DEVELOPMENT OF FEMALE SEX ORGANS DURING AESTIVATION IN THE ALFALFA WEEVIL, *Hypera postica* (Gyll.) (COLEOPTERA, CURCULIONIDAE)

By

Antonio A. Guerra

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INTRODUCTION

The alfalfa weevil was reported for the first time in Virginia in 1952. Since then, it has become the most serious pest of alfalfa, and its present distribution probably includes all of the counties in the state. The presence of great numbers of weevil larvae in alfalfa fields during the spring and early summer may result in the destruction of the first crop, serious damage to the second crop, and complete kill of the stand when infestations are severe.

The alfalfa weevil has been studied extensively by many workers in the western part of the United States, where this pest has been present since 1904 (Hamlin et al., 1949; Michelbacher and Leighly, 1940). More recently many others in the eastern states have also studied this pest. In spite of this large amount of research, a complete picture of its life history and habits is still lacking.

The alfalfa weevil remained in the west for approximately 50 years and according to Cook (1925) the heavy summer rainfall would not favor an eastward movement of the insect. On the other hand, Reeves (1917) presented evidence to support his theory that the weevil would be favored by more humid conditions and might find the eastern United States a favorable habitat. This state of confusion concerning the ecology of the alfalfa weevil became worse when the weevil suddenly appeared, causing severe economic losses, in zones where the natural habitat was predicted beforehand as being unsuitable for its survival (Manglitz and App, 1957; Gyrisco, 1958).

One of the little known aspects of the biology of this weevil, and perhaps the one most directly concerned with survival and conservation of the species, is that of its period of sexual maturation. The importance of this developmental phase is obvious, since its completion is necessary for the correct functioning of many other vital aspects of the insect's biology. Two of these vital aspects, namely, rate of oviposition and rate of egg hatch, have been referred to as the mechanisms governing the time of appearance and the population density of the larvae in the spring (Evans, 1959). The period of sexual maturation of this beetle seems to be the common denominator for many of its life processes. It undoubtedly plays a vital role in synchronizing the insect's life cycle to the rhythm of its fluctuating environment.

Associated with ovarian development is a poorly understood period of aestivation. This "diapause" of the adult insures that the larvae are present only in that season of the year which is favorable for their development and survival. Although the exact relationship between diapause and sexual maturation in this insect is not known, it is presumed that they interact by complementing each other as an adaptation for survival.

Preliminary tests conducted by the author indicated that the time required for sexual maturation in the adult female alfalfa weevil was influenced by aestivation. This, plus the lack of adequate ecological and physiological information about the weevil's responses to its environment, are enough to account for a complete re-examination of the work done in this field. Accordingly, the main objective of this investigation is to make a detailed study of the maturation in female alfalfa weevils under approximate field conditions. Some of the basic problems as components of this objective are summarized as follows:

1. The influence of various environmental conditions upon the length of time required for ovarian maturation.
2. The effects of certain environmental factors on aestivation.
3. The role of aestivation (diapause) in the maturation of ovaries.
4. The interaction of factors concerned in sexual maturation.

DEFINITIONS AND SPECIAL CONSIDERATIONS

Various terms which have been used rather broadly or which have controversial meanings, will often be mentioned in this paper. The author feels that at this point, these terms should be identified with the meanings in which they are used in this manuscript.

Environment - Andrewartha (1961) has stated: "The environment of an animal is everything that may influence its chance to survive and multiply". He believes that the term "environment" could be better defined and understood, if its definition is based on its ecological magnitude. Since the main problem is that of deciding on the boundaries and components constituting this concept, he concluded that all environmental factors could be grouped into four main components. These components are: weather, food, other animals and pathogens, and a place in which to live. Each component is susceptible to further subdivision.

Diapause - Ecologically, diapause is a stage in physiogenesis which has to be completed in order for morphogenesis to resume. In this sense the term diapause-stage should be used when referring to that stage in the life cycle in which morphogenesis is more or less arrested. Further, diapause-development is that physiological development or physiogenesis which proceeds during diapause as a necessary step for the normal resumption of morphogenesis. This infers that the resumption of morphological growth can be an indicator of the completion of the diapause stage (Andrewartha and Birch, 1954; Odum, 1959).

Aestivation - A state of dormancy in summer.

Hibernation - A state of dormancy in winter.

Voltinism - Voltinism is an expression of the number of generations of the animal per year. Voltinism in insects is a variable but never independent feature. According to Andrewartha and Birch (1954) in certain species every individual in every generation enters diapause; typically, there is only one generation a year. When this is the case, the life cycle is called "uni-voltine", and this type of diapause is referred to as "obligate. In this species, diapause occurs consistently and appears to be independent of (or synchronized so nicely to the environment, that it cannot be related to) any external stimuli. On the other hand, there are many species in which only a few or no individuals enter diapause for one or several generations, and then a generation occurs in which most or all individuals enter diapause. In this case the life cycle is called "multi-voltine", and the diapause "facultative".

REVIEW OF LITERATURE

Snow (1928) reported on the relationship of ovulation and age of adult females of this weevil in the Great Basin Area. He stated that some of the beetles of the first generation were ready to lay eggs by October and he concluded that the weevils emerging in spring and summer remain immature for about four months after emergence. In order to get anatomical evidence to prove his point, Snow made large collections of adults from a variety of environments and at all seasons during several years; and by means of dissections he attempted to follow ovarian development throughout the year. As a result of this work, he described the female reproductive organs and arbitrarily separated ovarian development into five stages.

Evans (1959) reported that soon after emergence the adult weevils fed slightly on alfalfa, and then migrated into neighboring fields containing sod where they burrowed into protected locations. This aestivation lasted for about three months, he stated, and served the dual purpose of protecting the adults during the hot, dry summer and providing the waiting period necessary for sexual maturation of the females. Evans also reported that mating probably took place soon after the return migration to alfalfa, since adults which he had collected in mid-October oviposited immediately in the laboratory. Michelbacher and Leighly (1940), however, indicated that the alfalfa weevil female reaches its sexual maturity two months after emergence.

Manglitz (1958) concluded that in Maryland the exodus of these weevils from alfalfa fields during the summer, may be in response to high temperatures. He also stated that this aestivation period may also be associated with the inability of the female weevils to lay eggs for several months after reaching the adult stage.

Evans (1959) found that the time necessary for the genitalia to develop varies with respect to temperature, and he mentioned that the effects of temperature on physiological activity are well-known and have been described by mathematical equations such as those of Arrhenius and Van't Hoff.

Yakhontov (1934) demonstrated that within the temperature range of 53.2 to 77 degrees (F.) a period of about two months was required after emergence for the maturation of female weevils. Michelbacher and Leighly (1940) concluded that the temperature range favorable for sexual development was between 50 and 77 degrees (F.). These workers based their conclusions on data obtained from several parts of California and Europe. Evans (1959) suggested that oviposition will probably also be affected by the same temperature range as that of sexual maturation, about 45 to 77 degrees (F.), and he explained that the increasingly higher temperatures in late spring and summer inhibit oviposition and thus regulate the seasonal activities of the alfalfa weevil.

Cook (1925) presented evidence in support of a theory that the weevil would be limited in its distribution eastward by heavy summer rainfall while Reeves (1917) believed that conditions in the east would not prevent the insect from becoming established there. Later, work by Sweetman and Wedemeyer (1933) tended to corroborate Reeves' work in that the survival and development of nearly all stages studied were favored consistently by conditions of high moisture.

Koehler and Gyrisco (1961) found in a study of all stages of the alfalfa weevil under controlled environments, that high humidities were more favorable for egg hatch than low humidities. Low temperatures together with low humidities were the most destructive factors to the pre-pupal and pupal stages.

Huggans and Blickenstaff (1962) reported that alfalfa weevil larvae which had been subjected to less than ten hours of light per day developed into adults which laid viable eggs less than 30 days after adult emergence. Those larvae subjected to more than ten hours of daylight required about three months from adult emergence to oviposition.

