

71-7297

LOUGHNER, George Emmanuel, 1943-
FACTORS INFLUENCING MATING OF
THE EUROPEAN CORN BORER, OSTRINIA
NUBILALIS.

Iowa State University, Ph.D., 1970
Entomology

University Microfilms, Inc., Ann Arbor, Michigan

FACTORS INFLUENCING MATING OF THE
EUROPEAN CORN BORER, OSTRINIA NUBILALIS

by

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Entomology

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1970

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INTRODUCTION

In recent years restrictions on the use of certain chemicals have necessitated that alternative methods be found to regulate pest populations. Through extensive research the European corn borer control program has been able to employ integrated control methods for some time (Brindley and Dicke 1963). Recently, however, the pest management or ecological approach to insect control has gained favor among modern economic entomologists. The ultimate goal of this approach is the more effective and judicious use of the chemical and biological programs necessary to maintain pest populations at tolerable levels. The underlying principle of this program is to adjust control operations to fit more closely the biology of the pest. To apply this program a basic knowledge of the factors that contribute to the fluctuation of numbers in the pest population is necessary.

To aid further research on the nature of corn borer populations, I studied the mating habits of this pest. I made a twofold approach. I first briefly surveyed some of the biotic factors that might influence mating success. Secondly, I identified some of the abiotic factors that influence mating. To aid interpretation of results, the environmental conditions under which both studies were made

were similar. Male and female precopulatory behavior is described also as well as other behavioral phenomena concerned with mating.

REVIEW OF LITERATURE

In 1917 the European corn borer, Ostrinia nubilalis, was identified as an introduced pest of corn. Many attempts have been made since that time to explain the biology of this pest in the field (Huber, Neiswander and Salter 1928; Stirrett 1938; Everett, Chiang and Hibbs 1958; Chiang, Jarvis, Burkhardt, Fairchild, Weekman and Triplehorn 1961; Sparks, Chiang, Triplehorn, Guthrie and Brindley 1967). However, no attempt has been made to study the factors that influence mating of the European corn borer under controlled conditions.

The fact that external stimuli can effect certain behavioral patterns in animals is well recognized among workers in behavioral biology. Attempts to isolate, identify, and interpret these stimuli, however, involves much conjecture in that an organism is never completely isolated from the environment (Brown 1960). Nevertheless, workers in this field generally agree that there are two abiotic stimuli that seem to influence behavior patterns more than others. These are light and temperature. Good reviews of their influence on animal behavioral patterns is given by Marler and Hamilton (1966) and Beck (1968). My literature review is limited mainly to the influence of these abiotic factors on the mating of the European corn borer.

Light

A few visual observations on the mating of the European corn borer have been reported. Vinal and Caffrey (1919) observed that the adult will mate within 24-hr after eclosion of the imago and that mating occurs in the late afternoon or evening. O'Kane and Lowry (1927) report that the adults mate at or shortly after dusk. Caffrey and Worthly (1927) report seeing adults in copulo at all hours of the day, but usually in the late afternoon or evening. Weather conditions related to these matings, however, were not given.

Recently behavioral studies have progressed on other nocturnal lepidopterans. Allen and Hodge (1955) observed that the tobacco hornworm, Protocarce sexta, mates sometime after midnight. The pink bollworm, Pectinophora gossypiella, mates between 2 and 5 A.M. (Lukefahr and Griffin 1957). Callahan (1958) observed that the corn earworm, Heliothis zea, usually mates between 1 A.M. and dawn. Shorey (1962) reports that the average time for mating among the cabbage looper, Trichoplusia ni, was 2 A.M.

Under laboratory conditions Shorey (1966) found that the proportion of T. ni that mated was inversely correlated to light intensity within a range of from 0.3 to 300 lux at 26 C.

Dustan (1964) observed the Oriental fruit moth,

Grapholitha molesta, mating in the late afternoon and early evening. Under a light cycle however, mating occurred 2-3 hr before the dark period. This rhythm once established persisted under continuous light (George 1965).

A light-phased circadian rhythm of male responsiveness to a female pheromone was observed by Shorey and Gaston (1965) among several species of Noctuidae.

Temperature

Preliminary work by Sparks (1963) on the mating of the European corn borer indicated that successful copulation was achieved when the adults were exposed to a falling temperature that was in phase with a 14-hr light, 10-hr dark cycle.

Callahan (1958) observed that H. zea adults mate only when the temperature is below 85 F. Shorey (1966) found that T. ni adults do not normally mate below 14 C, but showed a correlation between temperature and percent mating within a range of 10 to 15 C.

Temperature has also been shown to effect cyclic activity patterns in insects. A locomotor activity rhythm was shown in the spider beetle, Ptinus tectus (Bentley, Gunn and Ewer 1941). Roberts (1962) observed a locomotor activity rhythm in the cockroaches Leucophaea and Periplaneta when entrained to a sinusoid temperature cycle. The eclosion rhythm of the moth Anagasta seems to be primarily controlled by tempera-

ture (Scott 1936). The adults emerged in the hours following the daily temperature maximum.

The fruit fly, Dacus tryoni, also shows a sensitivity to temperature cycles in the pupal stage (Bateman 1955), emergence being concentrated around the temperature-rise phase of the thermoperiod. The pupae of this species were found to be insensitive to photoperiod, but egg and larval stages could be entrained to a photoperiod to effect emergence.

Schurr and Holdaway (1966) observed a 24-hr rhythm of oviposition by the European corn borer when exposed to natural environmental fluctuations of light and temperature. This rhythm, however, was lost under constant conditions of light and temperature. Combinations of photoperiod and thermoperiod have been studied by Pittendrigh (1958) on Drosophila emergence. Under simultaneous cycles of temperature and light, emergence occurred at dawn (lights on) and low temperature. When the minimum temperature occurred during the scotophase, emergence still occurred at dawn. However, when the minimum temperature occurred during the photophase, emergence was at this minimum temperature and not necessarily at dawn.

Humidity

The role that humidity plays in governing mating has been established for a few insects. Sparks (1963) observed

mating among European corn borers exposed to relative humidities of between 45 and 95 percent. In laboratory studies Callahan (1958) found that Heliothis zea mated at 80 percent relative humidity. Shorey (1966) reported that 46 percent of Trichoplusia ni females mated at a low relative humidity of 44 percent.

GENERAL METHODS AND MATERIALS

Adults of the European corn borer, belonging to a strain two generations removed from the field, were reared in the laboratory from larvae maintained on an artificial diet (Guthrie, Raun, Dicke, Pesho and Carter 1965). Reed, Showers and Huggans (in press) showed that more pupae could be recovered from this diet by improved handling methods. These methods were adopted during the course of the investigation. Larvae were reared under constant conditions of light (150 foot candles) and temperature (80 F). After 20 days, pupae were removed from diet-dishes, placed in individual 0.5-oz plastic cups which were then capped with paper lids, and returned to an emergence incubator programmed for simultaneous cycles of light and temperature. A daily photophase of 16-hr was used (16 L). Light intensity and temperature during the photophase were 175 foot candles and 85 F, respectively. The temperature during the 8-hr scotophase (8 D) was 65 F. Relative humidity during the 24-hr cycle averaged 75 percent. Light was provided by 40-w fluorescent lamps.

Pupae were checked each day to determine the number of adults that had emerged. When adults were found they were grouped according to day of emergence and held an additional 24-hr in the same incubator. For the purposes of these experiments adult age is the number of days after emergence

of the imago. All adults used in these experiments were 2 days old. During subsequent experiments, however, the pupae were incubated under conditions that would result in the eclosion of the imagoes satisfactory for a particular experiment. These conditions will be described in the discussion of specific experiments.

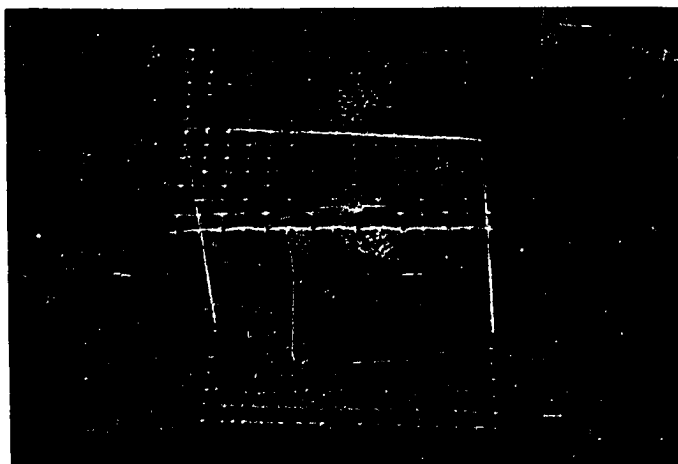
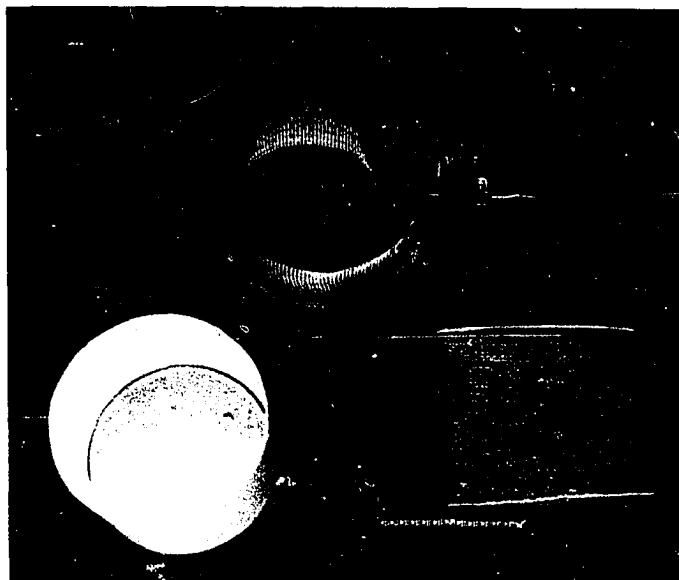
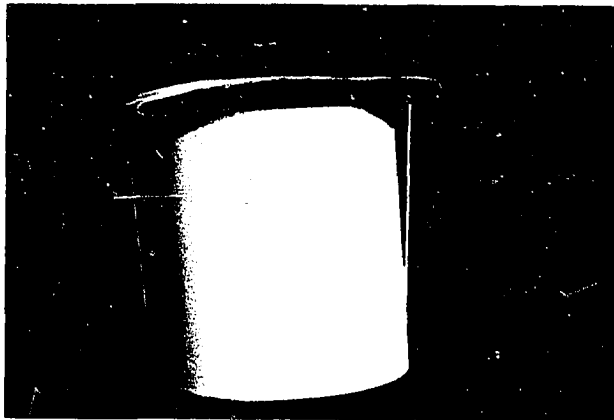
Adults were transferred from the plastic cups to mating cages in a 60 F room. The mating cages were then placed in a test incubator programmed for a particular experiment. Adults were then provided water by spraying the cages with a fine spray.

