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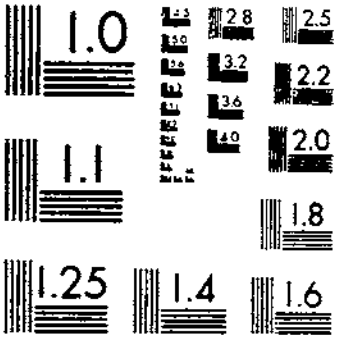

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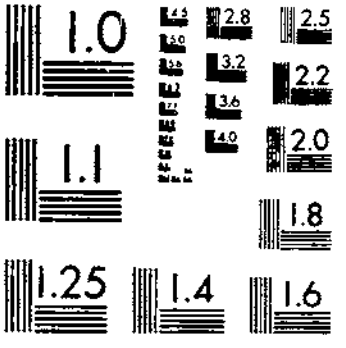

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Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE
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Contents

	Page
Methods	2
Controlled photoperiods	3
Constant photoperiods	4
Increasing and decreasing photoperiods	5
Parental effect on diapause of progeny	7
Temperature studies	9
Boll age	10
Field studies	11
Discussion and conclusions	12
Summary	15
Literature cited	15

Washington, D.C. Issued March 1964

Factors Inducing Diapause in the Pink Bollworm

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The pink bollworm (*Pectinophora gossypiella* (Saunders)) is found in all the major cotton-producing countries of the world except Russia, Peru, Union of South Africa, and Nicaragua. It can inhabit such a wide geographical range undoubtedly because it enters diapause before the seasonal onset of unfavorable environmental conditions. This diapause occurs in the fourth instar. Boll age and cool autumn temperature are associated with the induction of diapause, as reported by many authors in different regions.

Squire (*23, 24*)[2] concluded from work conducted in the Lesser Antilles that diapause of the pink bollworm was initiated by a nutritional factor, because a higher percentage of larvae in diapause were found in older bolls than in young, immature ones. Chapman and Hughs (*6*), at Presidio, Tex., found that the proportion of larvae in diapause was four times greater in 40-day-old bolls than in 20-day-old bolls, when both boll groups were infested on the same date. Vanderzant and Reiser (*28*) reported that diapause was not affected by changes in the protein, carbohydrate, and fat contents of a purified casein larval diet. However, Bull and Adkisson (*4*) stated that a diet containing 5 percent of oil induced more larvae to enter diapause than one containing 1 percent of oil.

Storey (*25*) and Bishara (*2*) reported that cool weather in autumn was an important factor in the induction of diapause. Chapman and Hughs (*6*) found a higher percentage of larvae in diapause in bolls collected during late September than in bolls of the same age collected in August. They attributed this difference to the lower temperatures in September at Presidio. Bedford (*1*) in the Sudan, Nadkerny (*17*) in central India, and Bishara (*2*) in Egypt, all reported that the initiation of diapause was associated with crop maturity. This association might well include effects of both boll maturity and seasonally lower temperature. It is clear, however, that cool temperature is not required to initiate diapause, because

[1] Portion of a dissertation presented by the senior author as a partial fulfillment of the requirements for the degree of doctor of philosophy in entomology at Texas A. & M. University.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 15.

diapause larvae have been collected before autumn; also they were reported in Puerto Rico (Fife *9*), West Indies (Squire *23*), and Hawaii (Busck *5*), where the average temperature in the coldest month of the year did not fall below 70° F. during a 40-year period.

It is obvious that factors other than boll age and temperature must be involved in initiating diapause in the pink bollworm, when one considers the diapause habits of the insect throughout its distribution. In the equatorial region of Africa between the north and south 10° latitudes, diapause larvae were not found in a concerted search made by Bredo (*3*), Geering (*12*), Kirkpatrick (*14*), Maisiour (*15*), Smee (*21*), and Taylor (*27*). In this region the planting date is timed to permit the crop to mature during the dry season, but neither the dry season nor the maturation of the crop is sufficient to induce pink bollworm diapause.

