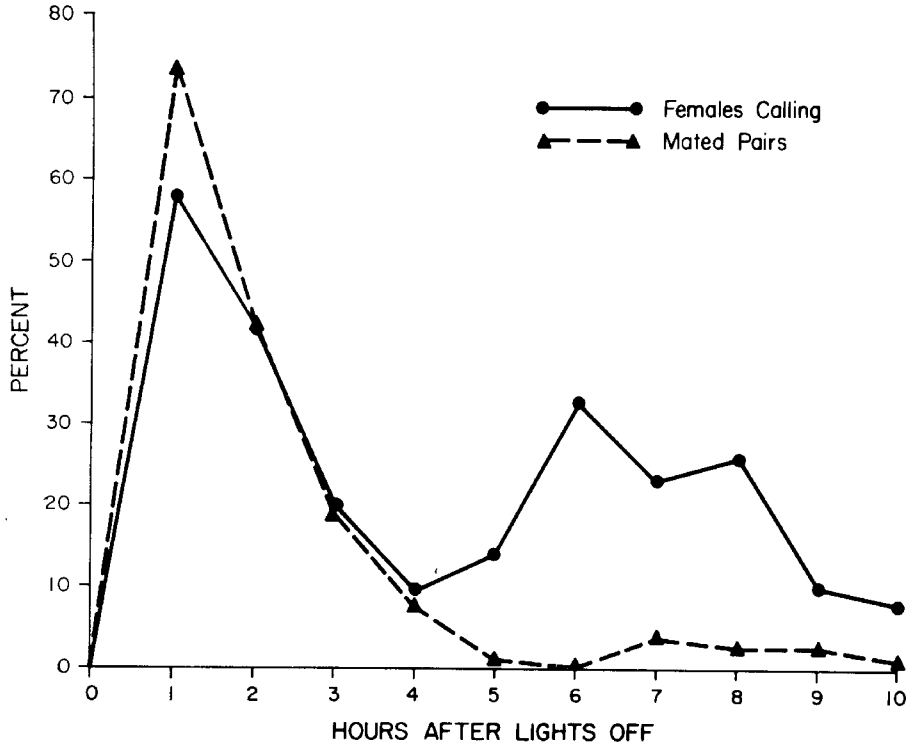

Fig. 1. Periodicity of female calling behavior and mating in the tomato pinworm.

of lights-off, but did exhibit some persistent activity during the hour after lights-on (11% of the 3-night-old moths). Moths ceased to call by the 2nd h of the photophase.

The duration of individual calling bouts was not recorded, but individual females frequently remained in the calling posture for 2-3 h during the first 4 h of darkness. This was especially true of females during the 3rd night.

Mating Periodicity

Mating behavior was determined in the environmental cabinets described. Hourly observations were made of 10 individual male-female pairs of virgin moths in 7-dram vials or of 10 virgin pairs in a 12.6-liter plexiglass cage. At each observation the mating pairs were removed from the experiment and replaced with a virgin pair, each individual of which had been held to that point under test conditions. The moths were in their 2nd to 5th night following eclosion. Four replications were conducted of each of the single-pair and multiple-pair matings.

The copulatory periodicity of the moths is diagrammed in Fig. 1. Sexual activity, like female calling, was greatest during the 1st h of darkness, and very little copulation occurred after the 3rd h. Males ran or walked in their approach to calling females. Approach was generally from behind or at ca. 90° to the female and was accompanied by rapid wing fanning. Females were not observed to fan their wings when males approached. The copulatory strikes of the males were made laterally with males beside the females. Moths remained in copula from 30 min to 2 h.

Bioassay of Male Response to the Sex Pheromone

One hundred 2-night-old virgin female moths were aspirated into a flask containing 10 ml of anhydrous ether and held for 24 h. The liquor was filtered and adjusted with ether to 10 ml. Six graded concentrations of the extract expressed in female equivalents (FE) were prepared by using acetone (less volatile and easier to use for bioassay) as the diluent. These preparations were stored at 0°C until tested.