Pint-size cylindrical cardboard ice-cream containers lined with 19-mesh wire screen were used as mating cages. The bottoms of the containers were left intact but the cardboard top was replaced with a 4 inch square of screen held in place by a rubber band (Figures 1 and 2). Cages used for evaluating mating success under variable light intensities at different temperature conditions were modified as described by Shorey (1964). The bottom of the container was removed and the container placed over the cell of a Photovolt photometer (model 200) with screen liner and screen top in place. By adding more screen tops to create various thicknesses and by moving the container varying distances from the light source, the desired light intensities were obtained within the container.

Figure 1. Mating cage used in experiments designed to evaluate mating success under various light intensities at three different temperature conditions

Figure 2. Exploded view of mating cage in Figure 1

Figure 3. Screen-wire mating cage



When visual observations were required the screen liner was removed and the ends fastened so as to form a cylinder. A screen square was added to each end of the cylinder and held in place by a rubber band (Figure 3).

The various environmental conditions to which the adults were exposed were provided by a walk-in incubator that could be programmed to automatically control temperature, humidity, and light for a 24-hr period. If longer exposures were needed the regimes could be maintained manually. Temperature control was accurate to within ± 0.5 F and relative humidity to within 2 percent. Light was provided by fluorescent lamps. Temperature and humidity records were recorded on a Bendix hygrothermograph which was checked every week for accuracy with a standardized mercury thermometer and a sling psychrometer.

At the termination of a test the adults were removed from the test incubator and placed in a 40 F room to immobilize them. They could then be examined for success in mating. The criterion for successful mating was the presence of a spermatophore in the bursa copulatrix of the female (Pesho 1961). Adults still joined in copulo at the end of the test period were not considered mated successfully unless a spermatophore was found in the bursa copulatrix.

Definitions

The following words are used in connection with the discussion of this study. The definitions used differ slightly from some of those suggested by Aschoff, Klotter and Wever (1965), Beck (1968), Bruce (1960), Halberg (1959), and Went (1944).

Circadian rhythm--A rhythm persisting in the absence of an entraining synchronizer. The period varies slightly from 24-hr.

Cycle--The whole of certain specified changes recurring within an organism or within the environment.

Dark-phase--That part of a rhythm devoid of illumination.

Diel rhythm--A rhythm existing only in the presence of an 24-hr entraining synchronizer. Under controlled conditions the entraining synchronizer is varied to produce a long (28-hr) or short (22-hr) day.

Endogenous rhythm--A circadian rhythm, a term suggesting that the rhythm comes from within the organism.

Entrainment--The coupling of an activity of, or in, an organism to a given synchronizer.

Exogenous rhythm--A diel rhythm, a term suggesting that the rhythm comes from outside (e.g., the environment) the organism.

Free-running--A rhythm persisting in the absence of an entraining synchronizer. The period varies

drastically from 24-hr.

Light-phase--That part of a rhythm provided with illumination.

Period--The length of time occupied in a cycle by a particular event, e.g., the mating period.

Phase--A specified part of a rhythm, e.g., the high-temperature phase, scotophase, or photophase.

Photophase--See light-phase.

Photoperiod--A recurring alternation of illumination and darkness.

Rhythm--A regularly recurring quantitative change.

Scotophase--See dark-phase.

Synchronizer--A particular aspect of a photoperiod, thermoperiod etc., e.g., scotophase, photophase, high temperature, low temperature, temperature change (rise or drop), lights-on, lights-off.

Thermoperiod--A recurring alternation of high and low temperatures.

Transient--A carry-over response by an organism to a previous rhythm while being entrained to a new rhythm.

RESULTS

Biotic Effects on Mating

Crowding

An experiment was designed to determine the number of pairs of adults that could be put into a pint-size mating cage without having a crowding effect on mating. Combinations of 1, 2, 3, 4, and 5 pairs of adults were placed in the mating cages and exposed for 24-hr to cycling conditions of light and temperature. A daily photophase of 16-hr was used. Light intensity and temperature during the photophase was 250 foot candles and 85 F, respectively. The temperature during the 8-hr scotophase was 67 F. The relative humidity ranged from 75 to 85 percent. These conditions of photoperiod and thermoperiod approximate those of the early weeks in June when the first moth flights occur. A maximum of five pairs was chosen because no more than this would be used in subsequent single-cage experiments. Later experiments were designed so that two screen mating cages could be joined, thus increasing the maximum number of adult pairs to ten.

Table 1 shows the percentage of females mated. For the purposes of the following experiments it was concluded that there was no difference in mating due to crowding under these conditions.

Table 1. Influence of crowding on mating^a

Pairs per cage	Percentage of mating	
	Mean	Range
1	75	0-100
2	63	0-100
3	75	66-100
4	56	25-75
5	75	60-80

^aExperiment performed twice using two cages of adults per treatment.

Mating frequency

An experiment was designed to determine if an adult male or female will mate more than once during a 24-hr period. Adults were caged in different sex ratios and exposed to cycling conditions of 16-hr L at 85 F and 8-hr D at 67 F. Relative humidity ranged from 75 to 85 percent.

Results (Table 2) indicated that an individual of either sex mates only once during a 24-hr period.

Multiple matings

In the previous experiment the paired adults were left together for 24-hr. During this time it was determined that neither the male nor the female will mate more than once. An experiment was designed to determine if the female will mate more than once in 48-hr.

Table 2. Mating frequency in a 24-hr period

Sex ratio	Spermatophores
1 male to 5 females ^a	1
5 males to 1 female ^b	1

^aExperiment performed three times.

^bExperiment performed four times.

Adults of the same age were exposed to a 24-hr cycle of 16-hr L at 85 F and 8-hr D at 67 F. Relative humidity ranged from 75 to 85 percent. Adults were provided water once a day.

Of 41 females examined, none had mated twice. The experiment was repeated except that the females were examined for spermatophores only after all the females had died. At the end of 11 days 17 females had mated and, of these, 2 (11.7 percent) had mated twice.

Adult age

An experiment was designed to determine if the age of the imago is a factor influencing mating success. Adult males and females were paired as to age to determine how long after emergence adults would be able to mate. Age, for the purposes of this study, was established as the number of hours after eclosion of the imago. Age groups

studied were 1-6, 24-30, 48-54-hr old at the beginning of the experiment. Adults of the same age group were exposed to cycling conditions of 16-hr L at 85 F and 8-hr D at 67 F. Relative humidity ranged from 75 to 85 percent. The experiment was performed five times with five pairs of adults per age group.

The results (Table 3) show that there is no difference in the ability of adults, in the age groups tested, to mate successfully.

Table 3. Effect of age on mating

	Age (hr)		
	1-6	24-30	48-54
Females mated	17	18	18
Females unmated	8	7	7

Age combinations

An experiment was designed to determine if there would be a difference in mating success when adults of different ages were placed together. Adults were mixed in various age combinations up to 5 days of age. Adult age was the number of days after eclosion of the imago. Adults were exposed to cycling conditions of 16-hr L at 85 F and 8-hr D at 67 F. Relative humidity ranged from 75 to 85 percent. The

experiment was performed twice using five pairs of adults per cage.

Results indicated that there is no difference in mating success when adults of different ages are placed together (Table 4).

Table 4. Effect of age combinations on mating

Age combinations	Rep.	Number of females	Spermatophores present
1-day-old male and 1-day-old female	1 2	5 5	3 4
2-day-old male and 1-day-old female	1 2	5 5	4 4
1-day-old male and 2-day-old female	1 2	5 5	5 3
2-day-old male and 2-day-old female	1 2	5 5	4 3
1-day-old male and 3-day-old female	1 2	5 4	4 4
3-day-old male and 1-day-old female	1 2	5 5	5 4
1-day-old male and 5-day-old female	1 2	5 5	4 4
5-day-old male and 1-day-old female	1 2	5 5	4 4