Andrewartha and Birch (1954) stated that when diapause affects the adult, there may be a failure to ripen eggs or sperm and that diapause may be manifested in an extended pre-oviposition period. They discussed some of the factors which might be involved in the inception and duration of diapause. Among the factors discussed were temperature, humidity, and photoperiodism.

Photoperiodicity is chiefly important in relation to behavior and as a stimulus for those mechanisms which regulate life cycles to keep certain stages of the organism in step with the seasons (Andrewartha and Birch, 1954). Light has been referred to as a token stimulus by Frankel and Gunn (1940). They stated that light is an indicator of circumstances which may be favorable or unfavorable and the organism possessing the appropriate adaptations will respond accordingly. They continued that photoperiod is a very precise timekeeper and acts as an environmental clock in indicating seasonal changes in temperature, moisture, food, etc.

Wilde, Duintjer and Mook (1958) concluded that Leptinotarsa decemlineata responds to photoperiodicity as a long-day insect for the completion of diapause and that the effect of short photoperiods results in the ovi-sorption and degeneration of the ova present in hibernating females.

Hasegawa (1952) reported that the diapause pattern in Bombyx mori was dependent upon the physiological constitution of the females of the preceding generation. Fukuda (1952) believed that this effect in the silkworm was determined by the function of the neurohormone system in the pupal stage.

Very little is known about the influence of light on the mating and oviposition of insects. Andrewartha and Birch (1954) reported that *Dacus tryoni* mated only at dusk. The beetle *Aphodius howitti* copulates at dusk when illuminance drops to about two lumens per square foot. Another crepuscular species is the hepialid *Oncopera fasciculata*.

Oviposition in many insects can be stimulated by exposing them to a certain light intensity or to darkness. Isely and Ackerman (1923) found that the codling moth laid a majority of its eggs during darkness.

The cotton boll worms *Earias* and *Amsacta* exhibited the same response to darkness or twilight (Pruthi, 1940).

The following points may be made in summarizing some of the aspects of the biology of the alfalfa weevil that will be discussed in this paper: (1) An undetermined length of time is required for sexual maturation of female weevils, with virtually nothing known about environmental influences upon this development; (2) Assumptions on the effect of certain environmental factors on the inception and duration of the aestivation period have not been experimentally supported; (3) The role of aestivation in ovarian development is suspected but experimental data are not available; and (4) Environmental factors have been considered separately in explaining the sexual maturation rhythm of the alfalfa weevil, resulting in an accumulation of data in which the estimates are not free of bias.

METHODS AND MATERIALS

The investigation reported herein was initiated in the fall of 1960 with a series of preliminary observations in the insectary. These studies were continued in the spring of 1961 when full-grown larvae and pupae were collected by sweeping with a 15-inch beating net. The collections were taken from an alfalfa field about three miles west of Blacksburg, Virginia. The samples were held and transported in one gallon, paper ice cream cartons which had been modified slightly to provide ventilation. These larvae were removed from the cartons as they crawled to the top and the pupae were separated from the rest of the material in the containers. The larvae and pupae were placed in battery jars. In the case of the larvae, fresh alfalfa was introduced periodically until pupation occurred.

As soon as the adults emerged, they were placed in one gallon, glass battery jars. Three or four "Masonite" panels were placed in the battery jars; these panels had wire staples driven into them so that when they were stacked one on top of the other about one-fourth inch separated them. This arrangement of panels provided crawling space and hiding places for the weevils. Water was offered in vials of four dram capacity stoppered with cotton wicks. Fresh alfalfa bouquets were maintained in the battery jars The jars were covered with cheese cloth which was secured with rubber bands.

Approximately 300 adult weevils were introduced into each jar. The jars were labelled with date of collection, date of adult emergence, and other appropriate information.

Four populations of alfalfa weevils of known age and physical condition were used in this study. The date of emergence of each population is given below. Each population made up the percentage of the total number of weevils collected as indicated.

Population	Date of Emergence	Percentage of Total
1	May 9, 1961	10
2	May 16, 1961	15
3	May 23, 1961	70
4	September 3, 1961	5

The observations in this study were made on about 4,000 weevils collected in May 1961, and about 200 collected the following September. The adults emerging from these larvae were distributed in the battery jars as previously described.

The test weevils in the battery jars were placed in an open insectary where conditions of temperature, humidity, and light were closely approximated. These battery jars were placed within 100 feet of a weather station[a/]. Relative humidity and photoperiod information was provided by the Department of Agricultural Engineering at Virginia Polytechnic Institute.

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a/ Official Weather Bureau Station

Adult female weevils were dissected at various lengths of time after emergence for measurements and observations of growth and development of the sex organs. Samples of approximately 200 females were taken from the battery jars for dissections one day after emergence and at monthly intervals thereafter until egg laying. The beetles were preserved until subsequent dissection, in a fixative consisting of 4:1:1 parts by volume of 95 percent ethanol, glacial acetic acid, and chloroform, respectively.

The dissection technique used in this work was slightly modified from that of Snow (1928). The weevils were partially imbedded in paraffin with the dorsum exposed. After removal of the wings, the thoracic and abdominal terga were teased caudad, exposing the internal organs. The digestive tract was severed at both ends and carefully removed. Finally, the fat body was extracted as completely as possible. Representative specimens that had been satisfactorily dissected with intact sex organs were preserved in 70 per cent ethanol at four degrees (C.) for future photographing. Measurements were taken through a binocular microscope with an ocular grid which had previously been calibrated.

The photographs were taken with an Exacta 35 mm. camera with bellows attachment. The film was Kodak Tri-X and the light source was one strobe unit held at a 30 degree angle above and 16 inches from the insect.

RESULTS

The results of this investigation are presented somewhat in the chronological order in which events occurred in the field and insectary. They are based on data obtained during the entire year of 1961 and from January to May 1962. Although this study was chiefly concerned with adult females during summer diapause, the author felt that by studying the weevil throughout the year he could avoid the pitfall of trying to explain an effect in an adult stage without considering the other earlier stages.

Resumption of activity in the spring. The first populations of active adults were observed in the field in the last week of February in 1961 and again in 1962. At this time, after a hibernation period of about two or three months, the insect had re-initiated feeding and mating. In the last week of March, eggs were found within the green stems of alfalfa. The average minimum and maximum temperatures for the last week in February 1961 were 33^{o} and 58.6^{o} (F.), respectively (Plate I). In Plate I it can be seen that the photoperiod increased daily from 11.03 to 11.27 daylight hours.

Pre-imaginal stages of the spring generation. Light to medium infestations of first and second instar larvae were first found by the author on April 7, 1961, and heavy infestations of larvae followed throughout April and May. Pupation was first noticed in the field and in the laboratory on April 29, 1961. It is shown in

Plate I that the average maximum temperatures rose from 58.6° (F.) at the beginning of March to about 70° (F.) in the third week of May. Relative humidity averaged about 60-70 per cent for this period (Plate II). The photoperiod gradually increased from 12.6 daylight hours on April 1 to 14 38 hours on May 23, 1961 (Plate I).

Sexual growth before diapause. Dissections of adult females which were collected and preserved within a few hours after emergence indicated that the sex organs in this group were small but, in general, all the component parts were well defined and represented.