Bioassays of the extracts were conducted by placing groups of 10 virgin males of known age in 1.8 x 44-cm plexiglass tubes connected in groups of 15 to a common manifold (Sower et al. 1973). Compressed air filtered through activated charcoal and a molecular sieve was metered through each tube at 1.0 liter/min via the manifold. A pheromone sample was applied to the surface of a stainless steel applicator that was then inserted through a 0.5-cm diam hole at the upwind end of each tube. Upwind movement to within 4 cm of the applicator and behavior associated with sexual excitation were observed and timed. Only those males more than 4 cm from the applicator at the time of sample introduction were used in computing the percent responding. Response was recorded at 15 and 30 sec after introduction of the sample. Males were allowed to acclimate to test conditions for at least 15 min before each bioassay.

The tests in the olfactometer were conducted to determine three parameters of male biology: the responsiveness of various ages; the daily periodicity of the response; and the dose-response characteristics in the olfactometer. The olfactometer was illuminated (1.3 lux) from below by rheostat-dimmed

incandescent bulbs shielded by a diffuser of white bond paper supported by 0.64-cm plexiglass.

Six age groups of males (1st to 6th nights after eclosion) were tested at 10^{-1} FE. Each age group was assayed beginning 30 min before lights-out and every hour until lights-on. Fifty males (5 tubes) were assayed per age group per night. Each age X time was tested 5 times (250 males, 25 assays).

Response curves for 2-, 3-, and 4-night-old males are shown in Fig. 2. Newly emerged males in their first night showed little sexual excitation or upwind movement when exposed to the female extract. On their 2nd night, the males were attracted to the pheromone source, but excited wing fanning and copulatory attempts were absent. Males in their 3rd night exhibited the highest level of excitation, attraction, and numbers of copulatory strikes. Responses declined on the 4th and each subsequent night.

Males became responsive within 0.5 h of lights-out. The 3-night-old males exhibited a high level of response throughout the scotophase with peak activity at 0.5 and 7.5 h. This tendency toward bimodal activity was present in all age groups tested. Males did not seem to exhibit decreased or inhibited response to the pheromone due to multiple exposure.

A dose-response curve was constructed from data obtained by exposing randomly mixed populations of 3- to 6-night-old males to 6 concentrations (10^{-1} to 10^{-6} FE) of female extracts at 30 min into the scotophase. Each dose was assayed 15 times. In our olfactometer, 10^{-2} FE produced the greatest upwind response (Fig. 3, average of 15 and 30 sec counts). The number of males attracted to within the 4-cm area decreased at 10^{-1} FE, but the level