When latitudes of the different pink bollworm-infested regions of the world are considered in relation to the time of induction of larval diapause in the respective areas, some correlations can be noted that suggest a response to photoperiod, or day length. The reports show that in areas with similar latitudes there is very little difference in the time larvae begin entering diapause. In latitudes from 26° to 32° N. (Brownsville, Tex., 26°; Torreón, Coahuila, Mexico, 26°; Presidio, Tex., 29°; Egypt, 30°; Punjab, 30°; Waco, Tex., 32°) diapause is initiated shortly after the summer solstice, when the days begin to shorten, and the proportion of the population in diapause increases as the season advances. Wolcott and Sein (*30*), Squire (*23*), and Geering (*12*) showed that in tropical latitudes between 10° and 22° N., diapause was initiated about September, and the proportion of larvae in diapause increased until December. As previously stated, diapause has not been reported at the Equator, where day length remains constant throughout the year.

The work reported herein was conducted at Brownsville, Tex., to study the effects of different factors on pink bollworm diapause, especially the effect of photoperiod, which was studied under controlled light, temperature, and humidity. The effect of photoperiod on this insect had not been fully studied previously.

METHODS

Studies with controlled photoperiods were conducted in bioclimatic cabinets especially designed for temperature and humidity control, as described by Flitters et al. (*11*). Light in these cabinets was provided by the lighting arrangement reported by Sowell and Rouse (*22*), but 64-inch lamps were used because the cabinets were small. Photoperiods were regulated by time switches. This installation provided approximately 2,500 foot-candles of light at the tops of cotton plants placed in the cabinets.

Plants used in the experiments were grown in 5-gallon heavy-gage metal cans and held in the greenhouse until bolls were suitable for use in the experiments. The date of anthesis was recorded on a small tag affixed to the flower stem for determination of boll age at the time of initial infestation. The plants were infested after placement in the cabinets under controlled photoperiods and held for 30 days for

development of a pink bollworm generation. After completion of the experiments, the plants were removed, pruned back to about 8 inches from the soil surface, and held in the greenhouse for regrowth and production of bolls for use in later experiments.

Eggs used to infest bolls were deposited by moths from diapause larvae, which had been held in storage in infested seed cotton at 50°–55° F. The moths oviposited on small cotton leaflets in oviposition chambers. These leaflets were cut into pieces containing six to eight eggs each. Each piece was placed on one end of a narrow strip of masking tape and the other end of the tape affixed to a cotton boll.

Ten days after the bolls were infested a small plastic-screen bag containing small pieces of soft paper was placed over each boll and securely tied around the petiole. The bags served to retain the larvae that cut out of the bolls for pupation. The larvae pupated in the pieces of paper. The bolls and bags were examined for larvae and pupae after a 30-day developmental period. Larvae were placed in petri dishes with soft paper for webbing and observed for an additional 5 days, or a total of 35 days from hatching. Larvae that had not pupated by the end of this 35-day period were assumed to be in diapause, since the developmental period for short-cycle larvae is 15–18 days under such conditions.

A weighted least square analysis of variance was made of the data obtained under controlled photoperiods to determine statistically significant differences in effect among different photoperiods, age of boll when infested, and the interaction between photoperiod and boll age. The number of larvae that entered diapause was expressed as a percentage of the total number observed. Percentages were then transformed to the arc sine $\sqrt{\text{percentage}}$ to equalize the variance. The means were tested for significance by using the Duncan multiple range test.

To determine the seasonal occurrence of diapause larvae in the field, samples of firm, green bolls were collected and examined at approximately weekly intervals beginning on July 5. Since a cultural-control program, enforced in the lower Rio Grande Valley, requires that stalks be destroyed by August 31, it was necessary to take the September and October samples from plants in screen cages, which measured 6 by 6 by 36 feet. All fourth-instar larvae in the boll samples were removed and placed in petri dishes; those not pupating within 10 days were assumed to be in diapause.

CONTROLLED PHOTOPERIODS

Under controlled photoperiods the experiments were designed to determine the effects of different constant day lengths on the induction of pink bollworm diapause. Subsequent experiments compared effects of increasing and decreasing photoperiods. As the work progressed, the results obtained led to an investigation of parental effect on diapause of the F_1 progeny. The effects of reduced temperature and boll age on larval diapause under different photoperiods were also evaluated.