The ovaries, with two ovarioles each, were whitish in color and of about the same length as the egg tubes which were transparent, slender and straight. The two egg tubes of each ovary unite into a paired oviduct slightly smaller in length which in turn unites with the paired oviduct of the opposite ovary to unite into the unpaired oviduct or vagina. The vagina is connected to the bursa copulatrix to which is attached dorsally the spermatheca, a dark, chitinized structure situated either to the right or left of the bursa copulatrix. To one side of the spermatheca is the spermathecal gland, a semi-transparent and oval-shaped structure with tapered ends. The weevils of this age were classed as age "0" and a detailed account of the measurements for the parts mentioned above is included in Tables 1 and 2. The shape and size of the sex

Table 1. Measurements of the designated structures at monthly intervals in adult female alfalfa weevils. Blacksburg, Virginia 1961

Age in months	Length in millimeters: Ovaries Min.	% [a]	Max.	%	Egg Tubes Min.	%	Max.	%	Paired Oviducts Min.	%	Max.	%
0	.390	10	.520	50	.390	20	.520	60	.260	80	.390	20
1	.715	13	.975	13	.520	88	.650	13	.390	50	.455	30
2	.715	4	1.235	4	.520	10	.760	4	.455	76	.520	10
3	.715	14	1.235	5	.585	5	.780	10	.390	19	.650	5
4	.780	9	1.235	3	.585	3	1.040	6	.494	3	.650	9
5	1.235	7	1.820	13	.715	17	1.950	3	.390	53	.455	47
6	1.500	8	1.950	6	.910	11	3.770	6	.325	3	.455	6

[a] Percentage of weevils with the designated measurements of the total number (Approximately 200) of weevils measured in each age group.

Table 2. Measurements of sexual structures which did not grow following emergence of adult alfalfa weevil females.

Structure	Millimeters			
	Length		Width	
	Min.	Max.	Min.	Max.
Vagina	0.260	0.390	0.208	0.520
Spermatheca	0.312	0.364	0.364	0.365
Spermathecal gland	0.390	0.572	0.260	0.286
Bursa copulatrix	0.845	0.910	0.715	0.780

organs (although slightly smaller) are similar to those in age "1" which are shown in Plate VI. The stage of development in the weevils in group "0" correspond to an early step of the "small stage" as described by Snow (1928).

The main activity of newly emerged adults was that of feeding; no mating and very little movement was observed. Beginning in mid-June the feeding activity decreased gradually until June 23, when feeding completely stopped. On this date, weevils were collected from battery jars and preserved for dissections. As a result of these dissections, it was concluded that weevils in the three populations whose ages were 44, 37, and 30 days, respectively, and which had entered diapause on the same date, were in the same stage of sexual development. Due to these similarities, dissections were continued only on the group which emerged on May 23, 1961.

Morphogenesis took place at a slow rate (Table 1, Plates V and VI) during the first month of adult life although the weevils were active. However, the growth of the ovaries can be seen by comparing them with the size of ovaries exhibited in weevils of age "0" (Table 1). The egg tubes and the paired oviducts showed a slight increase in size over those structures in the weevils of age "0". The rest of the sex structures showed no change in size. Their minimum and maximum measurements are shown in Table 2.

<u>Environmental factors before diapause</u>. The average maximum temperatures increased from about 67° (F.) on May 22 to 81.5° (F.) on June 14, 1961, as indicated in Plates I and II. From June 14 to June 21 the temperatures dropped steadily with a maximum reading of 73° (F.) on June 21, 1961, and the average maximum temperature remained at 73° (F.) until the last of June.

The length of daylight increased gradually from 14.40 to 14.75 hours (Plates III and IV) during this period. The longest days of the year, 14.75 hours of daylight, occurred on June 20, 21 and 22.

<u>Sexual growth during diapause</u>. All weevils in the battery jars entered into diapause on June 23, 1961, and remained in this state until October 23, 1961. The populations of weevils and ages of adults when diapause ended were:

Population	Adult Age When Diapause Ended
1	5 months and 15 days
2	5 months and 8 days
3	5 months
4	1 month and 20 days

Throughout the diapause period weevils were dissected to ascertain the stage of sexual development. The results are summarized in Tables 1 and 2, and are photographically shown in Plates VII and VIII. There was only a slight increase in size of ovaries from July through September, but in October some growth was noticeable.

This slow rate of development is even more conspicuous in the egg tubes which were about the same size in September as at the time of emergence. However, following development of the ovaries in October, the egg tubes also exhibited a sudden burst of growth. Diapause ended on October 23, 1961, with the resumption of feeding, flight, and copulation.

The morphological changes observed in the sex organs at the end of diapause are summarized as follows: (1) There was a slight increase in the length and width of the ovarioles, but the egg tubes remained short and straight (Plate VIII, fig. 1). This stage corresponded to Snow's "medium stage." (2) The ovarioles somewhat enlarged and with the egg tubes starting to form a loop as they extended (Plate VII, fig. 2), corresponding to a late "medium stage" of Snow. (3) Ovarioles and egg tubes enlarged and broadened with developing ova visible in these two structures as beads or segments (Plate VIII, fig. 3). This is an early stage of Snow's "segmented stage."

Environmental factors during diapause. The average maximum temperatures and average per cent relative humidity for this period are shown in Plate II. It should be mentioned that the inception of diapause occurred on June 23, when the average maximum temperature was 73° (F.) and remained so for a period of 16 days. It should also be pointed out that the average minimum temperature on October 23 when diapause ended was 34.7° (F.), a considerable drop from previous minimum temperatures. Average relative humidity percentages remained rather constant over the entire period. On June 23, the length or daylight was shorter than the previous day for the first time in the year. On June 22 there were 14.75 hours of daylight and on June 23 there were 14.72 daylight hours (Plate III). By October 23 there were only 10.97 hours of daylight.

Sexual growth after diapause. Results from measurements of weevils six months old are presented in Tables 1, 2, and 3. Twelve and five-tenths per cent of the weevils had a sexual growth similar to the most developed state observed in weevils which were five months old (Plate VIII, fig. 3). Ten per cent of the dissected weevils had fully developed ovaries which were club-shaped at the anterior end; egg tubes were looped twice or more; progressive stages of ovulation were observed; and paired oviducts were distended and contained from two to four nearly full sized eggs. This stage is shown in Plate IX, fig. 1 and corresponds to an early stage of the "large stage" described by Snow. Approximately 76 per cent of the six-month-old weevils possessed fully developed ovaries, elongated and looped egg tubes, and greatly distended paired oviducts containing up to 40 full size eggs. This stage is shown in Plate IX, figs. 2 and 3.

Table 3. Length in millimeters of the designated structures in mature female alfalfa weevils. Blacksburg, Va.

Ovaries				Egg tubes			
Min.	%[a/]	Max.	%	Min.	%	Max.	%
1.755	78	1.950	18	2.73	63	3.770	25

a/ Percentage of mature female weevils which had the designated measurements.

It was previously stated that mating occurred on October 23, and it is clearly demonstrated that female sex organs were still underdeveloped at that time (Plate VIII, figs 1, 2, and 3).

Eggs were first observed on November 23, but coloration and physical condition of the eggs indicated that oviposition had occurred one or two days before. Over 95 per cent of the eggs deposited at that time hatched. Date of emergence and age when egg laying occurred were as follows:

Population	Date of Emergence	Age at First Laying
1	May 9, 1961	6 months 14 days
2	May 16, 1961	6 months 8 days
3	May 23, 1961	6 months
4	September 3, 1961	2 months 16 days

Environmental factors after diapause. Between October 23 and November 23, 1961, as indicated in Plates I, II, III and IV average maximum temperatures dropped more or less steadily from 69° to 48.5° (F.), while the average minimum temperatures for this period varied between 40.7° and 34.7° (F.). Relative humidity gradually increased from 65 to 67.5 per cent. Length of daylight decreased from 10.97 hours on October 23 to 9.95 hours on November 23.

<u>Survival of the overwintering adults</u>. A few of the overwintering adults survived through the summer and lived through part of the fall. A number of these old adults were collected from the field and readily laid eggs when held under near optimum conditions (74° to 75° degrees Fahrenheit).

