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Diapause Studies of the Bollworm, Heliothis Zea (Boddie) and the Tobacco Budworm, Heliothis Virescens F.

Jacob R. Phillips
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DIAPAUSE STUDIES OF THE BOLLWORM, HELIOTHIS ZEA (BODDIE) AND THE TO-
BACCO BUDWORM, HELIOTHIS VIRESCENS

Louisiana State University, Ph.D., 1965
Zoology

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DIAPAUSE STUDIES OF THE BOLLWORM, *HELIOTHIS ZEA* (BODDIE) 
AND THE TOBACCO BUDWORM, *HELIOTHIS VIRESCENS* F.

A Dissertation

Submitted to the Graduate Faculty of the 
Louisiana State University and 
Agricultural and Mechanical College 
in partial fulfillment of the 
requirements for the degree of 
Doctor of Philosophy 

in 
The Department of Entomology 

by 
Jacob R. Phillips 
M.S., University of Arkansas, 1961 
January, 1965
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ABSTRACT

The bollworm, *Heliothis zea* (Boddie) and the tobacco budworm, *Heliothis virescens* F. overwinter in the soil as diapausing pupae. The onset of diapause is influenced by both climatical and host conditions. This study was conducted to determine the effect of certain temperatures and photoperiods on the induction of diapause and diapause characteristics of the two species.

Laboratory cultures of the two species were established from adults that were collected in the vicinity of Baton Rouge, Louisiana. Larvae were reared on an artificial medium throughout the course of the study. Parent stocks of both species were reared under continuous illumination and a constant temperature.

Diapause induction studies were conducted by holding the larvae in temperature and photoperiod controlled cabinets until pupation occurred. Two different photoperiods each at four different temperatures constituted the controlled environmental conditions.

Physiological characteristics of diapause and non-diapause pupae were determined the sixth day following pupation. Oxygen consumption was measured by the direct method employing standard Warburg manometry. Total crude lipids were extracted by homogenizing the pupae in chloroform-methanol. Separation of the miscible chloroform-methanol was achieved by the addition of magnesium chloride. After
evaporation of the chloroform the crude lipid samples were held in desiccators until constant weights were obtained. Pupae were dissected in Ringer's solution to determine the progression of gonadal development.

The earliest observable indicator of diapause in both the bollworm and tobacco budworm was found to be the retention of larval eye spots in the post genal region of the pupae. In non-diapause pupae these spots migrated entad, disappearing the sixth day following pupation. Other characteristics of diapause in the two species were found to be: arrest of gonadal development in the female, cessation of spermatogenesis in the male, lighter colored adults that emerged from the diapause condition and an approximate three-fold decrease in oxygen consumption.

Diapause was induced by 10-hour photoperiods and prevented by 14-hour photoperiods. High temperature counteracted the short photoperiod effect while low temperature counteracted the long day effect. The sensitive stage was found to be the larva and the responsive stage the pupa. The tobacco budworm was more responsive to photoperiod at the different temperatures than the bollworm. Pupae of both the bollworm and tobacco budworm can remain in diapause for at least twenty months.

There was a parental effect that reduced the incidence of diapause in \( F_1 \) and \( F_2 \) progeny. This effect was apparently influenced by both a time interval and temperature.
INTRODUCTION

The occurrence of diapause is a common feature in organisms that inhabit inconstant environments. Diapause is characterized by the temporary cessation of growth or reproduction, by reduced metabolism and often by enhanced resistance to adverse climatic factors such as cold, heat, and drought.

The arrest of growth in organisms with a facultative diapause is ultimately governed by the environment. This phenomenon may be influenced by a number of factors such as temperature, and the conditions of the host. However, these factors must be regarded as somewhat imperfect indicators of season for they are intrinsically variable and their influence on the organism more or less immediate. Photoperiod on the other hand changes during the season with mathematical accuracy. Thus the response can be so adapted that the organism can enter diapause before the advent of unfavorable seasonal conditions.

Both the bollworm, *Heliothis zea* (Boddie) and the tobacco budworm, *Heliothis virescens* F. undergo a facultative diapause (Chamberlin and Tenhet, 1927; Phillips and Barber, 1929; Barber, 1937; Ditman *et al*., 1940; Danilyevsky, 1956; Komarova, 1959). The environmental factors and their interrelationships that lead to the onset of diapause in these two species is still somewhat obscure.
With regard to the bollworm, research by American workers (Phillips and Barber, 1929; Ditman et al., 1940) indicates that the onset of diapause is due principally to low temperatures prevailing during larval development and to the maturity of the host. Foreign authors (Danilyevsky, 1956; Komarova, 1959) discount the relative importance of low temperatures and host maturity in the induction of diapause in the old world species, Chloridea obsoleta F. [Heliothis armigera (Hbn.)]. They maintain that the onset of diapause is a response to the seasonal change in the length of day. However, Komarova (1959) found the effect of photoperiod to be "well marked" only at a temperature of 23°C.

The present study was undertaken to determine: (1) The influence of photoperiod and temperature on the induction of diapause in the bollworm and the tobacco budworm, (2) the characteristics of diapause in the two species.
REVIEW OF LITERATURE

Research has established that the bollworm, *Heliothis zea* (Boddie), and the tobacco budworm, *Heliothis virescens* F., enter "hibernation" as pupae in the autumn (Riley, 1871; Malley, 1893; Quaintance and Brues, 1905; Garman and Jewett, 1914; Chamberlin and Tenhet, 1927; Phillips and Barber, 1929; Barber and Dicke, 1937; Barber, 1937).

