

DIAPAUSE INDUCTION IN THE BOLL WEEVIL

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Abstract

This study examines the diapause response of naturally occurring boll weevil populations in north Mississippi, using weevils collected throughout the growing season (shortly after oviposition in squares) and held under simulated natural environments of temperature and photoperiod. Results indicate that boll weevils enter diapause throughout the growing season, starting with the first generation in July. The percentage of weevils in diapause increases as the season progresses, achieving an average maximum rate of 98.7% late in the year. A greater proportion of males achieve diapause than females at any given time except late in the season, when convergence in the diapause response occurs between the sexes. Models are presented of percent diapause of males and females as a function of julian date of emergence, useful in predicting weevil diapause in Mississippi. More mechanistic models are presented of percent diapause as a function of daylength, which have potential application over a wide region of the cotton Belt.

Introduction

The cotton boll weevil, *Anthonomus grandis* Boheman, was introduced into Texas from Mexico in 1892. The movement of this insect from Central America to the north and east through the United States is well documented (Burke et al. 1986). By 1922, the insect had spread across the eastern Cotton Belt. A decade later, crop destruction resulting from this pest greatly curtailed cotton production in the southeast, an event that forced production to the southwestern U.S. (Frisbie et al. 1989). Adequate control of the insect was not possible until after World War II with the introduction of organo-chlorine insecticides. These chemicals helped restore cotton production to the southeast.

As the boll weevil moved across the eastern Cotton Belt, its ability to survive seasonal weather and host-related changes became important topics for study. Although early investigators recognized the ability of the boll weevil to hibernate (e.g., Hunter and Hinds 1904, Sanderson 1907, Hinds and Yothers 1909), Brazzel and Newsom (1959) formally characterized weevil diapause as a physiological state of reduced metabolism, atrophied reproductive system, increased fat content, and decreased body water content. This definitive research acted as a catalyst for other studies,

but despite several decades of the research, diapause in the boll weevil remains a curiosity of great importance. Although well studied, the process is not well understood. There are several reasons for this ambiguity.

Research reports on diapause of the boll weevil are contradictory and often confusing. Researchers have applied different experimental designs to study the process. For example, weevil strains, rearing conditions, and methods of diapause determination vary among studies. Within studies, rearing conditions often vary between life stages. Most studies examine the progeny of colony weevils reared on artificial diets under static photoperiods and temperatures. Laboratory tests are sometimes unreplicated, and broad conclusions are drawn from poorly designed tests or from preliminary data. Based on experimental designs and conclusions, there appears to be a lack of understanding or appreciation among investigators for the process of diapause itself -- its phases and dynamic nature. Researchers typically study the effects of several token stimuli on diapause induction, and selectively use the results from one test to support conclusions, while ignoring results from other tests that contradict these findings. Thus, relevant data are sometimes overlooked. There is a lack of control of environmental stimuli that potentially affect diapause in the field, and this weakness makes replication difficult if not impossible. Results on the environmental stimuli that potentially influence the process are poorly correlated with field conditions. To further complicate matters, some believe the insect does not diapause at all, rather it overwinters in a state of quiescence (Guerra et al. 1982; 1984). These combined problems make it difficult to distinguish meaningful, scientifically sound results from those that are not.

Reports concerning the role of photoperiod on boll weevil diapause underscore the problem. Photoperiod plays a dominant role in diapause of most insects (Tauber et al. 1986), and several studies have examined its influence on the boll weevil. One of the earliest and most influential studies is that of Earle and Newsom (1964). Based on laboratory studies using colony weevils from Louisiana, they found that an 11-hour fixed daylength induces diapause (59-83%) and a 13-hour daylength suppresses it (6-10%, Table 6). These findings have generally been confirmed by others, but problems in experimental design, interpretation, and lack of reproducibility have led to lasting confusion over the precise role of photoperiod in diapause induction.

For example, Sterling (1966) reported that 8, 10, 11, 12, 14, and 15-hour fixed daylengths induce diapause in colony weevils in Texas and that 12.5, 13, and 16-hour daylengths suppress it. In a subsequent study, Sterling (1972) examined the influence of "long" (15 hour), "intermediate" (13 hour), and "short" (11 hour) daylengths on eggs, larvae/pupae, and emerging adults. Overall, he found an increased percentage of weevils in diapause with decreasing

daylength in each stage; but because diapause rates were so high under all conditions (46-92%), he concluded that photoperiod was unable to override the dietary influences on the process, even though no dietary tests were performed! Lloyd et al. (1967) concluded that an 11-hour photoperiod induces diapause during the immature stages, even though rates never exceeded 45% (their results are suspect for other reasons as well). Using colony weevils reared on artificial diet and fed squares as adults, Harris et al. (1967) also reported that an 11-hour photoperiod initiates diapause and a 13-hour inhibits it, even though their rates were less than 34%.