Constant Photoperiods

Constant 16- and 10-hour photoperiods were selected for the initial experiments because they represented the day-length extremes occurring in the U.S. Cotton Belt. Effects of these two photoperiods were compared in one experiment under a constant temperature of 80° F. and 70-percent relative humidity and in another experiment under a mean temperature of 81.5° and 64-percent relative humidity, the latter simulating the diurnal fluctuating temperatures and humidities recorded at College Station, Tex., during the first 28 days of June 1956.

Larvae developing in these two experiments showed a higher proportion entering diapause in 10-hour day lengths than in 16-hour day lengths, with no difference between the two temperature-humidity conditions, as shown in table 1. Although approximately 60 percent of the larvae entered diapause under the 16-hour light regime, a definite response to photoperiod was indicated by the much higher percentage in diapause in the short day-length treatment. The data indicated that the induction of diapause was affected by boll maturity, but to a less extent than by photoperiod, since the differences in percentage of larvae in diapause among the boll age groups were not so great as those between long and short photoperiods.

TABLE 1.—*Effect of constant long and short photoperiods on induction of pink bollworm diapause* [1]

Age of bolls when infested (days)	16-hour day		10-hour day	
	Larvae observed	Larvae entering diapause	Larvae observed	Larvae entering diapause
	EXPERIMENT 1			
	Number	*Percent*	*Number*	*Percent*
1–10	20	55.0	37	81.1
11–20	140	50.7	122	91.0
21–30	162	68.5	224	99.1
31–40	25	60.0	74	100
41–50	0	0	6	100
Total or average	347	59.9	463	95.7
	EXPERIMENT 2			
1–10	88	51.1	25	72.0
11–20	222	64.4	38	92.1
21–30	158	61.8	243	86.4
31–40	14	57.1	352	100
Total or average	482	60.6	658	93.5

[1] In experiment 1, constant 80° F. and 70-percent relative humidity; in experiment 2, fluctuating temperature with mean of 81.5° and 64-percent relative humidity.

The proportion of larvae entering diapause under constant 16-hour days, as shown in table 1, remained unchanged in other experiments in which the intensity and spectral properties of light were varied and the temperature increased. Results of experiments under constant photoperiods of 10, 12, 14, and 16 hours show that the 14-hour photoperiod resulted in a very high proportion of nondiapause larvae, whereas those photoperiods shorter or longer than 14 hours increased the percentage entering diapause (table 5). The photoperiods, listed in the order of ascending proportions of diapause larvae in each, were 14, 16, 12, and 10 hours. The differences in proportion of diapause larvae among the various photoperiods were significant except between 10- and 12-hour days.

Increasing and Decreasing Photoperiods

During the early studies with a single bioclimatic cabinet, similar photoperiod treatments were repeated under the same temperature in a period extending over both summer and winter. It was assumed that a controlled photoperiod should have the same effect on the induction of diapause during summer and winter. Actually, it was observed that a higher percentage of the larvae entered diapause in summer than in winter. In checking possible factors affecting this phenomenon, it was noted that the plants had undergone a change in photoperiod when they were removed from the greenhouse and placed in the bioclimatic cabinet. During summer, when a high percentage of the larvae entered diapause, the plants had been moved from a long photoperiod to a shorter photoperiod. In winter, when the plants were moved from a short to longer photoperiod, the percentage of the larvae entering diapause was reduced. Based on these observations the effect of changing photoperiods on pink bollworm diapause was investigated.

Examination of published data that indicated a change in diapause response of pink bollworm larvae after midsummer suggested that the seasonal incidence of diapause in nature might be primarily governed by an increase or decrease in day length rather than by actual duration of the photoperiod. Experiments based on this hypothesis were conducted to determine the effects of different ranges of increasing and decreasing photoperiods, which were simulated simultaneously in two cabinets. The College Station mean temperature of 81.5° F. and 64-percent relative humidity were maintained in all experiments.

The initial experiment compared effects of photoperiods increasing from 13.5 to 16 hours at the rate of 15 minutes every 3 days, or an average of 5 minutes per day, with those of photoperiods decreasing from 13.5 to 11 hours at the same rate. Another experiment included photoperiods increasing from 12.5 to 16 hours and decreasing from 16 to 12.5 hours with both light regimes changing at the rate of 10 minutes per day.