Factors that may govern the onset of this phenomenon were not suggested until Phillips and Barber (1929) reported the incidence of "hibernating" pupae to be greater when the larvae were reared on dough stage corn. Later, Ditman *et al.*, (1940) found diapause (here diapause, not hibernation) to be initiated by low temperatures during the larval period. Larvae reared at 18.9°C. produced pupae, some groups of which entered diapause to the extent of 50 per cent. There is some difficulty in understanding the authors' conclusions. They stated that the maturity of the corn fed to the larvae did not exert any influence on the incidence of diapause. Yet, in one of their tests the only indicated variable was that of the maturity of corn given to the larvae for food, in this instance dough stage corn was compared with milk stage corn. The results show a four-fold increase in diapause within the group reared on dough stage corn over that of the group reared on milk stage corn.
Danilyevsky (1956) and Komarova (1959) reported that the onset of diapause in the old world species *Chloridea obsoleta* F. (*Heliothis armigera* (Hbn.)) was due to short days in the autumn. However, Komarova found that temperatures above and below 23°C neutralized the photoperiod effect.

Indeed, the aforementioned findings associate the onset of diapause in the bollworm and tobacco budworm with three environmental variables: (1) photoperiod (2) temperature and (3) food.

Extensive reviews, concerning arthropod diapause, by Lees (1955) and de Wilde (1962) discuss research that show these three factors to be the most important in governing diapause in other insects.

**Photoperiod**

The many species of arthropods known to have photoinduced diapause include *Bombyx mori* (Kogure, 1933), *Grapholitha molesta* (Kickson, 1949), *Diataraxia oleracea* (Way and Hopkins, 1950), *Metatetranychus ulmi* Koch (Lees, 1953), *Leptinotarsa decemlineata* Say (de Wilde, 1954), *Ostrinia nubilalis* (Hbn.) (Beck and Hanec, 1960), *Pectinophora gossypiella* (Saunders) (Bull and Adkisson, 1960), and *Anthonomus grandis* Boh. (Earle and Newsom, 1963). The direction of response was the same in nearly all these species. Long days cause uninterrupted development while short days initiate diapause. This uniformity satisfies the ecological requirement for dormancy to occur at one particular time of the year, namely
autumn. It is worth noting that all these species occur in the temperate zone and are phytophagous, thus their dependence upon a reliable indicator of season is obvious.

**Ratio of Light to Dark in Controlling Diapause.**

The role of light versus dark in controlling diapause has been studied in many different species of insects. There is a variety of results and interpretations (Adkisson, 1964; Beck, 1962; Dickson, 1949; Lees, 1953, 1955; de Wilde, 1962).

Dickson found the ratio of hours of light to dark could not explain the photoperiodic control of diapause in *Grapholitha*. The most meaningful results were obtained when the combined number of hours of light and dark per cycle were approximately 24-hours. Diapause was always prevented in *Grapholitha* by photoperiodic regimes having a 9-hour dark period, regardless of the length of the light period which preceded it.

In the mite *Metatetranychus ulmi* Koch (Lees, 1953) and the larva of *Ostrinia*, (Beck, 1962) the induction of diapause was most strongly correlated with the periodic occurrence of a 12-hour dark period. The dominant action of the dark period was best demonstrated in cycles of about 24-hours duration. Adkisson (1964) reported results similar to this with *Pectinophora*. He found the critical dark period to be 10.75 hours in cycles of about 24-hours duration.

Tanaka (1950) in research with the pupal diapause of the moth *Antheraea pergni*, showed that the induction of diapause revolves
around a dark period of 11 hours, regardless of the duration of the accompanying light period.

One may conclude from these findings that (1) the expression of diapause is greatest under light-dark cycles of 24-hours total duration, (2) the ratio of light to dark appears to be of no consequence and (3) the duration of the dark period appears to be critical in determining the diapause status of the insect.

Mode of Action of the Photoperiod.

In his review, de Wilde (1962) discussed research that had demonstrated the action of the photoperiod to be directly on the insect rather than being mediated indirectly through the host on which the insect was feeding. However, exceptions may exist in the parasitic insects *Cheilonus annulipes* and *Habrobracon brevicornis* (Andrewartha, 1952) and apparently the subterranean aphid discussed by de Wilde (1962) is an exception. More research is needed before the relationships of these insects to their hosts are clearly defined.

The necessity of simple or compound eyes for receiving the light stimulus has not been proven (de Wilde, 1962). Research involving cauterization of the eyes, painting the eyes with black lacquer, and covering the head or body has revealed that tissue other than the eyes may be involved in light reception. Research by Lees (1959, 1960) suggests the possibility of direct action on the brain by the photoperiod.

Postulating a photoperiodic reaction system, de Wilde (1962) suggests that if the assumption is made that the effect of each pho-
The periodic cycle is composed of a light reaction and a "dark process", it may then be supposed that, in the photodynamic process, a pigment is involved. Such a pigment may either transfer radiant energy to a chemical process and remain unchanged or may itself take part in a photochemical reaction. However, de Wilde favors the idea of a periodic excitatory state of the brain, produced by light both directly and by photoreceptors, and acting together with an "internal clock" mechanism that determines responsiveness to light. He believes that this mechanism is probably located in the brain and suboesophageal ganglion.