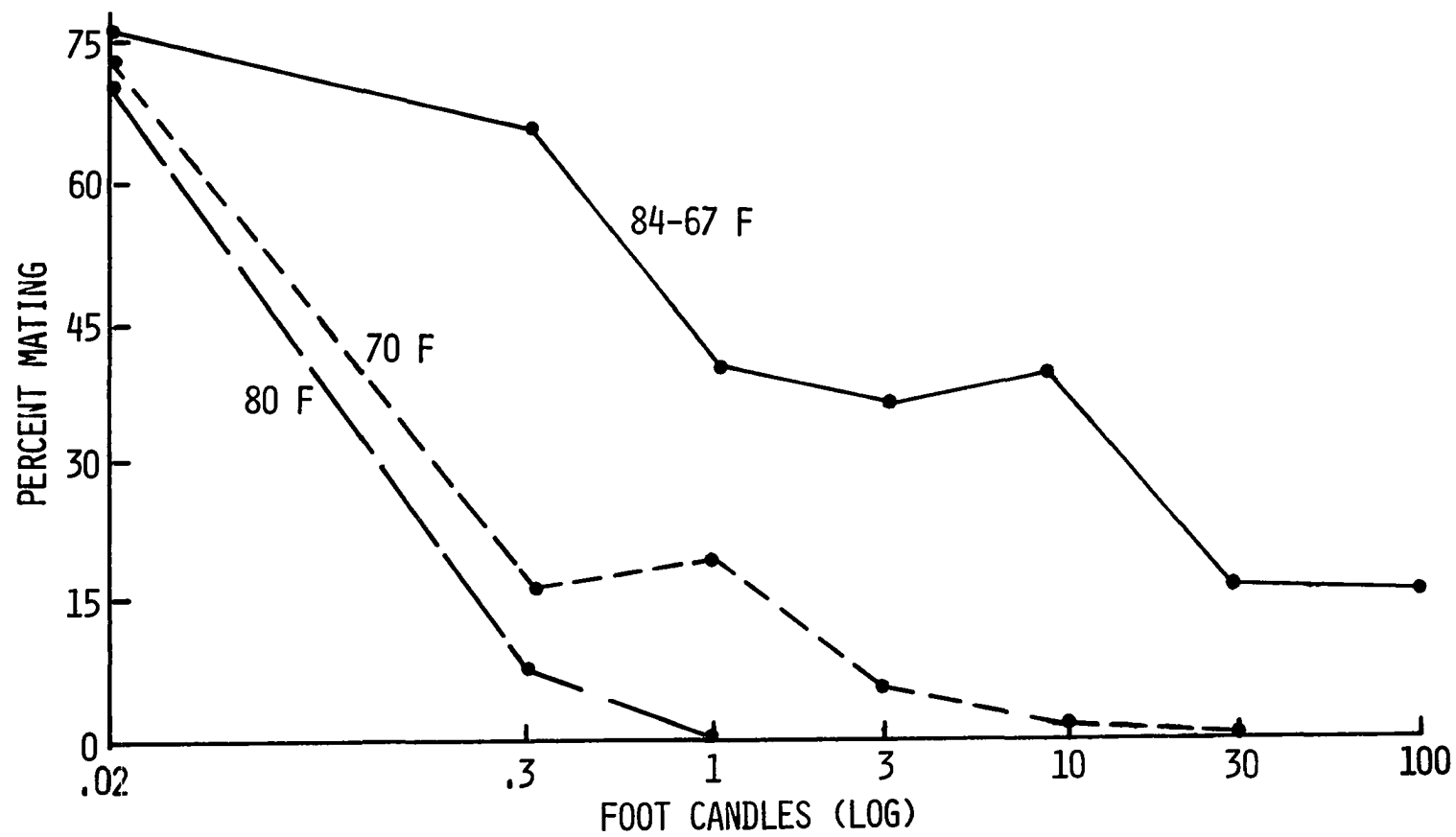
Abiotic Effects on Mating

Light

An experiment was designed to determine the influence of various light intensities at different temperature regimes on mating success. Constant temperatures of 80 F and 70 F as well as a 24-hr thermoperiod of 16-hr at 84 F and 8-hr at 67 F were used. Each light intensity was maintained at a constant level throughout a 24-hr period. These light intensities were 0.02, 0.3, 1, 3, 10, 30, and 100 foot candles, respectively. Cardboard pint-size containers with screen liners were used as mating cages (see General Methods). All adults used were less than 24-hr old at the beginning of any experiment. The experiments were performed ten times for each intensity using five pairs of adults. Arcsine transformations of the proportion mated were used in the chi-square analysis (Steel and Torrie 1960).

The relationship between mating and light intensity at these temperature conditions is shown in Figure 4. These results indicate that the effect of light is less dramatic in influencing mating at a cycling temperature and most influential at a constant temperature of 80 F. There is a highly significant difference between mating success occurring under constant temperatures and mating success occurring under cycling temperature. A partition of the sum of squares of the temperature x light interaction

Figure 4. The effect of light intensity on mating at three different temperature regimes



indicates that the variance is due mainly to temperature, light being less influential. A quadratic curve best expresses the relationship between constant and cycling temperature, further indicating the diminishing influence of light.

Temperature-drop

The previous experiment revealed that a 17 F drop in temperature was significant in influencing mating success. An experiment was designed to determine if there is a relationship between the amount of temperature-drop and mating success.

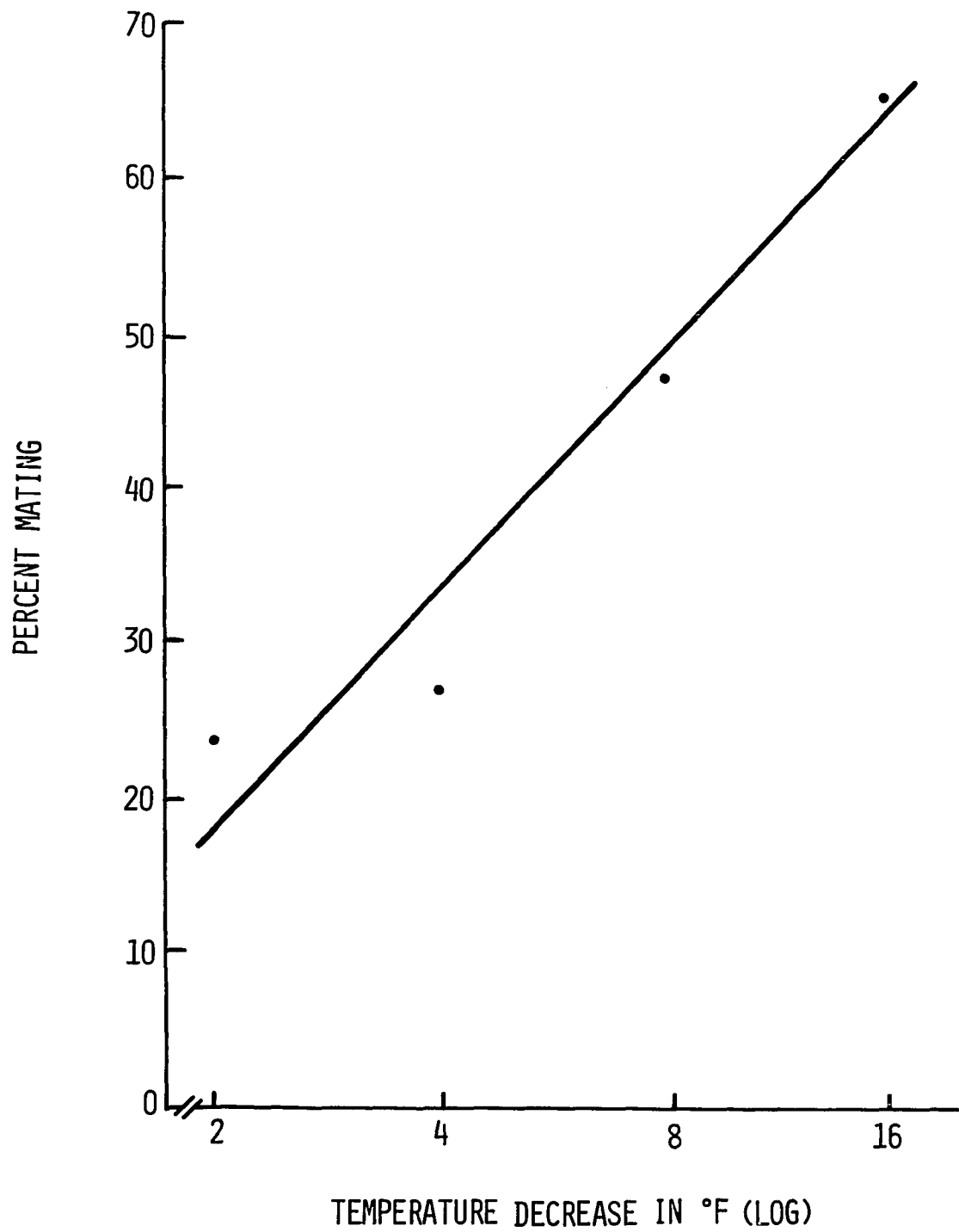
Adults were exposed to various 8-hr temperature-drops during a 24-hr photoperiod of 16-hr L:8-hr D. Relative humidity ranged from 75 to 85 percent. The temperature fell when the light went off. The temperature-drops were of 2, 4, 8, and 16 F within the range of from 86 to 70 F. The experiment was performed five times, using 20 pairs of adults each time. Arcsine transformations of the proportion mated were used in the chi-square analysis (Steel and Torrie 1960).

Results indicated (Figure 5) that mating is correlated with temperature within the range of from 86 to 70 F according to the general equation

$$\hat{Y} = 26 + 1.8 X$$

having a sample correlation coefficient of $r = 0.98$.

Figure 5. Effect of various temperature-decreases and percent mating. Temperature range, 86-70 F. $r = 0.98$, significant at the 0.05 level



Humidity

An experiment was designed to determine the effect of low relative humidity on mating-success. Previous experiments were carried out at relative humidities within a range of from 75 to 85 percent. This range was assumed optimum for the previous experiments.

The effect of low relative humidities on mating success was studied by placing anhydrous calcium chloride in an incubator. In this manner the humidity ranged from 35 to 45 percent under fluctuating conditions of light and temperature. A daily photophase of 16-hr was used. Light intensity and temperature during the photophase were 250 foot candles and 85 F, respectively. Temperature during the 8-hr scotophase was 67 F. The experiment was performed three times using 35 pairs of adults each time.

Table 6 shows the number of females that mated and that the average mating success was 50 percent.

Lower humidities were obtained by preparing a slush of potassium acetate and placing a bell jar over this slush with the cages of adults supported on a wire screen. In this manner relative humidities of 22-23 percent were obtained (Hydrodynamics, Inc. 1967). Temperature and light cycles were as above. The experiment was performed three times.

At this low humidity 8 out of 30 (27 percent) females mated (Table 6).

Table 6. Influence of humidity on mating

	Females mated	Females unmated
35-45 percent relative humidity		
Replicate		
1	18	17
2	16	19
3	18	16
Percentage of females mated--50 percent		
22-23 percent relative humidity		
Replicate		
1	3	7
2	2	8
3	3	7
Percentage of females mated--27 percent		

Wind

An experiment was designed to determine the influence of windspeed on mating success. Adults of mixed ages were exposed to various wind speeds during the scotophase. A daily photophase of 16-hr was used. Light intensity and temperature during the photophase were 250 foot candles and 84 F, respectively. Temperature during the 8-hr scotophase was 67 F. Relative humidity ranged from 75 to 85 percent.

Windspeed from a 16 inch fan was measured with an anemometer. Windspeed was constant during the scotophase. The controls experienced no wind. The experiment was performed twice using 5 pairs of adults.

A constant windspeed of 3 mph during the scotophase had no affect on mating success. However a constant windspeed of 10.3 mph during the scotophase completely inhibited mating (Table 7).

Table 7. Influence of wind on mating

	Treatment		Control	
	Females mated	Females unmated	Females mated	Females unmated
<hr/>				
	3 mph		0 mph	
Replicate				
1	4	1	4	1
2	4	1	4	1
<hr/>				
	10.3 mph		0 mph	
Replicate				
1	0	5	4	1
2	0	5	4	1

Environmental rhythms

Laboratory observations indicated that mating occurred after the temperature had dropped from 85 to 67 F and the light intensity had decreased from 250 to 0 foot candles.