Results of these experiments, as shown in table 2, indicated that the decreasing photoperiods were effective in inducing diapause, whereas larvae developing during increasing photoperiods showed a relatively low percentage in diapause. The range over which increasing photoperiods promoted the development of nondiapause larvae is shown in

table 3. Increasing day lengths within 12 to 16 hours was effective in reducing the number of larvae going into diapause, whereas those above and below these ranges were conducive to diapause (table 3).

TABLE 2.—*Effect of changing photoperiods on induction of pink bollworm diapause*

Age of bolls when infested (days)	Increasing photoperiod from 13.5 to 16 hours [1]		Decreasing photoperiod from 13.5 to 11 hours [1]	
	Larvae observed	Larvae entering diapause	Larvae observed	Larvae entering diapause
	Number	*Percent*	*Number*	*Percent*
1–10	0	0	5	100
11–20	174	19.5	327	88.1
21–30	272	38.5	128	95.3
Total or average	446	31.2	460	90.2
	Increasing photoperiod from 12.5 to 16 hours [2]		Decreasing photoperiod from 16 to 12.5 hours [2]	
1–10	23	30.4	25	72.0
11–20	401	20.7	38	92.1
21–30	172	22.7	243	86.4
31–40	0	0	104	100
Total or average	596	21.6	410	89.5

[1] 5 minutes per day.
[2] 10 minutes per day.

TABLE 3.—*Effect of various ranges of increasing photoperiods on induction of pink bollworm diapause*

Photoperiod during larval development (hours)		Average daily rate of increase	Larvae observed	Larvae entering diapause
Beginning	End			
		Minutes	*Number*	*Percent*
9	11	5	472	96.2
10	12	5	349	90.0
10	16	15	349	90.0
12	14	5	242	33.5
12.5	16	10	596	21.6
14	16	5	166	22.3
16	18	5	420	93.6
16	20	15	171	90.0

Data from other experiments show that the larval response to photoperiods increasing from 13 to 16 hours, when the parents emerged and oviposited in a shorter photoperiod, was not significantly different from the larval response to a constant 14-hour photoperiod (see table 5). The response resulting in diapause was significantly higher in decreasing photoperiods from 16 to 13 hours than in increasing photoperiods from 13 to 16 hours, but was significantly lower than in extremely long photoperiods increasing from 16 to 20 hours.

Parental Effect on Diapause of Progeny

A review of data obtained in the early experiments indicated a possibility that the parents exerted some effect on their progeny as a result of photoperiod to which the parents were exposed. To investigate such possible parental effect, pink bollworms were subjected during pupation, emergence, and oviposition to different photoperiods and their F_1 progeny were subjected in turn to other photoperiods in several experiments, as shown in table 4.

TABLE 4.—*Parental effect on induction of diapause in F_1 progeny when parent pink bollworms were subjected during pupation, emergence, and oviposition to different photoperiods and F_1 progeny were subjected in turn to other photoperiods*

Experiment No.	Photoperiod in which—		Progeny observed	Progeny in diapause
	Parents exposed	Progeny exposed		
	Hours	*Hours*	*Number*	*Percent*
PROGENY EXPOSED TO INCREASING PHOTOPERIODS				
1	10	13–16	452	8. 4
	15	13–16	310	15. 2
2	10	13–16	303	6. 3
	12	13–16	234	6. 0
	15	13–16	506	19. 2
PROGENY EXPOSED TO DECREASING PHOTOPERIODS				
3	10	16–13	137	73. 7
	15	16–13	200	71. 0
PROGENY EXPOSED TO CONSTANT PHOTOPERIODS				
4	10	10	68	81. 0
	12	10	40	90. 0
5	10	12	161	75. 2
	12	12	138	87. 2
6	10	14	128	5. 5
	12	14	313	8. 0
7	10	16	160	50. 0
	12	16	147	41. 5

In experiment 1 of this series, cotton bolls infested with diapause larvae were soaked with water to stimulate pupation. They were then divided and the respective halves held under constant 10- and 15-hour photoperiods during pupation, adult emergence, and oviposition. The F_1 larvae from both parent groups developed in photoperiods increasing from 13 to 16 hours. The same procedure for exposing parents with the progeny developing in different photoperiods was used in subsequent experiments.