With all the misunderstanding generated from the literature, perhaps none is more important than the one proclaiming that the weevil does not diapause at all, especially in tropical or subtropical environments (e.g., Guerra et al. 1984). While the animal may use other strategies such as quiescence to overcome periods of adversity, we reject the no-diapause hypothesis based on the preponderance of evidence. While the details of the evidence are confounded, previous work clearly has value. In addition, the boll weevil could not have moved northward without an adaptation to endure harsh environments for long periods independent of its host. It is solely dependent on commercial cotton at the northern latitudes, and cotton is absent from the landscape for 7-9 months of the year. The speed at which the insect moved through the U.S. alone indicates a predisposition to diapause in tropical, as well as temperate, zones.

This study evaluates diapause induction of the boll weevil under simulated natural environments of temperature and photoperiod over the course of the growing season. It places primary emphasis on the effect of photoperiod on diapause.

Materials and Methods

Experimental Procedures

Boll weevils used in all experiments originated from native populations spontaneously oviposited in flower buds (squares) in commercial cotton. Punctured squares were collected from fields in Union Co., MS during the summers of 1989-90 and 1992-94. These fields were located within 3 miles of each other. In 1995 punctured squares were collected in Webster Co., MS.

To minimize age differences among weevils within each collection date, only green, punctured squares were removed from plants prior to flaring. Weevils were primarily first and second instar larvae at the time of collection, although a few eggs or later larval stages were present. The oviposition date of most weevils was estimated at 3-8 days prior to the collection date. Punctured squares were collected on similar dates in July through September each year, although differences in the availability of punctured squares and the availability of

environmental cabinets among years prevented the replication of all collection dates each year. Collection dates in 1989 were July 27, August 18, and September 6; in 1990: July 31 and August 17; in 1992: August 1 and September 12; in 1993: August 18, September 9 and 20; in 1994: July 19 and August 19; and in 1995: August 3, 15, and 29.

Rearing. Squares were brought to the laboratory immediately after removal from plants and were divided among clear plastic boxes (27 x 40 x 10 cm) that served as rearing containers for the weevils. Squares were placed on hardware cloth supported by damp sponges 4 cm from the bottom of the boxes. Screened holes at the ends of the containers allowed for ventilation within. High humidity was maintained to keep the squares from drying.

Weevils were reared in computer-controlled cabinets that simulated field temperatures and daylengths incremented from the date of collection. The computer controlled the temperature and lights dynamically using the following instructions. A daily temperature profile was derived from 20-year averages of daily maximum and minimum temperatures for Stoneville, MS (Hull et al. 1982) and the daily times of sunrise and sunset for Mississippi State, MS. Daily minimum and maximum temperatures in the cabinets were set at sunrise and 1500 hour, respectively. A sine curve described the instantaneous temperatures between sunrise and T1 (75% of the time between 1500 hours and sunset). Two exponential functions described the decline in temperatures between T1 and sunrise the next day. The first function apportioned 75% of this decline between T1 and 2400 hours, and the second function apportioned the remaining 25% between 2400 hours and sunrise. Cabinet temperatures were held at 15-minute values derived from these equations. The computer adjusted the cabinet temperature every 10 seconds by reading two thermocouples, comparing the observed and expected temperatures, and regulating the cabinet heater to maintain the expected value. The A/C compressor ran continuously. Thermocouples were positioned inside of squares held in rearing containers with developing weevils.

Four 25-w, Standard incandescent light bulbs were turned on and off each day at sunrise and sunset (time resolution = one min). To simulate increasing and decreasing light intensity during the early morning and late evening, eight 20-w florescent light bulbs (four Coolwhite and four Daylight) were turned on and off about 47-71 minutes after sunrise and before sunset, respectively, depending on the Julian date. The daily time delay between incandescent and florescent lights simulated the sun's relative position 15° above the horizon, and was determined by a polynomial equation ($R^2 = 0.99$).