Research by Williams (1947, 1952, 1956, 1961) has demonstrated diapause to be an endocrine deficiency syndrome of the prothoracic glands. The continued development in many insects depends on the action of the brain in activating the prothoracic glands causing them to secrete the growth hormone, ecdysone. Without the presence of ecdysone, growth cannot proceed. The direction of development to the larva, pupa, or adult is mediated by another hormone, the juvenile hormone. These findings along with results by Van der Kloot (1955) and Cloutier et al., (1962) establish quite positively that in a diapausing larva or pupa, arrested development is caused by the failure of the brain to activate the prothoracic glands. In view of these findings and results of research by de Wilde (1961), it appears that diapause during the growing stages is due to a temporary absence of neurosecretory activity in the brain while in adult diapause there may be an active inhibition of the corpora allata.
Seasonal Forms Controlled by Photoperiod.

Seasonal polymorphism in insects is often day-length dependent. Müller (1955) found photoperiod to be the primary cause for the seasonal dimorphism of *Araschnia levana* (L.) (forms *levana* and *prorsa*). Pease (1962) found photoperiod to influence the seasonal forms of *Ascia monuste* L.

Other examples of seasonal polymorphism controlled by photoperiod are given by de Wilde (1962). These include the jassid *Euscelis plebejus* (Fallen), the pear sucker, *Psylla pyri* (Linnaeus), and the aphid *Megoura viciae* Buckton.

In the case of the nymphalid butterfly, *Araschnia levana*, and similar examples, the photoperiodic form determination is connected with diapause. The spring form *levana* is a lighter pigmented individual than the summer form *prorsa*. There is a noticeable absence of melanin in the wings of *levana*. It is of value to mention here, observations made by Quaintance and Brues (1905) on the bollworm, *Heliothis zea*. They found that rearing the larva under cold conditions caused the resulting adult to be very dark-pigmented. However, no especially dark moths were reported in the spring brood whose pupae had been subjected to the lower temperatures of winter. These observations suggest that some condition contributes to the control of pigment formation in the bollworm adult other than low temperatures.

The Sensitive Stage.

The reviews by Andrewartha (1952) and de Wilde (1962) present
the results found by many investigators concerning the stage or stages of arthropods that are sensitive to the photoperiod. These results show that the sensitive stage may be, excepting the pupal stage, from egg to adult, however, in most cases sensitivity is intensified in a limited number of stages or instars. Research discussed in these reviews show in most cases the sensitive and responsive stages are different.

The sensitive and responsive stage may in some cases be so remote in time that it may be referred to as a delayed photoperiodic response. For example, in Polychrosis botrana the photoperiod acting on the egg may influence diapause in the pupa. The extreme case is Bombyx mori in which expression of the photoperiodic response is delayed by one complete generation.

Temperature and the Onset of Diapause

In most long day species, high temperatures tend to suppress diapause, while low temperatures have the opposite effect. This type of response is shown by Diataraxia (Way and Hopkins, 1950) and Metatetranychus (Lees, 1953).

When Diataraxia is reared at 34°C all the pupae develop without arrest if the larvae have been exposed to a long photoperiod, and diapause is prevented in one-half (approximately) of the pupae from larvae which were reared under a short photoperiod. Conversely, at low temperatures of 12 and 15°C over 90 percent of the pupae enter diapause even though the larvae have experienced a long photoperiod.
Very similar results have been obtained in *Metatetranychus*.

The action of high temperature in suppressing the onset of diapause has been described in many other insects, including *Pieris brassicae* (Maercks, 1934), *Loxostege sticticalis* (Steinberg and Kamensky, 1936), *Araschnia levana* (Müller, 1955), and *Ostrinia nubilalis* (Beck and Hanec, 1960).

This rule is by no means universal. In *Grapholitha* both high and low temperatures prevent diapause (Dickson, 1949). In *Bombyx mori* diapause is induced by high temperatures and averted by low temperatures (Kogure, 1933). In *Antheraea* and *Harrisina* photoperiod has assumed so dominant a role that the arrest of growth is essentially independent of temperature (Tanaka, 1944; Smith and Langston, 1953).

Simmonds (1948) and Andrewartha (1952) concluded that temperature influenced the processes of growth through the medium of metabolism. Lees (1955) however, suggested that temperature should be regarded as a "token" stimulus in the same sense as photoperiod. This suggestion by Lees has, according to de Wilde (1962), lost much of its appeal since the temperature may affect the dark process in one case and the light reaction in another. This seems to be substantiated by Goryshin (1955) who found that low temperature periods during the light cycle are rendered equivalent to darkness in their effect on the diapause response of *Acronycta*. 
Physiological Characteristics of the Diapause Condition

Accumulation of fat accompanied by a decline of the ratio of water to dry matter, a decrease in the level of oxygen consumption, the cessation of oogenesis and spermatogenesis are characteristics that have been found to be associated with the diapause condition.

The hypertrophy of the fat body is evident in many insects with an adult diapause. Fat may form up to 56 percent of the dry weight in the noctuid Scolispterix libatrix (Sacharon, 1930) and up to 60 percent in diapausing females of Culex pipiens (Buxton, 1935). Fat and glycogen are stored abundantly in cells of the tracheal organ in Gastrophilus (Levenbook, 1951). An accumulation of fat has also been found in diapausing Anthonomus grandis (Brazzel and Newsom, 1959). An excess of dry weight due to fat has been reported in the diapausing larvae of Pectinophora gossypiella (Squire, 1940) and Ostrinia nubilalis (Beck and Hanec, 1960). However, Ditman et. al. (1940) did not find any differences in dry weight and fat when comparing diapause and non-diapause pupae of Heliothis zea.

