MATING RESPONSES RELATED TO ANTENNA CONDITION IN THE CORN EARWORM MOTH, HELIOTHIS ZEA (BODDIE)

by

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I. INTRODUCTION

This project was designed to test the mating response related to the condition of the antenna in the corn earworm moth, Heliothis zea (Boddie). In pursuit of these tests, the antennae of male and female moths of the corn earworm were amputated and mating experiments were performed.

Virgin male and female moths were raised from pupae to permit accurate determination of adult age before amputation or mating were attempted. The sexes were determined in the pupal case and reared in separate chambers to insure virginity.

Behavior tests were performed in large plastic chambers where the humidity was maintained with ease (see Fig. 1). Mating testing was performed under room lighting level at 13.3 ft-candles and in a subdued-light metal cabinet with less than 0.5 ft-candle light intensity.

II. MATERIALS AND METHODS

A. AMPUTATIONS OF MOTH ANTENNAE

Anesthetics were employed to permit handling of moths with minimum damage to body structures while the amputations were performed. Ether was the first anesthetic tried, for a 3-min exposure for the moths. This technique was effective in deactivation of moths, but was fatal to 70% of the moths exposed. Less

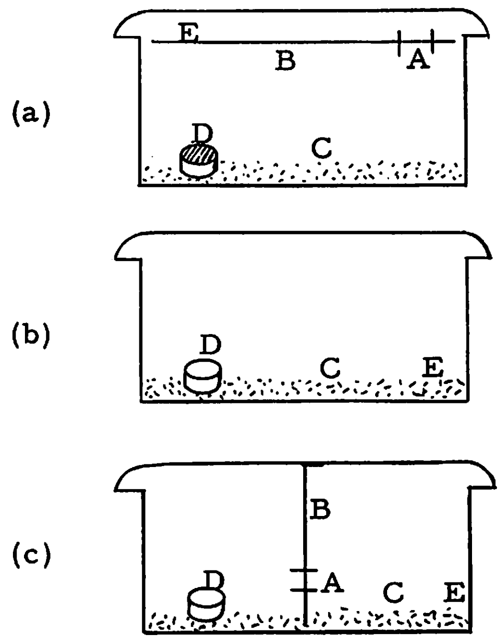

FIG. 1. --(a) Large plastic box used as mating chamber (A, small cut section in plastic; B, plastic sheet; C, vermiculite; D, female cage; E, location of liberated males); (b) chamber without longitudinal dividing sheet; (c) chamber with the sheet.

than 3 min failed to deactivate the moths very well, and the moisture caused the moth to stick to the cotton wet with ether.

Carbon dioxide was applied next to deactivate the moths. Short exposure failed to immobilize the moths and longer exposure produced high mortality after amputation. The abdomen of these moths was abnormally swollen and often burst along intersegmental membranes.

Refrigeration has been used effectively by Callahan in his research with Heliothis.[1] The best results, in my experimentation, were obtained when moths were refrigerated to 0-5°C. No unusual mortality can be associated with this technique in so far as I can ascertain. A few minutes of below-zero exposure, however, frequently resulted in death. Chilled moths were placed in a trough in modeling clay with the antennae and head exposed; a second clay piece covered and restrained the body during amputations. Some mating experiments involved moths with the entire antennal filament amputated. Other moths had both antennal filaments amputated. Still another series of moths (all males) were amputated in 1-mm increments, removed from the apical tips. Some moths had 1 mm removed, others 2 mm, and so on until a total of 7 mm had been removed.

B. MATING-BEHAVIOR CHAMBERS

Females were housed in chambers that restrict the moths from normal flight movements with wings during one series of experiments. Other female moths were housed in larger cages without such restrictions of flight movements with wings. The smaller cage was 1.5 cm high and 5 cm in diameter. The larger chamber was 5 cm^3.