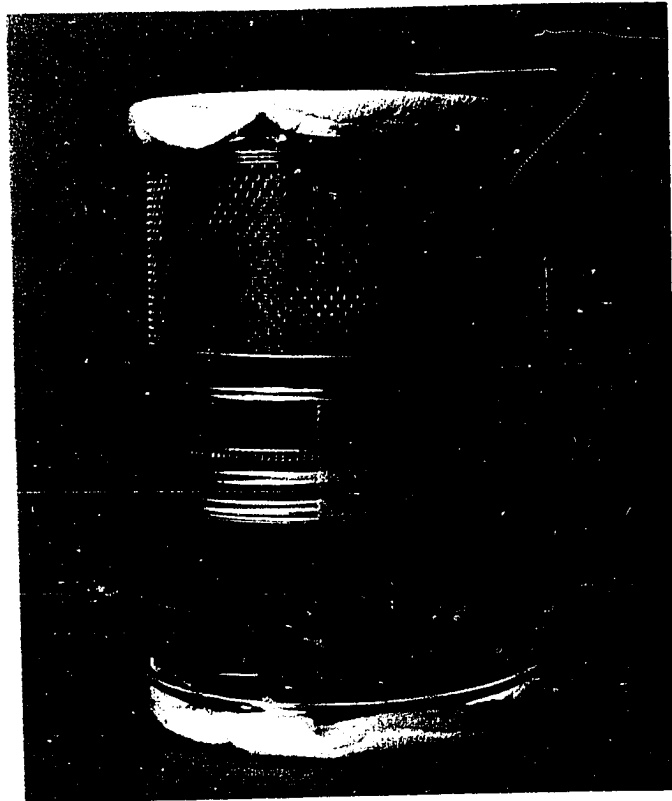
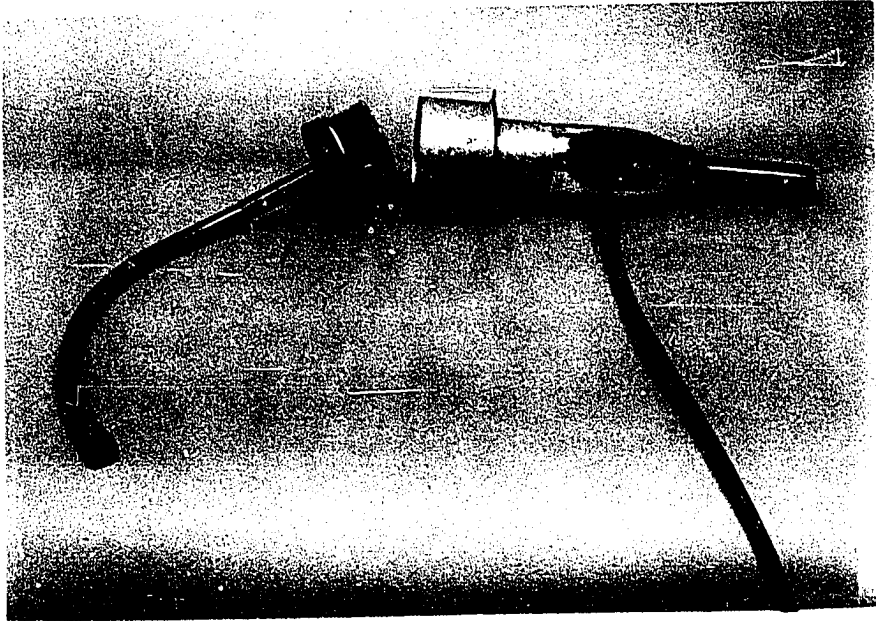
It was hypothesized, from these observations, that this cyclic change in environmental conditions might serve as a time clue for mating. To test the hypothesis, the variables, light and temperature, were segregated and tested for their influence on mating success.

Imagoes used for these experiments were laboratory-reared and were 6-7 generations removed from the parent stock collected from the field. The eggs of this stock were incubated under constant conditions of light (175 foot candles) and temperature (80 F). The larvae from these eggs were reared under the same constant conditions of light and temperature. The adults emerged under constant conditions of light (150 foot candles) and temperature (85 F). Males and females were collected separately in pint-size screen cages by means of a suction apparatus (Figure 6). An excess (11 or 12) of males was provided for every 10 females. Sexes were kept separate during entrainment by placing a Mason jar-lid between the cages (Figure 7). The cages were held together by placing a piece of masking tape around no more than half the diameter of the cages (Figure 7). This method kept handling to a minimum during subsequent tests because all one had to do to allow the sexes to intermingle was to remove the lid from between the cages.

Adult moths were provided with water by fitting one-half of a petri dish filled with cotton saturated with water

Figure 6. Suction apparatus used for collecting adults

Figure 7. Mating cages showing placement of jar-lid to keep the sexes separated. The tape holding the cages together goes no more than $1/2$ way around the cages. In this way the lid is easily removed allowing the sexes to intermingle



over the end of the cage. This system provided water for six days.

All cages for each experiment were placed in an incubator at the same time so that all adults experienced the same pre-treatment entraining time and conditions. During test periods the sexes were allowed to intermingle for 2-hr after which they were removed to a 40 F room for examination for a spermatophore in the bursa copulatrix of the female. At the end of one 2-hr test period another pair of cages were joined for 2-hr and so on until the end of the experiment. The number of females mated out of 10 was recorded. Results are presented graphically in each time period as the mean percentage of mating for three replicates.

Cycling temperature An experiment was designed to determine the effect of a 24-hr thermoperiodic cycle on mating success. A square wave thermoperiod of 16-hr at 80 F and 8-hr at 65 F was used. Constant light was provided by a 15-w fluorescent lamp baffled to produce a diffuse illumination of about .001 foot candles. Relative humidity ranged from 85 to 95 percent. Imagoes were entrained to 2 temperature drops before the experiments were initiated. At the beginning of the third temperature drop, the lid separating the sexes in one pair of cages was removed to allow the adults to intermingle for a 2-hr period. This procedure was followed every 2-hr for 36-hr for the remaining

cages.

The results (Figure 8) indicated that a temperature-phased diel rhythm of mating exists. Mating occurs soon after the temperature drops from 80 to 65 F and continues throughout the 8-hr low temperature phase. No matings occurred during the high temperature phase although a mating response to temperature rise may be present as indicated by some mating at the end of the tenth hour.

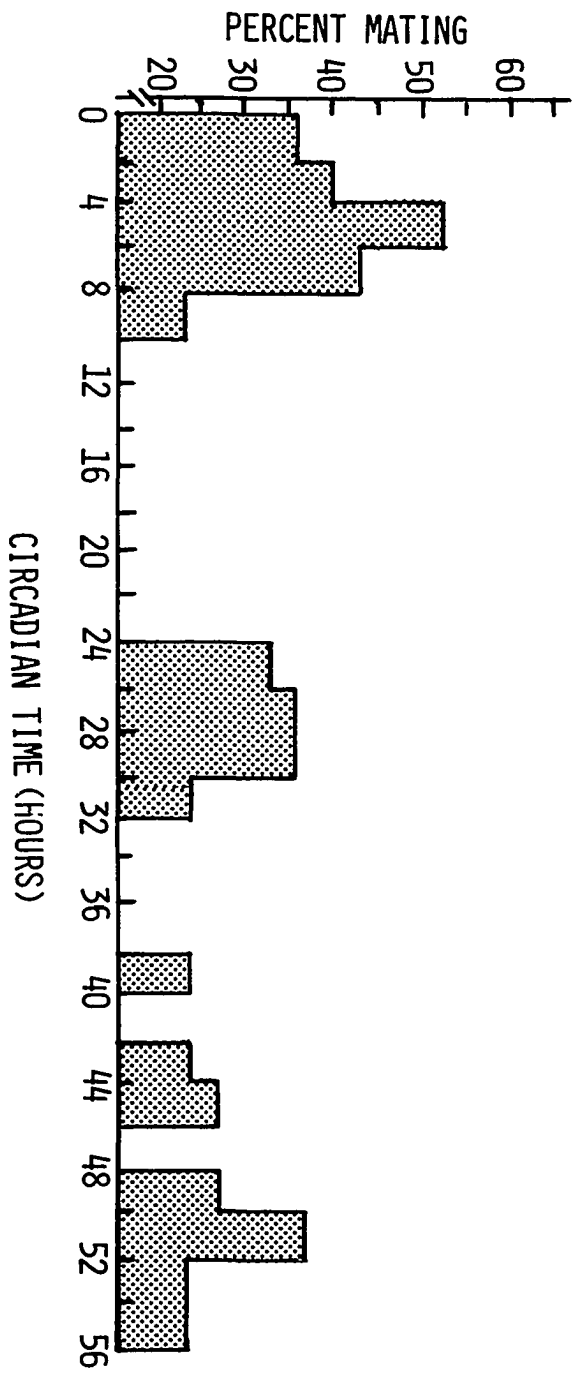
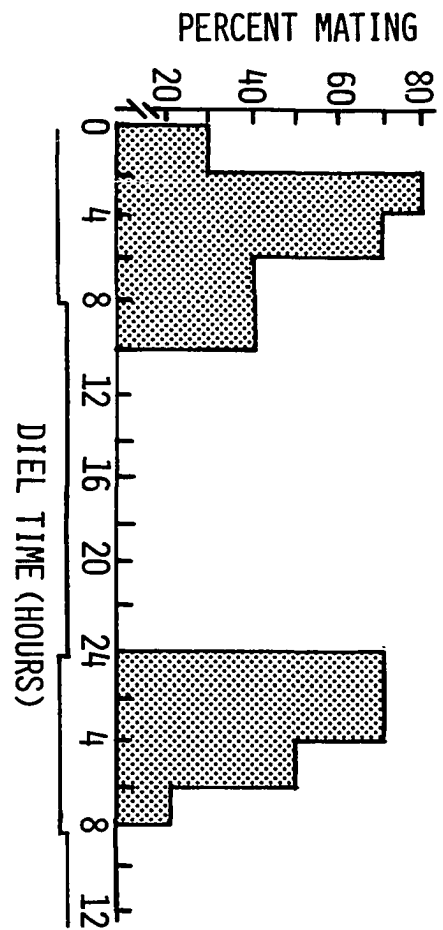
Since a diel rhythm of mating occurred each time the temperature fell from 80 to 65 F, it was hypothesized that a circadian rhythm of mating might be present in the corn borer. To test this hypothesis adults were entrained as above. At the third temperature-drop the incubator was programmed to hold low temperature (65 F) conditions for 56-hr. The procedure for handling adults was as described.

Results (Figure 9) indicated that mating, per se, is a circadian response to temperature-drop. Using mating peaks as standard reference points, the circadian rhythm expressed a 23-hr period under constant conditions. Beyond 36-hr the rhythm starts to wane.

To demonstrate the controlling influence of temperature or light Bunning (1964) suggested that a phase shift is the easiest and most convincing way. To shift the phase, the temperature was dropped so that the low temperature phase occurred in the middle of the second high temperature phase

Figure 8. The influence of a temperature-phased diel rhythm on mating. n = 10 females per time period

Figure 9. Temperature-phased circadian rhythm of mating. n = 30 females per time period



of the original cycle. The new cycle was 8-hr of low temperature (65 F) followed by 16-hr of high temperature (80 F).

Re-entrainment of mating to the new temperature cycle was accomplished within 24-hr (Figure 10). Mating transients are present in the middle of the new high temperature phase which is also the same time period that a mating peak was recorded in the original temperature cycle.