Physiological Status of Weevils

Boll weevils were collected from rearing containers each day of the emergence period. Adults were placed in clear

plastic containers (15-cm diam by 6-cm deep) that were provisioned with fresh squares daily for up to 35 days. Most weevils were dissected at 14-21 days post eclosion. They were classified as diapause-induced or reproductive using internal characteristics similar to those applied by Brazzel and Newsom (1959) and Earle and Newsom (1964). Observations (10-100x) were made of the body fat, testis size and appearance, amount of sperm in the vas deferens and seminal vesicles, amount of food in the gut, physiological age of females (Grodowitz and Brewer 1987), ovarian condition, minimum and maximum number of developing follicles, presence of follicular relics, number of eggs in the lateral and common oviducts, and presence or absence of sperm in the spermatheca. While these characteristics provided information on the general condition of the weevil, most were not needed as criteria for separating diapausing from reproductive states. As discovered by earlier investigators (Brazzel and Newsom 1959, Earle and Newsom 1964), two characteristics proved most useful in identifying the physiological status of boll weevils -- the sizes of the gonads and fat body.

Females. The age-grading index of Grodowitz and Brewer (1987) is useful in defining the physiological status of females; however, because of the similarity between ovaries of sexually immature females and those exhibiting diapause, this characteristic could not be used alone in distinguishing diapause, especially in young adults. Diapause-induced females typically have small ovaries with little or no follicular development. This condition is similar to newly emerged females with N1 ovaries (Grodowitz and Brewer 1987). Depending on temperature, it takes females several days to acquire the ovarian characteristics associated with reproduction (Isely 1932, Cole 1970). For this reason, the size of the fat body was used in combination with physiological age of females. Fat body development was measured on a relative scale from 0 (no visible fat) to 6 (maximum fat). The range was divided into six equal parts.

The computer program that determined female physiological status used the following set of rules. Females with parous ovarian development (P1-P4) were classified as reproductive regardless of fat body size. Fat body development of most reproductive females ranged between 1-3, although females with parous ovaries occasionally had enlarged fat bodies (>3). The reduced activity levels of confined weevils may have contributed to this state. Females were also classified reproductive if they had an egg(s) in the lateral oviduct(s) but showed no evidence of follicular relics. Females with nulli-parous ovarian development (N1-N3) and fat body size >3 were classified as diapause-induced. Virtually all these females had N1-N2 ovaries. Occasionally, follicular relics were present at the base of ovarioles that otherwise were classified nulliparous (primarily N1 and N2). This evidence suggests that follicular development and ovulation had occurred but then stopped; thereafter, the ovary

returned to a nulliparous state. Females exhibiting these traits were also classified as diapause-induced regardless of fat body size (fat body size typically was >3). Lastly, females with nulliparous ovarian development (N1-N3) and fat body size <4 were classified as non-reproductive. These females may have been sexually immature at the time of dissection (pre-reproductive), or they may have been diapause-induced but lacked the morphological traits associated with the diapause syndrome.

Males. Two criteria were used to determine the physiological status of males: testis size and the appearance of sperm bundles in the testis lobes. Testis size was a relative measure based on the combined diameters of both lobes of one testis applied against the entire length of the abdomen. A very small testis was <3/8 the abdomen length, a small testis was 3/8-1/2 the abdomen length, medium 1/2-3/4, and large >3/4. Testis size was rarely <1/4 or >1, the length of the abdomen. Occasionally males did not become reproductive regardless of age.

The computer program that determined the physiological status of males used the following rules. Males <10 days old were not classified. Those with small to very small testes were classified diapause-induced regardless of other conditions. Most of these weevils had fat bodies >3 and showed prior evidence of sperm production. Remaining males with medium to large testes were classified reproductive, unless they lacked sperm bundles in the testes lobes, in which case they were classified as non-reproductive.

Analytical Procedures

Mean emergence dates of boll weevils were calculated for groups sorted by sex, year, and collection date (SAS Institute, 1989). Frequency counts were obtained for non-reproductive, reproductive, and diapausing weevils in each group. The proportions of reproductive and diapausing adults were calculated after excluding non-reproductive weevils. Percentages of diapausing males and females were plotted by year (dependent variable) at their respective mean emergence dates (independent variable). A logistic equation was used to describe this relationship, having the form:

$$F(x) = k/(1+\exp(a*(b-x))) \quad (1)$$

where $F(x)$ is the percentage of boll weevils attaining diapause given emergence on Julian date x , and a , b and k are parameters to be estimated. The parameters can be described as (a) the slope of the curve, (b) the mid-point of the curve along the x-axis (Julian date), and (k) the upper asymptote of the curve.