Males were liberated in a large plastic box 25 x 32 x 11 cm. A sheet of Dow Handi-wrap was used as a divider, as shown in Fig. 1. A cut section was made at one end large enough to accommodate the male walking or in flight. The substrate used in the cage was vermiculite, which provided the water storage for maintaining the highly humid environment required.[2]

C. ENVIRONMENT CONTROL

Temperature was tested under normal Berkeley, California, diurnal fluctuation with a small range of from 55 to 75°F and in an incubator with a constant 30°C. Successful rearing of Heliothis is accomplished in moist conditions at about 85°C.[2]

Humidity was measured with a hygrometer (Model B Serdex made by Bacharach Industrial Instrument Co.) placed inside the large plastic box. Humidity was found to be 95 to 96% at all times. Only distilled water was used to supply vermiculite with water.

Light intensity was measured on a Unittic Model 66 exposure meter. The light entensity in the closed cabinets and incubators was under 0.5 ft-candle.

Feeding dishes, for the moths, were petri plates with 10% honey solution. Feeding dishes had to be changed daily owing to fungus growths, which were favored in these dark, warm, moist chambers.

Controls were used with each experimental procedure. The controls were repeated concurrently with the experimental tests to insure reliability of each test.

III. MATING EXPERIMENTS INVOLVING FEMALES IN FLIGHT-RESTRICING CAGES

Callahan has proposed that part of the mating "call" behavior of female moths is a rapid vibration of her wings.[3] The habit of wing vibration in "calling" may aid in sex pheromone dispersal as well as being a releaser of male copulatory behavior.

A. PROCEDURES

A series of experiments was conducted with virgin female corn earworm moths in glass petri dish bottoms 5 cm in diameter and 1.5 cm deep with wire mesh covers. The females could move horizontally and walk normally, but were inhibited from normal wing movements.

The virgin males were housed in larger plastic boxes in which the female cage was inserted. Males with normal antennae, males with progressive increments of antenna amputated, and males with total antennectomies were liberated in separate experiments with the constrained females. Experiments were conducted using one, two, three, and four males at one time in conjunction with single caged females. In a further series of experiments, females, in their confining cage, were placed on top of the plastic sheet while the males were liberated on the vermiculite below the plastic sheet.

B. OBSERVATIONS

Females were mainly active the hours around midnight. Males were unrestricted and to a certain extent exceeded the activity level of the females. Males both flew and walked at random throughout the plastic box, but in no case did the males exhibit different or excited behavior around the female cage. The wire mesh was wide enough to allow males to copulate with females if they had chosen to do so. The greater the degree of amputation of the male antennae, the greater was the amount of activity observed; the activity of the males was most intense between 10 p.m. and 2 a.m. Both sexes sat facing upright during daylight hours as though staring at the artificial lights. Daytime activity was observed when the moth was disturbed or for feeding.

Females deposited eggs on the screen cover as the most villous surface.[2] However, these eggs were few in number and had the typical squeezed-in, pointed appearance of infertile eggs.[2] No hatching occurred from any experiment in this series.

C. CONCLUSIONS

The experimental results indicate that females do not behave in a normal manner when in restricted quarters. Since successful reproduction depends on a complex series of behavioral events with both sexes responding to each other, and since this series is probably initiated by the "calling" of the females which involves using their wings,[3] one can conclude from my experiments that females in flight-restricting cages cannot release responses from males leading to successful copulation. If there is a female sex pheromone attractant for males in *Heliothis zea* (Boddie), it is apparently inoperative in situations where females are inhibited in their wing beating. Either the wings waft the attractant molecules, or the heat generated by the body during intense wing activity broadcasts a signal,[1] or the wing scales themselves may be involved[1] in attraction of males by female moths. Evidently some factor of attraction or combination of factors is inoperative in the flight-restricted female moth.

IV. UNILATERAL AMPUTATION OF FEMALE ANTENNA

This experiment was performed to parallel the unilateral amputation of the male antenna. The methods and materials as employed on the males were employed in the amputated females.

In experiments with and without plastic dividers, and in experiments with temperature control (with the incubator) and diurnal temperature fluctuations, there were no successful matings of females which had been unilaterally amputated.