Cycling light An experiment was designed to determine the affect of a 24-hr photoperiodic cycle on mating success. An incubator was programmed for a square wave photoperiodic regime of 16-hr L and 8-hr D at a constant 65 F. Humidity ranged from 85-95 percent. Illumination during the photophase was from four 40-w fluorescent white lamps and from two 40-w Sylvania Gro-Lux lamps. To aid cage manipulation, a 15-w fluorescent lamp baffled to produce diffuse illumination was used during the scotophase. Light intensity during the photophase and scotophase was 175 and .001 foot candles, respectively. Light quality during the photophase is shown in Figure 12. Light quality during the scotophase shifted to the blue region but light intensity was too low to obtain an accurate tracing. The light quality tracings were made with an ISCO Spectroradiometer recorder and scanner, Model SRR.

Adults were entrained by two photoperiods before

Figure 10. Influence of a shift of the temperature cycle on mating. xxx = original temperature cycle. Solid line represents new temperature cycle. n = 10 females per time period

Figure 11. Influence of a shift of the light cycle on mating. ///// = original scotophase. Shaded area = new scotophase. n = 10 females per time period

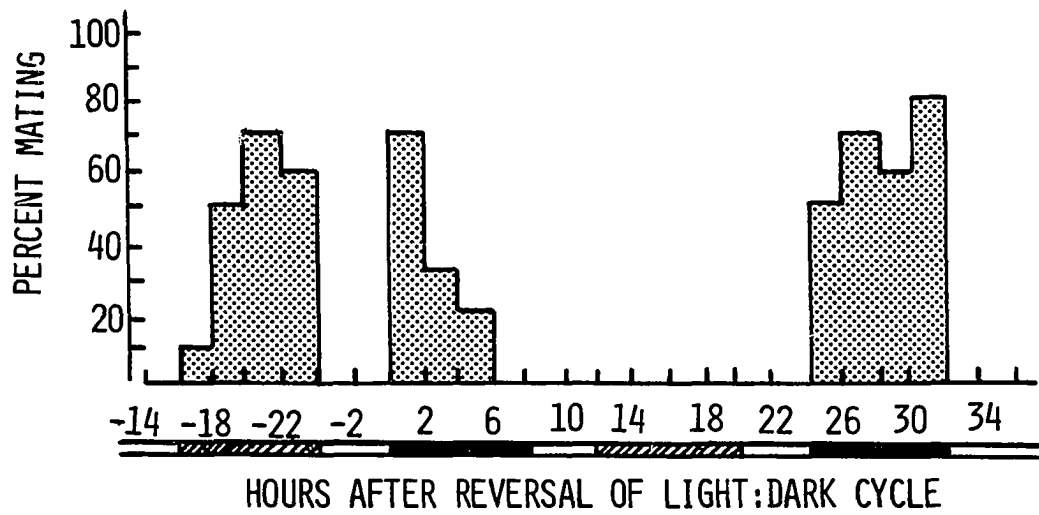
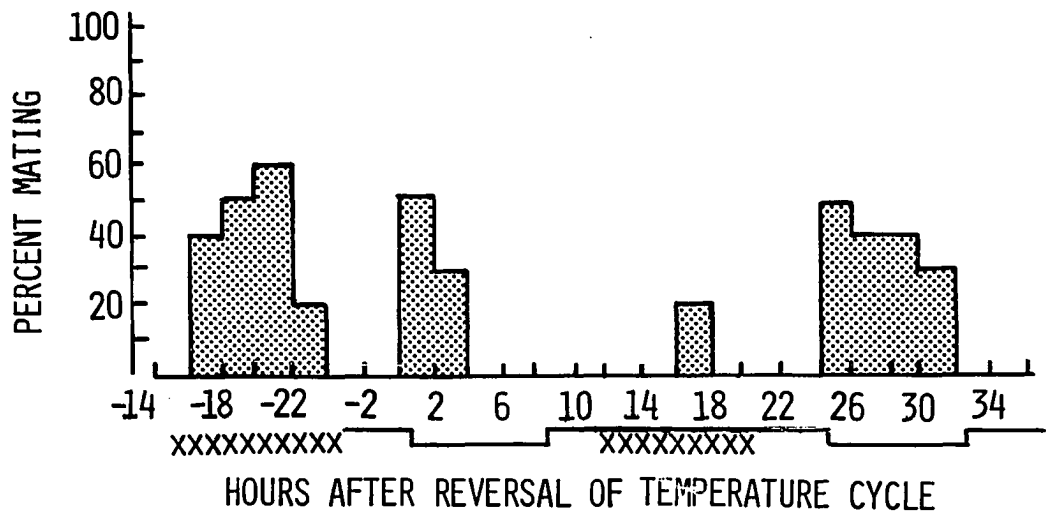
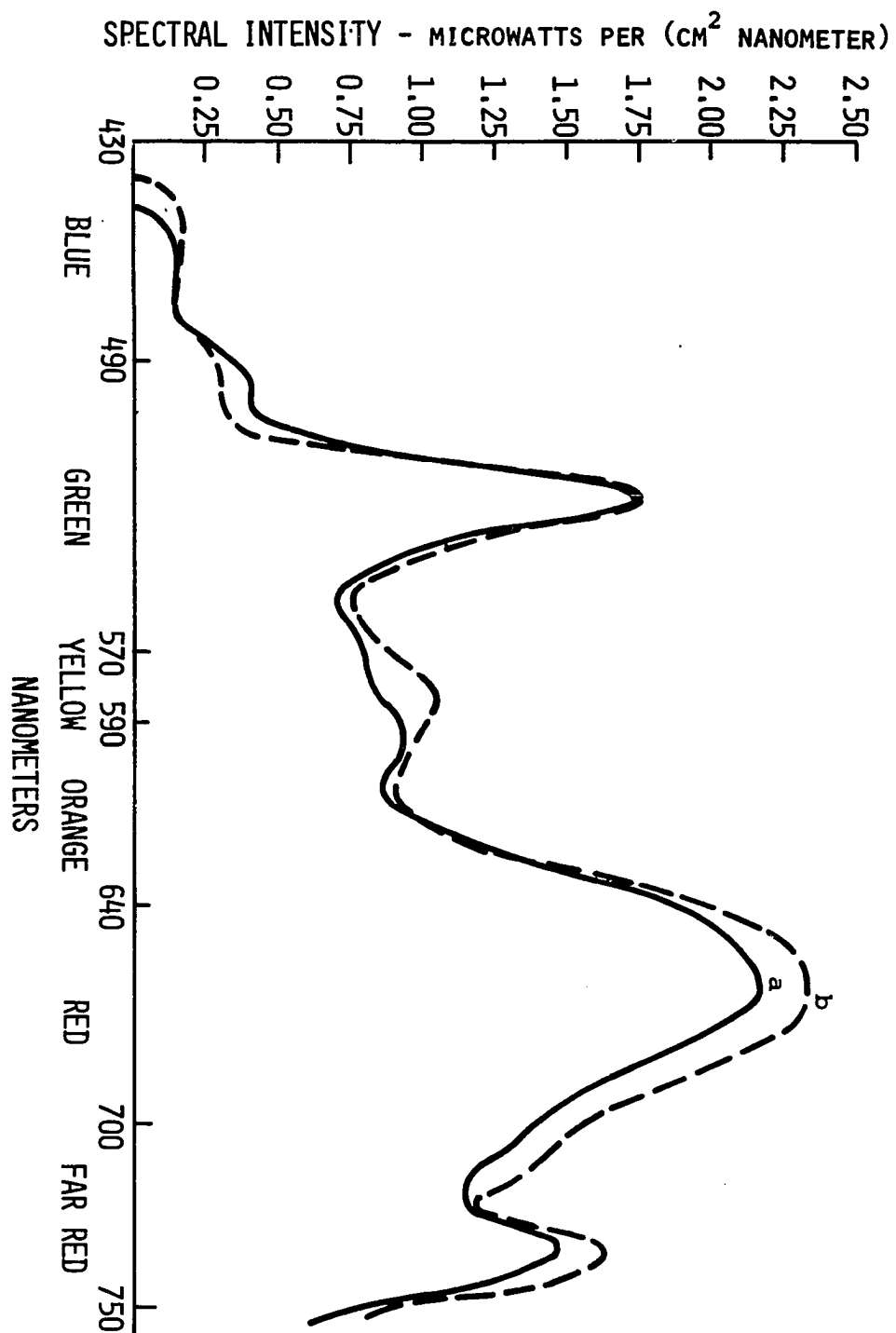


Figure 12. Light quality spectrum of the photophase during cycling light (a)
and cycling light and temperature (b)



initiating the experiments. At the start of the third scotophase, the adults of one cage were allowed to intermingle for 2-hr. At the end of the 2-hr adults from another cage were allowed to intermingle for 2-hr. This procedure was followed every 2-hr for 36-hr.