To examine the influence of daylength on diapause, we identified the daylength (sunrise to sunset) associated with the Julian date of mean emergence for weevils in each group, and substituted this independent variable in the

relationship above. Logistic (2) and Weibull (3) functions were used to describe percent diapause as a function of daylength, having the forms:

$$F(x) = k/(1+\exp(a*(b-x))) \quad (2)$$

and

$$F(x) = 100*\exp(-(x/a)^b) \quad (3)$$

where $F(x)$ is the percentage of weevils attaining diapause given emergence on a day with daylength x , and a , b and k are parameters to be estimated. This relationship is important because photoperiod is the most common environmental cue regulating diapause in insects (Tauber et al. 1986), and for this reason, equations 2 and 3 have potential application in predicting diapause throughout the Cotton Belt. To explore this application, these equations were used to compare the diapause response of boll weevils from north Mississippi with those from the Rio Grande Valley of Texas. A third-degree polynomial equation (4) described the relationship between daylength and Julian date for Starkville, MS and Brownsville, TX. Equation (4) was substituted for x in equations 2 and 3, transforming the mechanistic relationship of percent diapause versus daylength to the utilitarian relationship of diapause versus Julian date.

Results and Discussion

Diapause is a physiological, morphological, and behavior response of insects to environmental stimuli that occurs in advance of adverse conditions. It requires a period of receptivity to environmental cues that trigger this multifaceted response, and a period of preparation during which time the animal develops the diapause syndrome. Because the boll weevil overwinters as an adult, it potentially has a considerable amount of time in which these events can occur. In fact, the process of diapause initiation can begin as early as the egg stage, as thought by Earle and Newsom (1964).

Seasonal Manifestations of the Diapause Response

Cotton grown in north Mississippi typically produces squares the size preferred by boll weevils for oviposition (about 5-mm diam or larger, Lincoln et al. 1963) beginning in mid-June. First-generation weevils emerge from these squares during July. Using this chronology for initiating field populations, our results indicate that a portion of each generation acquires the diapause syndrome, and that the percentage of boll weevils attaining diapause increases progressively during the season (Figure 1). Illustrating the diapause response of a population at the time of emergence is a meaningful way to represent the process because the diapause syndrome is acquired during the adult stage, and diapause control applications directed against the weevil must precede the acquisition of the syndrome in order to prevent adults from leaving the field and entering overwintering sites.

The logistic equation (1) did a good job describing percent diapause relative to Julian date, depicting a flattened “S-shaped” curve that tails out at the beginning and end of the growing season (Figure 1, solid line; $R^2 = 0.884$ for males and 0.909 for females). Although some diapause is predicted at early dates, most of the diapause response falls within a range of dates that is biologically meaningful. For example, diapause predictions of 1 and 98% are observed on Julian dates 177.2 and 287.0 (June 26 and Oct 14) for males and 197.8 and 290.5 (July 17 and Oct 17) for females. The upper asymptote of the curves is 98.7% (Table 1A, parameter k), indicating that, on average, some members of a population will never initiate diapause, even late in the year. This observation is consistent with reports from the literature (e.g., Brazzel and Hightower 1960, Lloyd and Merkl 1961, Mitchell and Mistic 1965, Sterling 1971).

At any given time, a greater proportion of males achieve diapause than females, although gender differences decrease toward the end of the season due to a common upper asymptote of the curves (Figure 1A and B). According to equation 1, males emerging on Julian dates 217.6, 230.4, and 243.4 (August 6, 18, and 31) attain diapause at rates of 25, 50, and 75%. Predicted rates of diapause on these dates are 7.1, 22.1, and 51.5% for females. Viewed in another way, emerging females acquire diapausing rates of 25, 50, and 75% on 232.0, 242.8, and 253.8 (August 20, 31, and September 11) -- a 14, 12, and 10-day delay for females relative to males at these rates. An initial delay in the appearance of diapause in females (e.g., 21 days at 1%), and convergence in the diapause response between the sexes at the end of the season (e.g., a common value of k), results in a greater rate of increase in the diapause response of females relative to males (Table 1A, parameter b). A greater diapause response of males relative to females at fixed daylengths is reported in the literature (Earle and Newsom 1964, Cobb and Bass 1968, Mangum et al. 1968).