As shown in table 4, the percentage of larvae entering diapause in experiment 1 was lower when parents were exposed to the shorter photoperiod. Similarly in experiment 2, parent exposure to 10- and 12-hour photoperiods resulted in a significantly lower percentage of diapause larvae than did parent exposure to a 15-hour photoperiod when all F_1 larvae developed in photoperiods increasing from 13 to 16 hours. These two experiments illustrate the pattern found in nature during early summer, since moths that emerge before the summer solstice (increasing day lengths) are exposed to shorter photoperiods than those in which their progeny develop.

In experiment 3, any parental effect was obscured by the results of the decreasing photoperiods in which the progeny developed. The percentage of larvae entering diapause was more than 70 percent from parent exposure to both 10- and 15-hour photoperiods, with the progeny developing in photoperiods decreasing from 16 to 13 hours.

Parental effect was consistently indicated to a slight extent in experiments 4, 5, and 6 (table 4), when the progeny were exposed to constant temperatures. The percentage of F_1 larvae in diapause was lower for parent exposure to 10-hour photoperiods than for parent exposure to 12-hour photoperiods, when the progeny developed in constant 10-, and 12-, and 14-hour photoperiods.

Data showing parental effect together with data from other experiments conducted at 81.5° F. with constant, increasing, and decreasing photoperiods are summarized in table 5.

TABLE 5.—*Summary of data on effect of several photoperiods on induction of pink bollworm diapause at mean temperature of 81.5° F.*

Photoperiod (hours)	Larvae observed	Mean larvae in diapause [1]
	Number	*Percent*
14	571	5.6 a
13 to 16 [2]	801	7.5 a
13 to 16	1,840	23.6 b
16	1,256	64.5 c
16 to 13	1,030	65.4 c
12	299	87.3 d
16 to 20	983	91.7 d
10	864	95.7 d

[1] Means followed by different letter are significantly different at 1-percent level.
[2] Photoperiod in which parents emerged and oviposited was shorter than 13-hour photoperiod at beginning of development of their progeny.

Temperature Studies

After finding that certain photoperiods averted diapause under optimum temperature for larval development, a series of experiments was conducted to investigate the interaction between photoperiods and reduced temperatures. In the first two experiments larvae were reared in two cabinets with increasing photoperiods of 13 to 16 hours, which were known to be favorable for pupation. Eggs used to infest the plants were obtained from moths that emerged and oviposited in a 12-hour photoperiod. The test temperature maintained in one cabinet fluctuated daily from 55° to 85° F. with a mean of 70°, and the College Station temperature of mean 81.5° maintained in the other cabinet was the standard. The test temperature was selected to allow the completion of larval development within a 28-day test period. After this 28-day period, the infested plants were held at a constant temperature of 80° for an additional 12 days to permit pupation of the nondiapause larvae. At the end of this latter period the larvae remaining were considered to be in diapause.

Data from two replicates of the temperature treatments, which gave very similar results, are shown in table 6. The percentage of larvae entering diapause under the reduced temperature averaged approximately 65 percent as compared with 7 percent for the standard, thus indicating that cool temperatures may be of consequence in inducing diapause under a photoperiod otherwise conducive to pupation.

TABLE 6.—*Effect of reduced temperature with increasing photoperiods of 13 to 16 hours on induction of pink bollworm diapause*

Age of bolls when infested (days)	Reduced temperature (70° F.)		Standard temperature (81.5° F.)	
	Larvae observed	Larvae entering diapause	Larvae observed	Larvae entering diapause
	Number	*Percent*	*Number*	*Percent*
1–10	120	54. 2	65	1. 5
11–20	233	69. 5	141	9. 2
21–30	84	69. 1	118	6. 8
Total or average	437	65. 2	324	6. 8

A similar experiment with reduced temperature compared larval diapause under day lengths increasing from 13 to 16 hours with that under day lengths decreasing from 16 to 13 hours. Larvae developing during increasing day lengths showed 57.9 percent in diapause, whereas those developing during decreasing day lengths showed an increase to 88.6 percent in diapause, as given in table 7. Thus, a response to decreasing photoperiods was still evident under the reduced temperature; however, the difference in diapause between increasing and decreasing photoperiods was not so great as in other experiments (tables 2 and 4) with a higher temperature.