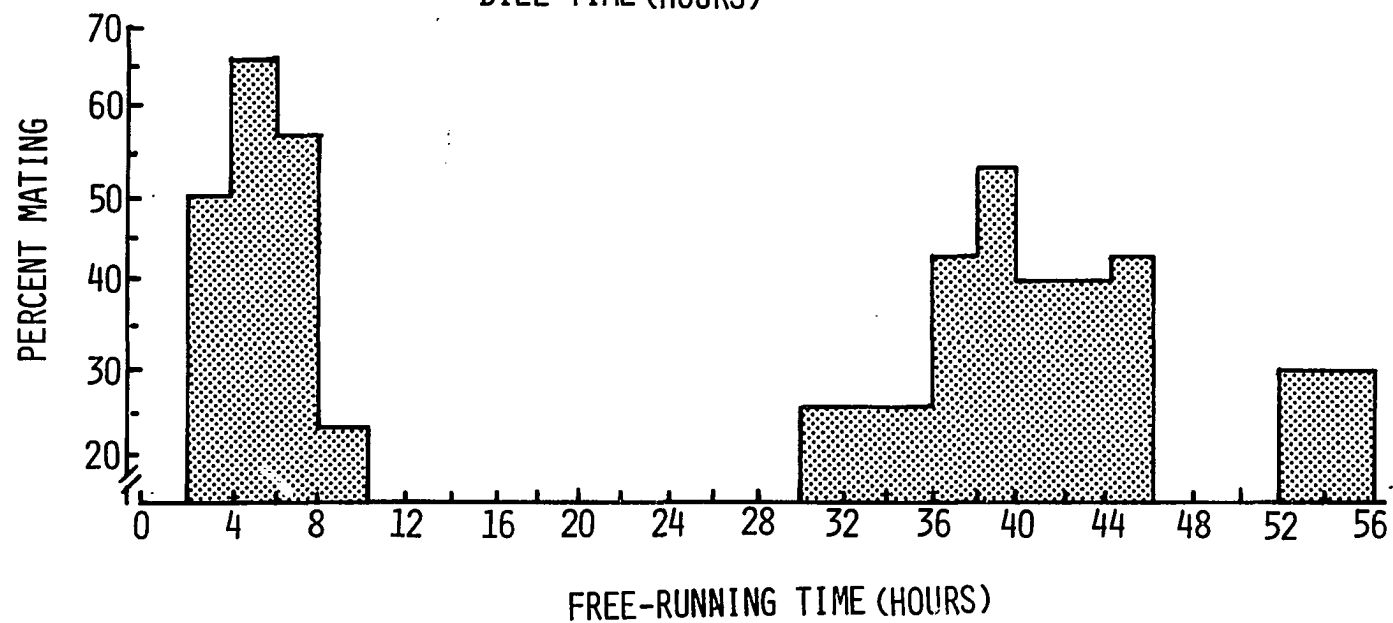
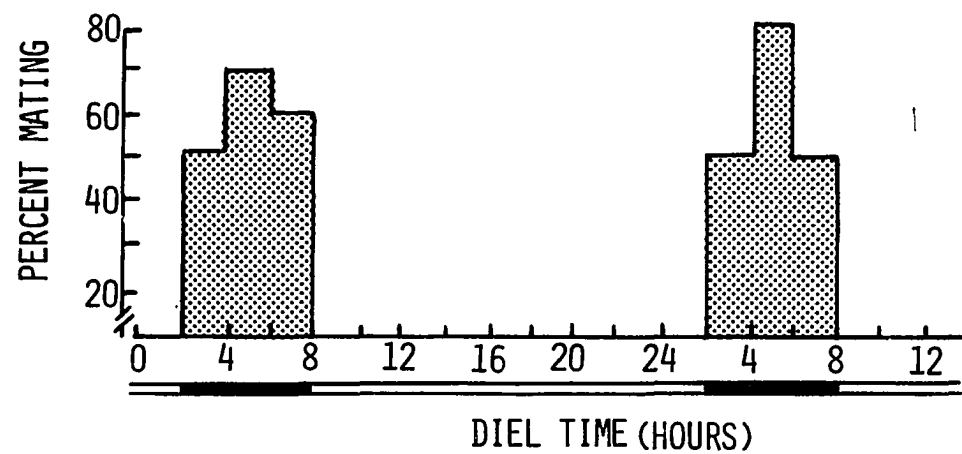
Results (Figure 13) showed that mating occurs soon after the beginning of each scotophase, indicating a dark-phased diel rhythm of mating. Since a diel rhythm of mating was shown to exist, it was hypothesized that a circadian mating response to cycling light may be present in the European corn borer.

To test this hypothesis, adults were entrained for two photoperiodic cycles as before. At the start of the third scotophase, adults of one cage were allowed to intermingle for 2-hr. At the end of 2-hr another cage of adults was allowed to intermingle for 2-hr and so on until the end of the test. This procedure was followed for 56-hr under constant scotophase conditions.

Results (Figure 14) indicated, however, that there is no circadian mating response entrained by a 24-hr photoperiod. Instead, mating peaks occur 34-hr apart. Mating was initiated 4-hr after the start of the original scotophase with a mating peak registered 8-hr later. This mating peak is 10-hr past the mating peak recorded during the entraining photoperiod.

Figure 13. Influence of a 24-hr light cycle on mating. Shaded area is the scotophase. n = 10 females per time period

Figure 14. Free-running light-phased rhythm of mating. n = 30 females per time period



From these results it appears that the change from photophase to scotophase (lights-off) acts mainly as an exogenous synchronizer for mating. To test this hypothesis the lights-off was shifted so as to occur 12-hr before the start of the scheduled scotophase. The new photoperiod was 16-hr of light and 8-hr of darkness.

Results (Figure 11) indicated that re-entrainment is immediate, with no transients occurring within a 24-hr cycle. The lack of transients during the new cycle may indicate that a circadian rhythm mechanism is not present and that lights-off acts only as an exogenous synchronizer.

Cycling light and temperature An experiment was designed to determine the entraining capabilities of simultaneous light and temperature cycles to effect mating of the European corn borer.

An incubator was programmed for simultaneous 24-hr cycles of light and temperature. A daily photophase of 16-hr was used. Light intensity and temperature during the photophase was 175 foot candles and 80 F, respectively. Temperature during the 8-hr scotophase was 65 F. To aid cage manipulations a 15-w fluorescent lamp baffled to produce a diffuse illumination of .001 foot candles was used during the scotophase. Light quality during the photophase is shown in Figure 12. Adults were entrained for two cycles. At the beginning of the third cycle con-

stant conditions (.001 foot candles and 65 F) were maintained for 56-hr. At the start of constant conditions adults were allowed to intermingle for 2 hr, as stated above.

Results (Figure 16) indicated that there is no circadian rhythm present when simultaneous light and temperature cycles are used as the entraining synchronizers. Mating peaks, per se, occur with no regularity after initiating constant conditions, even though indications of such regularity are given when a 24-hr diel rhythm of simultaneous light and temperatures are the synchronizers (Figure 15). However, mating is grouped into distinct blocks of several hours duration (Figure 16). The significance of these blocks will be discussed later.

Mating Behavior

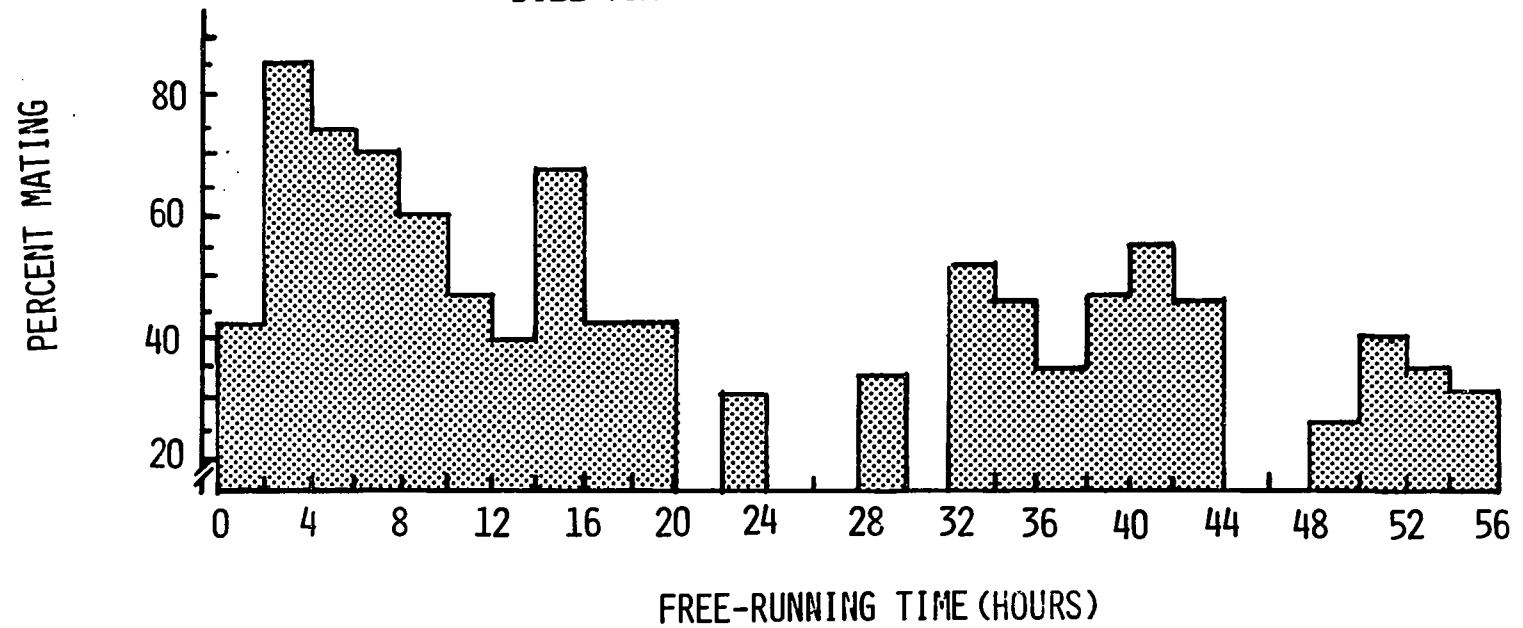
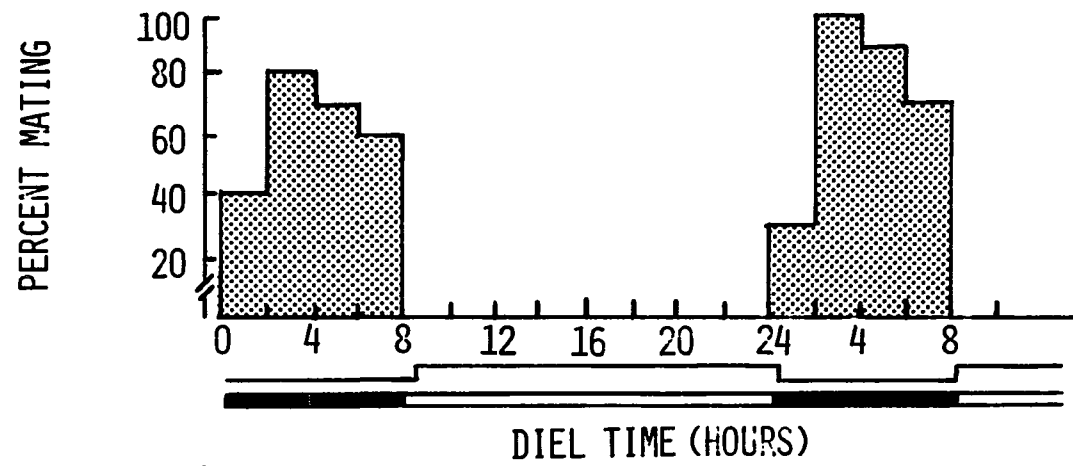
This section on behavior is included because, during the course of the study it was observed that certain behavioral actions by the male corn borer can affect mating success and because female precopulatory behavior had not been described adequately.

Male precopulatory behavior and copulation

Copulation and precopulatory behavior have already been well described by Poos (1927). The only addition to be made is that of male behavior during unsuccessful copulatory attempts. A male unable to couple with a female

Figure 15. Influence of simultaneous light and temperature cycles on mating. Solid line is the temperature rhythm. Shaded area is the scotophase. n = 10 females per time period

Figure 16. Free-running rhythm of mating under constant conditions after adults were entrained to a light temperature cycle. n = 30 females per time period



will sometimes kick the female with the near prothoracic leg. This appears to be a disturbance action that causes the female to move the tip of the abdomen, as some females will be resting with the tip of the abdomen against the substrate. In one observation, however, the male, after several unsuccessful attempts to couple, forcefully pushed against the female abdomen with his head. The female immediately flew off. If kicking is not successful the male will often turn 180 degrees and try to copule from the other side of the female. This turning is seen more often than kicking. A male is usually successful after 5-6 copulatory attempts, although Poos (1927) reported 16 unsuccessful attempts for one male.