Schneiderman and Williams (1953, 1954) found that respiratory metabolism of cecropia pupae was strongly suppressed during diapause. Oxygen consumption of pupae in diapause was only 1.4 percent that of the mature larvae. Such pupae were found to be relatively insensitive to carbon monoxide and cyanide poisoning. This suggests that the cytochrome oxidase system must be almost completely inactive. Oxygen consumption increased markedly as diapause was terminated.
Similar results have been found in diapausing *Ostrinia* (Beck and Hanec, 1960) and *Anthonomus grandis* (Brazzel and Newsom, 1959).

The cessation of reproductive activity has been reported in *Anthonomus grandis* by Brazzel and Newsom (1959). These authors found cessation of spermatogenesis and atrophied ovaries to be associated with the diapause condition. Beck and Hanec (1960) reported the earliest indicator of diapause in the larvae of *Ostrinia* to be retarded gonadal development. Ovisorption was found to be associated with the diapause condition of *Leptinotarsa decemlineata* (de Wilde, 1959).
METHODS AND MATERIALS

Laboratory Rearing of *Heliothis* *zea* and *H. virescens*

Laboratory cultures were established and maintained from bollworm and tobacco budworm adults that were collected in the vicinity of Baton Rouge, Louisiana. The larvae were reared on an artificial medium developed by Berger (personal communication). See Appendix I.

The rearing procedures were as follows:

Eggs were collected on cheesecloth. The cheesecloth with the eggs were collected daily and placed in plastic boxes until hatching occurred. Each newly-hatched larvae was transferred to a 10-dram vial containing 15-20 ml of media and plugged with cotton. A size "00" camels hair brush was used to facilitate transferring the larvae to the vials. After pupation the pupae were placed in one-half gallon ice cream cartons that contained a three or four inch layer of moist vermiculite. The ice cream cartons were covered with cheesecloth that had two cheesecloth strips attached. These strips served as platforms for the newly-emerged moths during wing expansion. Male and female moths, at a ratio of 1 to 2, were placed in oviposition cages. These consisted of large lantern globes or one-gallon, wide-mouth glass jars with the bottoms cut out. Both types of cages proved to be satisfactory. These cages were placed on moist vermiculite that
was covered with a double layer of paper toweling. The vermiculite was moistened as frequently as necessary in order to maintain a high humidity inside the cages. Small jars (25 ml. wide mouth), containing cellucotton saturated with a 10% aqueous honey solution, were placed in the cages to provide food. The cages were covered with cheesecloth and an appropriate size petri dish. During the night a light source was provided by using an E and C microscope lamp (Curtin No. 14078) equipped with a blue frosted filter. Light intensity was adjusted to 5 ft-candles by using an autotransformer (Sargent No. S-30940).

Diapause Induction

Larvae were reared in 95 mm X 25 mm glass vials plugged with cotton. They were held in temperature controlled (± 1°C) incubators which were equipped with a light. The lighting was provided by a 14-watt daylight fluorescent lamp wired to a timer to produce the desired dark and light periods in a 24-hour cycle. Each vial received from 20 to about 300 ft-candles of light, depending on its position in the incubator. The minimum intensity of light in this study was well above the thresholds that have been determined for other species of insects. Lees (1955) reported thresholds for some species to be well under 5 ft-candles.

Two photoperiods, each at four different temperatures constituted the test conditions for this study. The photoperiods were 10-hours and 14-hours duration. Constant temperatures at each of the
two photoperiods were 27°, 24°, 21°, and 18° ± 1°C. The test larvae remained under these conditions from not over 10 hours after hatching until pupation was completed in all the test individuals.

The study of the effects that these conditions may have on the two species in question was repeated six times except at 27°C when it was repeated twice. One-hundred larvae of each of the two species constituted a repetition.

Larvae to be used for this study were obtained from parent stock that, as larvae, were reared under continuous illumination and at a temperature of 27° ± 2°C.

Respiratory Measurements

The respiratory rates of pupae that, as larvae, had been reared under all the different photoperiod and temperature conditions were measured on the sixth day following the day pupation occurred.

The respiratory rates of 36 pupae from each of two repetitions were measured. Three male and three female pupae were used from each test condition. Oxygen consumption was measured during a four-hour period. Readings were taken every 30 minutes.

The volumes of the pupae were determined by measuring the amount of water displaced by groups of ten pupae repeated ten times. An average volume was calculated from these measurements.

Oxygen consumption was measured by the direct method employing standard Warburg manometry as outlined by Umbreit et al. (1957). The following conditions were constant throughout:
Center well:

Potassium hydroxide, 20 percent solution 0.6 ml.
Absorptive surface - pleated filter paper 10 sq. cm.

Main compartment:

One test pupa
Equilibration time after adding test pupa 30 minutes
Temperature 27°C

The thermobarometer contained all components except the test pupa.

Crude Lipid Determinations

The total crude lipids were extracted by the method of Polch et al. (1957). Homogenates were prepared from ten, five male and five female, pupae. The pupae were weighed and then placed in a 50 ml. homogenizing flask that contained 20 ml. of chloroform-methanol (2:1). The contents were homogenized for three minutes with a Virtis homogenizing mill. The homogenate was then filtered using a Büchner form fritted glass funnel. The residue was washed three times with chloroform-methanol. The filtrate was collected in 100 ml. graduate cylinders.