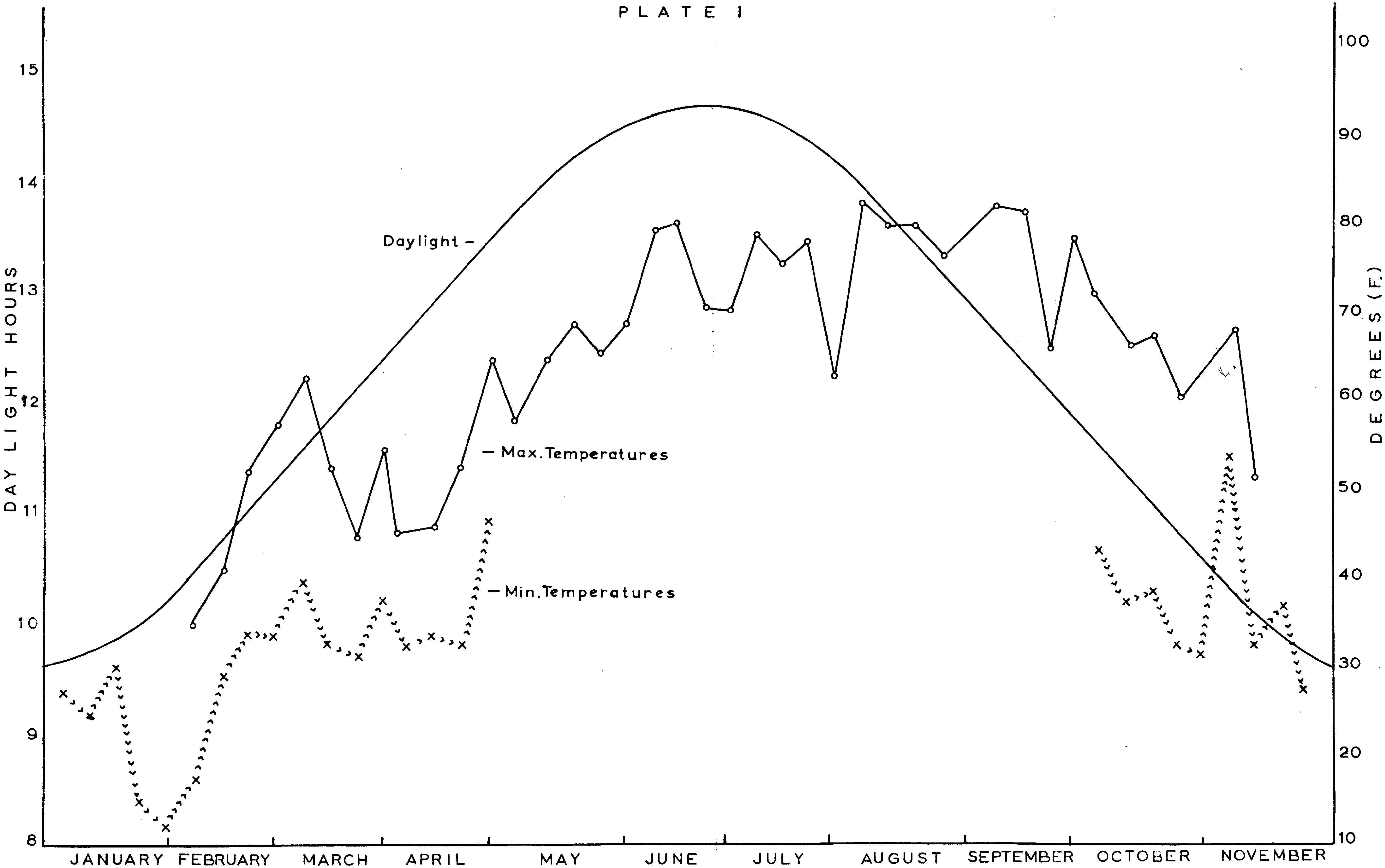
In late August, 1961 a number of third and fourth instar larvae were collected from the field and reared to adulthood in the insectary The date of emergence was September 3, 1961, and it was from this collection that the test weevils in population 4 were taken. These weevils did not enter into diapause as did the other three populations.

This summer generation developed under the following environmental characteristics: According to Plate I, these weevils came from eggs laid about the last week in July or early in August when the temperatures averaged under 77° (F.) which has been considered by many workers as the upper limit for sexual functioning (Michelbacher and Leighly, 1940; Evans, 1959). The larvae developed when the average maximum temperatures were above this upper limit but well within the developmental range. Average per cent relative humidity fluctuated between 65 and 67.5 (Plate II). Photoperiodism decreased from about 14 hours (July 31, 1961) to 12.9 hours on September 3, 1961 (Plate III).

EXPLANATION OF PLATE I

Hours of daylight and average maximum and minimum temperatures from January to December 1961. Blacksburg, Virginia.

PLATE I

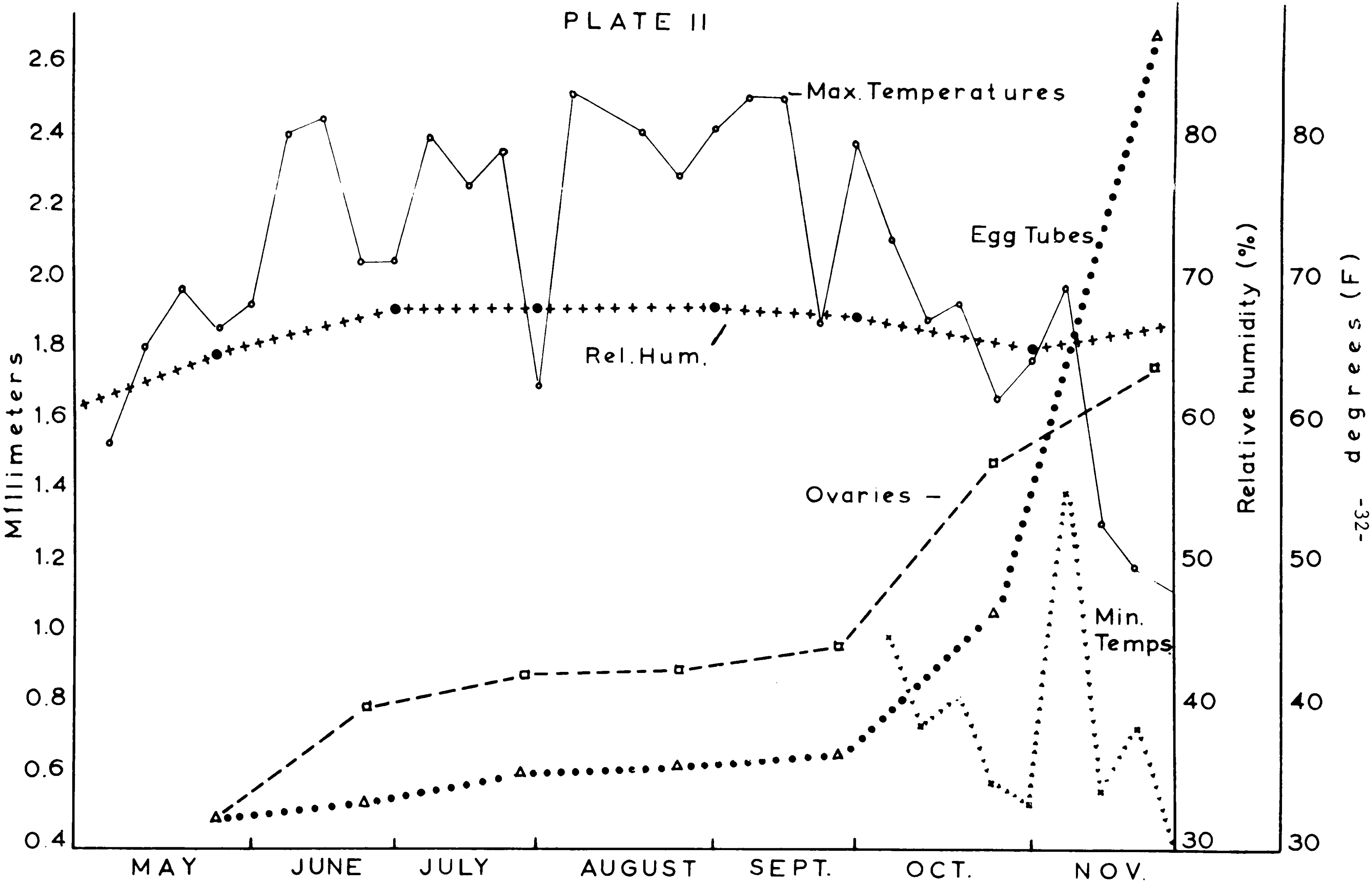


15
14
13
12
11
10
9
8
DAY LIGHT HOURS
100
90
80
70
60
50
40
30
20
10
DEGREES (F.)
Daylight -
- Max. Temperatures
- Min. Temperatures
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER

EXPLANATION OF PLATE II

Average maximum and minimum temperatures, average per cent relative humidity and the average sizes of the ovaries and egg tubes of female alfalfa weevils from May 23 to November 23, 1961.

