VELVETBEAN CATERPILLAR: RESPONSE OF MALES TO VIRGIN FEMALES AND PHEROMONE IN THE LABORATORY AND FIELD


ABSTRACT

Observations of the sexual behavior of Anticarsia gemmatalis (Hübner) were made in the laboratory and field during 1976. The calling behavior of the female is characteristic of many moth species in that the tip of the abdomen is curved upward when calling. In the laboratory, males fly in a zig-zag path toward calling females or to pheromone extracts. Clasper extension by some males was observed as they approached the pheromone source. Females began calling ca. 2 hours after sunset and this behavior continued intermittently throughout the scotophase. Mating and increased age greatly reduced the attractiveness of females to males in field traps. Pheromone was obtained from both non-calling and calling females. In field bioassays, extracts from females collected before and after dark were effective in attracting males, with extracts taken at 4 and 6 hours after dark capturing the most males. Seven trap designs were evaluated for effectiveness at capturing A. gemmatalis males, but only the electric grid trap proved efficient and warranted further use.

RESUMEN

El comportamiento sexual de Anticarsia gemmatalis (Hubner) fue observado en el campo y en el laboratorio en 1976. El comportamiento de la hembra en atraer a los machos es semejante a el de muchas otras especies de polillas, eso es, el punto del abdomen se encueva para arriba. En el laboratorio los machos vuelan en un rumbo de zigzag hacia las hembras atractantes y hacia extractos de feromonas. Se observaron la extensión de los agarradores de unos machos cuando estos acercaron el fuente de la feromona. Las hembras empezaron a “llamar” (atraer machos) cerca de 2 horas después de la puesta del sol y este comportamiento siguió intermitentemente durante el período de oscuridad. El apareamiento y la edad avanzada reducen mucho la actractividad de las hembras en trampas. Se obtuvieron la feromona de hembras que llamaba y los que no llamaba. En bio-ensayos de campo, los extractos de las hembras que se capturaron antes y después del período de oscuridad fueron efectivos en atraer a los machos. Los extractos de las hembras capturadas 4 y 6 horas después volverse oscuro atrayeron al máximo número de machos. Se evaluaron 7 diseños de trampas por efectividad en capturar machos de A. gemmatalis. Solamente la trampa de rejilla eléctrica resultó bastante eficiente para continuar uso.

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Greene et al. (1973) made observations in field cages on mating and oviposition behavior of the velvetbean caterpillar, *A. gemmatalis* (Hübner). They suggested the existence of a female sex pheromone, after noting that males frequently would fly around females whose abdomen tips were pointed dorsally. Because insect sex pheromones are providing new methods of controlling insect pests, we initiated investigations to confirm the presence of a sex pheromone in *A. gemmatalis* and to identify parameters which may affect female attractiveness and male response.

**Procedures and Results**

Insects used in these experiments were reared on an artificial diet according to the methods of Greene et al. (1976). Insects were sexed in the pupal stage (Butt and Cantu 1962) and placed in pans of moist vermiculite. Males and females were allowed to eclose in screenwire cages in separate rooms. Adults were maintained on 10% sucrose solution. Cages containing moths to be used in behavioral observations were held in greenhouse rooms which allowed penetration of sunlight (temperature ca. 24°C, RH ca. 55%). Female moths which were extracted for pheromone were held in a screenwire cage (63 cm x 61 cm x 91 cm), under a light cycle of 12:12, photophase: scotophase (temperature ca. 24°C, RH ca. 55%).

**Behavioral Observations:** Observations of pheromone release behavior were made in the greenhouse room containing the males. Individual virgin females were confined in screenwire cylinders (4.5 cm, diameter x 12.7 cm, length) suspended within a larger cage (57 cm x 54 cm x 184 cm) containing ca. 100 virgin males. Observations of the calling behavior and the subsequent responses of males were made between 1730 and 0200 h on 7 consecutive nights. Sunset occurred at about 1800 h during these tests.

Males became active ca. 45 min. after sunset. This activity was characterized by apparently indiscriminate flight around the cage, followed by walking on the sides of the cage, and rapid fluttering of the wings. Females exhibited similar behavior except they were unable to fly because of their confinement. Obvious sexual activity was not noted until ca. 2 hours after dark. Female calling consisted of wing fanning followed immediately by dorsal elevation of the abdomen tip. Females repeated the calling behaviors in an unbroken sequence for a time period varying between a few seconds to several minutes. These calling sequences were repeated by each female several times during the observation period. Calling was observed only when females were on the vertical walls of the cage. During this activity, males flew toward calling females. Walking or non-calling females did not elicit male attraction.