There is considerable variation in the diapause response of boll weevils among seasons. For example, percent diapause of males and females was considerably lower than expected during the mid-season in 1989, although more typical rates were observed during the early and late season (Figure 1, circles). The diapause response was much steeper in 1995 than expected, starting lower and ending higher than normal (Figure 1, hexagons). These seasonal deviations increased the variability in the diapause response along the curve. This variation is not due to differences in photoperiod, as day-lengths remain stable from year to year in a given location. It is not due to temperature differences among years, as immature and adult weevils were held under near identical temperatures while in the laboratory. With the exception of 1995, all boll weevils originated from a small area, probably ruling out genetic differences among populations. The reason for these deviations is under investigation.

Diapause as a Function of Daylength

Equation 1 is useful in predicting percent diapause throughout the growing season in north Mississippi; however, it does not describe the underlying mechanism(s) responsible for the process and thus has limited value in determining diapause outside this specific region of the Cotton Belt. A more meaningful relationship is percent diapause versus daylength. Photoperiod is the most common environmental cue regulating diapause in insects because of its stable seasonal relationship at any location. Decreasing daylengths are used by insects to warn of approaching environmental change harmful to their survival. To describe this relationship, we regressed percent diapause versus daylength observed on the day of emergence. The logistic equation (2) did a good job describing this relationship for females (Figure 2B, solid line; Table 1B, $R^2 = 0.914$), but it predicted higher diapause of males at the longer daylengths of the early season relative to the Weibull function (Figure 2A, dashed line versus solid line). For this reason, the Weibull function (3) was chosen to represent the process for males (Table 1C).

Regression analyses indicated that daylengths of 14.32, 13.68, 13.32, 12.88, and 12.06 hours produce male diapause rates of 1, 25, 50, 75, and 95%, respectively. Females attain the same rates given 14.32, 13.25, 12.91, 12.55, and 11.83 hours of daylight. It is difficult to compare these results with those from the literature because of the variation among studies. Earle and Newsom (1964) reported that the critical photoperiod for boll weevils (e.g., the daylength eliciting 50% of a population into diapause) falls between 12 and 13 hours. Using these results as a guide to better define the critical photoperiod, Mangum et al. (1968) examined the diapause response of males and females at 4 fixed daylengths between 12 and 13 hours. Just over half of the males (52-57%) achieved diapause under a 12.3-hour daylength. Diapause never exceeded 48% among females, even under the shortest daylength examined. From these results, it appears that the critical photoperiod falls close to 12.5 hours for males and 11.8 hours for females -- shorter times than those reported in the present study.

Seasonal trends in diapause can be re-examined by inserting the polynomial equation (4) for x (daylength) in equations 2 or 3. This analysis retains the mechanistic relationship between diapause and daylength, while providing an intuitive, Julian date approach for examining the diapause response over the course of the season. We used this approach to compare expected diapause of boll weevils from north Mississippi and the Rio Grande Valley of Texas. The Valley was chosen because it represents an extreme southern latitude in the U.S. Cotton Belt, and reports suggest that the boll weevil may not diapause at this location (Guerra et al. 1982, 1984). Daylengths were obtained for both areas (Figure 3), and equation 4 was fit primarily to the portions of the curves with decreasing daylengths (Table 2). We assumed that increasing

daylengths do not induce diapause because this situation generally implies improving conditions for the growth of cotton and thus the boll weevil.

These analyses produced virtually identical diapause response curves in Mississippi to those given by equation 1 (Figure 1A and B, dotted lines versus solid lines). They also revealed a higher proportion of Texas populations achieving diapause compared to Mississippi between Julian dates 176 and 213 (June 25 and August 1) (Figure 4). This condition resulted from the shorter daylengths observed in Texas during this period (Figure 3). Decreasing daylengths occur only at the end of the cotton-growing season in south Texas, leaving developing weevils little exposure to diapause-inducing photoperiods. June 25 represents one of the earliest dates of weevil emergence that will permit exposure to decreasing daylengths during development. This date also represents the beginning of harvest, which extends through the end of August. Harvest is nearly completed in the Valley by August 1. According to the model, males will acquire diapausing rates of 20 and 49%, and females 6 and 20%, between June 25 and August 1. Weevils may have trouble achieving these projected rates because of the lack of nutrition after eclosion. Other implications of the model are discussed below.