PLATE II


Max. Temperatures
Egg Tubes
Rel. Hum.
Ovaries
Min. Temps.
Millimeters
Relative humidity (%)
degrees (F)
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
80
70
60
50
40
30
MAY
JUNE
JULY
AUGUST
SEPT.
OCT.
NOV.

EXPLANATION OF PLATE III

Hours of daylight and average sizes of ovaries and egg tubes of female alfalfa weevils from May 3 to November 23, 1961.

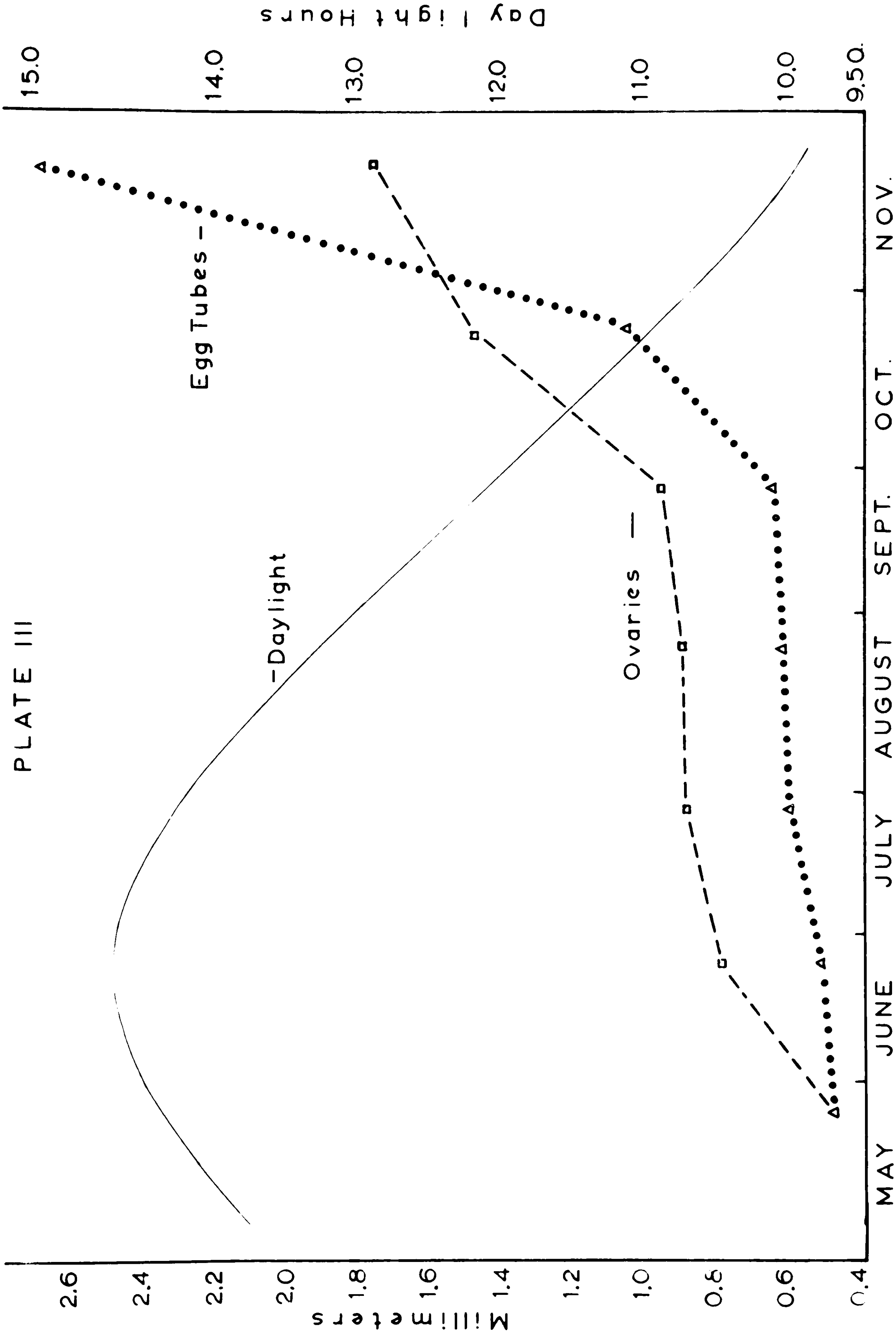
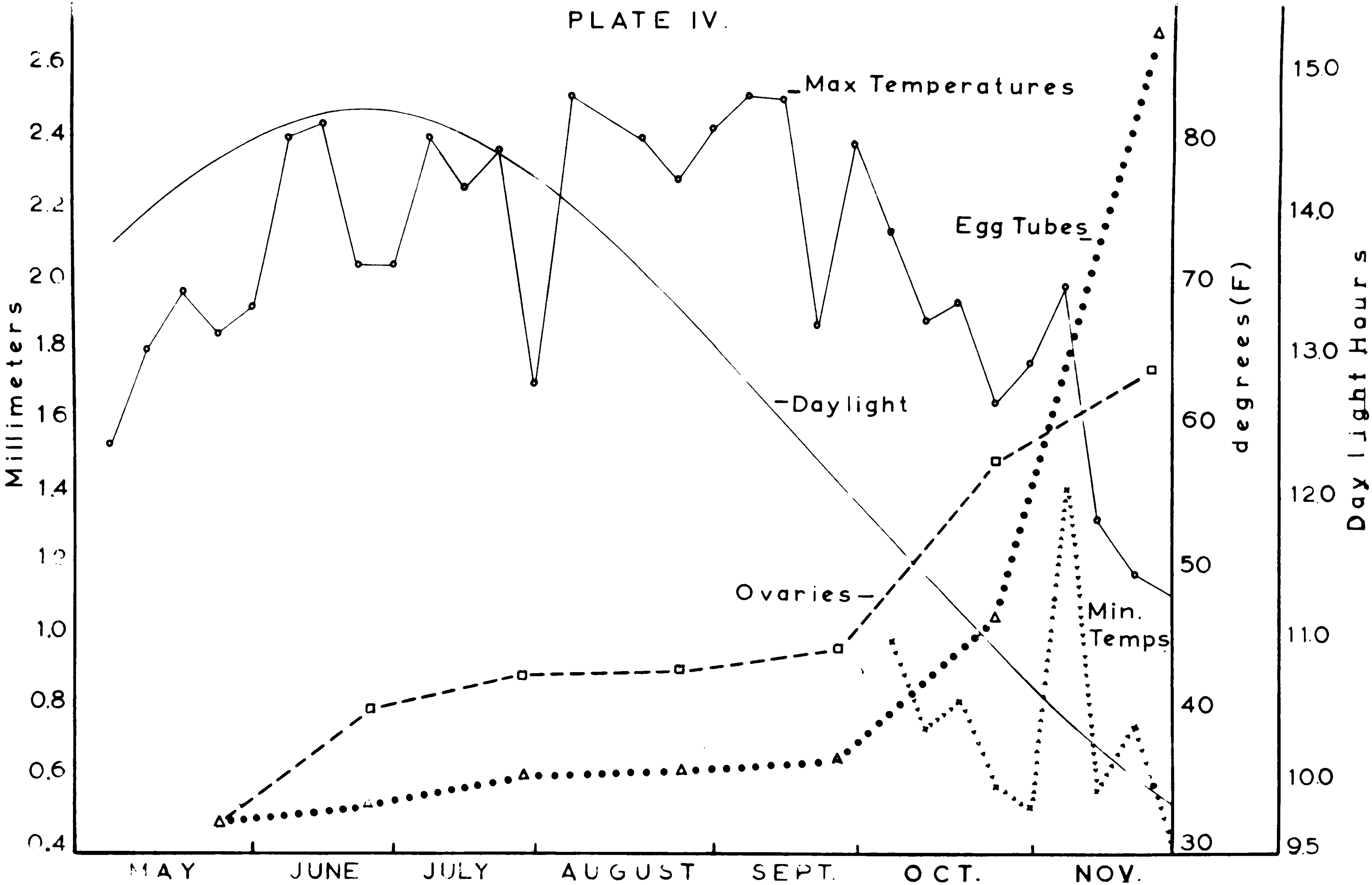