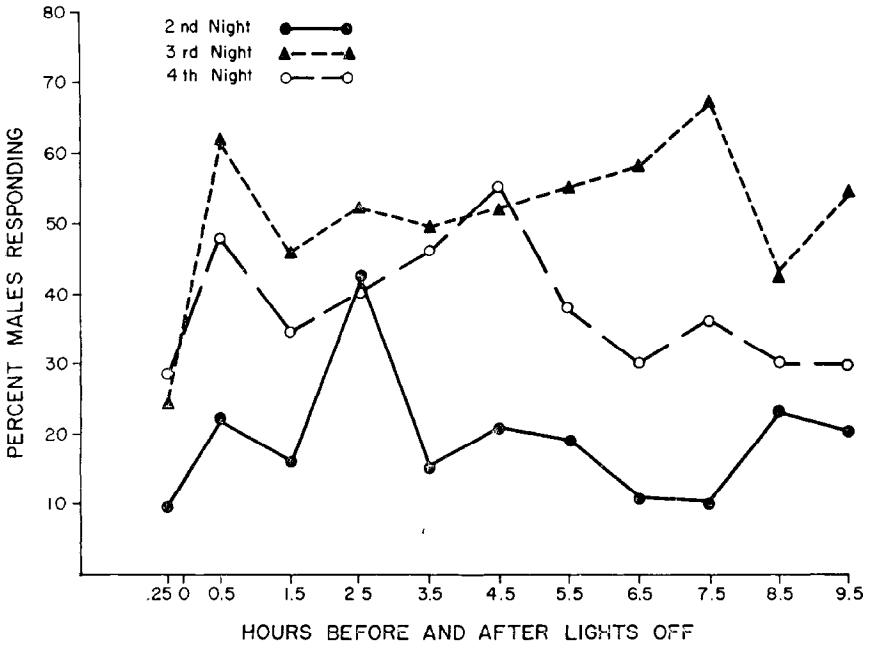

Fig. 2. Response of male tomato pinworm to a 10^{-1} FE extract of the female sex pheromone at hourly intervals from 0.5 h before lights-out until 0.5 h before lights-on. Males were in their 2nd, 3rd, or 4th night after eclosion.

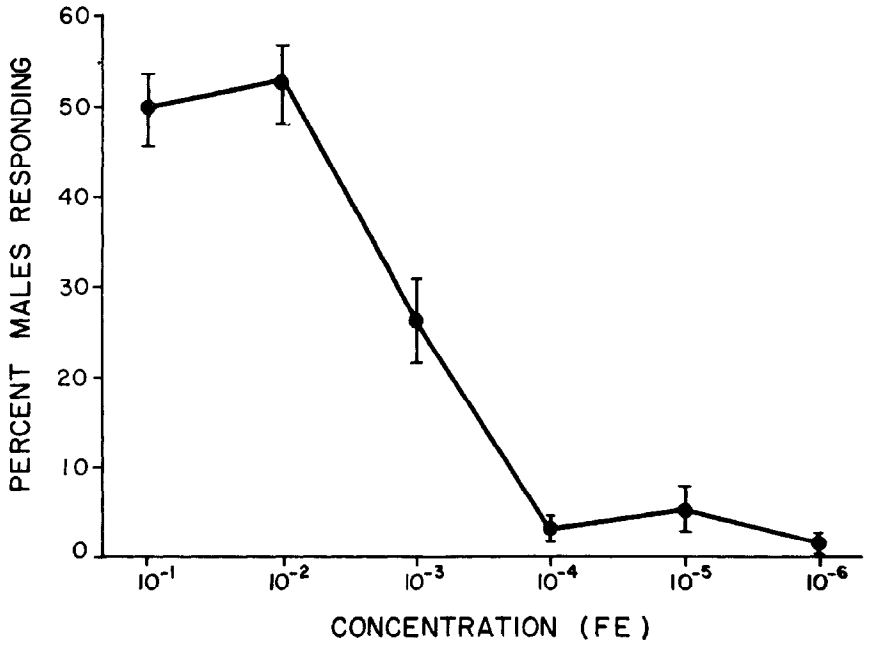

Fig. 3. Attraction response in an olfactometer of male tomato pinworm to 6 concentrations of the female sex pheromone (expressed in female equivalents = FE).

of excitation, wing fanning, and copulatory attempts increased. At concentrations below 10^{-2} FE attraction rapidly decreased as did the other behaviors.

During these preliminary bioassays, we began to suspect that TPW males were more responsive when the olfactometer was illuminated from above and below than when it was illuminated only from below (necessary to provide backlight for observation). We therefore constructed 2 plywood boxes (15 x 56 x 66 cm) each equipped with five 40-watt showcase light bulbs. These bulbs were covered by a diffuser consisting of a sheet of 0.13 mm teflon sandwiched between a sheet of clear prismatic polystyrene and a sheet of 0.64-mm plexiglass. Light intensity from each box was regulated by a rheostat. The bioassay device rested on the lower box and the upper unit was 40 cm above. Tests (25 replicates with 10 males/replicate) were then conducted at 2 light levels (0.2 and 1.3 lux) with light from below only or from both above and below using a dose of 10^{-1} FE at 0.5 h of the scotophase. At 0.2 lux, the mean ($\pm$SE) response with light below was 63.5$\pm$ 3.4%; the response with light above and below was 83.1$\pm$3.3% (significant, $p<0.01$, $F = 28.3$). The respective means at 1.3 lux were 71.8$\pm$3.7% and 87.1$\pm$3.9% (significant, $p<0.01$, $F = 12.09$). TPW males are more responsive in the olfactometer to the female sex pheromone when illuminated from below and above.