There are several assumptions in the use of the model in regions outside of the Midsouth. We assume that photoperiod is the primary mechanism for diapause induction in the boll weevil, and that the weevil responds to this mechanism in a similar manner across the Belt. Based on the variability in the diapause response observed in virtually all studies (e.g., Figure 1), we know that photoperiod is not the only mechanism involved. The weight of the evidence supports it as the primary mechanism; however, weevils may have adapted to the specific range of daylengths in their area. In this case, the model will have to be calibrated for different regions of the Belt. We also assume that increasing daylength does not induce diapause, as this situation generally implies improving conditions for cotton and the insect rather than deteriorating conditions. To the best of our knowledge, the influence of increasing daylengths on diapause induction has never been examined in the boll weevil. Similar to this assumption, the application of the model in other regions implies that the rate of change in daylength during the life of the insect (prior to the onset of diapause) does not affect the diapause response *per se*. Longer maximum daylengths at the summer solstice (around June 21-22), and more rapid declines in daylengths moving toward the winter solstice, will occur at more northern latitudes of the Cotton Belt. These phenomena are clearly seen in Figure 3, comparing photoperiods from northern Mississippi and southern Texas. While the rate of change in daylength may not influence diapause induction *per se*, it may influence the depth of the diapause response, and thus the amount of time required to satisfy diapause development (e.g., the duration of diapause). For example, weevils in north Mississippi

may retain their diapause status longer than those from south Texas. Based on these assumptions, the model indicates that a small portion the population potentially diapauses in south Texas. The proportion is much higher in north Mississippi.

References

- Brazzel, J.R., and L.D. Newsom. 1959. Diapause in *Anthonomus grandis* Boh. J. Econ. Entomol. 52: 603-611.
- Brazzel, J.R., and B.G. Hightower. 1960. A seasonal study of diapause, reproductive activity and seasonal tolerance to insecticides in the boll weevil. J. Econ. Entomol. 53: 41-46.
- Burke, H.R., W.E. Clark, J.R. Cate, and P.A. Fryxell. 1986. Origin and dispersal of the boll weevil. Bull. Entomol. Soc. Am. 32: 228-238.
- Cobb, P.P. and M.H. Bass. 1968. Some effects of photoperiod, temperature, and food on the induction of diapause in the boll weevil. J. Econ. Entomol. 61: 624-625.
- Cole, C.L. 1970. Influence of certain seasonal changes on the life history and diapause of the boll weevil, *Anthonomus grandis* Boheman. Ph.D. Diss., Texan A&M University. 114 pp.
- Earle, N.W. and L.D. Newsom. 1964. Initiation of diapause in the boll weevil. J. Ins. Physiol. 10: 131-139.
- Frisbie, R.E., J.K. Walker, Jr., K.M. El-Zik, and L.T. Wilson. 1989. Perspective on cotton production and integrated pest management, pp. 1-9. In R.E. Frisbie, K.M. El-Zik, and L.T. Wilson (eds.), Integrated Pest Management Systems and Cotton Production. John Wiley and Sons, New York.
- Grodowitz, M.J., and F.D. Brewer. 1987. Ovarian anatomy and physiological age-grading of the female boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae). Ann. Entomol. Soc. Am. 80: 642-651.
- Guerra, A.A., R.D. Garcia, and J.A. Tamayo. 1982. Physiological activity of the boll weevil during the fall and winter in subtropical areas of the Rio Grande Valley of Texas. J. Econ. Entomol. 75: 11-15.
- Guerra, A.A., R.F. Garcia, P.R. Bodegas, and M.E. De Coss. 1984. The quiescent physiological status of boll weevils (Coleoptera: Curculionidae) during the noncotton season in the tropical zone of Soconusco in Chiapas, Mexico. J. Econ. Entomol. 77: 595-598.
- Harris, F.A., E.P. Lloyd, H.C. Lane, and E.C. Burt. 1967. Influence of light on diapause in the boll weevil. I. Dependence of diapause response on the spectral composition of the light used to extend the photoperiod. J. Econ Entomol. 60: 1565-1567.
- Hinds, W.E. and W.W. Yothers. 1909. Hibernation of the Mexican cotton boll weevil. U.S. Dept. Agric. Bur. Entomol. Bull. 77.
- Hull, D.D, M.A. Brown, G. Rench, J.S. Hursh, and C.D. Ranney. 1982. Stoneville, Miss., weather normals, 1960-79. U.S. Dept. Agric., Agric. Res. Serv., ARM-S-25.
- Hunter, W.D. and W.E. Hinds. 1904. The Mexican cotton boll weevil. U.S. Dept. Agric. Bur. Entomol. Bull. 45.
- Isely, D. 1932. Abundance of the boll weevil in relation to summer weather and to food. Ark. Agric. Exp. Stn. Bull. No. 271.
- Lincoln, C., G.C. Dowell, W.P. Boyer, and R.C. Hunter. 1963. The point Sample method of scouting for boll weevil. Ark. Exp. Stn. Bull. 666.
- Lloyd, E.P., and M.E. Merkl. 1961. Seasonal occurrence of diapause in the boll weevil in Mississippi. J. Econ. Entomol. 54: 1214-1216.
- Lloyd, E.P., F.C. Tingle, and R.T. Gast. 1967. Environmental stimuli inducing diapause in the boll weevil. J. Econ. Entomol. 60: 99-102.
- Mangum, C.L., N.W. Earle, and L.D. Newsom. 1968. Photoperiodic induction of diapause in the boll weevil, *Anthonomus grandis*. Ann. Entomol. Soc. Am. 61: 1125-1128.
- Mitchell, E.R., and W.J. Mistic, Jr. 1965. Seasonal occurrence of diapause and hibernation of the boll weevil in North Carolina. J. Econ. Entomol. 58: 309-312.
- SAS Institute. 1989. SAS user's guide: statistics. SAS Institute, Cary, N.C.
- Sanderson, E.D. 1907. Hibernation and development of the cotton boll weevil. U.S. Dept. Agric. Bur. Entomol. Bull. 63, Pt I.
- Sterling, W.L. 1966. Factors involved in the seasonal and geographical regulation in the boll weevil, *Anthonomus grandis* Boheman. M.S. Thesis, Texas A&M University. 113 pp.
- Sterling, W.L. 1971. Winter survival of the boll weevil in the high and rolling plains of Texas. J. Econ Entomol. 64: 39-41.