TABLE 7.—*Effect of increasing and decreasing photoperiods with reduced mean temperature of 70° F. on induction of pink bollworm diapause*

Age of bolls when infested (days)	Increasing photoperiod from 13 to 16 hours		Decreasing photoperiod from 16 to 13 hours	
	Larvae observed	Larvae entering diapause	Larvae observed	Larvae entering diapause
	Number	*Percent*	*Number*	*Percent*
1–10	36	52. 8	26	76. 9
11–20	201	53. 7	146	88. 4
21–30	72	72. 2	100	92. 0
Total or average	309	57. 9	272	88. 6

Another experiment was conducted to determine the stage of larval development at which the response to cool temperature takes place to induce diapause. The experiment was carried out in 14-hour photoperiods. Larvae were exposed to the cool-temperature regime during different stages of their development from the first week through 20 days after hatching. During the remaining development, they were exposed to the College Station temperature of 81.5° F. Results shown in table 8 indicate that the primary response to the cool temperature occurred during the first and second instars, with the first-instar exposure resulting in the highest percentage in diapause.

TABLE 8.—*Diapause response of different larval stages to reduced mean temperature of 70° F. when pink bollworm larvae were held in 14-hour photoperiod continuously and mean temperature of 81.5° before and after reduced temperature*

Duration of reduced temperature (day)	Approximate larval stages exposed	Larvae observed	Larvae entering diapause
		Number	*Percent*
1–7	1st instar	299	66. 6
7–14	2d and 3d instar	342	40. 4
10–16	3d and 4th instar	215	16. 3
14–20	Prepupal	149	14. 1

Boll Age

No interaction occurred between photoperiod and boll age, as indicated by a statistical analysis of the data obtained under different photoperiods. This analysis included data on effects of boll age as shown in tables 1, 2, 6, and 7, and also those from several other experiments discussed previously.

The data obtained on effect of boll age are summarized in table 9. Larvae that developed in bolls infested when less than 20 days old had the lowest proportion to enter diapause. In bolls infested at 21 to 40 days of age, the percentage of larvae entering diapause increased with age of the bolls. There was no significant difference in percentage of larvae entering diapause between bolls 1 to 10 days of age and bolls 11 to 20 days old when infested; however, the percentages entering diapause in boll groups infested at 21 to 30 and 31 to 40 days of age were significantly different from each other and from those in the younger bolls. The effect of boll age undoubtedly was due to a progressive change in nutrition of the boll and, as indicated by these data, was independent of photoperiod.

TABLE 9.—*Summary of data on effect of boll age on induction of pink bollworm diapause in various experiments*

Age of bolls when infested (days)	Larvae observed	Mean larvae in diapause[1]
	Number	*Percent*
1–10	842	40. 1 a
11–20	3, 381	44. 0 a
21–30	2, 604	54. 8 b
31–40	817	79. 3 c

[1] Means followed by different letter are significantly different at 5-percent level.

FIELD STUDIES

A study to determine the seasonal aspect of pink bollworm diapause in the field was conducted in 1959. Periodic collections of fourth-instar larvae from firm, green bolls were made from July 5 through October 28, as shown in table 10. Mean monthly temperatures at

TABLE 10.—*Seasonal aspect of pink bollworm diapause in green bolls in field*

Date of collection	Mature larvae observed	Larvae in diapause	Date of collection	Mature larvae observed	Larvae in diapause
	Number	*Percent*		*Number*	*Percent*
July 5	23	0	Sept. 3	190	30
July 12	26	0	Sept. 23	190	83
July 19	61	2	Sept. 29	285	84
July 26	75	3	Oct. 7	313	91
Aug. 2	90	4	Oct. 14	140	95
Aug. 9	99	6	Oct. 21	86	95
Aug. 16	107	4	Oct. 28	33	94
Aug. 23	264	17			

Brownsville during this period were July 83.5°, August 84.9°, September 83.9°, and October 76.7° F., with no marked drop in temperature until after the middle of October. Thus, it is apparent that the seasonal change in temperature during the observation period was very slight and could have had no marked effect on the induction of larval diapause before the middle of October.

Diapause larvae were first found in bolls collected on July 19. The percentage of the population diapausing after that date increased each week until 95 percent were in diapause in the collection made on October 14 (table 10). Since the effect of temperature was negligible before the middle of October, this increase in diapause larvae was attributed primarily to the effect of decreasing photoperiods beginning after June 22. As the days progessively decreased in length, a higher percentage of the larvae entered diapause.