PLATE III
Day light Hours
15.0
14.0
13.0
12.0
11.0
10.0
9.50
Millimeters
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
MAY
JUNE
JULY
AUGUST
SEPT.
OCT.
NOV.
Egg Tubes
-Daylight
Ovaries

EXPLANATION OF PLATE IV

Hours of daylight, average maximum and minimum temperatures, and average sizes of ovaries and egg tubes of female alfalfa weevils from May 3 to November 23, 1961.

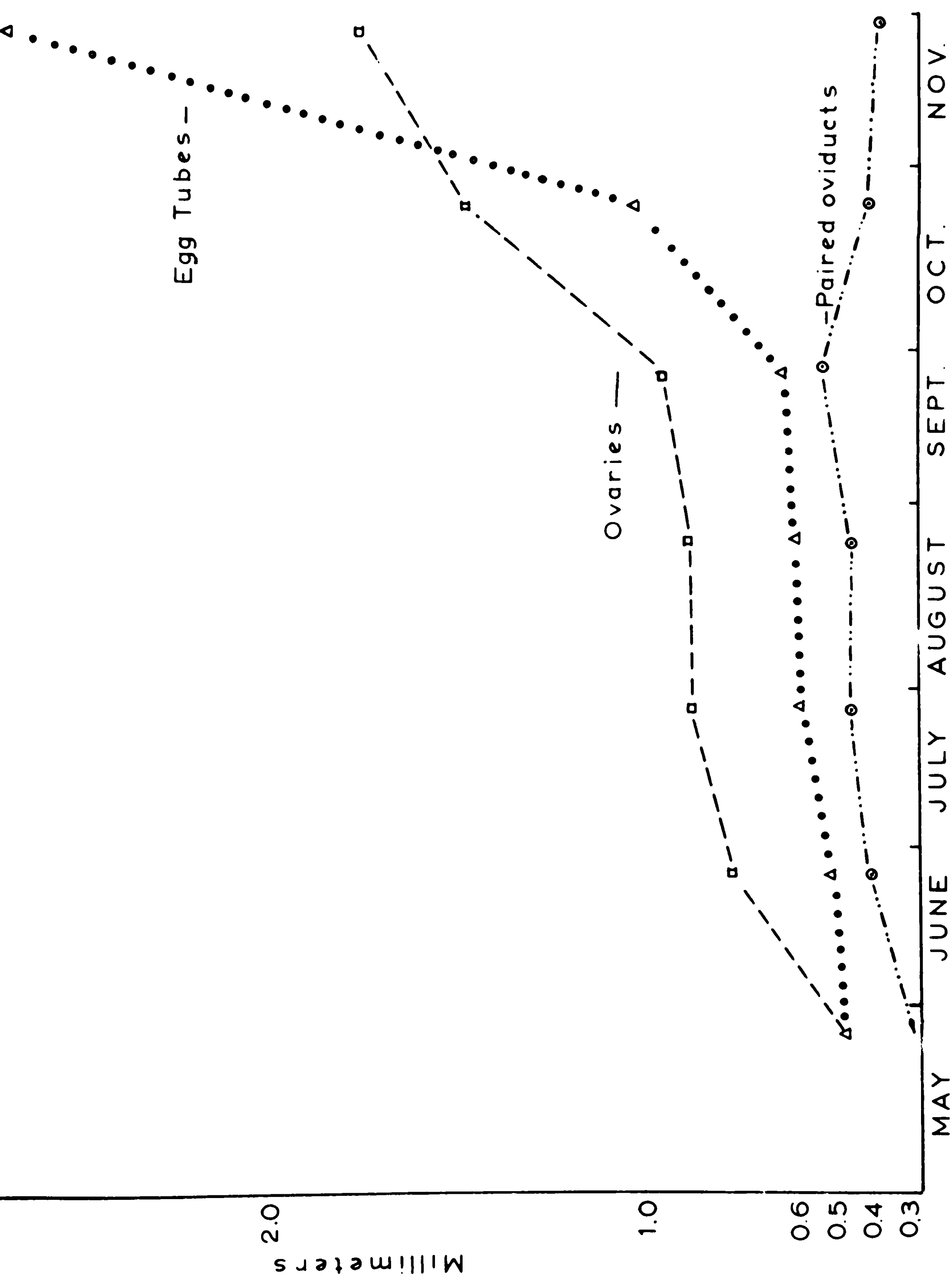
PLATE IV.


Max Temperatures
Egg Tubes
Daylight
Ovaries
Min. Temps
Millimeters
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.0
0.8
0.6
0.4
degrees (F)
80
70
60
50
40
30
Day Light Hours
15.0
14.0
13.0
12.0
11.0
10.0
9.5
MAY
JUNE
JULY
AUGUST
SEPT.
OCT.
NOV.

EXPLANATION OF PLATE V

Average size of ovaries, egg tubes and paired oviducts of alfalfa weevil females from May 23 to November 23, 1961.

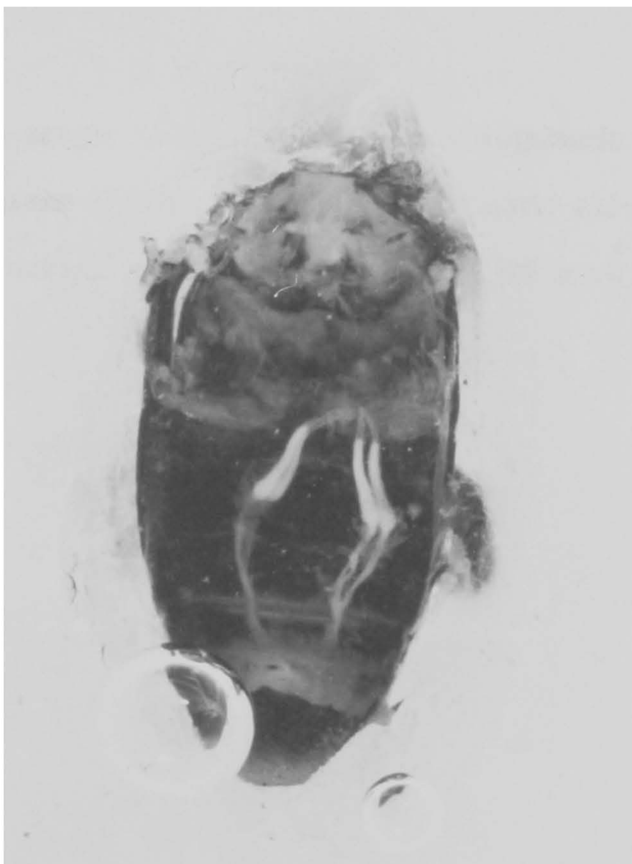
PLATE V


Egg Tubes
Ovaries
Paired oviducts
MAY
JUNE
JULY
AUGUST
SEPT.
OCT.
NOV.
2.8
2.0
1.0
0.6
0.5
0.4
0.3
Millimeters

EXPLANATION OF PLATE VI

Photograph showing size and shape of sex organs of female alfalfa weevil one month after adult emergence. (May 23 to June 23, 1961) (About 20 times actual size.)