Male response consisted of flying a zig-zag path toward the female. Flight was initially erratic but became more direct as the male approached the female. Upon reaching the female, the male flew around her several times, and either landed or flew away. As males hovered near the female, the claspers were usually extended.

**Extraction of Pheromone:** Calling females were vacuumed into a 1-liter jar containing sufficient ether to just cover the insects. The jar was checked often to insure that the females were always covered with solvent. Then they were extracted by soaking for 30 min. and the insect carcasses
were removed by filtration. The collection vessel was carefully rinsed twice with small portions of ether. This wash was added to the original filtrate by pouring it through the filtration apparatus (Mitchell et al. 1974). The excess ether was removed by distillation under atmospheric pressure through a 375 mm Vigreux column. This concentrated extract and the distillate were stored at -60°C until bioassay. The number of females obtained during each collection varied due primarily to the availability of insects. On various occasions, as few as 5 and as many as 75 females were collected. Collection required a minimum ca. 25 ml of solvent and the volume of residue after distillation was ca. 1 to 2 ml for each 100 insects extracted.

A bioassay of the concentrated materials was conducted by placing 25 female equivalents (FE) on a 9 cm Whatman #1 filter paper and presenting the paper to caged males. The cage and conditions were the same as those employed while making behavioral observations. The bioassay began at 2 h past sunset and the cage contained ca. 100 males. Using this procedure, ether extracts from calling and non-calling females collected at night were shown to be attractive and male responses were typical of the behavior shown by males approaching calling females.

**FIELD BIOASSAYS:** Traps of 7 different designs were tested to determine the most effective trap for capturing males: an electric grid trap (Mitchell et al. 1972); a double-cone trap similar to that of Kael and Shorey (1972); an omnidirectional trap designed for pink bollworms, *Pectinophora gossypiella* (Saunders) (Sharma et al. 1973); a screenwire trap used for the tobacco hornworm, *Manduca sexta* (L.) (Mitchell et al. 1972); a sticky trap used for the sugarcane borer, *Diatraea saccharalis* (Fabr.) (Perez and Long 1964); a Pherocon 1C sticky trap (Zoecon Corp., Palo Alto, CA); and a large walk-in trap constructed from screenwire used for capturing the lesser peachtree borer, *Symnathedon pictipes* (Grote and Robinson) (Gentry and Blythe 1978). Traps were baited with 3 virgin females (2- to 3-days old) and placed ca. 30 m apart in a soybean field near Gainesville, FL. Captured males were removed daily and the traps were rotated once position. Each collection was considered a replicate (4 replicates). Results obtained in this test indicated that the electric grid was the only trap effective against the VBC. The electric grid trap captured 5.9 ± 0.1 (X ± S.E.) moths/trap/night. All other traps captured no VBC moths. The ineffectiveness of traps other than the electric grid for capturing fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and the beet armyworm, *S. exigua* (Hübner), was noted by Tingle and Mitchell (1975). Therefore, all other field assays were conducted using electric grid traps.

The relative attractiveness of laboratory-reared and feral females was studied to insure that laboratory-reared females would provide reliable information. Individual feral and laboratory-reared females of equivalent age were placed in electric grid traps. The mean capture of males/trap/night by traps baited with feral females was 6.5 ± 1.3 and 4.6 ± 0.5 (X ± S.E.) by traps baited with laboratory-reared females (15 replications, 3 traps/female type, for 5 consecutive nights). The mean capture by feral and laboratory-reared females was not significantly different (t-test, t=0.407). Unbaited traps captured no males.

The influence of age of females on pheromone production was evaluated in 2 tests. One test utilized 3 laboratory-reared females as bait. Age groups
tested were 1, 7 days post eclosion. A replicate was considered to be the
capture of males by one trap/night (3 replicates/age group). The 2nd test
utilized baits consisting of 20 FE of crude extract obtained from non-calling
laboratory-reared females of different ages. Females were collected 2 hours
after dark. Each age was replicated 4 times. All females used in these tests
were aged in screenwire cages in the greenhouse. A pan containing female
pupae was placed in a cage and allowed to remain over night. The following
day the pan containing unecdosed pupae was transferred to a second cage
leaving the newly ecdosed females behind. Therefore, each cage contained
females which had ecdosed during a single night.