The findings in this study agree with data from experiments in the bioclimatic cabinets. The first generation developing after June 22 should include some individuals that enter diapause resulting from decreasing photoperiods, as indicated in the experiments under controlled photoperiods. The collection made on July 19 represented this generation.

DISCUSSION AND CONCLUSIONS

The foregoing data indicate that diapause in the pink bollworm is governed primarily by photoperiod, with boll age and autumn temperature exerting a secondary effect. Whether the effect of photoperiod was exerted directly on the insect or indirectly through effect on the cotton plant is not known. The data may be interpreted to clarify the onset of diapause in different parts of the world where previous reports have appeared to be contradictory.

The absence of pink bollworm diapause between the north and south 10° latitudes is undoubtedly due to the almost constant day length throughout the year in that area. The photoperiod at the Equator is approximately 13 hours, including visible light before sunrise and after sunset. This light period corresponds closely to the 14-hour constant photoperiods maintained in the bioclimatic-cabinet experiments that produced very few diapause larvae.

In areas removed from the Equator, diapause may be initiated or averted by changes in day length. The seasonal variation in photoperiod is dependent on latitude, but during the vernal and autumnal equinoxes there are 12 hours of sunlight everywhere. During the season between the spring equinox and the summer solstice, increasing photoperiods are favorable for the production of nondiapause larvae. Immediately afer the summer solstice, larval response to decreasing photoperiods induces diapause in a small proportion of the population. This response increases with the decrease in photoperiod through the autumnal equinox. After the autumnal equinox, when the photoperiods become shorter than 12 hours, nearly all larvae should be in diapause despite temperature favorable for pupation. This conclusion is substantiated by the field studies at Brownsville and by studies of Fife (*10*) and Storey (*26*) in different areas.

In latitudes above 32° N. a very strong effect on pink bollworm diapause results from the combined larval response to photoperiods above 14 hours, decreasing photoperiods, and cool, late-season temperature. The growing season is relatively short with fewer generations developing than in areas near the Equator. Such conditions occur in the northern cotton-growing areas of the United States. In these areas cotton bolls are not available for infestation until after July 1, and since the crop-production season is usually terminated by frost, it is essential that a high percentage of the larvae enter diapause before frost if the insect is to survive. Such survival requirements are fulfilled by the larval response to the photoperiods and late-season temperature occurring in the area. These factors result in diapause of a high proportion of each generation developing in bolls.

Although the ability to enter diapause early in the growing season insures perpetuation of the pink bollworm in the higher latitudes, it effectively limits the actively breeding population. This reduction in the active population due to diapause, coupled with effective cultural-control practices and fewer generations in these areas, may explain why the insect has been able to exist in the High Plains area of Texas since 1927 and in Oklahoma since 1947, but has caused very little damage. Similar circumstances apparently occur in northern China (Efimov and Miftakhov *8*) and in Turkey, Yugoslavia, and Italy (Nonveiller *18*, Scavone *20*), all located north of the 32° latitude and having less effective cultural-control practices than in the United States. It appears, therefore, that the pink bollworm populations in latitudes above 32° N. may be sufficiently restricted to prevent serious economic damage.

A relationship between photoperiod and natural spread of the pink bollworm is suggested as a result of a review of the worldwide spread, although other factors such as wind direction may be involved. Introduction of the pink bollworm into cotton-growing areas, far removed from known infestations, has been traced to the importation of infested cottonseed; but once the insect has become established in a new area, it spreads gradually away from the Equator.

The pink bollworm was introduced into North America and became established in the Laguna area in northern Mexico. From there it spread northward into the United States, but areas south toward the Equator have remained free from infestations even though extensive cotton production exists there. In South America a similar situation occurred after the introduction of the pink bollworm into Brazil. Most of this country is located south of the Equator, and the infestations moved away from the Equator into Paraguay and Argentina. Venezuela and Colombia, adjacent to Brazil but north of the Equator, are believed to have become infested from seed imported from the Leeward Islands, which lie to the north of both Venezuela and Colombia. The insect has not become a serious pest in Venezuela and Colombia.