Sterling, W. 1972. Photoperiodic sensitivity in the ontogeny of the boll weevil. *Environ. Entomol.* 1: 568-571.

Tauber, M.J., C.A. Tauber, and S. Masaki. 1986. *Seasonal Adaptations of Insects.* Oxford Univ. Press, New York.

Table 1. Parameter estimates and R^2 values for (A) the logistic equation (1) describing the relationships between percent diapause of male and female boll weevils versus Julian date of emergence, and (B) the logistic equation (2) and (C) the Weibull function (3) describing the relationships between percent diapause of male and female boll weevils versus daylength on the day of emergence.

A. Diapause Versus Julian Date (Eqn 1)				
Sex	k	a	b	R^2
Male	98.7141	0.0865	230.114	0.884
Female	98.7283	0.1023	242.553	0.909

B. Diapause Versus Daylength (Eqn 2)				
Sex	k	a	b	R^2
Male	97.8228	-2.8542	13.3123	0.888
Female	97.7309	-3.2546	12.9205	0.914

C. Diapause Versus Daylength (Eqn 3)			
Sex	a	b	R^2
Male	13.5121	26.1629	0.880
Female	13.1207	25.3256	0.901

Table 2. Parameter estimates and R^2 values for the polynomial equation (4) describing the relationships between daylength and Julian date for Starkville, MS (Julian dates 152-319) and Brownsville, TX (dates 166-304).

Location	a	b	c	d	R^2
MS	-3.10379	0.243234	1.050943e-03	1.3222677e-06	0.884
TX	0.87852	0.179896	-7.802525e-04	9.8755672e-07	0.999