Female precopulatory behavior

The female corn borer for the most part plays a passive role in mating. The female probably releases a copulatory stimulant or attractant at falling temperatures and decreasing light intensity. This stimulant apparently is distributed throughout the environment by the air. In only one observation was a female seen actively moving her wings in what might be considered a dispersal action. The female is sometimes seen to move the abdomen in a pumping action but usually the female will rest passively with the tip of the abdomen exposed (Figure 17) as the male tries to couple with her.

Figure 17. Female with tip of abdomen exposed

Figure 18. The male copulatory probe



The male copulatory probe

In observing the male abdominal copulatory probe, apparently there is no distance (Figure 17) or position (Figure 18) orientation by the male. The probe appears to be a trial-and-error method to successful copulation. Males rarely successfully copulate with a female on the first probe. If the male is not successful to couple after a few probes from one side of the female the male will turn around 180 degrees and try to couple from the other side of the female.

Time spent in copula

Although this experiment was done before the experiments on rhythms as a determinant for the hourly interval to be used in those studies, it is included here because it is considered a behavioral phenomenon.

This experiment was designed to determine the length of time spent in copula by the European corn borer under laboratory conditions. A ratio of 2 females to 3 males in pint-size screen cages were exposed to a 24-hr cycle of 16-hr L at 85 F and 8-hr D at 67 F. Light intensity during the dark phase was increased to 0.02 foot candles to facilitate observations.

Table 8 shows that the average time spent in copula was 2-hr and 27 minutes for 9 pairs. The time of actual copulation was taken. Precopulatory activity was not in-

Table 8. Time spent in copula

Copulation		Total time ^a
Initiated	Completed	
9:24 A.M.	11:02 A.M.	1:38
11:05	12:15 P.M.	1:10
11:05	2:12	3:07
11:35	1:37	2:02
11:38	2:15	2:37
12:45 P.M.	2:45	2:00
1:00	3:15	2:15
1:02	2:20	1:18
2:10	4:08	1:58
Average time 2:27		

^aHours and minutes.

cluded because this varies on the males' ability to couple with the female.

DISCUSSION

Those adults entrained to a 24-hr temperature cycle of 80 to 65 F expressed a circadian rhythm of mating when placed under constant conditions of 65 F. This is the first time a temperature-phased circadian rhythm of mating has been observed in insects.

Under an 80 to 65 F thermoperiodic cycle mating is variable in relation to time after the beginning of the low temperature phase. However, with replications it was possible to show that the population exhibits a weak peak mating-period at about the fifth hour into the low temperature phase. Under constant conditions of low temperature, this mating peak occurred on a circadian time scale with a period of 23-hr. Reversal of the thermoperiodic cycle showed, through the presence of transients, the endogenous entraining capabilities of the temperature cycles. The fact that the transients appeared in the same time period of mating as in the original temperature cycle also supports the endogenous synchronization to a temperature cycle. However, the continuance of a rhythm after the phase has been shifted is, perhaps, the best criteria for establishing the presence of a circadian rhythm. There are two other criteria that are important also. One is that a slight deviation from an exact 24-hr period be seen. If the rhythm is exactly 24-hr, then factors other than those under investigation may be

responsible for the rhythm. The second criteria is that the rhythm gradually loses its synchronization after a time. All these qualifications were recognized in this study and present strong evidence that the temperature-phased mating observed is a true circadian rhythm.

Uvarov (1931, p. 92) wrote "there is little doubt that the copulation of insects is regulated by temperature but there are no exact observations on this point". Although many insects have been observed to mate either above or below certain temperatures, the exact influence of temperature on mating was not understood. This was perhaps due to the lack of information on the interaction of light and temperature as it influences insect mating behavior. The majority of the following discussion is an attempt to explain this interaction.

A mechanism of entrainment of circadian rhythms has been proposed by Pittendrigh and Bruce (1959). Essentially this theory suggests two oscillators, A and B, which are capable of being synchronized with natural environmental rhythms. Oscillator A is sensitive to entrainment by light rhythms. It also drives oscillator B which is sensitive to temperature. But B's phase and period is due more to its entrainment by A than to direct entrainment by temperature. Oscillator B is however responsible for the overt expression of the rhythm.

For the purposes of this paper the theory of Tyshchenko (Danilevsky, Goryshin and Tyshchenko 1970) is more applicable if it can be applied to activity rhythms and not just to developmental rhythms. This theory, in attempting to describe the phase relations of the oscillators A and B, suggests that these oscillators are independent of each other, but capable of interacting to influence each other. The theory suggests that it is only when A and B are interacting that spontaneous activity will occur. Conversely the absolute separation of A and B induces rhythmic activity.

Exposure of adult European corn borers to a 16-hr light: 8-hr dark photoperiod revealed, under constant conditions of light and temperature, that mating is asynchronous (spontaneous) and that the mating peaks, per se, were irregularly spaced in time. However, definite time-periods (blocks) of continuous mating are present. These blocks of continuous mating reflect on the ability of oscillator A to measure time accurately. When oscillator A was exposed to diel rhythmic conditions, a purely exogenous response was observed. But oscillator A was unable to duplicate accurately the duration of the light and dark periods of the diel rhythm under constant conditions. The fact that oscillator A is unable to duplicate the length of the periods of a photoperiodic cycle is shown in the length of time these blocks of continuous mating occur and by the fact that these

blocks extend into the photophase of the entraining photoperiod. These results suggest that oscillator A is imperfectly endogenous. That is, oscillator A becomes synchronized to the external environmental rhythms but this synchronization is inaccurately expressed in time under constant conditions of light and temperature. This imperfectly endogenous characteristic of oscillator A is also seen in the free-running rhythm of mating after the adults were exposed to simultaneous light and temperature cycles. When both light and temperature cycles were the synchronizers, the mating rhythm registered under constant conditions was due to an interaction of oscillators A and B. This interaction affected mating peaks that were displaced only a few hours from those mating peaks resulting from the circadian thermoperiod. It appears then that the spontaneous mating affected by oscillator A actually masks the endogenous mating rhythm affected by a thermoperiod. The masking of the thermoperiodic rhythm by the photoperiodic rhythm produces the arrhythmic mating peaks observed under constant conditions. The arrhythmic mating is, in other words, caused by the interaction of oscillators A and B. This same interaction effect may be responsible for the loss of the rhythm in oviposition of the corn borer reported by Schurr and Holdaway (1966). However it is not known if the mating and oviposition rhythms are paced by the same oscillators or different oscillators.

The interaction of oscillators A and B occurs when the endogenous oscillator B is influenced by the imperfectly endogenous A oscillator. Danilevsky et al. (1970) has pointed out that a precise distinction between exogenous and endogenous rhythms is impossible. Endogenous rhythms can be suppressed by exogenous responses to favorable or unfavorable environmental conditions. In the European corn borer an interaction between the two rhythms is caused by the imperfectly endogenous A oscillator.

The influence of favorable and unfavorable environmental conditions and their interaction with endogenous rhythms is observed in the influences of different light intensities at several temperature conditions. At 80 F and 0.02 foot candles, no synchronization of oscillators occurred and mating was possible only because of the favorable lower light intensities. Under the same 80 F, light intensities greater than 0.02 foot candles prohibited mating. At a constant 70 F, mating occurred at light intensities up to 10 foot candles.

These results may be explained by the partial activation of oscillator B at 70 F. Pittendrigh and Bruce (1959) believed that the rhythm observed in their insect, Drosophila pseudoobscura, is really a reflection of the activation of oscillator B. Partial activation then suggests that either no mating would be observed or the

frequency of mating in the population would be reduced. One way for the frequency of mating in a population to be reduced would be the absence of an event in a sequential series of activities necessary for mating. For example, laboratory observations revealed that the flight activity of the adults increases during a drop in temperature (84-67 F). This flight activity then decreases as the temperature levels off at the lower level (67 F). This increase and decrease in flight activity is always observed before adult coupling occurs. This flight activity of the adults may then be considered an overt measure of the activity state of the insect, and may also be considered a part of a sequence of behavioral events necessary for successful mating in the population. If this behavioral sequence is broken then the mating potential within the population will be reduced. The results observed in this experiment indicate that the sequence of events was broken by the lack of flight activity in adults exposed to constant 70 F and 30 foot candles. Although occurrence of maximum flight activity in the population is dependent on the complete activation of the B oscillator, flight activity is ultimately controlled by light intensity. Nevertheless, exposure to a constant 70 F resulted in a partial activation of oscillator B by increasing the receptibility of B for synchronization. The synchronization to temperature-drop was never accomplished however because of the constant

temperature conditions. However, because of the partial activation of B, mating was able to occur under light conditions which prohibited mating at a constant 80 F; a temperature where no activation of oscillator B could occur because of the high temperature. Complete activation of oscillator B was accomplished when B was exposed to a temperature fall, as in a thermoperiod. With complete activation of B the influence of light is diminished somewhat below light intensities of 100 foot candles. It is apparent then that the endogenous B oscillator has two limiting factors. One of these limiting factors is the governing influence of light intensity and the other is partial activation of B by a low (70 F) constant temperature. When the results of the influence of temperature-drop on mating are reviewed, it appears that oscillator B also has a quantitative factor about it. This quantitative characteristic is in the amount of temperature-drop needed to completely activate oscillator B. Since oscillator B both governs and reflects the activity state of the insect, the activity state of the insect will then depend on the amount of temperature-drop. Allowing for individual variation we see that with a small temperature-drop of from 86 to 84 F only a few adults mated. With a large temperature-drop of from 86 to 70 F most of the adults mated. These results agree with the suggestion of an increased activity

state in the insect brought on by a temperature-drop. The greater the temperature-drop the more the activity state is increased and this results in increased mating. Bentley et al. (1941) showed that with a drop in temperature the activity state of the spider beetle, P. tectus, is increased and is expressed in the form of increased locomotor activity. In the European corn borer an increased activity state is observed as increased mating, if the light intensity is low.

The interaction of light and temperature along with the increased activity state of the corn borer results in a spontaneous or free-running rhythm. This means that feral adults would be free of any governing influences of a rhythm and could mate at anytime of the day if the temperature fell and illumination decreased. The author has observed such a phenomenon with feral adults. These adults were collected in the field on a clear day when the temperature was approximately 85 F. When these adults were brought into a 70 F laboratory, male copulatory movements towards the females were noted in a matter of minutes. Shortly thereafter many adults were copulating. O'Kane and Lowry (1927) reported seeing feral European corn borer adults mating on "cool, cloudy, dark days". These observations and the above data give some support to the suggestion that feral adults may mate whenever weather conditions permit, regardless of the time of day. The exact number mating, however, depends to

some extent on the temperature drop. The relationship between mating and temperature-drop shows that with only an 8 F drop approximately 50 percent of the adult population may mate. This figure will, however, be influenced by the "darkness" of the day. What effects this behavior will have on population numbers in terms of the increased percentage of females that could be mated in a given time and the resulting increase in the number of eggs laid is not known. However, since a male mates and transfers only one spermatophore in a 24-hr period this population-numbers increase may be an unrealized potential.