Separation of the miscible chloroform-methanol extract into two immiscible layers, with subsequent partitioning of the lipids into the chloroform layer, was achieved by the addition of magnesium chloride solution (0.2 M aqueous) to the chloroform-methanol extract. The magnesium chloride solution was added at the rate of about one-fifth
volume of the solution per volume of extract. Partitioning was allowed to proceed for 24 hours.

The upper layer was siphoned off and the interface washed gently two times with a rinse solution. The rinse solution was prepared by mixing chloroform, methanol, and water in a separatory funnel in the proportions 8:4:3 by volume. A biphasic system results when the mixture is allowed to stand. The two phases were collected separately. The upper phase constituted the rinse solution.

The chloroform extract was then poured into 500 ml. round bottom flasks, previously weighed, and the chloroform evaporated using a rotary evaporator. Flasks containing the crude lipids were then placed in desiccators containing calcium chloride and held under vacuum until a constant weight was obtained.

For moisture content determinations five female and five male pupae were placed in aluminum weighing pans. The pupae were then split longitudinally with a razor blade and weighed. The weighing pans with the pupae were dried in vacuo until constant weights were determined.

The crude lipid and moisture determinations were repeated with two repetitions of each photoperiod and temperature condition.
RESULTS

Some Physiological Characteristics of Diapause

In undertaking a study of the factors involved in the induction of diapause, the first problem encountered was that of identifying pupae in diapause. Since diapause in the bollworm and tobacco budworm occurs in the pupal stage, the simplest criterion of diapause is a failure of adult emergence within a reasonable, but necessarily arbitrary, period of time following pupation.

On the day pupation occurred, larval eye spots were found to be present in the post genal region of the pupae. When pupae were removed from the rearing conditions and held at $27^\circ \pm 2^\circ C$, these pigment spots migrated entad, in some pupae, disappearing by the end of the fourth day. In other pupae the pigment spots did not disappear during the same period of time. The retention of the larval eye spots was found to be a valid characteristic of a diapause pupa for both the bollworm and the tobacco budworm. Illustration of this characteristic is shown in Figure 1. The retention of larval eye spots as a characteristic of diapause has been reported by Shumakov and Yakimovich (1955) for the old world specie of the bollworm, Chloridea obsoleta F. (Heliothis armigera Hbn.).
FIGURE 1a. Retention of larval eye spots by diapausing *Heliothis zea* pupa six days following pupation (12.5x).

FIGURE 1b. Loss of larval eye spots by non-diapausing *Heliothis zea* pupa six days following pupation (12.5x).
Gonadal Development.

Diapause and non-diapause pupae of both sexes were dissected in Ringer's solution, the sixth day following pupation, to determine the stage of gonadal development. Figure 2 shows the comparative development of the ovaries in diapause and non-diapause pupae. Well developed ovaries were present in non-diapause pupae, whereas ovary development of female pupae in diapause had not proceeded beyond formation of the lateral oviducts.

The testes of both diapause and non-diapause pupae of the bollworm and tobacco budworm were found to be fully developed in six-day old pupae. However, spermatogenesis had proceeded to the point of sperm bundle formation in non-diapause pupae, whereas in diapausing pupae only cyst cells containing spermatogonia were present in the testes. Figure 3 illustrates the progression of spermatogenesis in diapause and non-diapause pupae.

The arrest of gonadal development in female pupae and the cessation of spermatogenesis were characteristics of diapause in both the bollworm and the tobacco budworm. The illustrations referred to are of the bollworm.

Dimorphism Associated with Diapause.

It has been known for some time that temperature has an effect on the color pattern of moths. Ford (1955) reported that more pigment was deposited in the wings of the moth, Heliothis peltigera Schiff, when reared at the lower of two temperatures.
FIGURE 2a. Ovaries of diapausing *Helothis zea* showing arrested gonadal development (100 x). Dissected six days following pupation.

FIGURE 2b. Ovaries of non-diapausing *Helothis zea* showing well developed ovarioles (100 x). Dissected six days following pupation.
FIGURE 3a. Testes smear of diapausing *Heliothis zea* showing progression of spermatogenesis to formation of cyst cells only (100x). Dissected six days following pupation.

FIGURE 3b. Testes smear of non-diapausing *Heliothis zea* showing presence of well developed sperm bundles (100x). Dissected six days following pupation.
The bollworm and tobacco budworm were reared under three different temperatures (24°C; 21°C; 18°C) during the course of this study. Female bollworm moths were darkest when reared at 18°C, somewhat lighter under 21°C, and much lighter under 24°C. The brown pigment was much more intense in the wings of the female emerging from the 18°C rearing condition. Low developmental temperatures intensified the green pigment in the male of the bollworm and in both the females and males of the tobacco budworm.

The diapause condition was found to completely neutralize the temperature effect on moth color of both the female and male bollworm moths. There was no distinguishable color difference between the moths at any of the three different temperatures. Figure 4 shows the effect diapause had on the color pattern of female and male bollworm moths. The neutralization of temperature effect on the color of moths was not as evident in the tobacco budworm. It appeared that the diapause effect was more pronounced in the case of the brown coloration than the green.

Metabolism.

Oxygen consumption, moisture content and total crude lipids were determined for both diapause and non-diapause pupae that were reared under the different photoperiod and temperature conditions. A field collected population of diapause bollworm pupae was included in this phase of the study.