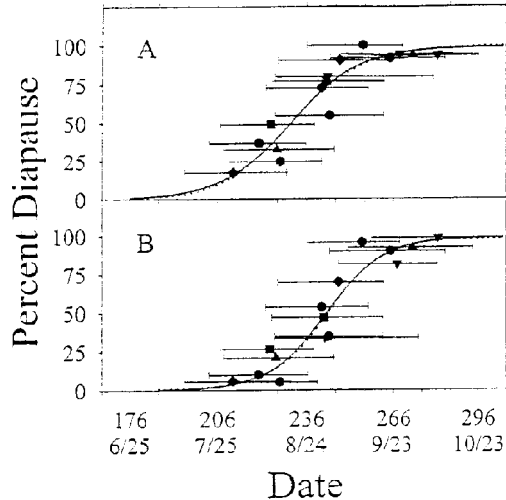
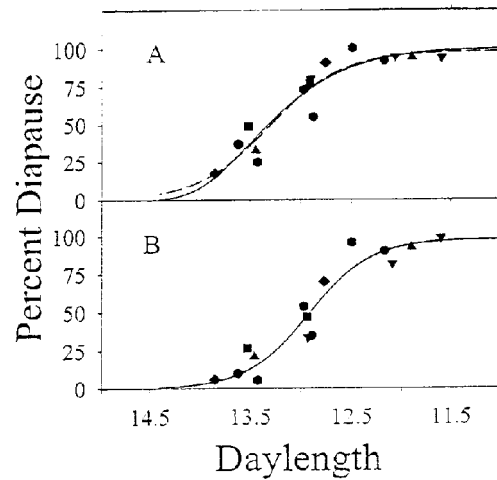


Figure 1. Percentage of (A) male and (B) female boll weevils attaining diapause as a function of Julian date at weevil emergence. Circles represent data from 1989, squares 1990, up triangles 1992, down triangles 1993, diamonds 1994, and hexagons 1995. The bars associated with the data represent the mean lifespans of weevils from oviposition (collection date minus 5 days) to dissection. Solid lines represent the logistic equation (1) fit to the data. Dotted lines represent predictions using equations 3 and 4 for males and equations 2 and 4 for females.

Figure 2. Percentage of (A) male and (B) female boll weevils attaining diapause as a function of daylength (hours between sunrise and sunset) at the time of weevil emergence (symbols are the same as Figure 1). Solid lines represent the Weibull function (3) fit to the data for males and the logistic equation (2) for females. Dashed line for males represents the logistic equation (2).



equation (2) for females. Dashed line for males represents the logistic equation (2).

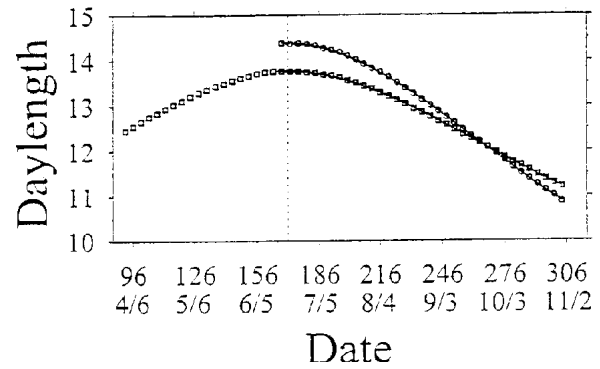


Figure 3. Daylength (hours between sunrise and sunset) for Starkville, MS (circles) and Brownsville, TX (squares) as a function of Julian date. Solid lines represent a polynomial equation (4) fit to the data during decreasing daylengths.

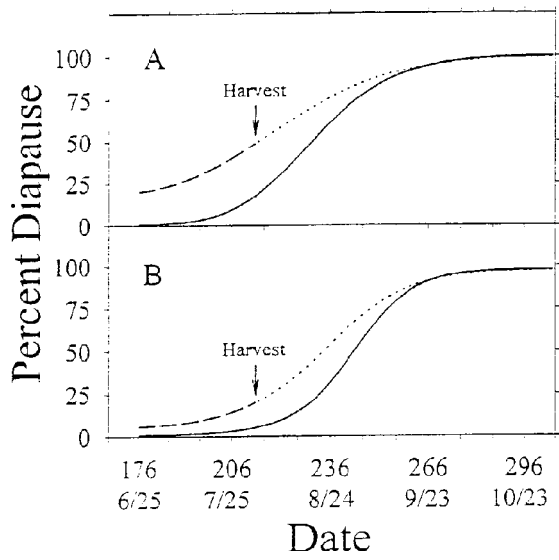


Figure 4. Predicted percentage of (A) male and (B) female boll weevils attaining diapause during the cotton-growing season in northern Mississippi (solid lines) and the Rio Grande Valley of Texas (dashed and dotted lines). Predictions generated using equations 3 and 4 for males and equations 2 and 4 for females.