PLATE VI



EXPLANATION OF PLATE VII

Photographs showing size and shape of sex organs of female alfalfa weevils during diapause. (June 23 to September 23, 1961). (About 20 times actual size.)

FIG. 1 2 months after adult emergence June 23 to July 23, 1961

FIG. 2 3 months after adult emergence July 23 to August 23, 1961

FIG. 3 4 months after adult emergence August 23 to September 23, 1961

PLATE VII

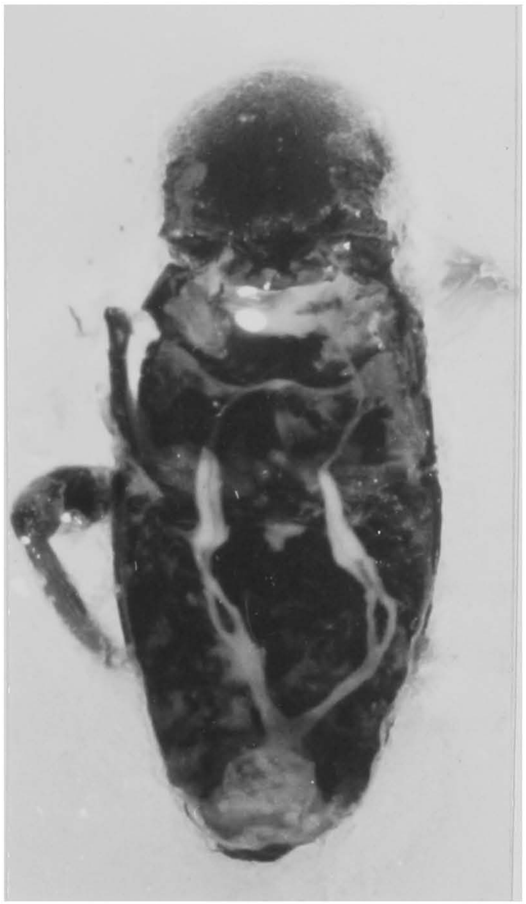


Fig. 1

Fig. 2

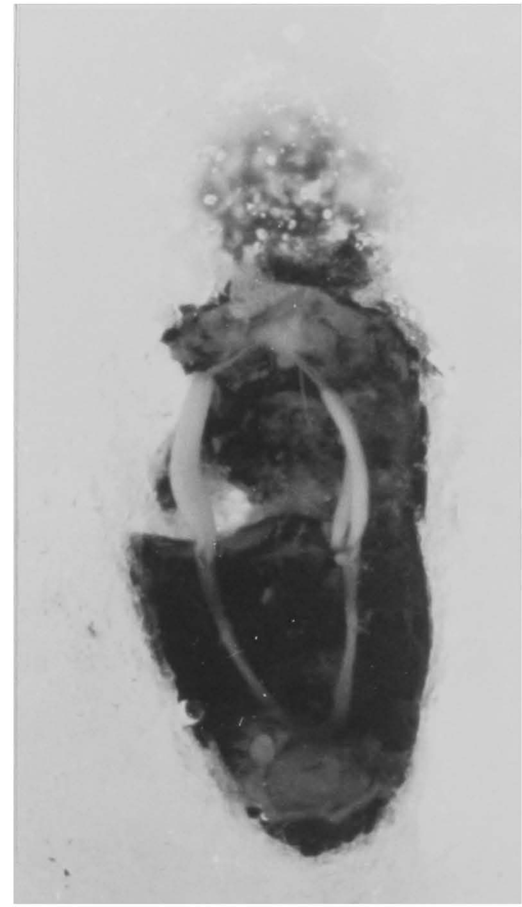


Fig. 3

EXPLANATION OF PLATE VIII

Photographs showing size and shape of sex organs of female alfalfa weevils at the end of diapause. (September 23 to October 23, 1961)

(About 20 time actual size)

FIG. 1. 5 months after adult emergence. Early stage

FIG. 2. 5 months after adult emergence. Medium stage

FIG. 3. 5 months after adult emergence. Late stage

PLATE VIII

Fig. 1

Fig. 2

Fig. 3

EXPLANATION OF PLATE IX

Photographs showing size and shape of sex organs of female alfalfa weevils after diapause. (October 23, to November 23, 1961)

(About 20 times actual size.)

FIG. 1. 6 months after adult emergence. Early stage. (Near maturation)

FIG. 2. 6 months after adult emergence. Fully developed sex organs, exposed after dissection.

FIG. 3. 6 months after adult emergence. Fully developed sex organs, as they appear in situ.

PLATE IX

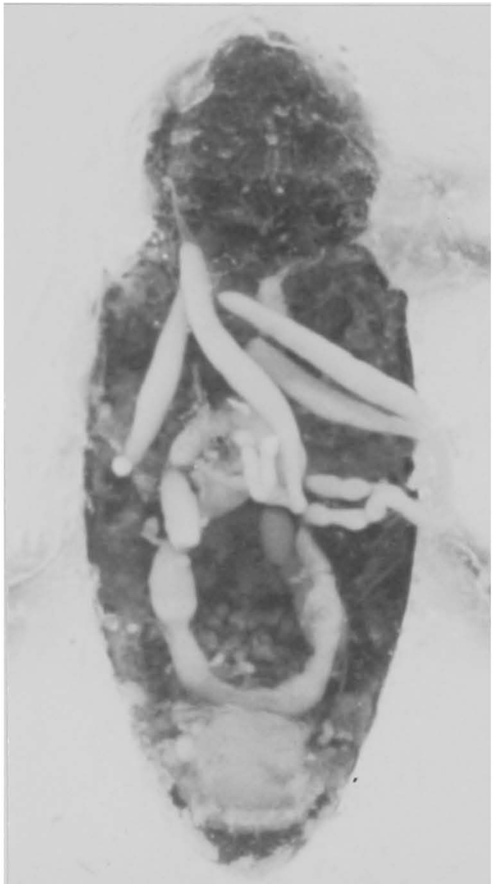


Fig. 1

Fig. 2

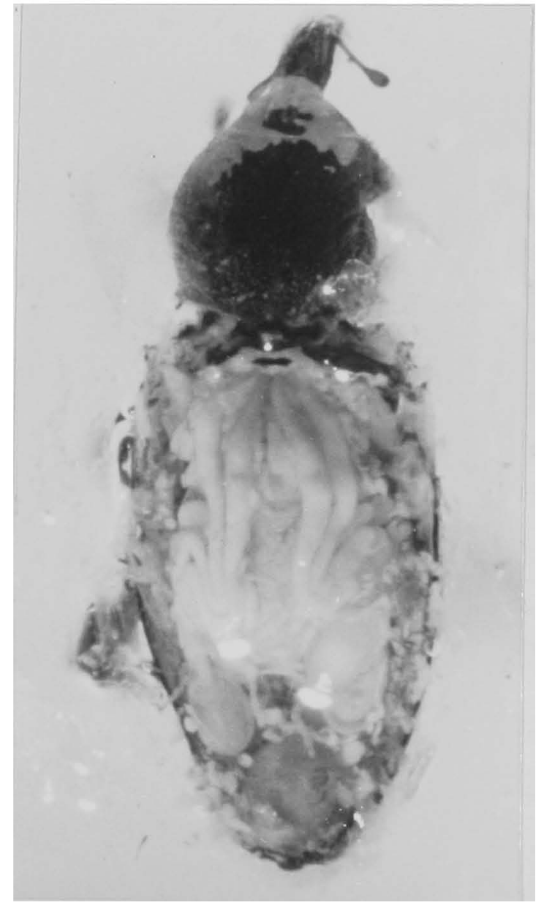


Fig. 3

DISCUSSION

When the question arose concerning the relationship between the period of sexual development and summer diapause, it was assumed that diapause of the adults regulated the rate of sexual development. Accordingly, both phenomena should be under the influence of a common stimulus. On the other hand, according to several workers, as mentioned earlier in this paper, diapause could be an effect of a slow rate of development. The question now becomes "which of the two, diapause or rate of sexual development, is the cause and which is the effect?" To answer this question it became necessary to study the life stages of the alfalfa weevil during hibernation, prior to diapause, during diapause, and after diapause. The opportunity to study weevils that did not enter diapause helped considerably to solve parts of this problem.