As regards mating rhythms in insects, of interest are the observations on male sexual responses to female pheromones. These sexual response rhythms have been shown to be photoperiodically entrained (Shorey 1965). These observations, however, do not necessarily mean that mating will also be cyclic. Nowosielski and Patton (1963) demonstrated that male crickets expressed a cyclic sexual excitability under a photoperiodic regime. However the frequency of copulation was too low during these same cyclic periods to provide adequate data on the daily distribution of copulation. Likewise, no coupling under controlled conditions was observed by Shorey (1965) among Trichoplusia ni. The results obtained from the present study on corn borer mating rhythms may help clarify the

position played by light and temperature in regulating insect mating.

Biotic factors such as mating age, age preference for a mate, and crowding under the described conditions had no affect on mating success. For mating age Caffrey and Worthly (1927) reported that the corn borer will mate as soon as 12-hr after eclosion. In comparison Trichoplusia ni, after eclosion of the imago, must mature for 2 day before mating will occur (Shorey 1964).

The frequency of multiple mating for females was low (11.7 percent) compared to the 46 percent (27 of 58) reported by Drecktrah and Brindley (1967). Among feral female corn borers, Pesho (1961) reported a range of 8 to 43 percent for multiple matings. The results reported here are within the frequency range described for multiple mating of feral females, and may therefore be more representative of field conditions.

It was observed that male precopulatory behavior towards the female can be a factor influencing mating. This behavioral factor usually results from "uncooperative" females however. Numerous observations revealed that the kicking of the female by the male was usually successful in getting the female to lift the tip of her abdomen. It was only when the male displayed force towards the female that copulation was not accomplished.

SUMMARY AND CONCLUSIONS

The main purpose of this investigation was to define some of the biotic and abiotic factors that might influence mating success of the European corn borer. The results of this laboratory investigation indicate that:

1. The combination of a temperature drop (from 85 to 67 F) and a decrease in light intensity (from 250 to 0 foot candles) are the main factors that synchronize the sexes for mating.
2. A temperature-phased circadian rhythm of mating is exhibited by the European corn borer under constant conditions of temperature and light.
3. Simultaneous cycles of light and temperature or light cycles alone did not effect a circadian rhythm of mating, but acted only as an exogenous synchronizer for mating.
4. A significant correlation was found between temperature-drop within the range of from 86 to 70 F and mating success.
5. At constant temperatures of either 70 F or 80 F light intensity, within the range of from 0.02 to 100 foot candles, has more influence on mating success than does temperature. Only under a 24-hr cycling temperature regime (84 to 67 F) is the influence of light intensity on mating decreased.

6. Wind above 10 mph prohibits mating.
7. The frequency of multiple mating among females was low but males will mate every day.
8. Both sexes will mate only once in 24 hr.
9. Age and crowding does not influence mating success under the described conditions.
10. The average time spent in copula was 2 hr and 27 minutes.
11. Male precopulatory behavior towards the female may be a factor influencing mating success.

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ACKNOWLEDGMENTS

I wish to express my appreciation to my major professor T. A. Brindley for his supervision and guidance throughout my graduate program.

I extend my appreciation to the personnel of the European Corn Borer Laboratory, Ankeny, Iowa, for their cooperation during this investigation.

APPENDIX

Table 9. Effect of light intensity on mating at different temperature regimes

	Foot candles							Total
	0.02	.3	1.0	3	10	30	100	
Constant 80 F								
Females mated	36	4	0	0	0	0	0	40
Females unmated	<u>16</u>	<u>49</u>	<u>53</u>	<u>52</u>	<u>50</u>	<u>53</u>	<u>53</u>	<u>326</u>
Total	52	53	53	52	50	53	53	366
Constant 70 F								
Females mated	37	8	9	3	1	0	0	58
Females unmated	<u>14</u>	<u>42</u>	<u>41</u>	<u>47</u>	<u>49</u>	<u>51</u>	<u>50</u>	<u>294</u>
Total	51	50	50	50	50	51	50	352
Cycling 84 to 67 F over 24-hr								
Females mated	38	32	20	17	20	8	8	143
Females unmated	<u>12</u>	<u>18</u>	<u>30</u>	<u>33</u>	<u>30</u>	<u>42</u>	<u>42</u>	<u>207</u>
Total	50	50	50	50	50	50	50	350

Table 9. (Continued)

	Foot candles						
	0.02	.3	1.0	3	10	30	100
Combined data of proportion mated for above data							
80 F	69.2	7.5	0	0	0	0	0
70 F	72.5	16.0	18.0	6.0	2.0	0	0
84 to 67 F	76.0	64.0	40.0	34.0	40.0	16.0	16.0

Table 10. Arcsine transformations of proportion mated from Table 9

Fahrenheit	Foot candles						
	.02	.3	1	3	10	30	100
80	56.3	15.9	0	0	0	0	0
70	58.4	23.6	25.1	14.2	8.1	0	0
84-67	60.7	53.1	39.2	35.7	39.2	23.6	23.6

Table 11. Chi square analysis of effect of light intensity on mating at different temperature regimes^a

Source	Degrees of freedom	Sums of squares	χ^2
Temperature	2	3127.08	
Constant vs fluctuating	1	2893.38	176.21*
80 vs 70 F	1	233.38	14.23*
Light	6	5617.45	
Linear	1	4435.57	270.13*
Quadratic	1	841.87	51.27*
Lack of fit	4	340.01	20.71*
Temperature x light	12	687.45	41.87*
Total	20	9431.98	

^aMade on arcsine transformations from Table 10.

*Significant at the 0.05 level.

Table 12. Partition of the sum of squares of the temperature x light interaction

Source	Degrees of freedom	Sums of squares	χ^2
Constant vs fluxuating temperature			
x linear	1	58.93	3.59
x quadratic	1	216.20	13.16*
80 vs 70 F			
x linear	1	53.82	3.28
x quadratic	1	126.71	7.54*
Residual	8	231.79	14.12**

*Significant at the 0.05 level.

**Significant just at the 0.05 level.

Table 13. Effect of temperature-drop, 86 to 70 F, on mating

	Temperature decrease (F)				Total
	2	4	8	16	
Females mated	24	27	47	65	163
Females unmated	<u>76</u>	<u>73</u>	<u>52</u>	<u>35</u>	<u>236</u>
Total	100	100	99	100	399

Chi square analysis of temperature-drop data^a

Source	Degrees of freedom	Sums of squares	χ^2
Temperature	3	388.91	
linear	1	364.65	44.41*
quadratic	1	16.81	2.05
residual	1	7.45	0.91

^aBased on arcsine transformations of proportion mated.

*Significant at the 0.05 level.

Table 14. Effect of temperature rhythms on mating

Diel		Circadian			
Females mated ^a		Females mated ^a			
hr	Rep. I	hr	Rep. I	Rep. II	Rep. III
2	3	2	8	0	3
4	8	4	4	4	4
6	7	6	5	5	6
8	4	8	4	6	3
10	4	10	3	2	2
12	0	12	1	1	0
14	0	14	1	2	1
16	0	16	0	2	2
18	0	18	1	0	0
20	0	20	4	1	1
22	0	22	2	1	0
24	0	24	1	3	2
2	7	26	4	2	4
4	7	28	1	6	4
6	5	30	3	4	4
8	2	32	5	2	0
10	0	34	4	0	0
12	0	36	0	0	1
		38	3	0	2
		40	2	0	5
		42	4	0	2
		44	2	1	4
		46	3	2	3
		48	2	0	3
		50	1	2	5
		52	5	0	6
		54	3	3	1
		56	3	1	3

^an = 10 females per replicate.

Table 15. Effect of light rhythms on mating

Diel		Free-running			
Females mated ^a		Females mated ^a			
hr	Rep. I	hr	Rep. I	Rep. II	Rep. III
2	1	2	1	1	1
4	5	4	6	4	5
6	7	6	6	7	7
8	6	8	3	8	6
10	0	10	2	3	2
12	0	12	2	2	2
14	0	14	1	1	1
16	0	16	1	1	1
18	0	18	0	1	1
20	0	20	2	3	1
22	0	22	1	2	1
24	0	24	1	2	1
2	2	26	1	0	2
4	5	28	0	2	1
6	8	30	0	0	1
8	5	32	3	4	1
10	0	34	3	4	1
12	0	36	3	4	1
		38	4	3	6
		40	7	6	3
		42	5	4	3
		44	1	6	5
		46	6	3	4
		48	0	1	1
		50	2	1	1
		52	0	1	2
		54	2	5	2
		56	3	5	1

^a_n = 10 females per replicate.

Table 16. Effect of phase shift on mating

Light		Temperature	
hr	Females mated ^a Rep. I	hr	Females mated ^a Rep. I
0	0	-14	0
-16	0	-16	0
-18	1	-18	4
-20	5	-20	5
-22	7	-22	6
-24	6	-24	2
- 2	0	- 2	0
0		0	
2	7	2	5
4	3	4	3
6	2	6	0
8	0	8	0
10	0	10	0
12	0	12	0
14	0	14	0
16	0	16	0
18	0	18	2
20	0	20	0
22	0	22	0
24	0	24	0
2	5	2	5
4	7	4	4
6	6	6	4
8	8	8	3
10	0	10	0
12	0	12	0

^an = 10 females per replicate.

Table 17. Effect of simultaneous light and temperature rhythms on mating

Diel		Free-running			
Females mated ^a		Females mated ^a			
hr	Rep. I	hr	Rep. I.	Rep. II	Rep. III
2	4	2	4	3	6
4	8	4	8	10	8
6	7	6	7	9	6
8	6	8	6	7	8
10	0	10	9	6	3
12	0	12	6	4	6
14	0	14	6	5	1
16	0	16	8	9	3
18	0	18	5	6	2
20	0	20	6	5	2
22	0	22	1	2	2
24	0	24	2	3	4
2	3	26	1	1	1
4	10	28	1	1	2
6	9	30	1	4	5
8	7	32	1	2	3
10	0	34	3	7	6
12	0	36	4	4	6
		38		2	5
		40		5	5
		42		4	7
		44		4	5
		46		1	3
		48		1	3
		50		3	2
		52		3	5
		54		3	4
		56		3	2

^an = 10 females per replicate.