The lack of pink bollworm infestation buildup in the equatorial regions of South America and Africa is undoubtedly related to the inability of the insect to enter diapause in areas between the north and south 10° latitudes. Diapause appears to be unnecessary in these

regions, because temperatures are favorable for continuous insect development; however, the occurrence of a dry season that may last for 6 months and the cyclic fruiting habits of plants growing in the Tropics tend to produce a host-free period or a long period in which fruiting hosts are scarce. The low population that exists in these regions is no doubt due to the absence of the diapause stage and the scarcity of food during the dry period. Thus, the equatorial zone appears to have served as an ecological barrier that the pink bollworm has been unable to cross and establish itself on the opposite side unless aided by man. The opposing seasons on the opposite sides of the Equator, long dry periods that restrict host abundance, and the absence of larval diapause in equatorial regions undoubtedly are major factors in limiting the natural spread of the pink bollworm across the Equator.

Insect response to changing photoperiods may not be restricted to the pink bollworm. A report by Katiyar and Long (*13*) shows that in Louisiana the sugarcane borer (*Diatraea saccharalis* (F.)) undergoes a diapause-initiation pattern similar to that of the pink bollworm, and its behavior thus suggests a response to photoperiod. Similarities in the spread and distribution pattern of the pink bollworm to that of other cotton insects can be seen in the following discussion.

The boll weevil (*Anthonomus grandis* Boheman) has long been a major pest of cotton in Central America, Mexico, and the United States. This insect is believed to have originated in southern Mexico or Central America. Its early, rapid spread was to the north away from the Equator. Only in recent years has this insect shown any tendency to move southward. It was first found in Venezuela in 1949 (Whitcomb and Britton *29*) and in Colombia in 1951 (Marin *16*), an indication that its southward movement has been very slow. Both these countries are located north of the Equator, and the insect has not shown any tendency to continue its southward movement across the Equator.

A false pink bollworm, *Sacadodes pyralis* Dyar, is found in Venezuela, Colombia, and several Central American countries. It is not found south of the Equator, but its northward movement appears to be following a pattern similar to that of the boll weevil and the pink bollworm.

Another cotton insect that has failed to become established across the Equator is a red bollworm, *Diparopsis watersi* (Rothschild). This insect is found only north of the Equator and for many years was confined to continental Africa, but recently it has spread northward to the Aden protectorate. A closely related species, *Diparopsis castanea* Hampson, occurs only south of the Equator. Neither species occurs in the equatorial regions of Africa, which include parts of the Belgian Congo, Uganda, Kenya, and Tanganyika (Pearson *19*). These insects may respond to photoperiod and this response may tend to restrict their movement and distribution.

An insect that is probably limited to a certain geographical region by its response to photoperiod is the Colorado potato beetle (*Leptinotarsa decemlineata* (Say)). DeWilde et al. (*7*) have shown that diapause is initiated in this beetle when the photoperiods are shorter than 15 hours. This insect has never been found in southern Texas. The Rio Grande Valley was an important potato-growing area for

several decades. At the present time only a few hundred acres are planted to this crop, but abundant wild solanaceous plants in this area could serve as host plants for the insect. Since the hours of sunlight in the Rio Grande Valley never exceed 15, the insect's response to such photoperiods may account for its absence in the area.

SUMMARY

Laboratory and field studies conducted at Brownsville, Tex., indicated that diapause in the pink bollworm (*Pectinophora gossypiella* (Saunders)) was controlled primarily by photoperiod, with boll age and temperature exerting a secondary effect.

Photoperiods progressively increasing from 12 to 16 hours and a constant photoperiod of 14 hours favored the development of nondiapause larvae, whereas decreasing photoperiods and constant ones above and below 14 hours were effective in inducing larvae to enter diapause. Parental effect on the F_1 progeny was also expressed in the progeny's response to photoperiod. Diapause was induced when the parents emerged in a photoperiod longer than that in which the progeny developed.

Larval response to a mean temperature of 70° F., compared with 81.5°, partially overcame the response to photoperiod. This effect of reduced temperature, which induced diapause, was found to take place in the first and second instars.

The incidence of diapause larvae in bolls infested after the bolls were 20 days old increased with age of the boll. This response to boll age, found to be independent of photoperiod, undoubtedly was due to a progressive change in nutrition of the boll.

It was concluded that the diapause habits of the pink bollworm, as affected by the factors discussed, largely determine the insect's distribution and seasonal abundance.

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