Field Test

In February 1976 a field test was conducted at Homestead, FL, to determine whether males could be captured in sticky traps (Pherocon® 1C)

baited with female extracts. Two traps each were baited with 10^{-1} or 10 FE placed on a piece of filter paper suspended within the trap. These traps, alternating doses, were placed at plant level (ca. 45 cm) and 10 m apart along a row in a tomato field. The experiment began at 6:00 AM and continued for 24 h. The traps were checked hourly and replaced at that time with new, newly baited traps. Two unbaited traps were also deployed.

The average number of males captured per trap each hour is shown in Table 1. A total of 2999 males were captured in the four traps, 66% of those by the traps baited with 10 FE. Unbaited traps captured 5 males. Males began to respond to the female extracts as the sun began to set (5:00-6:00 PM). The maximum response occurred after sunset, from 6:00 to 7:00, i.e., during twilight. Captures diminished rapidly after full dark. The bimodal response of males in the laboratory was not evident in the field captures.

Discussion

The tomato pinworm is crepuscular in its mating habits and may use a decline in light level as an environmental cue to synchronize mating activity. Males and females exposed to abrupt changes in light intensity in the laboratory displayed precopulatory and copulatory behaviors shortly after lights-out. Male response to pheromone in the field and our unquantified field observations of general moth activity revealed that this activity occurs primarily during the period immediately preceding and following sunset.

The crepuscular activity of TPW moths may be exploited by applying pesticides during or just before twilight since a major impact of pesticide sprays on this species is the death of the adults. Such a multiple-application of sprays obviously is not desirable, but they would probably be more effective if applied late in the day.

An interesting contrast exists between the calling behavior of females and male response in the laboratory (Figs. 1 and 2) and the mating curve (Fig. 1). Even though females call and the males are quite responsive throughout the night, virgin pairs do not mate after 3-4 h into the scoto-

TABLE 1. Mean Number of Male Tomato Pinworms Captured at Various Times in Traps Baited With Female Sex Pheromone in a Tomato Field at Homestead, FL in February.

Time	Mean capture/trap	Percent of total	Mean temp. (°C)
6:00-7:00 AM	16.3	2.0	15.2
7:00-12:00 Noon	0	0	23.9
4:00-5:00 PM	0	0	24.4
5:00-6:00 PM	98.3	12.1	22.8
6:00-7:00 PM	413.0	50.8	21.7
7:00-8:00 PM	95.0	11.7	20.6
8:00-9:00 PM	64.3	7.9	19.7
9:00-10:00 PM	59.0	7.3	19.2
10:00-11:00 PM	38.5	4.7	19.2
11:00-6:00 AM	28.5	3.5	18.6

phase. Possibly the females do not release pheromone during the latter hours of the scotophase even though they do adopt the calling posture. Female *Plodia interpunctella* (Hübner) that call during the scotophase release ca. 13 times more sex pheromone than those that call during the photophase (Nordlund and Brady 1974). There may also be a female receptivity rhythm as in *Ephestia cautella* (Walker) (Barrer and Hill 1977).

The increased response of male TPW in the olfactometer when overhead illumination was provided is apparently caused by the overhead orientation of the stimulus rather than the increase in intensity. It is very possible that this phenomenon would be observed in other species since the dorsal light response is a well-known orientation mechanism in flying and swimming animals (Fraenkel and Gunn 1961).

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MATING COMPETITIVENESS IN THE LABORATORY OF IRRADIATED MALES AND FEMALES OF *EPHESTIA CAUTELLA*[1]

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Abstract

Males or females of the almond moth, *Ephestia cautella* (Walker), a serious pest of stored commodities, were irradiated (I) with either 35 krad (a partially sterilizing dose) or 50 krad (a sterilizing dose) and combined with pairs of untreated (U) adults at I:U ratios of 1, 5, 10, 15, or 25. Doses

[1]Lepidoptera: Pyralidae.