The results in Table 1 show that almost a three-fold decrease
FIG. 4. Color phases of adult Heliothis zea associated with diapause and non-diapause conditions.
D = Diapause  ND = Non-diapause
TABLE I. Oxygen consumption, water content, and fat content of pupae reared under controlled temperature and photoperiod.

<table>
<thead>
<tr>
<th>Rearing Condition</th>
<th>( O_2 ) Consumption ( \mu l/mg ) dry wt./hr.</th>
<th>% Water</th>
<th>% Fat (Dry weight basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{Heliothis zea} )</td>
<td>( \text{Heliothis virescens} )</td>
<td>( \text{Heliothis zea} )</td>
</tr>
<tr>
<td>24°C Diapause</td>
<td>0.89</td>
<td>0.59</td>
<td>68.26</td>
</tr>
<tr>
<td>24°C Non-Diapause</td>
<td>1.73</td>
<td>1.49</td>
<td>69.40</td>
</tr>
<tr>
<td>21°C Diapause</td>
<td>0.53</td>
<td>0.50</td>
<td>67.10</td>
</tr>
<tr>
<td>21°C Non-Diapause</td>
<td>1.64</td>
<td>1.51</td>
<td>68.29</td>
</tr>
<tr>
<td>18°C Diapause</td>
<td>0.51</td>
<td>0.55</td>
<td>67.14</td>
</tr>
<tr>
<td>18°C Non-Diapause</td>
<td>1.61</td>
<td>1.44</td>
<td>67.94</td>
</tr>
<tr>
<td>Field collected (Diapause)</td>
<td>0.58</td>
<td>--</td>
<td>66.61</td>
</tr>
</tbody>
</table>
in oxygen consumption was associated with the diapause condition of both the bollworm and tobacco budworm.

It was noted, however, that the reduction in oxygen consumption by diapausing bollworm pupae reared at 24°C was less than for the other two temperatures. One may assume from these data that the diapause condition of the bollworm is not as intense in pupae reared at the higher temperature. This assumption is valid and will be discussed in more detail under a more appropriate section.

No consistent differences were found in moisture content or crude lipids that could be associated with the diapause condition. There was as much variation between groups of diapause pupae as there was between diapause and non-diapause pupae. These findings are similar to those reported by Ditman et al. (1940).

Effects of Photoperiod and Temperature on Incidence of Diapause.

The results in Table II show that diapause in the bollworm was initiated by a 10-hour photoperiod under temperatures of 18°, 21°, and 24°C. However, 27°C completely neutralized the short photoperiod effect and at 24°C the incidence of diapause was reduced 50 percent. This effect by temperatures of 27° and 24°C was not found in the tobacco budworm. These data show that the response of the tobacco budworm to the short photoperiod was almost the same under the four different temperatures. In contrast 18°C counteracted almost completely the long day effect of inhibiting the onset of diapause in both species. Figure 5 shows the interaction of photoperiod and temperature.
TABLE II. Incidence of diapause in pupae reared under different temperatures and photoperiods.

<table>
<thead>
<tr>
<th>Rearing Temperatures (°C)</th>
<th>Photoperiod</th>
<th>Number Pupae</th>
<th>Average Percent Diapause</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light (hr)</td>
<td>Dark (hr)</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>10</td>
<td>14</td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>10</td>
<td>181</td>
</tr>
<tr>
<td>24</td>
<td>10</td>
<td>14</td>
<td>569</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>10</td>
<td>556</td>
</tr>
<tr>
<td>21</td>
<td>10</td>
<td>14</td>
<td>572</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>10</td>
<td>554</td>
</tr>
<tr>
<td>18</td>
<td>10</td>
<td>14</td>
<td>539</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>10</td>
<td>588</td>
</tr>
</tbody>
</table>
Diapause induced by 18°C temperature-14-hour photoperiod was found to be as intense as the diapause condition at 10-hour photoperiods. Pupae of both the bollworm and tobacco budworm reared under these conditions were held in diapause for twenty months with 76 percent of the pupae surviving at the end of this period.

Normally diapause was terminated during this study by chilling the pupae at 4°C for 5 days and either returning the pupae to the original rearing conditions or holding them at 27°C ± 2°C for adult emergence. Chilling was found to be unnecessary for bollworm pupae entering diapause at 24°C-10-hour photoperiod. Under these conditions the average time of initial adult emergence was the 28th day following pupation and continued for an average of 97 days before emergence was complete in any one test group (repetition). In contrast only 23 percent of the tobacco budworm adults reared under the same conditions, emerged during the same periods of time.

The results in Table II show that the tobacco budworm was more responsive to photoperiod and less influenced by temperature than the bollworm.

Parental Influence on the Incidence of Diapause.

The incidence of diapause was determined in F₁ and F₂ progeny of diapause parents. Results in Table III show that diapause incidence, in both the F₁ and F₂ of both species, is greatly reduced when compared to that of their parents, and that there was a substantial increase in F₂ compared to F₁.
FIGURE 5. Interaction of Photoperiod and Temperature on the Incidence of Diapause
TABLE III. Percent diapause in two generations of progeny from diapausing parents. Parents reared at 18°C and a 14-hour photoperiod.