The results of these experiments are unexpected, particularly with the lower degree of activity observed with female moths. One would expect that the greater the activity level, the more sensory devices should be scanning the environment. If the loss of one antenna makes a female less capable of "calling" the male moth, it is a new facet of female antenna behavior.

V. UNILATERAL AMPUTATION OF MALE ANTENNA

Some writers have mentioned amputations of insect antennae.[5-10] Typically, reports of circuitous behavior or loss of some degree of sensitivity have been made.

Male moths in anesthesia owing to refrigeration to near 0°C were unilaterally amputated. These male moths were subsequently liberated in the large plastic box. Female moths were caged in a 12 x 12 x 3 cm wire mesh cage and inserted into the large box.

In the experiments with a plastic sheet divider, no mating took place even though males frequently entered the female end through the cut slit in the plastic sheet. Where no plastic sheet divider was used, mating did take place. Successful mating only occurred when the box was in the incubator at a constant 30°C.

The temperature factor here is as suggested previously.[2] The behavior of the males which have been unilaterally amputated may be explained as follows. The male moths are able to locate the female moths provided there are no complications or obstructions, such as the plastic divider.

VI. UNILATERAL AMPUTATION OF THE ANTENNA OF BOTH MALE AND FEMALE MOTHS

Following the experiments with unilateral antenna amputations of one sex and then the other, both amputated groups were observed together in mating experiments. The materials and methods are identical with the single-sex unilateral amputations.

No successful mating was achieved in any experiment in this series, regardless of environment or apparatus. Behavior of the moths was not unusual either in walking or flying.

Apparently the loss of one antenna from both male moths and female moths placed some inhibitions on one of the sexes or both sexes.

VII. SERIAL AMPUTATION OF MALE MOTH ANTENNAE

Callahan reports the nature of the antennae of Heliothis zea (Boddie); the antennae are described to have from 72 to 86 segments with various sensillae on the surface of the segments.[11] Since spines and other sensillae are repeated on each successive segment, I tried amputating 1 mm from the apex of each antenna of the male moths; subsequent mating observation was made. Serial removal of 1-mm increments from the apex of male moth antennae was made and mating observation noted.

The only successful mating in this experiment series involved males with amputations of not more than 1 mm. All those with amputations of more than 1 mm failed to mate. None of this series was exposed to the constant-temperature environment of the incubator. The greater the degree of antennal amputation of the male moth, the greater was their activity, including their tendency to fly when disturbed.

Further experimentation is essential before drawing any conclusions. Temperature-control work and different plastic divider experiments must be performed and the results studied. The successful mating of the males with 1 mm of antenna removed could have been accidental rather than a true response. It may be possible for corn earworm moth males to find females who are calling even with the amputation of 1 mm of their antennae.

VIII. TOTAL ANTENNECTOMY OF FEMALE MOTHS

As a follow-up of the total antennectomy of male moths, the female moths were amputated totally and mating behavior was observed. Amputations were performed on refrigeration-anesthetized females.

One experiment involving females with antennae totally amputated resulted in a successful mating. Most experiments in this series resulted in no mating.

The one successful mating could be random or laid to accident. The lack of successful mating in the balance of the experiments may be due to the female moths being unable to recognize the presence of the males near them.

IX. TOTAL ANTENNECTOMY OF MALE MOTHS

Both antennae have been amputated before and reports have been in the literature on some moths and beetles. To compare with other amputation experiments, total amputations of male moth antennae were performed on <u>Heliothis</u>. Amputations were performed on refrigerated male moths to eliminate damage to other sensory receptors.

Regardless of temperature, relative humidity, or plastic divider conditions, the male moths were unable to find and mate with normal female moths. Male moths were capable of walking or flying into the vicinity of the female cages and were observed sitting on top of the female cages, but not <u>in copulo</u>.

Although male moths were able to get into close proximity of the normal virgin female moths, they were inhibited from successful mating. Perhaps the male is unable to recorgnize that the female is close when totally amputated to the antennal base.

X. TOTAL ANTENNECTOMIES OF MALE AND FEMALE MOTHS

After performing total antennectomies of male moths and female moths separately, I decided to test both sexes together with total antennectomies and attempt to get mating under those circumstances.