The alfalfa weevil hibernates in Virginia as an adult as well as in the egg stage. Overwintering adult females are sexually mature. This is clearly indicated by the data obtained in this work where it can be observed that all weevils, regardless of date of emergence, became sexually mature at about the same date. The slight differences in sexual development were not significant because of the rapid rate of growth at that time. In addition, observations on dissected weevils collected on November 23, 1961 showed that nearly 80 per cent of the female weevils were capable of laying eggs at that time.

Hibernation in this species is temperature-dependent and is exhibited in the form of a simple "quiescence." Snow (1928), reported that during the winter the small amount of egg development is due to inactivity and small amount of feeding. However, when the limiting factor (low temperatures) was removed feeding and activity was resumed. This indicates that hibernation is merely a form of "quiescence." Hibernation ended in the last week of February in 1961 and 1962, when the overwintering adults resumed activity. This activity undoubtedly was a response to favorable temperature because the weevils returned to the quiescent state when temperatures dropped. During this period of spring activity, feeding and mating occurred about one month before egg laying took place. The lower temperature ranges are different for oviposition and hatching, but both are temperature dependent.

In late March or early April the larvae appear and it is in this stage that the stimulus determining the diapause tendency is impressed (Huggans and Blickenstaff, 1962). The work of Huggans and Blickenstaff suggests that exposure of the larvae to more than 10 hours of light per day may be the stimulus for diapause in this insect. However, as reported earlier in this paper, larvae were collected in August when photoperiods were over 13 hours per day (Plate III). These larvae developed into adults which did not enter diapause. Thus, it appears that a certain length of daylight is not the main stimulus for the tendency to enter diapause.

Larvae which develop in the spring are exposed to increasing daily photoperiods (Plate III). The author submits that this daily increase in hours of light per day is the stimulus for diapause. Although the insect enters diapause as an adult, the stimulus for the inception of diapause was received by the larva, and is exhibited on the first day that photoperiod (day length) decreases (June 23).

Duration of diapause apparently is not influenced chiefly by temperature. The weevils that emerged in September, although not in diapause, first exhibited mating and flight activity on the same date that the diapausing weevils did, although temperatures were previously favorable for this activity (Plate II). Thus, morphogenesis began in both populations on the same date. The stimulus for the beginning of mating and flight must have been a certain photoperiod (length of day). On this date, October 23, 1961, there were approximately 11 hours of daylight (Plate III).

The particular rhythm in the slow development of the sex organs during aestivation, corresponds to an expression of a "true" diapause which synchronizes the life cycle of the insect to the environment. Thus, diapause in the alfalfa weevil has the functional purpose of preventing the insect from becoming sexually mature when the season is not favorable for reproduction and survival. In fact, sexual maturation is not completed until some time after diapause has ended.

SUMMARY AND CONCLUSIONS

Laboratory studies and field observations were conducted to ascertain the development of the female sex organs of the alfalfa weevil during aestivation, to study the role of this aestivation on sexual development, and to investigate the factors responsible for this interaction.

Measurements of female sex organs were made in dissected weevils at monthly intervals to observe each of the developmental stages of sexual maturation. Results of this sexual development as it progressed after adult emergence, were presented by means of tables, graphs, and a series of photographs.

The importance of not confusing old and new weevils was recognized in this work, since their behavioral patters differ in many respects.

The nature and physiology of the aestivation of this weevil was discussed and evidence was presented to support the author's suggestion that this aestivation is a "true diapause." It was demonstrated in this paper that diapause synchronized the sexual growth of the alfalfa weevil to the seasonal changes of the year for species adaptation and survival.

Results obtained from observations of females fed on alfalfa can be summarized as follows:

1. Adult females within 24 hours after emergence had small but well-defined sex organs.

2. Female weevils which emerged in the spring showed a slow rate of growth of the sex organs during the first month of age which was just prior to entering into diapause.
3. During diapause sexual maturation was literally at a standstill. Only after five months of age were there any apparent changes in size or shape of the sex organs.
4. After diapause differentiation and growth of the ovaries began as flight, feeding and mating activities were resumed. Egg laying was observed about one month after diapause ended.
5. The stimulus for the tendency to enter diapause in evidently received in the larval stage, and it is suggested that this stimulus is a daily increase in the photoperiod. This is based on the fact that spring larvae are exposed to approximately the same number of hours of light per day as are summer larvae, but summer larvae develop into adults which do not enter into diapause.
6. The evidence as presented in this paper suggests that the triggering factor for the inception of diapause in the adult is a shortening of daylight hours since all weevils of the spring generation entered into diapause on June 23, 1961.
7. It was also shown that photoperiodicity could very well be the stimulus for resumption of morphogenesis, or in other words, the stimulus for ending diapause, since all weevils, including the summer generation which did not diapause, resumed activity on October 23, 1961.

8. Morphogenesis is temperature dependent, yet, during diapause growth did not occur eventhough optimum temperatures for morphogenesis prevailed. This indicates that a strong controlling factor overrides the effects of optimum temperatures. Thus, diapause is the cause of the slow rate of sexual growth.

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VITA

The author was born June 14, 1931, in San Antonio, Texas, as the second son and fifth child of Pilar Guerra and Antonia E. Alvarez. He obtained his elementary education at Monterrey, N.L., Mexico, and subsequently entered the Franco Mexicano High School in 1947. After graduating from high school, he entered the Instituto Technologico Y De Estudios Superiores De Monterrey in February 1950 where he had two years of preparatory schooling. After graduation from preparatory school, he continued his studies in the School of Agriculture at the same institute until entering the United States Army in 1952.

Following honorable discharge from the service, he returned to Monterrey Tech in 1954 and earned his B.S. degree in Agricultural Engineering in 1958.

In 1960 he was accepted as a graduate research assistant in the Department of Entomology at Virginia Polytechnic Institute. The author received his Master of Science degree in Entomology in 1962 from V.P.I.

He is a member of the Entomological Society of America and of Phi Sigma Society

Antonio A. Guerra

Author

ABSTRACT

The sexual development of the female alfalfa weevil during aestivation was studied in the field and laboratory. Interactions between this growth and aestivation as affected by some environmental factors were considered, and the successive developmental stages were observed, measured, and photographed at monthly intervals.

The nature of the diapause in this weevil was investigated and evidence was presented in support of the author's suggestion that the aestivation of this insect is a "true diapause". It is demonstrated that this diapause is functional in that it synchronizes sexual growth to seasonal changes as an adaptation for survival.

A comparison of sexual development in diapausing and non-diapausing weevils points out that considerable caution must be exercised in collecting weevils for investigations of this type.

The work reported in this paper was conducted in 1961 and from January to May 1962. A detailed account of climatic conditions during this period was included.

Sexual development was studied in the pre-diapause, diapause, and post-diapause periods. Speculation is made on the stimuli probably involved in the inception, duration, and end of diapause, as well as the stage of the weevil receiving the stimuli.

The possible action of photoperiod was emphasized and discussed with graphs and photographs complementing the data in showing the relationship between light and sexual development of the alfalfa weevil during aestivation.