<table>
<thead>
<tr>
<th>Generation</th>
<th>Rearing Temperature (°C)</th>
<th>Photoperiod</th>
<th>Number Pupae</th>
<th>Average Percent Diapause</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Light (hr)</td>
<td>Dark (hr)</td>
<td>H. zea</td>
</tr>
<tr>
<td>Parents</td>
<td>18</td>
<td>14</td>
<td>10</td>
<td>47</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>24</td>
<td>10</td>
<td>14</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>10</td>
<td>14</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>10</td>
<td>14</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>14</td>
<td>10</td>
<td>91</td>
</tr>
<tr>
<td>F&lt;sub&gt;2&lt;/sub&gt;</td>
<td>24</td>
<td>10</td>
<td>14</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>10</td>
<td>14</td>
<td>92</td>
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<td>18</td>
<td>10</td>
<td>14</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>14</td>
<td>10</td>
<td>90</td>
</tr>
</tbody>
</table>
Temperature and photoperiod at which the parents were reared had the greatest inhibitory effect on the incidence of diapause in F₁ and F₂ progeny. The bollworm was obviously more sensitive to the parental influence than the tobacco budworm.

The tobacco budworm response to the 10-hour photoperiod and 24°C temperature was altered considerably by the parental influence. During this study 94 percent of the pupae normally entered diapause under these conditions. The results show that only 19 percent of the pupae entered diapause under these conditions. This inhibitory effect at 24°C was expressed also in the F₂. This same effect occurs in the bollworm but is not considered as significant since only 40 percent of the bollworm pupae normally entered diapause under these conditions.
DISCUSSION OF RESULTS

Some Physiological Characteristics of Diapause.

In many respects the physiological characteristics of diapause in the bollworm and tobacco budworm were similar to characteristics that have been reported for other insects.

The arrest of gonadal development in the female, the cessation of spermatogenesis in the male, and the reduced level of oxygen consumption have been found to be a common denominator for diapause in many species of insects. The bollworm and the tobacco budworm do, however, depart from the usual in that neither an increase of the fat body nor a decrease in moisture content were associated with diapause, within the conditions imposed upon the two species during the course of this study.

The retention of the larval eye spots by a diapause pupa is a useful character. The character not only provides the earliest means for detecting the diapause condition but also allows for diapause recognition without waiting the necessary period of time for moth emergence or dissecting the pupa for internal characteristics. The value of this character for survey and population studies in the field is obvious.

The dimorphism found to be associated with diapause in the bollworm offers one explanation of light and dark colored moths observed in the field by Quaintance and Brues (1905) and Phillips
Quaintance and Brues suggested, as a result of their work, that some condition other than temperature was involved in effecting the color pattern of bollworm moths. It is now apparent that the effect diapause has on the color of bollworm moths (Figure 4) is an explanation of light colored moths in the field early in the spring, rather than dark moths which would normally be expected, considering the low temperatures to which the pupae had been subjected during the winter. Obviously genetic differences are involved in the response of these two species to the conditions that initiated diapause. Thus, the possibility of genetic differences being also involved in the color differences found in this study cannot be ignored.

Since it is known that the color of an insect is controlled by the neurosecretory cells of the brain (Van der Kloot, 1960), it would seem that these data indicate an effect on these cells by the diapause mechanism. This is substantiated by the fact that much lighter colored moths emerged from pupae that were chilled to terminate diapause and returned to the original rearing conditions than their counterparts that did not enter diapause under the same conditions.

Effect of Photoperiod and Temperature on Incidence of Diapause.

Like many other insects, short day regimes induced diapause in the bollworm and tobacco budworm and long photoperiods prevented diapause development. The two photoperiods (14-hours and 10-hours) used in this study occur in Baton Rouge, Louisiana, near the dates of summer and winter solstices.
It is known, however, that diapause pupae of the bollworm are present in the field during the second week of October (Phillips, unpublished data). This is shortly after the fall equinox (September 21) and day length is near 12-hours duration. Thus the diapause threshold can be assumed to be less than 12-hours but longer than the 10-hour photophase used during this study.

The results of this study show that the bollworm can be added to the list of insects in which diapause has been demonstrated to be suppressed by high temperatures. The response of the tobacco budworm to high temperatures was, however, quite different than that of the bollworm. It will be noted in Table III that the high temperatures, at the short photoperiod, used in this study had little, if any, effect on the incidence of diapause in the budworm. In contrast the lower temperature (18°C) counteracted the long-day effect of inhibiting diapause development in both species. The tobacco budworm was again more responsive to photoperiod than the bollworm.

The response of these two species to the photoperiod-temperature conditions substantiate observations made by Barber (1937). Barber reported that the tobacco budworm entered hibernation during a short period of time in contrast to the longer period of time the bollworm entered hibernation. Barber was able to find diapause bollworm pupae present in the soil throughout the year. This was not found to be true for the tobacco budworm.

Preliminary investigations indicated that both the penultimate and last instars of the bollworm were the sensitive stages to the
photoperiod-temperature condition. With this in mind it can be assumed that a brief cool period during the summer could cause a fortuitous occurrence of diapause in the bollworm. This effect by temperature and the fact that the pupae are able to remain in diapause for at least twenty months is one explanation of bollworm pupae, in diapause, being present throughout the year. It is of course obvious that the response of these two species to temperature at other photoperiod regimes than those employed in this study may be quite different. It seems, however, that the assumptions made, in view of what is presently known, are tenable.