After the amputations both sexes exhibited excited behavior, but this excited behavior failed to yield successful mating. Very few eggs were deposited by these female moths.

As a follow-up to the total amputation of male moths and female moths separately, the conclusions here are not unexpected with the total amputation of both sexes. Although the male moths and female moths were able to be very close with only a mesh layer between them, there were apparently some inhibitions to mating when the antennae were totally missing. Excited behavior failed to result in better mating. Oviposition was strangely inhibited in comparison with female moths who have normal antennae. Even unmated normal females lay large groups of eggs.

XI. SUMMARY AND CONCLUSIONS

Females which copulated were between 48 and 72 hr of adult age. Oviposition occurred at 72 hr of adult age in every case: the oviposition then continued for three to 5 successive nights. Incubation time for the eggs ranged from 48 hr in the 30°C incubator to 96 hr in room diurnal fluctuating temperatures. The most unusual behavior was observed in the totally amputated females; these female moths were older then they oviposited and oviposited over a longer period. The best mating condition in my cages was to use six female moths with four male moths[11] and an adult age of at least 24 hr when starting the experiments.

The environmental conditions most conducive to Heliothis zea

(Boddie) was a combination of a relative humidity of 95% or more at a temperature of 30°C in a dark chamber with 10% honey solution for food. The horizontal plastic sheet, although it seemed to limit male moths very little, may have some inhibiting effect unkown to this writer at this time; no successful mating was accomplished with the plastic divider in the horizontal position.

The vertical plastic sheet divider had a lesser effect on the moths; successful mating occurred except where large-scale amputations of male antennae were performed.

Activity was not a significant measure of mating potential by itself. The optimum environment had the effect of stimulating behavior, but larger amounts of amputation had the effect of stimulating behavior also. Optimum environment yielded mating, whereas large-scale amputation failed to yield mating. The optimum mating situation is obtained by providing optimum environment and having normal male and female moth antennae. It must be noted that even in the best field conditions, Heliothis zea (Boddie) has a low mating rate, 50% according to Callahan.[2] Mating was only successful in 33% of my experiments; these matings were produced by the controls (with and without plastic sheet dividers), by totally amputated female moths with normal male moths, by normal female moths with unilaterally amputated male moths, and by normal females with males amputated by 1 mm on both antennae.

Therefore, it seems to suggest that males may require most of their antennae for mating.

For more quantitative results, all the experiments are to be repeated in order to establish statistical reliability. The serial amputation series was not performed in the incubator and therefore lacks some validity.

Another type of antenna treatment series will concentrate on the removal of sensillae from *Heliothis zea* (Boddie) and establishing the effects on mating.[10]

For the purposes of comparison, other moths and beetles will be amputated in identical tests. It would be desirable to know how similar antennae of different species, different families, and even different orders of insects may function in mating. Some insect control mechanisms may be proposed based on more knowledge of antenna physiology.

DATA SUMMARY SHEET: X=TEST, 0=NOT APPLIED

Experiment	Diurnal Fluctuation	Incubator	Mating
Control, no plastic divider	x	0	no
Control, no plastic divider	0	x	yes
Control, horizontal plastic divider	x	0	no
Control, horizontal plastic divider	0	x	no
Control, vertical plastic divider	0	x	yes
Unilateral amputation of male antenna	x	0	no
Unilateral amputation of male antenna	0	x	yes
Unilateral amputation of female antenna	x	0	no
Unilateral amputation of female antenna	0	x	no
Total amputation of male antennae	x	0	no
Total amputation of male antennae	0	x	no
Total amputation of female antennae	x	0	yes
Total amputation of female antennae	0	x	no
Total amputation of male and female antennae	x 0	0 x	no no
1 mm amputation of male antennae	x	0	yes
2 mm amputation of male antennae	x	0	no
3 mm amputation of male antennae	x	0	no
4 mm amputation of male antennae	x	0	no
5 mm amputation of male antennae	x	0	no
6 mm amputation of male antennae	x	0	no
7 mm amputation of male antennae	x	0	no

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