In the tests using live females, the baits were placed in the field just be-
fore sunset and removed the following morning. Test females were held
during the day at 15°C under a photoperiod approximating that in the field.
Females dying during the day were replaced with females of equivalent age.
Pheromone extracts were tested by placing 20 FE in 0.25 ml of solvent on a
9 cm #1 Whatman filter paper and suspending it within the trap. Trap loca-
tion within the field was constant. At each collection, the treatment was
replaced with a new and different one; these treatments were rotated
throughout the field. Moth capture for the extracts were recorded hourly
(replicate). Extracts for each age group (1, 2, 3, 4, 5, 10 days old) were replicated
4 times. Tests were run between 2400 and 0300 h.

In tests using live females 1-7 days of age as baits, trap capture of
males was 45 ± 19, 66 ± 12, 37.3 ± 9, 43.3 ± 21, 11.3 ± 4, 10.6 ± 4, and 5 ± 1,
respectively (X ± S.E.). Linear regression analysis substantiated a decrease
of male capture with increasing age of the bait females (F = .05). In tests
using 20 FE of crude extract obtained from 1, 2, 3, 4 and 10-day-old females
capture of males was 3 ± 1.6, 2.5 ± 0.2, 2.3 ± 1, 3.5 ± 3 and 3.0 ± 0.6, respect-
ively (X ± S.E.). No significant difference in attractiveness of these baits
to VBC males was apparent. This was somewhat surprising in that there was
a significant decline in the number of VBC males captured in traps baited
with live females of different ages. Thus, one might assume that not only age
but age in conjunction with exposure to the changing environment caused the
decrease in female attractiveness. The extracts were taken from females
which were not exposed to these changes as they were held under constant
temperature, humidity, light cycle and food supply.

The nocturnal sexual activity of the VBC male was monitored with an
electric grid trap equipped with an automatic sample-changing device
(Mitchell et al. 1972) that captured males and segregated samples into
hourly intervals. The trap was baited with 3 laboratory-reared females. The
bait females were placed in the trap ca. 1 h before sunset (20:15 EDT) and
removed early the following morning. Captured male VBC were counted
daily. Each night’s capture was considered a replicate (8 replicates). Cap-
tures of VBC males commenced ca. 1 h after sunset and remained fairly
uniform throughout the night (X ± S.E. captures/h = 4.4 ± 0.8).

Female VBC were extracted with ether as described previously to de-
termine if the pheromone could be obtained at any time during the photo-
periodic cycle. Laboratory-reared females held under a 12:12 photophase:
scotophase were collected at 9 and 6 h before dark, immediately after the
lights were turned off and 2, 4, 6 and 9 h into the scotophase. Females were
extracted when ca. 3 days old. The extracts were bioassayed between 2400
and 0300 h by baiting individual electric grid traps with 20 FE of pheromone in 0.25 ml of solvent on a 9 cm #1 Whatman filter paper. Counts of captured VBC males were recorded hourly (replicate), and the filter paper was replaced with fresh bait. Each treatment was replicated 4 times. All treatments attracted some wild VBC males (2.5, 2.0, 2.0, 0.7, 5.6, 4.6 and 1.5 X males/treatment, respectively). However, significantly more males were attracted to pheromone extracts obtained from females 4 and 6 h into the scotophase (P < 0.05, Duncans Multiple Range Test).

The effect of mating on female attractiveness to wild males was determined by comparing the capture of males by traps baited with mated females to that of virgin females of equivalent age. Newly emerged virgin laboratory-reared females were placed in 4 liter paper cartons with virgin laboratory-reared males at a ratio of 1 female to 4 males. The carton tops were replaced with screenwire and the insects were held for 2 nights in the greenhouse under natural light. The following day, mated and virgin females were placed in traps ca. 1 h before sunset, and they were removed early the following morning. Each trap contained 1 female. Four traps per female type per night for 8 nights were used in this evaluation. Male captures were recorded daily (replicate). Females were dissected to confirm mating. The mean capture of males in traps baited with virgin females was 16.8 ± 0.6 (N ± S.E.) as compared to 1.5 ± 0.8 (N ± S.E.) males captured in traps baited with mated females (means differ significantly at the 1% level, student's t-test). The reduction in attractiveness of mated VBC females to feral VBC males is consistent with results reported for other moth species (Perez and Long 1964, Raulston et al. 1975).

This investigation has confirmed the presence of a female-produced sex pheromone in *A. gemmatalis*. The pheromone can be extracted with ether yielding an attractive extract to both laboratory reared and wild males when tested under both laboratory and field conditions.

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Literature Cited