Parental Effect on Diapause

In many respects the parental influence on the incidence of diapause in the bollworm and tobacco budworm is similar to what has been reported by Marcovitch (1924) and Lees (1960) concerning aphids. With aphids the inhibitory effect on sexual formation is related to a time interval rather than the number of generations from the fundatrix. Lees used the term "interval timer" to describe this phenomenon. Lees suggests that the physiological "clock" is set in motion in the fertilized egg rather than in the fundatrix.

It can be seen in Table III that the incidence of diapause in both the bollworm and tobacco budworm was greater in the F2 than in the F1 progeny. There was, however, less increase in the number of individuals entering diapause at the parental conditions. The high temperature was also noted to have an inhibitory effect on the inci-
dence of diapause in the F₁ and F₂ progeny.

If this phenomenon is related to a timing mechanism it certainly seems that its function is temperature-dependent. In this respect it was noted that a temperature higher (21°C) than that to which the parents were subjected shortened the interval for an increase in the percent diapause. At the same time, however, a still higher temperature (24°C) opposed the action of the short photoperiod. The increase in diapause of the F₂ under conditions at which parents were held suggests the time interval to be of considerable duration before the incidence of diapause reaches the level determined in this study. It should be noted here that low temperatures opposed the effect of the short photoperiod. These data suggest that the length of the refractory period was influenced more by temperature than photoperiod.
CONCLUSIONS

Results of this study allow the following conclusions to be made.

Diapause was characterized by (1) retention of larval eye spots in the post genal region of the pupae; (2) arrest of gonadal development in the female; (3) cessation of spermatogenesis in the male; (4) reduced oxygen consumption, and (5) lighter colored adults emerging from pupae which have undergone diapause.

Diapause was initiated by 10-hour photoperiods and inhibited by 14-hour photoperiods. High temperature (27°C) counteracted short photoperiod effects. In contrast low temperatures (18°C) counteracted the long day effect. The sensitive stage was the larva and the responsive stage the pupa. The tobacco budworm was more responsive to photoperiod at the different temperatures studied than the bollworm. Pupae of both the bollworm and tobacco budworm can remain in diapause for at least twenty months.

There was a parental effect that reduced the incidence of diapause in F₁ and F₂ progeny. This effect was apparently influenced by both a time interval and temperature. It is probably the "check" that prevents the bollworm from re-entering diapause in the early spring when it is exposed to environmental conditions that induce diapause.
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Wilde, J. de, C. S. Duintjer and L. Mook. 1959. Physiology of diapause in the adult Colorado beetle. I. The photoperiod as a con-


*Original article was not read.*
APPENDIX I

An Artificial Medium for *Heliothis zea* (Boddie)

For 100 grams of diet:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>22.0 ml</td>
</tr>
<tr>
<td>Casein, vitamin free</td>
<td>3.5 g</td>
</tr>
<tr>
<td>4M KOH</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Alphacel</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Wesson's salt</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>3.5 g</td>
</tr>
<tr>
<td>Wheat germ</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Vitamin solution</td>
<td>1.0 ml</td>
</tr>
<tr>
<td><strong>Contains:</strong></td>
<td></td>
</tr>
<tr>
<td>1.0 mg niacin</td>
<td></td>
</tr>
<tr>
<td>1.0 mg Ca pantothenate</td>
<td></td>
</tr>
<tr>
<td>0.5 mg riboflavin</td>
<td></td>
</tr>
<tr>
<td>0.25 mg thiamine HCl</td>
<td></td>
</tr>
<tr>
<td>0.25 mg pyridine HCl</td>
<td></td>
</tr>
<tr>
<td>0.25 mg folic acid</td>
<td></td>
</tr>
<tr>
<td>0.02 mg biotin</td>
<td></td>
</tr>
<tr>
<td>0.002 mg vitamin B-12</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.4 g</td>
</tr>
<tr>
<td>Antimicrobial agents</td>
<td></td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>0.05 ml</td>
</tr>
<tr>
<td>Methyl parahydroxybenzoate</td>
<td>0.15 g</td>
</tr>
<tr>
<td>Aureomycin</td>
<td>0.03 g</td>
</tr>
<tr>
<td>Agar, hot, dissolved in 62 ml. of water</td>
<td>2.5 g</td>
</tr>
</tbody>
</table>
JACOB R. PHILLIPS was born June 29, 1929 at Newport, Arkansas. He attended elementary school at Newport, Arkansas and high school at Grubbs, Arkansas and graduated in 1947. From September, 1947 to June, 1951 he attended the University of Arkansas where he received the degree of Bachelor of Science in Agriculture. On February 3, 1952 he married Carol D. Diffee of Newport, Arkansas. From 1952 to 1959 he was engaged in farming activities at Grubbs, Arkansas. In January, 1960 he returned to the University of Arkansas as a graduate student in Entomology. In June, 1961 he received the degree of Master of Science with a major in Entomology. He entered Louisiana State University in September, 1961 and was granted a graduate assistantship with the Department of Entomology Research, Louisiana Agricultural Experiment Station. In January, 1964 he was appointed to the staff of the Department of Entomology, University of Arkansas as an Assistant Professor. At present he is a candidate for the degree of Doctor of Philosophy.
EXAMINATION AND THESIS REPORT

Candidate: Jacob R. Phillips

Major Field: Entomology

Title of Thesis: Diapause studies of the bollworm, *Heliothis zea* (Boddie) and the tobacco budworm, *Heliothis virescens* F.

Approved:

[Signature]
Major Professor and Chairman

[Signature]
Dean of the Graduate School

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Date of Examination:

January 8, 1965